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ORIGINAL ARTICLE

P. Gosselin · M. Jangoux

From competent larva to exotrophic juvenile: a morphofunctional study of the perimetamorphic period of *Paracentrotus lividus* (Echinodermata, Echinoida)

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Abstract The perimetamorphic period in *Paracentrotus lividus* lasts for 8–12 days. It starts from the acquisition of larval competence, includes the change in form (metamorphosis) and the endotrophic postlarval life, and stops with the appearance of the exotrophic juvenile. All major postlarval appendages already occur in competent larvae being either grouped into the echinoid rudiment (terminal plates, early spines and primary podia) or scattered within the larval integument (genital plates and sessile pedicellariae). Competent larvae show particular behaviour which brings them close to the substratum. The latter is tested by primary podia protruding through the vestibular aperture of the larva. Primary podia are sensory–secretory appendages that are deprived ampullae. They are able to adhere to the substratum in order to allow evagination of the echinoid rudiment (i.e. metamorphosis) and substatum adhesion of the postlarva. Particular spines are borne by the postlarva; these are multifid non-mobile appendages forming a kind of protective armour. Like those of the larva, all characteristic structures of the postlarva (primary podia, multified spines and sessile pedicellariae) are transitory and regress either at the end of postlarval life (primary podia) or during early juvenile life (multifid spines and sessile pedicellariae). Other appendages that develop during postlarval life (i.e. podia with ampulla, point-tipped spines and sphaeridiae) are similar to those borne by the adults and become functional when the individual enters its juvenile life. Thus, the perimetamorphic period appears to be a fully fledged period in the life-cycle of *P. lividus*, and presumably in the life-cycle of any other sea-urchin species.

P. Gosselin (✉) · M. Jangoux
Laboratoire de Biologie Marine,
Université de Mons-Hainaut,
B-7000 Mons, Belgium
Fax: +32/65/37.34.34;
e-mail: Pol.Gosselin@umh.ac.be

M. Jangoux
Laboratoire de Biologie Marine,
Université Libre de Bruxells (CP 160/15),
B-1050-Brussels, Belgium

M. Jangoux
Centre Régional d'Etudes Côtières (CREC),
Université de Caen, F-14530 Luc-sur-Mer,
France

A. Introduction

Many sea-urchin species have a planktonic planktotrophic larva, the so-called echinopluteus, whose morphology, development and biology has been intensively studied over the past 150 years (for review see Dawidoff 1948; Hyman 1955; Strathmann 1975). In most species, echinoplutei take a few weeks to become competent larvae, which means larvae able to enter metamorphosis (Chia 1978). Competent echinoplutei usually have eight arms and a well-developed echinoid rudiment (Strathmann 1978). The echinoid rudiment occurs inside an epidermic invagination or vestibule that is located on the left-hand side of the larval body. It corresponds to the lower part of the body of just postmetamorphic individuals (Burke 1987). Competent echinoplutei show particular behaviour which brings them close to the substratum (Strathmann 1978). They will metamorphose providing they are adequately stimulated by environmental factors (Highsmith 1982; Pearce and Scheibling 1990). Metamorphosis usually lasts less than 1 h, and implies the evagination of the echinoid rudiment and the resorption of most larval tissues (Burke 1987). The resulting individual resembles a miniaturized adult that is as yet deprived of a mouth and anus (Burke 1987). This postlarval endotrophic stage lasts a few days during which the postlarva undergoes some major changes before becoming an exotrophic juvenile (Gordon 1926; Hinegardner 1969).

The present work deals with the common European sea-urchin *Paracentrotus lividus*. It aims to describe precisely the changes in behaviour and external morphology presented by individuals from the stage of larval competence to the appearance of exotrophic juveniles, and to assess their biological significance.

B. Materials and methods

Adult *Paracentrotus lividus* (Lamarck, 1816) were collected intertidally on a rocky shore at Morgat (Brittany, France) in June 1995. There were maintained in marine aquaria (33‰, 19° C) at the biological station of Luc-sur-Mer (Normandy, France) and fed to satiety with kelp (*Laminaria digitata*). Spawning was induced by injecting 2 ml of 0.5 M KCl into the body cavity of individuals. Fertilized eggs were transferred at a concentration of 250 embryos/l into large plastic bags of 200 l capaciy. Natural sea-water previously filtered (15 μm) and allowed to settle for 48 h was used for rearing the larvae. The water was aerated and stirred constantly, and larvae were fed using cultivated planktonic algae (*Phaeodactylum tricornutum*). Competent larvae were induced to metamorphose by placing them into marine aquaria containing adult sea-urchins and their food according to the procedure of Gosselin and Jangoux (1995).

For scanning electron microscopy (SEM), competent larvae, postlarvae and early juveniles were fixed either for 4–10 h in Bouin's fluid without acetic acid at room temperature or for 30 min at 4° C in 3% glutaraldehyde in cacodylate buffer (0.2 M, pH 7.4). Fixed larvae were dehydrated in a graded series of ethanol concentations, dried by the critical-point method, mounted on aluminium stubs, coated with gold and observed with a Jeol JSM-6100 scanning electron microscope. For the examination of skeletal structures, specimens were wholly or partially cleaned of their associated soft tissues, either in 10% (v/v) common bleach (*eau de javel*) for 10–30 min at room temperature or in 1% trypsin solution in phosphate buffer (pH 7.0) for 30 min at 37° C. Partially digested individuals, cleaned rods (competent larvae) or ossicles (postlarvae, juveniles) where air dried and processed as before.

C. Results

I. Competent larvae

In our rearing conditions, larvae become competent around the 18th day after fertilization. They then measure about 500 μm long and 300 μm wide and are at the eight arms stage (Fig. 1). The larval body can be divided into a ciliated oral region and a glabrous aboral region separated by the circumoral ciliary band (Figs. 1A, 3). The oral region overlaps noticeably onto the aboral region on both sides of the larval body, so forming the left and right lateral fields (Fig. 1D). The anus and the hydropore are both included in the aboral region (Figs. 1A, C). At this stage the plutei have two pairs of epaulets, one anterior and the other posterior. The two epaulets of a same pair make contact under the anus (Fig. 1A; *AE*) and under the hydropore (Fig. 1C; *PE*), but the pairs are separated by the aforementioned lateral fields (Fig. 1D). Hence, the four epaulets form a nearly continuous ciliated ring perpendicular to the long axis of the larval body.

On their right-hand side, competent larvae present one to four sessile pedicellariae of the ophiocephalous type. These pedicellariae are set alone or in pairs on each side of the right lateral field, just under the epaulets (Figs. 1A, D, 3). They are supported by two ossicles that develop on contact with the skeletal rods one sustaining the right posterodorsal arm and the other the anterolateral and postoral right arms. Later on, these ossicles will be integrated into the test of the postmetamorphic individual where they will form the DE and AB genital plates, respectively (Fig. 1D). In the same way, the future CD genital plate grows on contact with the basal part of the dorsal arch, progressively surrounding the larval hydropore (Fig. 1C). Finally, BC and EA genital plates appear independently of the skeletal rods: the BC plate in the centre of the right lateral field and the EA plate in the inferior region of the larval body (Fig. 1D). In competent larvae, red pigment cells are associated with each future genital plate. Moreover, these plates support small ectodermic protrusions corresponding to future spines. These two characteristics make them easy to locate.

All other ossicles that will compose the test of postmetamorphic individuals develop within the echinoid rudiment. Most of them mineralize independently except for the A and E terminal plates. The latter are born by the skeletal rods sustaining, respectively, the left posterodorsal larval arm and the left anterolateral and postoral larval arms. They mineralize from the extremity of a short excrescence carried by the respective rod as opposed to genital plates DE, AB and CD which develop on contact with the larval rods. The position of A and E terminal plates, set back from the rod, allows them to be incorporated into the echinoid rudiment.

At competence, the echinoid rudiment distends somewhat on the left side of the larval body (Fig. 3). It is connected to the outside by an opening located in the left lateral field, the vestibular pore. This pore remains closed for the length of the pelagic life. On account of the development of numerous ossicles within the echinoid rudiment, the vestibule is relatively opaque. However, the first five podia could be spotted; they are distributed on the periphery of the echinoid rudiment, alternating with five groups of point-tipped spines (Fig. 1B). Spines and podia have their distal extremities directed towards the centre of the vestibular cavity.

Before acquiring competence, reared plutei swim near the air/water interface, their arms oriented towards the surface. Competence is characterized by a typical behaviour that larvae repeat systematically 2–4 times min when placed in a dish containing a small volume of water. First, they swim near the surface in their usual position (swimming phase; Fig. 2A). Then, they suddenly interrupt the ciliary beatings of their epaulets and go down until they reach the bottom of the dish (sinking phase; Fig. 2B) (during this phase, the epaulets' cilia remain perpendicular to the long axis of the body and act like a small parachute). Just after coming in contact with the substratum, the larvae pull the epaulets' cilia down to the bottom, bend their preoral lobe forwards and spread out their postoral and posterodorsal arms located on the left side. This last distortion allows the opening of the vestibular pore and the partial protruding of the primary podia (control phase; Figs. 2C, 4). These transformations are classically regarded as allowing an assessment of the quality of the substratum by the podia (see for example, Burke 1980). Primary podia first contact the substratum through the peripheral part of their therminal disc and then through all the apical surface of the disc. Without the appropriate stimulation, plutei fold back their podia

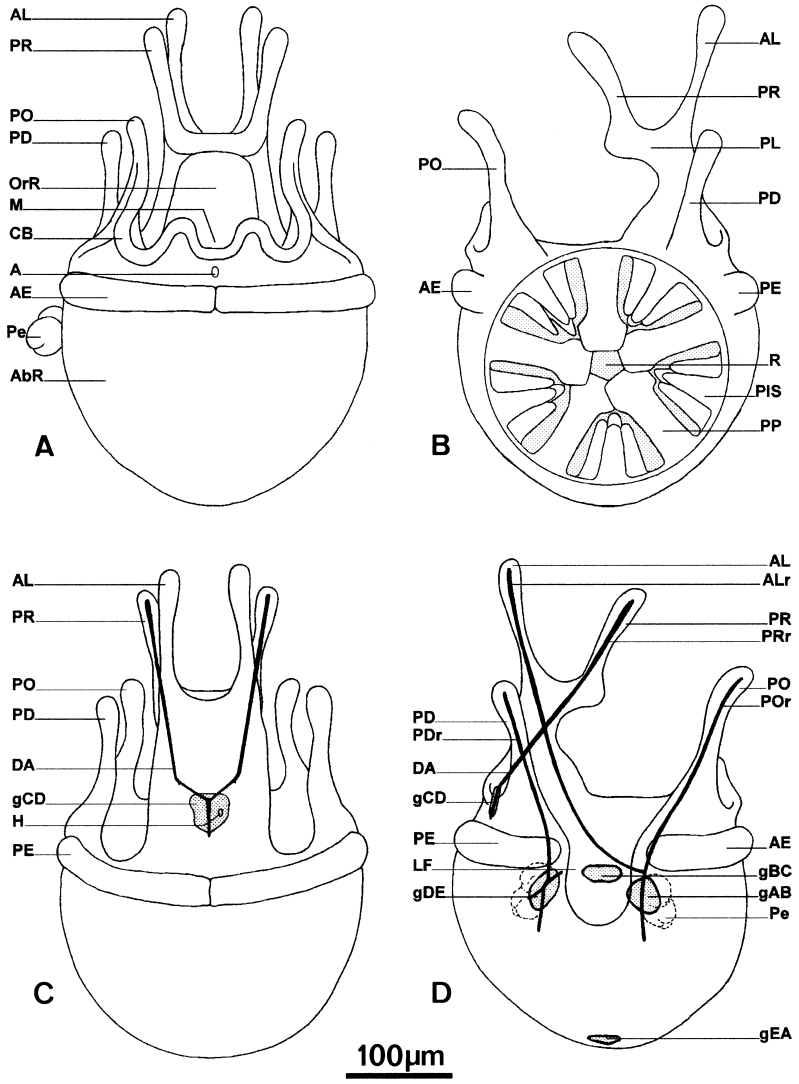


Fig. 1A–D *Paracentrotus lividus*. Diagrammatic representation of a competent echinopluteus. **A** Ventral view. **B** Profile view (left-hand side) showing through the appendages (primary podia and spines) of the echinoid rudiment located inside the vestibule. **C** Dorsal view showing through the dorsal arch and its relation with the CD genital plate, pierced by the hydropore. **D** Profile view (right-hand side) showing through the skeletal rods and the localization of the five genital plates. *A* Anus, *AbR* aboral region, *AE* anterior epaulet, *AL* anterolateral arm, *ALr* anterolateral rod, *CB* ciliary band, *DA* dorsal arch, *gAB*, *gBC*, *gCD*, *gDE*, *gEA*, AB–EA genital plates, *H* hydropore, *LF* lateral field, *M* mouth, *OrR* oral region, *PD* posterodorsal arm, *PDr* posterodorsal rod, *Pe* pedicellaria, *PE* posterior epaulet, *PIS* point-tipped interambulacral spine, *PL* preoral lobe, *PO* postoral arm, *POr* postoral rod, *PP* primary podium, *PR* preoral arm, *PRr* preoral rod, *R* echinoid rudiment

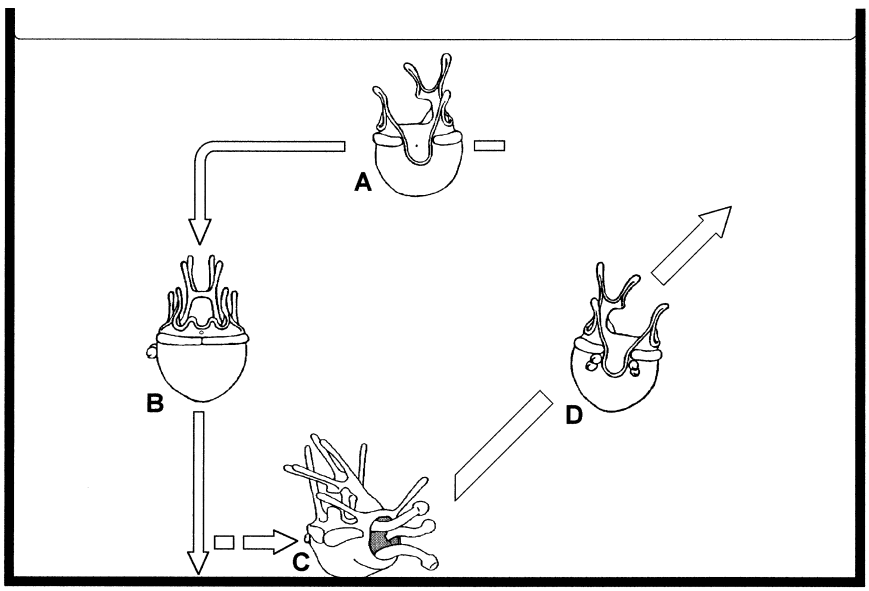
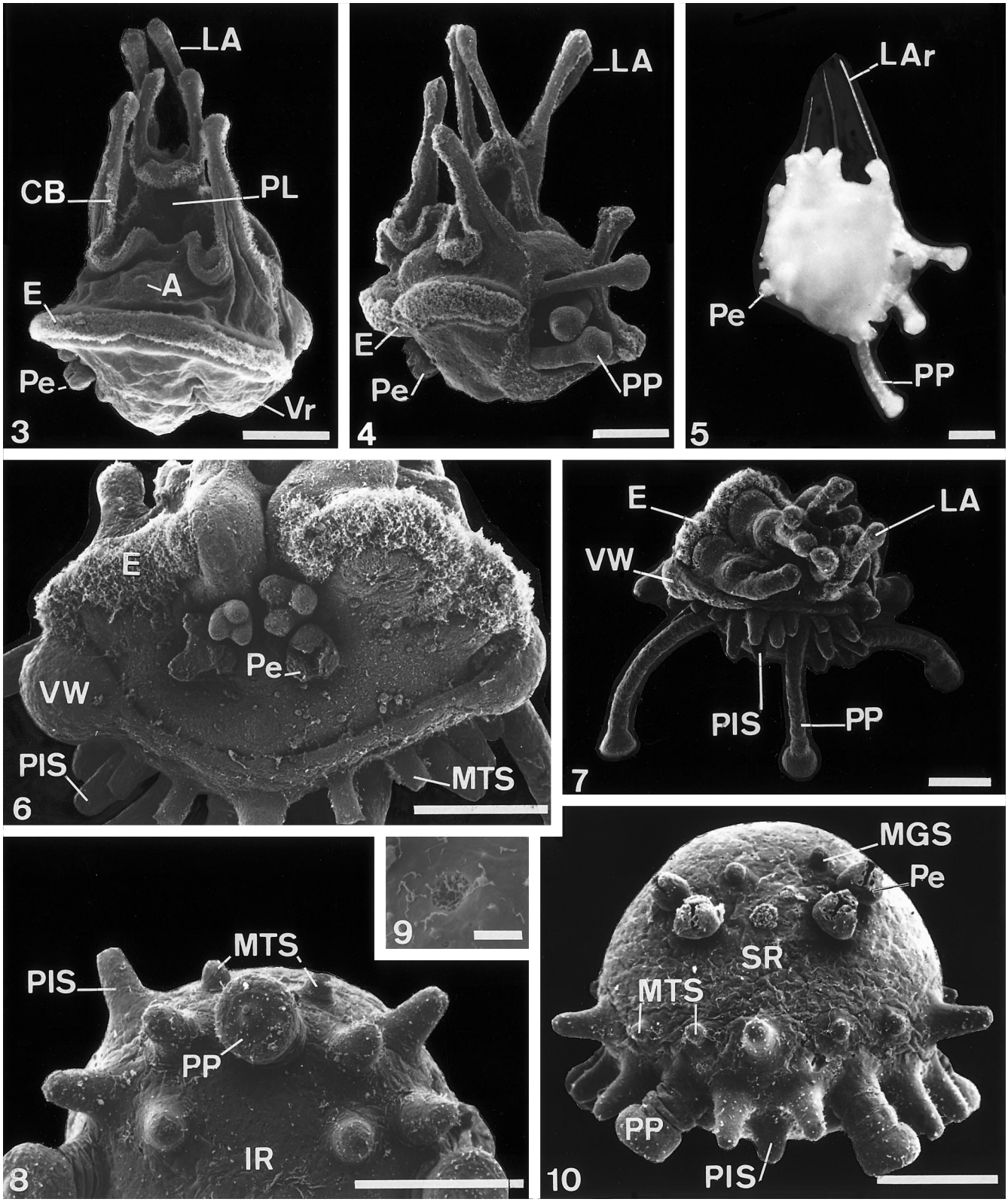


Fig. 2A–D *P. lividus.* Behavioural sequence shown by competent larvae in rearing conditions. **A** Swimming phase (near the air/water interface). **B** Sinking phase. **C** Control phase. **D** Ascending phase. Not to scale



Figs. 3–10 *P. lividus*. Competent larvae, metamorphic larvae and postlarvae. *A* Anus, *E* epaulet, *IR* infracoronal region, *LA* larval arm, *LAr* larval rod, *MGS* multified genital spine, *MTS* multifid terminal spine, *Pe* pedicellaria, *PIS* point-tipped interambulacral spine, *PL* preoral lobe, *PP* primary podium, *SR* supracoronal region, *Vr* vestibular region, *VW* vestibular wall. *Bars* 10 μm for **Fig. 9**, 100 μm for **Figs. 3–8, 10**

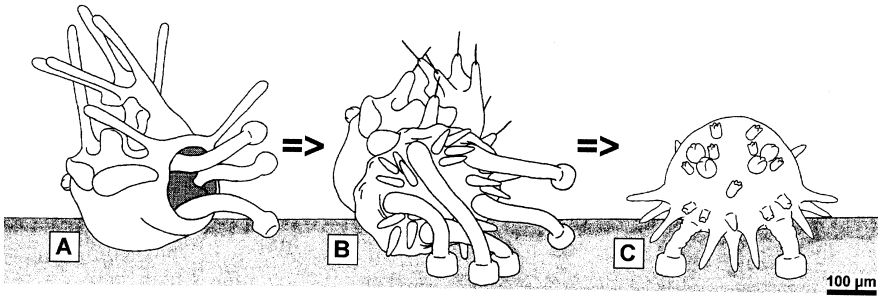


Fig. 11A–C *P. lividus.* Metamorphosis. After a positive recognition of the substratum during a control phase (**A**), the larva opens its vestibule, allowing the protrusion of the echinoid-rudiment's spines and primary podia that hold in to the substratum (**B**), and transforms in less than 1 h into a spherical postlarva (**C**). *Bar* 100 µm

into the vestibule, replace their arms in their original position and go back up to the surface following an oblique direction, thanks to the beating of the epaulets' cilia (ascending phase; Fig. 2D). However, if the plutei meet a suitable stimulation, they will engage the process of metamorphosis.

II. Metamorphosis

Metamorphosis consists essentially of the evagination of the echinoid rudiment (Figs. 5, 7, 11B) and the resorption of the major part of the larval tissues. It will take place only after a positive recognition of substratum by the larva, as described before (Figs. 2C, 11A). When metamorphosis begins, the spines of echinoid rudiment lift up, distend the vestibular wall and, consequently, lead to the wide opening of the vestibular pore. The echinoid rudiment then protrudes reducing the vestibular wall to a small fringe underlining the bases of its spines and podia (Fig. 7). Thereafter, the vestibular wall spreads progressively over the rest of the larval body covering notably the epaulets (Fig. 6). At the same time the future genital plates move until they form an apical circle. The individuals then reveal their new symmetry: the part centred on what was the echinoid rudiment will become the lower surface and the opposite part, including the genital plates, will become the upper surface.

Tissue resorption is achieved assentially by the retraction of the epidermis that covers the larval arms. This process starts at the same time as metamorphosis and lasts about 30 min. The skeletal rods are gradually stripped (Figs. 5, 1B) and will generally be broken down. They comletely disappear 24 h after metamorphosis. The larval epidermis shrinks to the base of the rods before it is covered by the vestibular wall and finally lysed. Some parts of the larval epidermis, such as those associated with the genital plates and with their appendages (spines and pedicellaria), remain after metamorphosis. These parts never present in SEM any signs of deterioration. They are not lysed, but eventually merge together with the vestibular wall in order to form the aboral epidermis of the postlarva.

Metamorphosis is completed in about 1 h and ends with the formation of an endotrophic individual, the postlarva (Figs. 10, 11C). The spherical postlarva presents a clear radial symmetry. It will realize the major part of its imaginal ontogenesis to become an exotrophic juvenile in about 8 days. During metamorphosis and all the postlarval life, the primary podia are unremittingly active: they detach from the substratum, stretch out, retract and fix again to the substratum. Hence, the postlarvae are always moving, but do not go very far from the place they have initially selected.

The postlarval body can be divided in three regions: a midcoronal region, an upper supracoronal region and a lower infracoronal region. The infracoronal and coronal regions are directed towards the substrate forming the lower surface of the postlarva, and the supracoronal region towards the water column forming the upper surface. The supracoronal region is evenly covered with 10-µm-long cilia. It includes the genital plates, bearing the pedicellariae and the spines developed during the larval life, and the hydropore, which is a single and heavily ciliated orifice of about 10 µm diameter (Fig. 9). The coronal region is subequatorial and is very similar to what the adult coronal region will be. It includes ten zones: five interambulacral areas, each presenting a group of four point-tipped spines, and five ambulacral areas each presenting one central primary podium and two spines in aboral positions (Fig. 8). Each podium is formed by an extensible stem (from 80 to 500 µm in length) and by a terminal disc (ca. 60 µm wide) which includes a single, circular skeletal piece with an open-work design (Fig. 17). The primary podia are deprived of ampullae and their light directly communicates with the corresponding ambulacral canal. The infracoronal region covers the rest of the postlarval body. In the young postlarva, it is free of appendages and is outlined by a ciliary band.

◀ **Fig. 3** 18-day-old (18 d) competent larva in ventral view (the distortion of the integument indicates the dimension of the vestibular region which includes the echinoid rudiment)

Fig. 4 18 d competent larva. The primary podia emerge by the vestibular pore

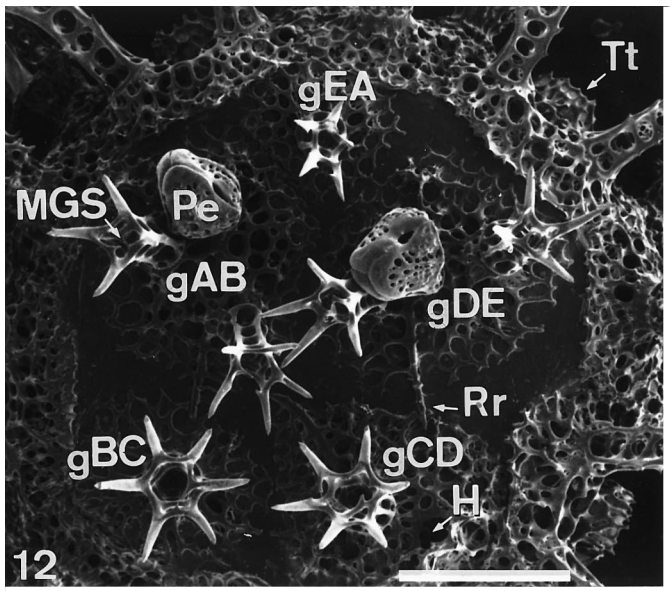
Fig. 5 General view of a metamorphic larva, photographied *in vivo*

Fig. 6 Upper region of a metamorphic larva (the vestibular wall spreads over the larval integument and the epaulets are twisted)

Fig. 7 General view of a metamorphic larva attached to the substratum

Figs. 8, 9 Detail of a part of the corona (**Fig 8**) and of the hydropore (**Fig. 9**) of a 1-day-old (1 d) postlarva

Fig. 10 Upper view of a 1 d postlarva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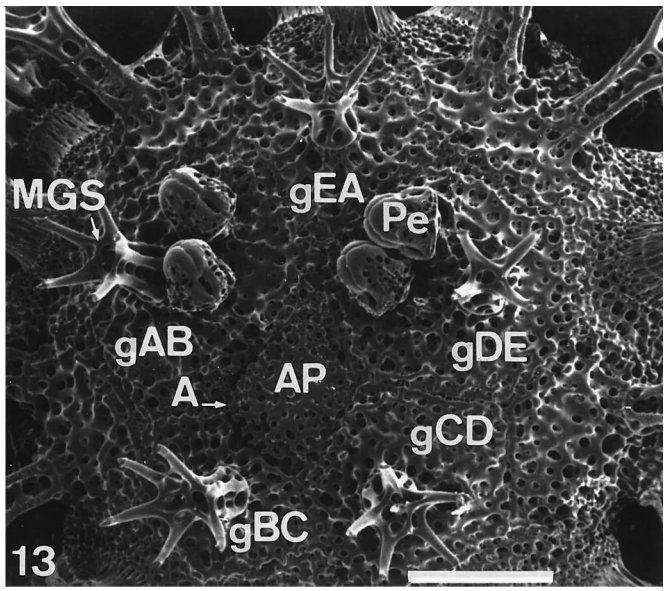
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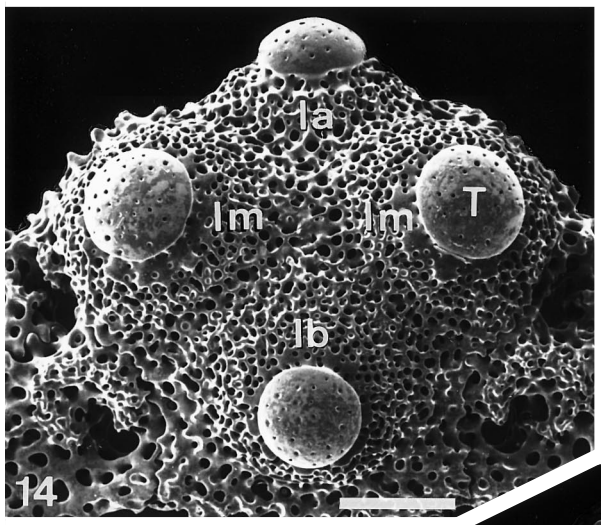
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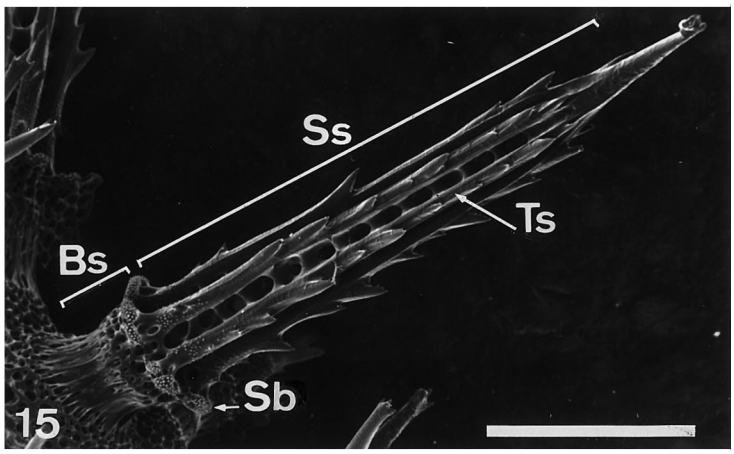
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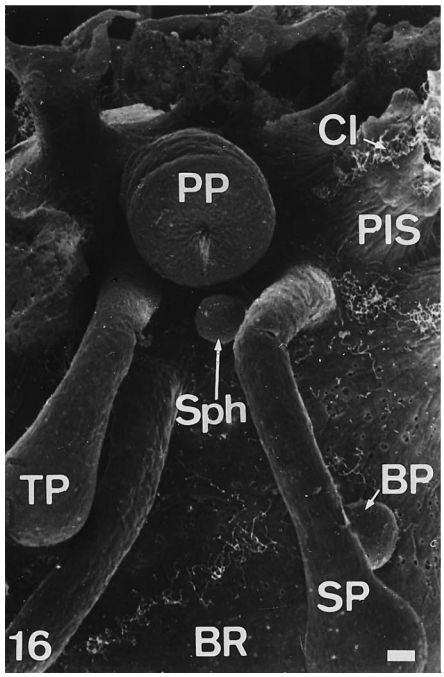
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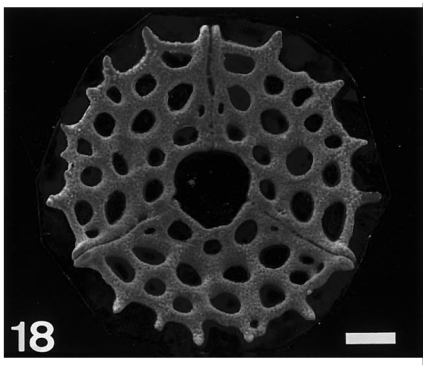
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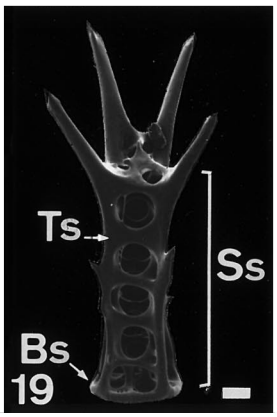
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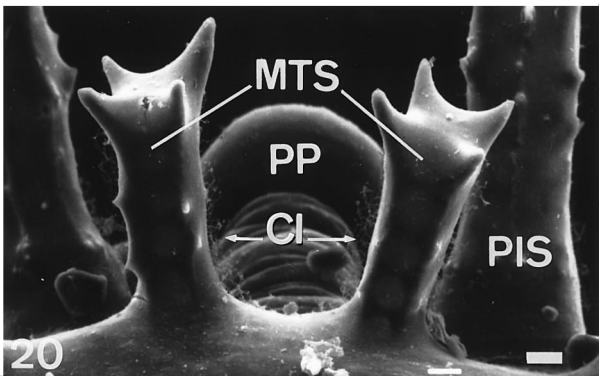
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III. Postlarval life

The postlarval stage results from metamorphosis and precedes the juvenile stage. It is very short (only lasting about 8 days), but is very active from an organogenetic point of view: at the end of its postlarval life, individuals will have acquired mobility and the ability to feed. During this period, the diameter of the test barely increases (it goes from ca. 280 μm to ca. 350 μm). Conversely, the coronal spines grow noticeably and an 8-day-old (8 d) postlarva can reach a total diameter (spines included) of ca. 800 μm against ca. 500 μm in a 1-day-old (1 d) postlarva.

1. Change in the supracoronal region

Being very rounded just after metamorphosis, the supracoronal region will progressively flatten and become of less importance while the coronal and infracoronal regions develop. If the supracoronal region makes up about two-thirds of the postlarval body just after metamorphosis, it represents only half of that 3 days later. The supracoronal region includes genital plates each bearing at least one spine, with the AB and DE plates bearing two spines and one (or two) pedicellaria(e) (Figs. 12, 13). Located in front of the interambulacral plates of the corona, the genital plates remain deprived of genital pores during the entire postlarval life.

In a 1 d postlarva (early postlarva), genital plates do not contact each other (Figs. 12, 21A). The widest plates (ca. 120 μm in length), AB and DE, can still present some relics of larval skeletal rods (Fig. 12). The genital plates present glabrous and non-mobile spines with a multiple apex, the multifid genital spines (MGS). These spines are formed by a stem of galleried stereom and a very inconspicuous base which fits exactly in a circle of skeletal processes borne by the underlying genital plate. Their stem is formed by four to six longitudinal trabeculae joined by stereomic bridges. The tip of each stem grows free and away from the axis of the spine, giving it its characteristic multifid aspect.

In a 6-day-old (6 d) postlarva (late postlarva), the five genital plates are attached to one another and touch the coronal plates (Figs. 13, 21B). The EA plate is the smallest (about 60 μm wide) and is pushed to the periphery of the apical region because of the development of the AB and DE plates (Fig. 13). The AB, BC, CD and DE genital plates delimit the future periproctal zone, where the first anal plate appeared (Fig. 13). The latter do not form sutures with the surrounding genital plates. At this stage, the MGS have reached their final length (ca. 60 μm).

2. Change in the coronal region

From the very first day of postlarval life the coronal plates make contact, forming the outline of a rigid test.

Interambulacral areas. The number of interambulacral plates remains unchanged during postlarval life. Each interambulacral area is composed of four plates forming a lozenge: one apical plate, two median plates and one basal plate (Figs. 14, 21). Each plate bears a tubercle, made of compact stereom, on which a spine with a pointed tip is articulated (point-tipped interambulacral spine or PIS). These spines are mobile and provided with two lateral ciliated bands ca. 60 μm length from their base (Fig. 16). Around the fourth day of postlarval life, the tips of the six longitudinal trabeculae forming the stem converge and merge into a single apical tip. After fusion, at the end of postlarval life, the PIS can reach up to 320 μm in length (Fig. 15). The PIS present a six-branched corwn-shaped basal part that is articulated with the tubercle of the underlying interambulacral plates (Fig. 15).

Ambulacral areas. In each ambulacral area, the number of plates increases from three to five during postlarval life. In a 1 d postlarva (early postlarva), each ambulacral area includes three skeletal plates: a pair of ambulacral plates and one terminal plate in an apical position (Fig. 21A). These three plates form an oval space from which the primary podium emerges. Just after metamorphosis, the ambulacral plates are free of appendages and independent one from the other and from the other surrounding plates. The terminal plate bears a pair of multifid spines (terminal multifid spines or MTS) and a short stereomic outgrowth, a so-called tongue, overtaking the basis of the podium (Figs. 12, 21). The MTS are similar to their homologues on the genital plates regarding their development and shape. However, MTS differ from MGS by their size (the stem and distal tip can reach up to 80 μm and 30 μm, respectively), their general aspect (their stem is made up of four longitudinal trabeculae instead

◀ **Figs. 12–20** *P. lividus*. Postlarval structures. *A* Anus, *AP* anal plate, *BP* buccal podium, *BR* buccal region, *Bs* basal part of the spine, *Cl* lateral ciliary band of the multifid terminal spine, *gAB, gBC, gCD, gDE, gEA* AB–EA genital plates, *H* hydropore, *Ia* apical interambulacral plate, *Ib* basal interambulacral plate, *Im* median interambulacral plate, *MGS* multifid genital spine, *PP* primary podium, *Rr* remnant of larval rods, *Sb* stereomic bud allowing the attachment of the muscles, *SP* secondary podium, *Sph* sphaeridia, *Ss* stem of the spine, *T* tubercle of a point-tipped interambulacral spine, *TP* tertiary podium, *Ts* trabecula of the spine, *Tt* tongue of the terminal plate. *Bars* 100 μm for **Figs. 12–15**, 10 μm for **Figs. 16–20**

Figs. 12, 13 Disposition of the skeletal plates in the supracoronal region in 1-day (**Fig. 12**) and 5-day (**Fig. 13**) postlarvae

Figs. 14, 15 Disposition of the interambulacral plates (**Fig. 14**) and skeleton of a point-tipped spine (**Fig. 15**) in a 4-day postlarva

Fig. 16 Detail of an ambulacral area in 7-day postlarva (note that the stem of the primary podium is regressing)

Figs. 17, 18 Apical rosette of the terminal disc of a primary podium in a 1-day postlarva (**Fig. 17**) and of a secondary podium in a 4-day postlarva (**Fig. 18**)

Fig. 19 Skeleton of a terminal multifid spine in a 1-day postlarva

Fig. 20 Aboral view of a pair of terminal multifid spines in a 4-day postlarva (one ciliary band runs on each side of these spines)

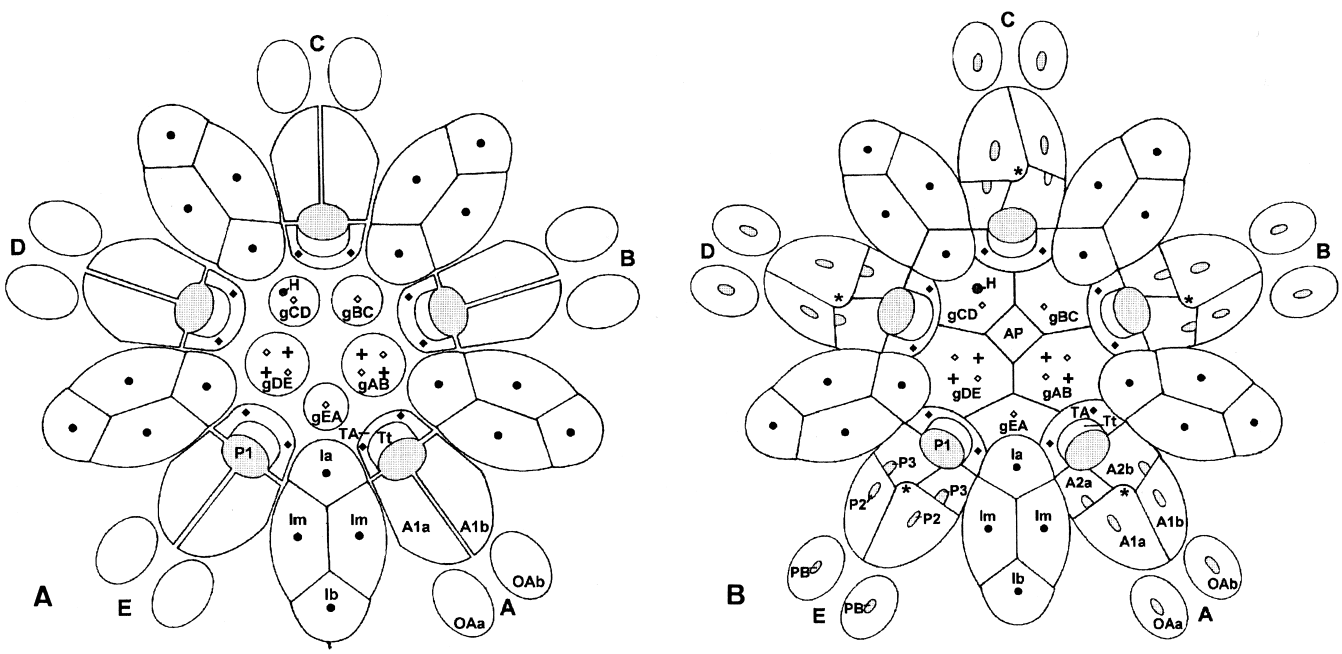


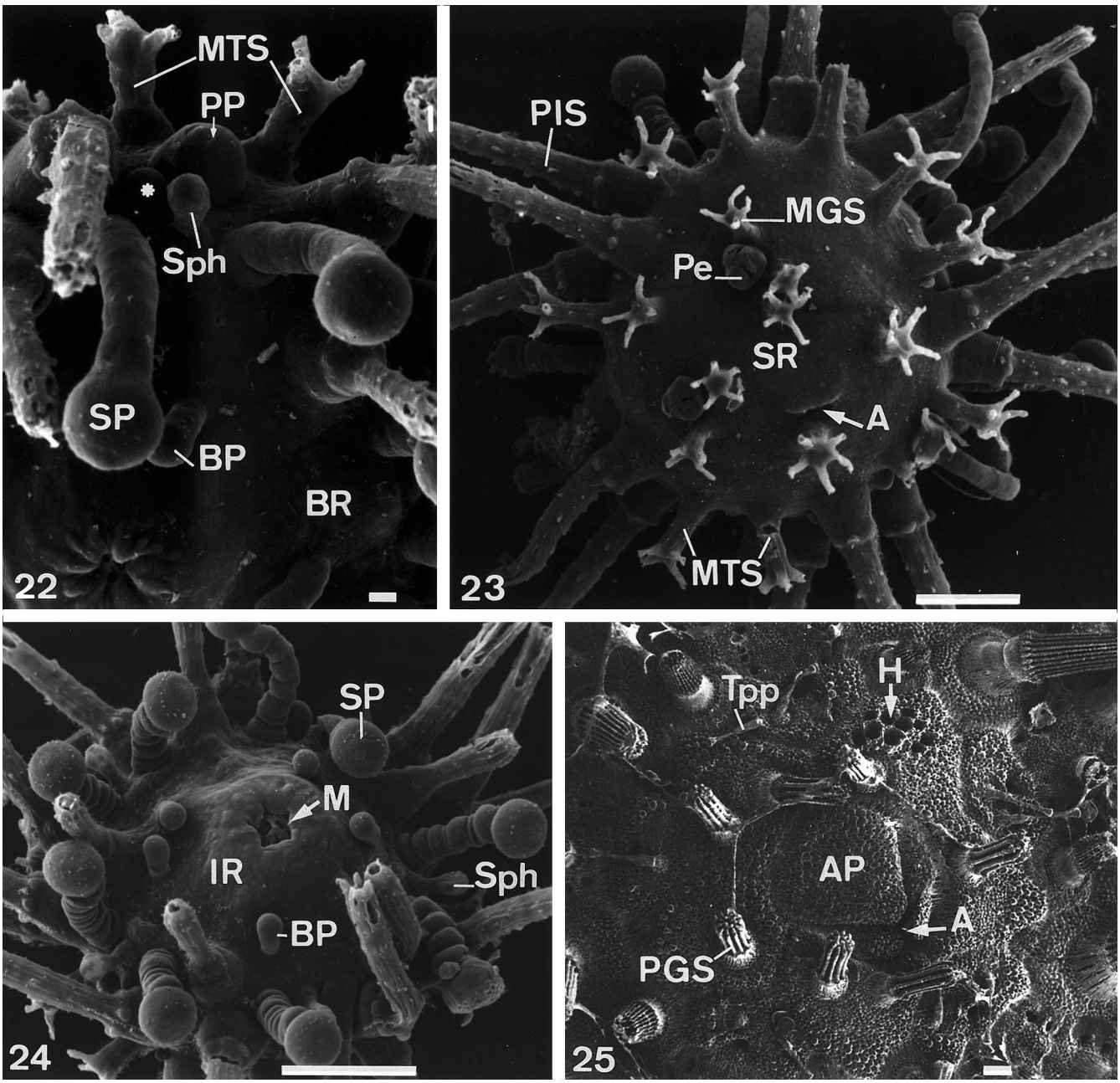
Fig. 21 *P. lividus*. Diagrammatic flattened views centred on the individual's upper surface showing the disposition of the skeletal pieces of the coronal, infracoronal and supracoronal regions in 1 d (**A**) and 5-day-old (**B**) postlarvae. Not to scale. *A, B, C, D, E* Refer to the corresponding ambulacrea, *A1a, A1b* primary ambulacral plates of the A ambulacrum, *A2a, A2b* secondary ambulacral plates of the A ambulacrum, *Ap* anal plate, *gAB, gBC, gCD, gDE, gEA* genital plates of the corresponding interambulacrea, *H* hydropore, *Ia* apical interambulacral plate, *Ib* basal interambulacral plate, *Im* median interambulacral plate, *OAa, OAb* oral plates located in front of the A ambulacrum, *P1* pore of a primary podium, *P2* pore of a secondary podium, *P3* pore of a tertiary podium, *PB* pore of a buccal podium, *TA* terminal plate of the A ambulacrum, *Tt* tongue of the terminal plate. The signs used on the diagrams show the position of a terminal multified spine (◆), a genital multifid spine (◇), a sessile pedicellaria (+), a tubercle where a point-tipped spine is articulated (●) and a sphaeridia (*)

of six; Fig. 19) and the presence of two lateral ciliated bands on the stem (as seen in the PIS, but not on the MGS; Fig. 20).

In a 6 d postlarva (late postlarva), a second pair of ambulacral plates, three or four supplementary podia (two secondary and one or two tertiary) and a sphaeridia develop in each ambulacral area. The newborn pair of ambulacral plates is located between the first and the terminal plate.Within each pair, the plates present an asymmetric development: for the first ambulacral plates, the largest are the plates A1a, B1b, C1b, D1a and E1b, whereas, for the second ambulacral plates, the largest are the plates A2b, B2a, C2a, D2b and E2a (Fig. 21B). Every first ambulacral plate presents a central pore from which emerges a secondary podium (Fig. 21B); there are therefore, two secondary podia per ambulacral area. Every second ambulacral plate also presents a pore, but it is located on the oral fringe of the plate and, therefore, partially limited by the apical side of a first ambulacral plate (Fig. 21B). Thus, a tertiary podium can develop during postlarval life on each second ambulacral plate of the same pair. If only one tertiary podium develops in each pair (Fig. 16), it will appear preferentially on the largest plate of the pair. Unlike the secondary podia, the tertiary podia does not always fully develop: they do not develop the ability to fix during postlarval life (Fig. 22). The terminal disc of secondary and tertiary podia is made up of a frame composed of five or six small independent skeletal pieces that support an apical rosette. The latter is formed by three trapezoidal pieces attached laterally, thus creating a central free space (Fig. 18). As opposed to the primary podia, the secondary and tertiary podia present an ampulla. A sphaeridia develops in the centre of each ambulacral area (Figs. 16, 22). These sphaeridiae are articulated on a discrete tubercle located on the largest plate of each first pair of ambulacral plates (Fig. 21B).

3. Change in the infracoronal region

During postlarval life, the infracoronal region presents five pairs of oral plates at its periphery and in an ambulacral position (Fig. 21). Their spatial organization leaves a free central place where the mouth will pierce. This region corresponds to the future peribuccal region of exotrophic individuals. In a 1 d postlarva (early postlarva), the infracoronal region is flat and appendage free, but can still be divided into a central glabrous zone surroundd by a peripheral ciliated zone (with ca. 10-µm-long cilia). In a 6 d postlarva (late postlarva), the oral region is convex and bears five buccal podia set in circle along the border of the two zones described above (Fig. 24). Each oral plate is pierced at its centre by a pore allowing the development of a podium. However, only one plate of each pair will eventually present a podium during postlarval life (plates OAa, OBb, OCb, ODa and OEb). The buccal podia are made up of a stem that can



Figs. 22–25 *Paracentrotus lividus.* Juveniles structures. *A* anus, *AP* anal plate, *BP* buccal podium, *BR* buccal region, *H* hydropore, *M* mouth, *MGS* multifid genital spine, *MTS* multifid terminal spine, *Pe* pedicellaria, *PGS* point-tipped genital spine, *PIS* point-tipped interambulacral spine, *PP* primary podium, *SP* secondary podium, *Sph* sphaeridia, *SR* supracoronal region, *Tpp* terminal pedunculate pedicellaria. *Bar* 100 μm

Fig. 22 Detail of an ambulacral area of a 1 d juvenile; the terminal disc of the primary podium is regressing. *Star* Position of a tertiary podium barely formed

Fig. 23 Aboral view of a 2-day-old juvenile; the anus is located along the genital plate BC

Fig. 24 Oral view of a 1 d juvenile

Fig. 25 Disposition of the apical skeletal plates of a 3-month-old juvenile

extend to 50 μm and a terminal disc (ca. 30 μm wide) that includes a single oval ossicle with an open-work design.

IV. Juvenile

The exotrophic juvenile stage is reached about 8 days after metamorphosis. The transition to the juvenile stage is marked by three major events: the piercing of the mouth, the piercing of the anus and the regression of the primary podia. As soon as the buccal orifice opens in the central zone of the infracoronal region, the five teeth of Aristotle's lantern are visible and mobile (Fig. 24). Simultaneously, the anal plate rises in front of the BC genital

plate immediately preceding the appearance of the anus (Fig. 23). The regression of the primary podia is marked first by the reduction of their stem (Fig. 16) followed by the regression of their terminal disc (Fig. 22). The regression of the primary podia is complete when the skeletal tongue born by the terminal plate is eventually resorbed. As opposed to the postlarva, the juvenile is mobile and capable of walking on the substratum.

After 3 months, the ambital diameter of the juvenile's test has increased to 5–20 mm (as against 350 μm in the late postlarva). Two supplementary anal plates have developed in the apical region. The genital plates are still deprived of genital pores, but the madreporite (genital plate CD) is pierced by eight to ten aquifer pores (Fig. 25). The genital and terminal plates have lost all the appendages developed during their larval and postlaral life (i.e. sessile pedicellariae and multified spines). On the other hand, these plates have developed one (or two) stereomic tubercle(s) on which a point-tipped spine is articulated (one to two spines per genital plate and always two spines per terminal plate). Furthermore, each terminal plate presents a pair of stalked trifoliate pedicellaria between its two spines.

The ambulacral and interambulacral areas extend considerably; the coronal region becomes the most important one in 3-month-old juveniles. At this stage, each ambulacral area includes at least 50 podia. All these podia emerge through a double orifice pierces in the ambulacral plates and their terminal disc includes a rosette formed by four trapezoidal skeletal pieces. The oral margin of the coronal region presents pairs of thickened skeletal pieces which result from the merging of the oldest skeletal plates. So, the early coronal plates of the postlarva, except the terminal plate, will lose their individuality in this process and the appendages they bore will disappear. Each of the thickened skeletal pieces develops a large skeletal process on their internal side. The processes of the two plates in front of the same ambulacral pair grow towards each other, prefiguring the auricles of the perignathic belt.

In addition to the five pairs of oral plates, the infracoronal region includes a great number of small plates without any particular spatial organization. Each oral plate bears an ophiocephalous pedicellaria with a short stalk (ca. 150 μm) and a buccal podium (there are ten buccal podia at this stage).

D. Discussion

The perimetamorphic period is an essential period in the life-history of *P. lividus*. It continues the larval period, precedes the juvenile period and assembles all the events for the transformation of a pelagic organism into a benthic one. These events may be grouped into three stages which are: (1) the competent stage at the beginning of which the larva acquires the ability to metamorphose and during which it searches for an adequate substratum on which to settle, (2) the metamorphic stage at the end of which the competent larva will be transformed into a benthic postlarva, and (3) the postlarval stage where the immediate postmetamorphic individual, which is endotrophic, prepares its passage to juvenile life (Table 1).

I. Competent stage

The competent larva presents specific morphological features. It has strictly larval elements, such as epaulets and larval arms that reached their full development, and the structures necessary for the building of the postlarval body are present and ready to be gathered. Indeed, the postlarval structures in competent larvae are not centralized. The most voluminous and obvious part of these structures are located on the left-hand side of the larva, at the bottom of the vestibular depression, where they form the echinoid rudiment (it includes notably the terminal plates, the first point-tipped spines and the first podia). The remaining structures (essentially the five geni-

Table 1 Successive steps in the life history of the sea-urchin *Paracentrotus lividus*

	Period in the life-cycle	Stages in the period	Characteristics	Duration[a]
Pelagic life	*Embryonic period*		From fertilization to 4-arms pluteus. Endotrophic	3 days
	Larval period		Differentiation of additional arms and of the rudiment. Exotrophic	15 days
Benthic life	*Perimetamorphic period*	Competent stage	Acquiring of the ability to metamorphose. Exotrophic	1 h to 4 days[b]
		Metamorphic stage	Change in form. Endotrophic	Less than 1 h
		Postlarval stage	Prejuvenile ontogenesis. Endotrophic	About 8 days
	Juvenile period		Differentiation of gonads. Exotrophic	About 6 months[c]
	Adult period		Ability to produce gametes. Exotrophic	Up to 9 years[d]

[a] According to our rearing conditions
[b] From the end of the larval period
[c] Gonads with mature gametes can be observed in individuals from up to 6 months old
[d] Nine years is the maximal longevity observed in the sea-urchin cultivation system

Table 2 Places of the main appendages borne by early postlarvae of euechinids (complied from literature data). (– not mentioned, *0* lack of structure, *BP* buccal podium, *MGS* multifid genital spine, *MTS* multifid terminal spine, *Pe* pedicellaria, *PIS* point-tipped interambulacral spine, *PP* primary podium, *SP* secondary podium, *Sph* sphaeridia, *Amb* ambulacral, *I-amb* interambulacral)

Family	Species	Post-larval age (days)	Oral region	Coronal region		Apical region					References
				Amb	I-amb	AB	BC	CD	DE	EA	
Echinidae	*Echinus esculentus*	1<	–	2 MTS 1 PP/2 SP	4 PIS	The apical region bears 2 MGS and 3 Pe. One of these pedicellaria is located on the EA genital plate					Mac Bride (1903)
	Paracentrotus lividus	2	0	2 MTS 1 PP/2 SP	4 PIS	2 MGS 1 or 2 Pe	1 MGS	1 MGS	2 MGS 1 or 2 Pe	1 MGS	Present work
	Psammechinus microtuberculatus	–	–	2 MTS 1 PP	4 PIS	2 MGS 2 Pe	1 MGS	1 MGS	2 MGS 2 Pe	0	Bury (1895)
	Psammechinus miliaris	–	5 BP	2 MTS 1 PP/2 SP 1 Sph	4 PIS	2 MGS 2 Pe	1 MGS	1 MGS	2 MGS 2 PE	1 MGS	Gordon (1926)
Echinometridae	*Heliocidaris crassispina*	ca. 2	0	2 MTS 1 PP/2 SP	4 PIS	2 MGS 1 Pe	1 MGS	1 MGS	2 MGS 1 Pe	2 MGS 1 Pe	Onoda (1931)
Temnopleuridae	*Mespila globulus*	3	0	2 MTS 1 PP/2 SP 1 Sph	4 PIS	0	1 MGS	1 MGS	0	1 MGS 2 PE	Onoda (1936)
	Temnopleurus hardwicki	1<	5 BP	2 MTS 1 PP/2 SP 1 Sph	4 PIS	–	–	–	–	1 MGS 2 PE	Fukushi (1960)
Toxopneustidae	*Lytechnius pictus*	1<	–	1 PP ? MTS	4 PIS	1 MGS	0	0	1 MGS	1 MGS	Hinegardner (1969)

tal plates and their appendages) are scattered in the larval integument (Fig. 1D).

Thus, the five genital lates and the five terminal plates that will form the apical system of juvenile and adult individuals are present in the competent larva. Five of these plates the B, C and D terminals and the BC and EA genitals, appear de novo whereas the other five, the A and E terminals and the AB, CD and DE genitals, each develop on contact with one of the five skeletal rods supporting the larval arms. These plates develop according to a general plan that seems to be followed by all regular sea-urchins studied so far [*Psammechinus microtuberculatus* (Blainville, 1825) (see Bury 1895); *Echinus esculentus* Linné, 1758 (see Mac Bride 1903); *Psammechinus miliaris* (Gmelin, 1763) (see Gordon 1926); and *Mespila globulus* A. Agassiz, 1863 (see Onoda 1936)]. David and Mooi (1996) recognized two sets of skeletal elements in echinoderms: the axial skeleton to which belong the terminal plats and the extra-axial skeleton including the genital plates. They showed that this dichotomy also occurs in competent larvae in the formation of the apical plates: the terminal plates, which are axial, develop inside the echinoid rudiment whereas the genital plates, which are extra-axial, grow outside of it. However, this duality is not respected when the premetamorphic genesis of these plates is considered, as B, C and D terminals and BC and EA genitals are born independently of the larval skeleton whereas the other terminals and genitals develop on contact with larval rods.

The presence of all the structures mentioned above is necessary but not sufficient to tell if the larva has reached the stage of competence. Larvae of *P. lividus* do not develop specific morphological features at competence as other planktonic larvae do including some echinoderm larvae (for example, Cirripedia, Walley 1968; Ascidiacea, Cloney 1978; Asteroida, Barker 1978; Crinoida, Jangoux and Lahaye 1990). Strathmann (1978) indicates that the reaching of competence in Echinoida is essentially marked by the appearance of new larval behaviour. Indeed, a change in the swimming behaviour of *P. lividus* larvae occurs at competence and allows them to reach the substratum. Soon after having contacted the substratum, larvae undergo some reversible body distortions in order to allow partial protrusion of some primary podia which then test the substratum. At this stage, the larva remains able to return to the water column and it is then able to delay its metamorphosis.

II. Metamorphic stage

When primary podia detect a suitable substratum, larvae of *P. lividus* engage the metamorphic process. Most larvae of marine benthic invertebrates adhere to the substratum prior to metamorphosis by means of a cement with a permanent action (see for example, larvae of Cirripedia, Bryozoa, Asteroidea, or Crinoidea; Walley 1968; Woollacott and Zimmer 1978; Barker 1978; Lahaye and Jan-

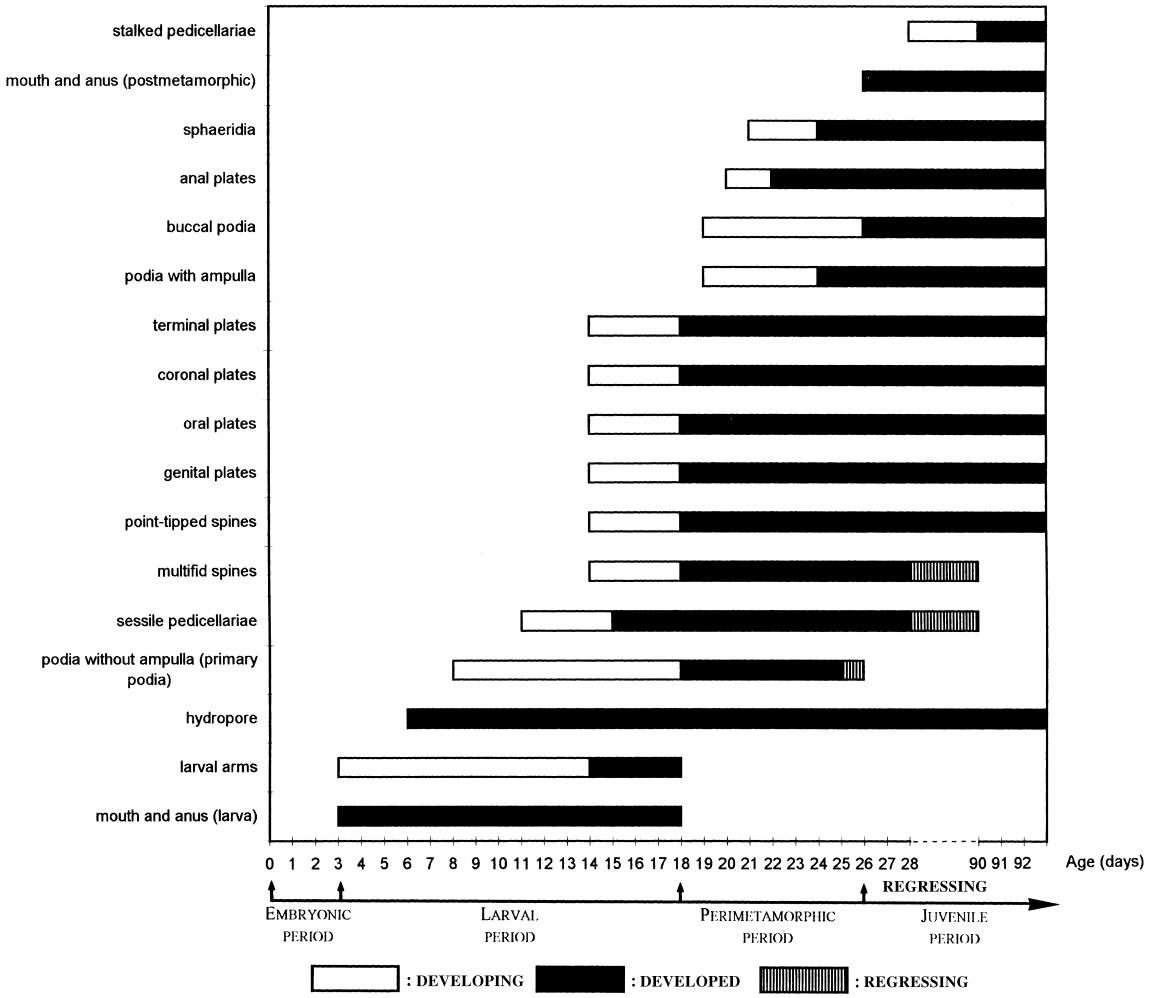


Fig. 26 Diagrammatic representation of changes in main morphological features from the embryonic to the juvenile period (*P. lividus*)

goux 1988, respectively). In the case of *P. lividus*, however, larval primary podia adhere only temporarily to the substratum in the sense that they can attach onto and detach from it throughout the competence stage as well as during the rest of the perimetamorphic period.

Metamorphosis occurs rapidly (about 1 h) and always proceeds in the same way: the echinoid rudiment comes out by the vestibular pore on the left larval flank while most of the larval integument concentrates on the right larval flank where it will be covered progressively by the vestibular wall. This occurs according to a general plan followed by all regular sea-urchins studied so far (see review by Burke 1987). The first signa of metamorphosis is the collapse of the epidermis covering the larval arms (Chia and Burke 1978). However, this process of resorption does not involve the entire larval epidermis, and preserved epidermis zones are associated with the genital plates and the appendages they support. These zones merge together and with the vestibular wall form the postlaral epidermis. This observation clearly supports the hypothesis of the double origin of the postlarval epidermis proposed by Emlet (1988).

III. Postlarval stage

The resulting postlarva has the general shape of a young adult. Indeed, it has podia, spines and pedicellariae. At this point, it is already possible to recognize the coronal, oral (infracoronal) and aboral (supracoronal) regions characteristic of the adult body. The hydropore remains visible during the metamorphic process and postlarval life, giving a reference point for orientation. However, the structure of some appendages borne by postlarvae (i.e. primary podia and multifid spines) are radically different from those of their adult homologues.

The primary podia are deprived of ambulacral ampulla, and the ambulacral system of the postlarva is very simple. The latter includes an oral ring from which emerges directly the five primary podia and the hydropore's canal (see review by Burke 1987). The particular behaviour of the postlarvae – they seem to stamp constantly – is related to the structural simplicity of its ambulacral system. Since there is no ampulla, any contraction of a podium must be counterbalanced by the extension of one or several other podia. The primary podia are sensory and adherent: they allow the competent larva to test the substratum and the metamorphic larva and postlarva to adhere to it. They have specific sensory–secretory equipment allowing for this double function (Flammang et al. 1997). Primary podia are transient organs, a

fact also showed in *Psammechinus miliaris* by Gordon (1926). They regress shortly before the opening of the mouth and anus, hence before the endotrophic postlarva becomes an exotrophic juvenile. At this point, the secondary and tertiary podia take over (these podia are locomotory and are provided with an ampulla).

As in several other species of Echinoida, postlarvae and young juveniles of *P. lividus* present multifid spines (Table 2). These spines seem to be the principal defence appendages of postlarvae. They are implanted on or in the immediate vicinity of the supracoronal region (proportionally the widest region of the postlarval body), where they form a kind of protective armour. As their extremities point in various direction, they are efficient while not being articulated and, therefore, do not require the development of a mucular structure. This is particularly adequate for the postlarva which is an endotrophic organism whose resources should be for the most part invested in the organogenesis of the future juvenile.

All appendages specific to the postlarval body, namely primary podia, multifid spines and sessile pedicellariae, will have disappeared in 3-month-old juveniles (Fig. 26). While primary podia are resorbed at the end of the postlarval life, multifid spines and sessile pedicellariae regress during the early juvenile life. The other appendages borne by postlarvae, namely buccal podia, point-tipped spines, podia with ampulla and sphaeridiae, are unspecific and close to their adult homologues. Some of these, such as buccal podia, will be definitively integrated into the juvenile body; others, such as secondary and tertiary podia, will regress during the first months of juvenile life. As a matter of fact, the only postlarval coronal plates that will be maintained are the terminal plates. They will form together with the genital plates the apical system of juvenile individuals.

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References

Barker MF (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

Burke RD (1980) Podial sensory receptors and the induction of metamorphosis in echinoids. J Exp Mar Biol Ecol 47:223–234

Burke RD (1987) Echinoderm metamorphosis: comparative aspects of the change in form. In: Jangoux M, Lawrence JM (eds) Echinoderm studies, vol 3. Balkema, Rotterdam, pp 81–108

Bury H (1895) The metamorphosis of echinoderms. Q J Microsc Sci 38:45–136

Chia FS (1978) Perspectives: settlement and metamorphosis of marine invertebrate larvae. In: Chia FS, Rice ME (eds) Settlement and metamorphosis of marine invertebrate larvae. Elsevier-North Holland, New York, pp 283–285

Chia FS, Burke RT (1978) Echinoderm metamorphosis: fate of larval structures. In: Chia FS, Rice ME (eds) Settlement and metamorphosis of marine invertebrate larvae. Elsevier-North Holland, New York, pp 219–234

Cloney RA (1978) Ascidian metamorphosis: review and analysis. In: Chia FS, Rice ME (eds) Settlement and metamorphosis of marine invertebrate larvae. Elsevier-North Holland, New York, pp 255–282

David B, Mooi R (1996) Embryology supports a new theory of skeletal homologies for the phylum Echinodermata. C R Acad Sci Paris 319:577–584

Dawidoff C (1948) Embryologie des Echinodermes. In: Grassé PP (ed) Traité de Zoologie, vol XI. Masson, Paris, pp 277–363

Emlet RB (1988) Larval form and metamorphosis of a ‘‘primitive’’ sea urchin, *eucidaris thouarsi* (Echinodermata: Echinoidea: Cidaroida), with implications for developmental and phylogenetic studies. Biol Bull 174:4–19

Flammang P, Gosselin P, Jangoux M (1997) The podia, organs of adhesion and sensory perception in larvae and post-metamorphic stages of the echinoid *Paracentrotus lividus* (Echinodermata). Biofouling (in press)

Fukushi T (1960) The formation of the echinus rudiment and the development of the larval form in the sea urchin, *Temnopleurus hardwickii*. Bull Mar Biol Sta Asmushi Tohoku Univ 10:65–72

Gordon I (1926) The development of the calcareous test of *Echinus miliaris*. Philos Trans R Soc Lond Ser B 214:259–312

Gosselin P, Jangoux M (1995) Induction of metamorphosis in *Paracentrotus lividus* larvae (Echinodermata, Echinoidea). Oceanol Acta 19:292–296

Highsmith RC (1982) Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: adults sand dollar beds. Ecology 63:329–337

Hinegardner RT (1969) Growth and development of the laboratory cultured sea aurchin. Biol Bull 137:465–475

Hyman LH (1955) The invertebrates: Echinodermata. In: The coelomate Bilateria, vol IV. McGraw-Hill Books, New York, pp 16–19

Jangoux M, Lahaye M-C (1990) The attachment complex of the dolilaria larvae of *Antedon bifida* (Echinodermata, Crinoidea). In: De Ridder C, Dubois P, Lahaye M-C, Jangoux M (eds) Echinoderm research. Balkema, Rotterdam, pp 99–105

Lahaye M-C, Jangoux M (1988) Morphologic externe et comportement des larves doliolaria d’*Antedon bifida* (Echinodermata, Crinoidea). Ann Soc R Zool Belg 118:183–189

MacBride EW (1903) The development of *Echinus esculentus* together with some points of the development of *E. miliaris* and *E. acutus*. Philos Trans R Soc Lond 195:285–337

Onoda K (1931) Notes on the development of *Heliocidaris crassispina* with special reference to the structure of the larval body. Mem Coll Sci Kyoto Imp Univ (B) 7:103–135

Onoda K (1936) Notes on the development of some Japanese echinoids with special reference to the structure of the larval body. Jpn J Zool 6:637–654

Pearce CM, Scheibling RE (1990) Induction of metamorphosis of the larvae of the green sea urchin, *Strongylocentrotus droebachiensis*, by coralline red algae. Biol Bull 17:304–311

Strathmann RR (1975) Larval feeding in echinoderms. Am Zool 15:717–730

Strathmann RR (1978) Larval settlement in echinoderms. In: Chia FS, Rice ME (eds) Settlement and metamorphosis of marine invertebrate larvae. Elsevier-North Holland, New York, pp 235–246

Walley LJ (1968) Studies on the larval structures and metamorphosis of *Balanus balanoides* (L.). Philos Trans R Soc Lond Ser B 256:237–280

Woollacott RM, Zimmer RL (1978) Metamorphosis of cellularioid bryozoans. In: Chia FS, Rice ME (eds) Settlement and metamorphosis of marine invertebrate larvae. Elsevier-North Holland, New York, pp 49–64