EMBRYONIC, LARVAL AND POSTLARVAL DEVELOPMENT OF THE TROPICAL CLAM, *ANOMALOCARDIA BRASILIANA* (BIVALVIA, VENERIDAE)

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ABSTRACT

Anomalocardia brasiliana (Gmelin, 1791) is a venerid clam, distributed from the West Indies to Brazil, which lives shallowly burrowed in muddy sands of mangrove lagoons in Guadeloupe. Development from induced spawning to metamorphosed juveniles is described by using light and scanning electron microscopy. The shell-field appears at the gastrula stage, 6 h after fertilization, and rapid embryonic development results in straight-hinge veligers, 18 h after fertilization. These swimming veligers develop to swimming-crawling pediveligers, then to benthic plantigrades with functional elongated gill filaments without interruption in 15 days. The transitional arched structures observed at the end of the pediveliger stage were called 'ctenidial crypts' to distinguish them from functional gill filaments which exist only in metamorphosed juveniles. Metamorphosis, which occurs without a special environmental cue, is completed with the differentiation of the siphons in 300 µm juveniles. Thus, there is no delay of metamorphosis in this species whereas a developmental hiatus has been described in most planktotrophic bivalves. Juveniles, 1 mm in shell-length with the triangular shape, pointed posterior end and brown zig-zag stripes on the shell, typical of *A. brasiliana* have been obtained 7 weeks after fertilization. However, a large variability of individual sizes and developmental stages within the same batches may indicate a high genetic variability.

INTRODUCTION

Anomalocardia brasiliana (Gmelin, 1791) is a venerid clam, distributed from the West Indies to Brazil (Warmke & Abbott, 1962; Abbott, 1974), and as far south as Uruguay, according to Rios (1985). Despite its small size of 45 mm maximum shell-length, *A. brasiliana* is used as food in Guadeloupe under the popular name of 'Chaubette' and in Brazil under the name of

'Berbigao'. The breeding cycle has been studied near Sao Paulo (Narchi, 1976) and Paraiba (Grotta & Lunetta, 1980, 1982) and the demography and growth were investigated in Guadeloupe (Monti, Frenkiel & Mouëza, 1991). *A. brasiliana* lives at water depths of 0.5–1.5 m, shallowly burrowed in muddy sand along the mangrove border. In Guadeloupe, it has disappeared from most areas where it used to be collected. The populations of *A. brasiliana* are sensitive to ecological variations and experience heavy mortalities due to heavy rains which cause large fluctuations in population distribution and size (Monti *et al.*, 1991).

Larval development has been described in several species of Eulamellibranchia providing basic knowledge for aquaculture or for plankton identification of larval stages (Loosanoff & Davis, 1963; Loosanoff, Davis & Chanley, 1966; Sastry, 1979). The developmental stages of some venerid clams have been described with as much detail as possible with light microscopy (Quayle, 1952; Ansell, 1962; D'Asaro, 1967; La Barbera & Chanley, 1970). However, the embryonic development could not be accurately studied with light microscopy, and most studies on the developmental patterns of bivalves lack descriptions of early stages as noticed by Verdonk & Van den Biggelaar (1983).

Modern studies using electron microscopy on larval and postlarval organogenesis are scarce for Bivalvia (Moor, 1983) and often focused on problems related to spat fixation (Cranfield, 1973, 1974). The larval stages of *Ostrea edulis* have been described by Waller (1981); shell morphogenesis has been studied in *Crassostrea virginica* by Carriker & Palmer (1979) and in *Spisula solidissima* by Eyster & Morse (1984). The lecithotrophic development of the pandoroidean *Entodesma cuneata* has been described by Campos & Ramorino (1981) and the planktotrophic development of the

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pectinid *Chlamys hastata* by Hodgson & Burke (1988). Recently, Beninger, Dwiono & Le Pennec (1994) described the developmental pattern of the gills in the fillibranch *Pecten maximus* and Gros, Frenkiel & Mouëza (1997) described the general development of the symbiotic lucinid clam *Codakia orbicularis*.

The family Veneridae has been considered by Ansell (1962) as a convenient group in which to study metamorphosis as it has evolved along unspecialized lines and lacks special adaptations. This statement applies to the whole development. The aim of this paper is to describe, with light and scanning electron microscopy, the developmental pattern of the tropical venerid clam, *A. brasiliana*, from spawning to metamorphosed juveniles possessing functional gills and siphons.

METHODS

Adult individuals of *Anomalocardia brasiliana* were collected in a shallow mangrove lagoon connecting to the 'Rivière salée' which separates the two main islands of Guadeloupe, in the French West Indies. For each spawning trial, about thirty individuals from 25 mm long were allowed to acclimate in 50-litre tanks at the laboratory temperature, i.e. 25°C in sea water at a salinity of 34‰ which is usual in their natural habitat. Water was renewed daily by replacing 1/5 of the volume before feeding. Food provided daily was a mixture of three algal species—*Nannochloris* sp., *Isochrysis tahiti* and *Pavlova lutheri*—for a final concentration of 500 cells μl^{-1}. All sea water used from spawning to metamorphosis was filtered through a 5 μm cartridge filter (Millipore) then UV treated (Proteco LM6-S86; $2 \times 80W$).

Various stimulations such as temperature fluctuations, osmotic stress and serotonin stimulation were used to induce spawning but, most often, gametes were obtained through spontaneous spawning after an intensive diet up to 3,000 algal cells μl^{-1}. Breeding clams were taken out of the tank as early as possible after spawning and the sea water was aerated by bubbling through a hydrophobic Gelman filter during the whole period of embryogenesis. Straight-hinge larvae were raised at a density of 10,000 litre^{-1} in 20-litre tanks. During larval development, sea water was changed and 0.5 ppm sodium hypochlorite (NaClO) added every day up to metamorphosis. Food, composed of the same three algae as used to feed the adults, were provided after each water change to a final density of 60 to 300 cells μl^{-1} from the early veliger up to metamorphosis.

Samples were fixed for scanning electron microscopy (SEM) in 2.5% glutaraldehyde in 0.1 M cacodylate buffer adjusted to pH 7.2 and 700 mOsm with NaCl. Samples were fixed at hour-intervals during embryogenesis, at day intervals during the larval stages and during metamorphosis. From the veliger stage, larvae were anaesthetized, before glutaraldehyde fixation, by adding one drop of phenoxyethanol to 25 ml of sea water containing the larvae, to allow observation of velum and foot. After fixation, all samples were rinsed in the same buffer and dehydrated through an ascending series of acetone solutions. Then, they were critical point dried using CO_2 as transitional fluid in a Biorad E 3000 critical point drier, gold-coated in a Biorad SC 500 sputter coater and observed in a Hitachi SEM 2500 at 20 kV. Periodic random samples of about 20 larvae were also taken to measure their maximum length and height and to make photonic pictures with a Leica Orthoplan fitted with a Leica Orthomat.

RESULTS

Adult individuals of *Anomalocardia brasiliana* spawned in the hatchery during most of the year. On a few occasions, spawning was obtained through serotonin stimulation. Successful spawning was obtained within 3 h from animals kept dry for 2 h or 3 h then put back in sea water. Osmotic stress obtained by putting the clams in fresh water then back in filtered and UV treated sea water, was a more effective stimulus. However, the best results were obtained by spontaneous spawning after an intensive feeding period of 20 to 30 days. The main problem is that spawning is not predictable; therefore frequent checking is necessary, at least twice daily, to take out the breeding clams as early as possible after spawning. For each experimental spawning, only 4 to

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Figs 1–6. Embryonic stages from fertilization to early gastrula. 1—phase contrast light microscopy; 2–6 SEM.

Figure 1. Spawned egg and sperm (arrow). The large germinal vesicle (V) and nucleolus (n) indicate that the egg is still not fertilized.

Figure 2. Sperm lying on the surface of an egg; short, arched head and middle piece with four round, large mitochondria (asterisk). MV: microvilli.

Figure 3. Two-cell embryo with unequal blastomeres (AB and CD). The polar body (arrow) is in the cleavage plane.

Figure 4. Four-cell embryo with three equal blastomeres (A, B, C) and a larger one (D), with the polar body (arrow) between them, is a common developmental pattern in bivalves.

Figure 5. Ciliated blastula is the first motile stage, 3 h after fertilization ($T_0 + 3$ h). Arrow heads: cilia.

**Figure 6.** The early gastrula, differentiated at $T_0 + 6$ h, is characterized by a large blastopore (B). Its circular margin is surrounded by some cilia (arrow heads).

Scale bars: **1.** 30 μm, **2.** 1 μm, **3–6.** 10 μm.

6 females out of a batch of 30 individuals spawned at the same time. Fecundity proved to be low and each spawning was limited to some 100,000 oocytes per female. In the same batch, some other animals may spawn weeks later provided they are properly fed and the same individuals may also spawn several times as maturation of the gonad is asynchronous (personal observation). Oocytes, 60 μm in diameter, have a thin vitelline coat and no prominent jelly-coat (Fig. 1). They have a large germinal vesicle and contain few vitelline platelets. The very active sperm is characterised by a short arched head, 2 μm long and 0.5 μm wide at its base, and a flagellum approximately 40 μm long (Fig. 2).
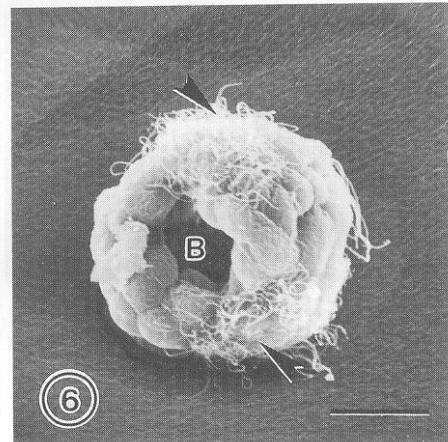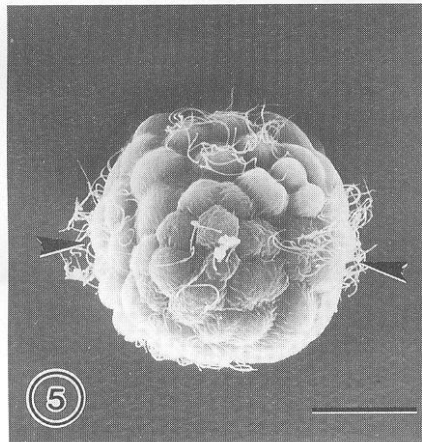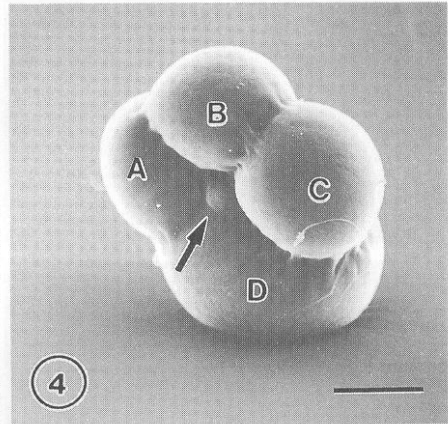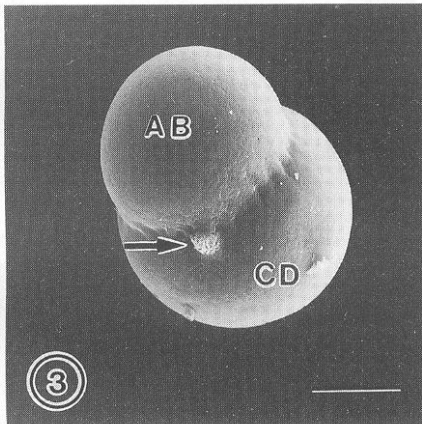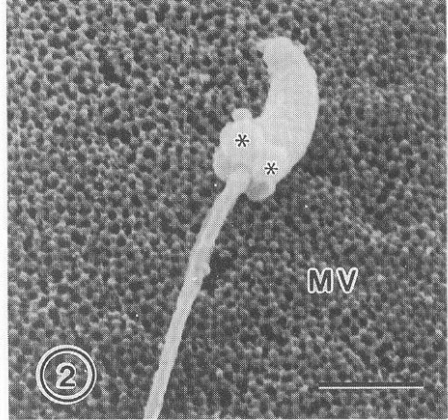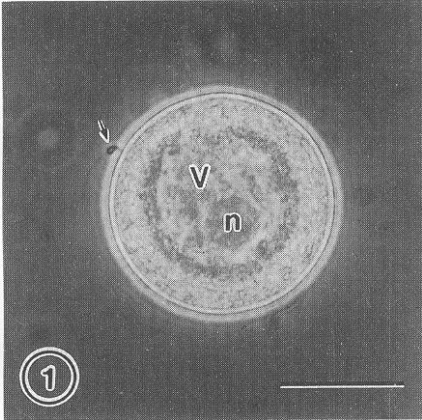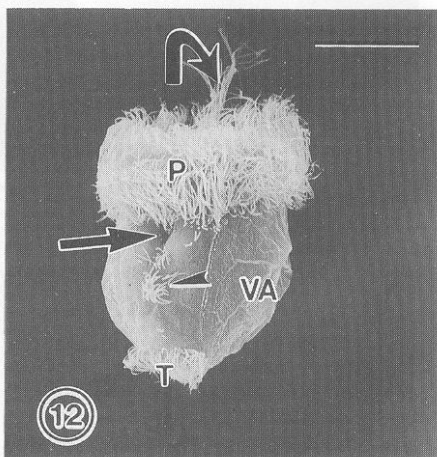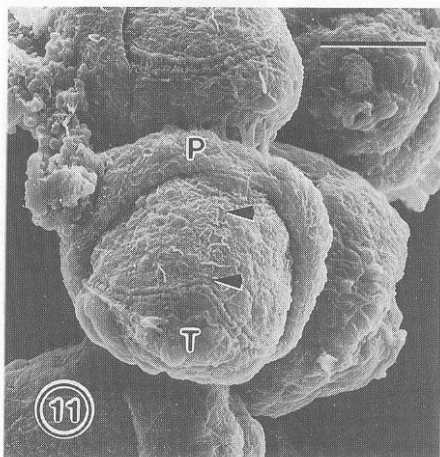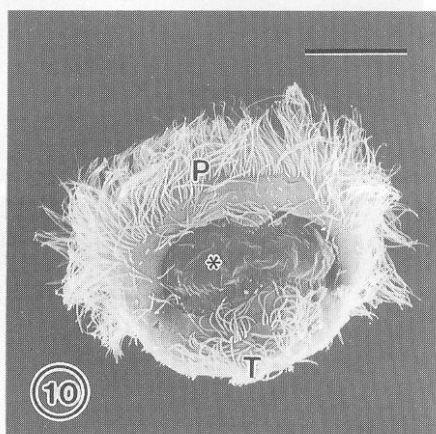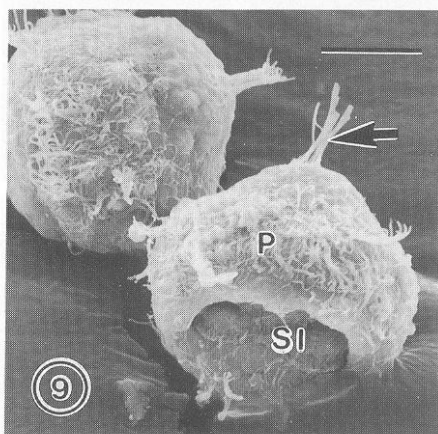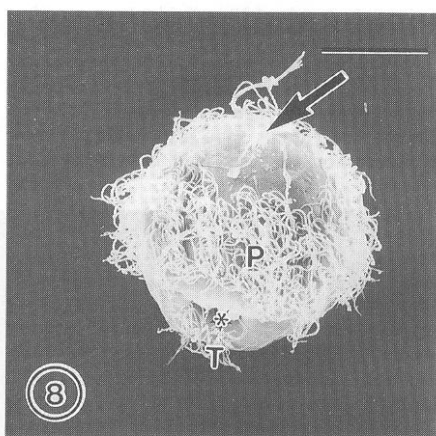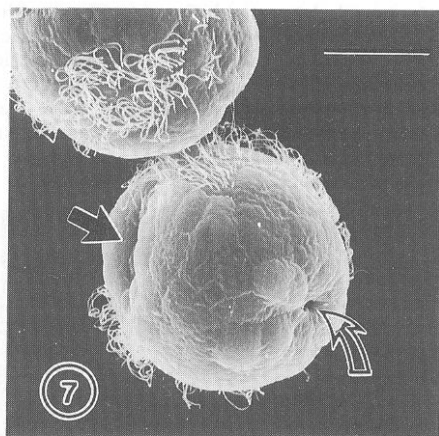
Figs 7–12. Differentiation of the shell from late gastrula to post-trochophore (SEM).

Figure 7. Late gastrula at $T_0 + 7.5$ h with a small round blastopore (curved arrow) anteriorly and crescent shaped shell-field invagination (arrow) posteriorly.

Figure 8. Early trochophore at $T_0 + 9$ h; the apical sense organ (arrow) in the middle of apical plate is surrounded by the prototroch (P). The telotroch (T) borders the anus. The shell field is indicated by an asterisk.

Figure 9. Later trochophore at $T_0 + 12$ h; postero lateral view to show the shell-field invagination (SI) which appears as a transverse pad. Arrow: apical sense organ; Op: prototroch.

Figure 10. A little latter, the wrinkled shell organic pellicle (asterisk) is dumbbell shaped. P: prototroch; T: telotroch.

Figure 11. The shell-pellicle is saddle shaped and the first hinge line, delineated by arrow heads, emphasizes the bilateral symmetry. P: prototroch; T: telotroch.

Figure 12. Intermediary stage between typical trochophore and early veliger; ventral view to show the mouth (arrow) immediately under the prototroch (P) as well as the postoral tuft (arrow head) and the telotroch (T). The apical sense organ is indicated by a curved arrow. VA: valve.

Scale bars: **7–12.** 10 µm.

Embryogenesis

Fertilization occurred naturally; fortunately no polyspermy seems to occur and we have obtained several batches of healthy larvae with this method. There is no polar lobe and the first polar body is detected 10 min after contact between oocytes and sperm, in a position opposite to sperm puncture, the second one is detected a few minutes later but is not always obvious. The first cleavage occurs 30 min after fertilization ($T_0 + 30$ min) and results in two unequal blastomeres AB and CD, the polar body being located in the plane of cleavage (Fig. 3). The second cleavage, at right angle to the first, produces a 4-cell stage observed at $T_0 + 1$ h, with equal size A-, B-, and C-blastomeres, and a larger D-blastomere (Fig. 4). Successive cleavages have a spiral pattern. Then, micromeres built a cap on the top of macromeres and some cilia appear on the embryo without a precise distribution at $T_0 + 3$ h (Fig. 5). Such a ciliated blastula is the first motile stage. At the initial stage of gastrulation, which starts at $T_0 + 6$ h by an invagination process, the large circular margin of the blastopore, making the border between ectoderm and endoderm, bears some cilia (Fig. 6). Originally located at the vegetal pole, the blastopore is displaced during the gastrulation process, so that it comes to lie on the ventral side caused by the outgrowth of the dorsal region of the embryo due to the development of the shell field (Fig. 7).

By $T_0 + 9$ h, the late gastrula had differentiated into a typical trochophore (Fig. 8) with a crown of motile cilia, the prototroch, which divides the trochophore into two areas. The anterior area constitutes an apical plate with the long ciliary tuft of the apical flagellum in its centre. The area posterior to the prototroch is occupied by the blastopore on the ventral side and the shell-field, lined on its posterior margin by a tuft of cilia, on the dorsal side. The newly secreted shell-material evaginates gradually and appears as a transverse pad in the early trochophore (Fig. 9); its subsequent spreading makes it dumbell shaped (Fig. 10) as described by Waller (1981). The shell field spreads out, and folds into right and left segments connected by a thicker band which represents the first hinge (Fig. 11). Behind it in the

postero-dorsal region, a tuft of cilia dorsal to the anus represents the telotroch.

In the late trochophore, the prototroch develops to form the velum with ciliary bands for locomotion and food gathering. These ciliary bands border the mouth only on its anterior margin. Its posterior margin is lined by a tuft of stiff cilia while the anus margin is surrounded by the telotroch (Fig. 12). The prodissoconch I progressively encloses the soft body parts, 16–18 h from fertilization.

Larval development

Typical straight-hinged D-shaped larvae have developed by 24 h and are 95 µm long and 72 µm high. The straight-hinge is 64 µm long on average (Fig. 13). Prodissoconch I is equivalve with both shoulders short. The slope of the anterior shoulder is rounded whereas the posterior shoulder is nearly straight. The surface of prodissoconch I presents a shallow alveolar pattern, up to acutely pointed triangles which take over up to the edge of prodissoconch I where they merge as arrow-heads (Fig. 14). These arrow-heads blend with a narrow, commarginally striated zone. Beyond this slightly cancellate zone, there are only commarginal lines which are characteristic of prodissoconch II. Thus the prodissoconch II begins to grow very soon after the formation of prodissoconch I. 24-h-old veliger larvae lacking vitelline supply are translucent and need to be fed immediately. Three-day old larvae (123 µm ± 5 long and 104 µm ± 5 high) still have a straight hinge. Prodissoconch II, which is commarginally striated, is growing (Fig. 15).

An apical flagellum and a fully functional velum are present until day eleven at which time these larval structures begin to regress prior to metamorphosis. The oval velar crown is composed of four discrete bands of cilia. Two are preoral, one is adoral and the fourth is postoral. The inner preoral band which has numerous short cilia is distinct from the outer preoral band (Fig. 16). The double row of the outer preoral band is the most conspicuous because of its width and of the size of its compound cilia (Fig. 17). The outer preoral band is composed of two distinct rows of compound cilia (Figs. 18 & 19). Each row consists
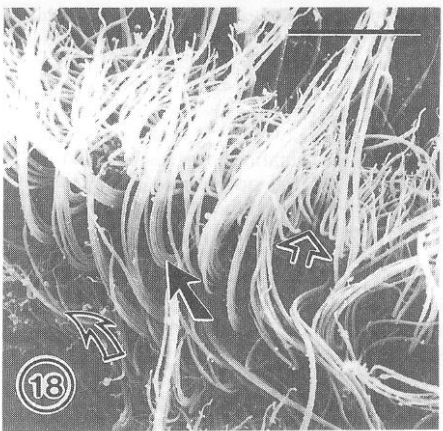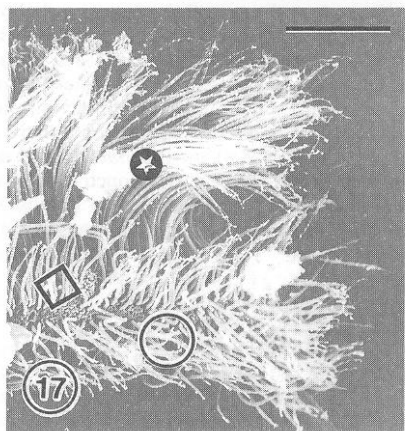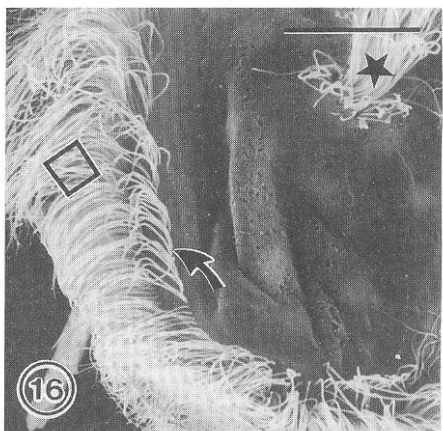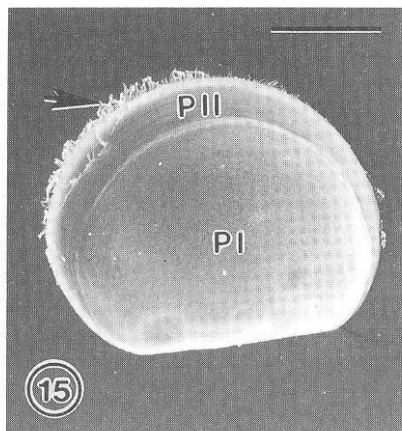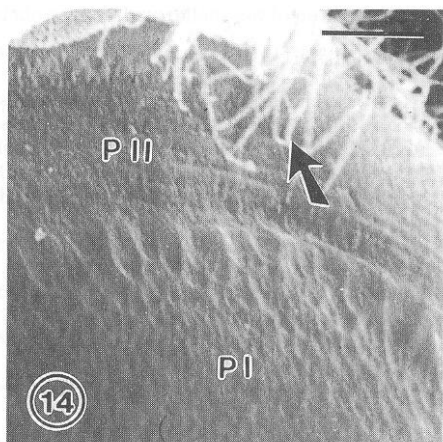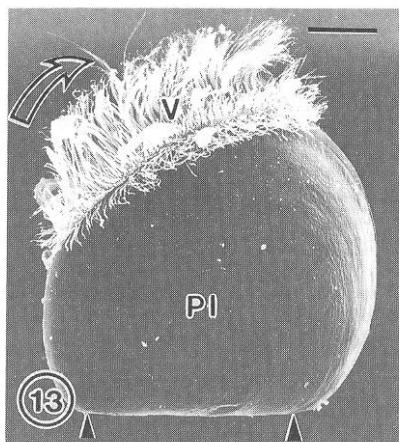
Figs 13–18. D-shape veliger larvae and velum (SEM).

Figure 13. Early veliger or D-larva at $T_0 + 24$ h enclosed within prodissoconch I (PI). The velum (V) and the apical sense organ (curved arrow) protrude between the shell valves when swimming, but may be completely withdrawn inside the shell. The straight hinge line is indicated by arrow heads.

Figure 14. Details of 2-day old veliger shell; the edge of prodissoconch I (PI) is characterized by 'arrow-head sculpture' merging with the shallow alveolar pattern of prodissoconch I. The prodissoconch II (PII), with commarginal growth rings, is well delineated from prodissoconch I. Arrow indicates velar cilia.

Figure 15. 3 day-old veliger with prodissoconch II (PII) well delineated from prodissoconch I (PI). Arrow indicates cilia from the velum which partially extends outside the shell.

Figure 16. Velum ciliary bands; inner preoral band composed of short cilia (curved arrow) and outer preoral band made up of long cirri (square). The apical sense organ is indicated by a black star.

Figure 17. Velum ciliary bands; out of four discrete ciliary bands, three are identified here, postoral (circle), adoral (square) with short cilia, and outer preoral (star) with long compound cirri.

Figure 18. Velum ciliary bands; inner short cilia (empty curved arrow) and outer preoral cirri which comprise 5 single cilia each (straight arrow); the adoral band (empty straight arrow) is partially hidden by the cirri and the postoral band is not shown.

Scale bars: **13.** 10 μm, **14.** 5 μm, **15.** 20 μm, **16.** 10 μm, **17–18.** 5 μm.

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of a unique line of independent cirri (Figs. 20 & 22) each one composed of 5 to 7 single cilia which stick to one another above their bases and remain in contact for nearly their entire length, before becoming separated at their tip. Cirri from the two rows of the outer preoral band are in register with each other (Figs. 21 & 22). The adoral and postoral bands have short single cilia which appear randomly distributed (Fig. 17). The arrangement of cilia belonging to the four bands of the velum is summarized on figure 23.

Five days after fertilization, the D-shape begins to get lost. The veliger larvae are 150 μm ± 5 in length and 137 μm ± 5 in height. The shell, which becomes oval in shape, is slightly umbonate with dissimilar shoulders, the anterior, being longer than the posterior. Seven-day old veligers are 175 μm ± 5 long and 156 μm ± 5 high. At that size, they enter metamorphosis which will be completed with the differentiation of siphons at a size of 300 μm. The foot rudiment which develops posteriorly to the velum is already recognizable through the prodissoconch (Fig. 24). The larvae are still swimming but they tend to gather near the bottom. The foot comes out, occasionally but is not yet functional. Inside its base there is a large triangular clear structure which seems to be the byssal gland just beneath the statocysts which contain a single statolith. It moves freely in the large ventral gap between the anterior and posterior adductors which are conspicuous through the translucent prodissoconch shell. Between days 8 and 10, the foot becomes functional in 180 μm ± 10 long and 175 μm ± 10 high veligers but the velum is still functional; thus the larvae swim and crawl for short intervals. In these veliconcha or pediveligers, gill development begins with the appearance of a double roundish structure made up of a central pillar ciliated on both sides and of a simple one anteriorly and posteriorly. These three pillars delimit two roundish ciliated crypts (Figs. 24 & 25).

Post larval development

From day 11, the central pillar of the ctenidial crypts grows to make up the second gill filament. The first filament formed by the anterior pillar of the anterior crypt anchored to the body wall remains short and rudimentary. The third and successive filaments will bud off from a block of tissue posterior to the ctenidial crypts situated on each side of the posterior mantle edge fusion which makes up the siphonal septum. At this stage, the incurrent and excurrent water flows are separated. The velum disappears; its nutritional and respiratory functions having been progressively taken over by the gills and its locomotory function by the foot. The larvae are now plantigrades which settle on the bottom of the culture vessel without any additional substratum. The movements of the foot become limited posteriorly by the siphonal septum (Fig. 26). The growth of the post-larval shell proceeds rapidly after settlement. The umbones overhang the straight part of the hinge and the post-larval dissonconch with commarginal ridges is clearly delineated from the larval prodissoconch which has a mean size of 200 μm. From day 15, the umbones, which have moved forward, overhang the anterior edge.

The siphons are differentiated in these post-larvae of at least 300 μm in shell-length, which have 4 to 6 gill filaments. The exhalant siphon extends first, at the posterior part of the ventral edge of dissoconch; then it grows out progressively (Fig. 27). When extended, this siphon may be nearly half as long as the shell. The inhalant aperture is detected by the differentiation of four short tentacles peeping out at the ventral gap of the shell near the base of the exhalant siphon (Figs. 28 & 29). Some tentacles appear also on the exhalant siphon under the tip of the primary siphon which remains thin. From day 30, both siphons are coalescent from the level of the tentacles (Fig. 30). The inhalant siphon, which has numerous conspicuous tentacles, remains shorter than the exhalant.

The tip of the foot constitutes the propodium covered by long cilia which make contact with any hard surface to crawl on. The byssal gland opens onto the heel of the foot and a pedal groove leads forwards from the duct opening up to the tip of the foot (Fig. 31). During locomotion, while the foot is
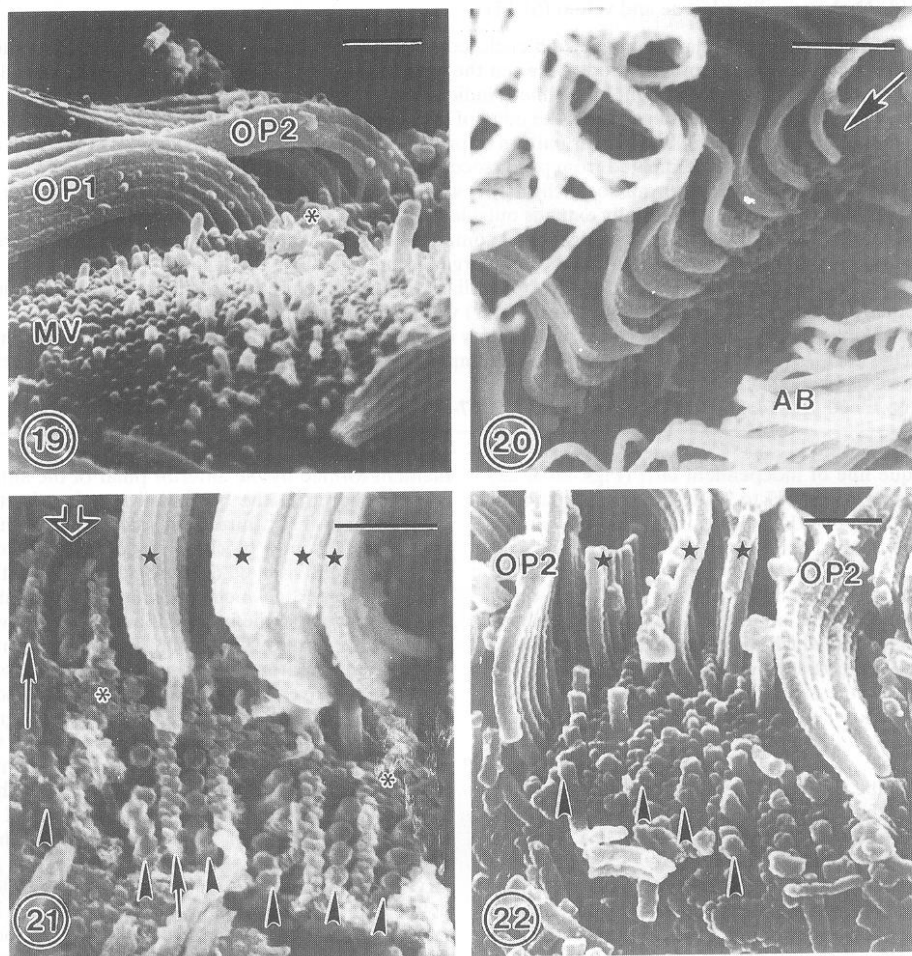
Figs 19–22. Details of the arrangement of cilia belonging to the outer preoral band of the velum (SEM).

Figure 19. The outer preoral band consists of a double row of cirri composed of 5 to 7 cilia. The internal row (OP1) is separated from the external row (OP2) by a narrow space (asterisk). MV, microvilli.
Figure 20. The external row (like the internal one) of the outer preoral band (arrow) is composed of numerous independent cirri which are layed out in a line. AB, cilia from the adoral band.
Figure 21. Cirri of the external row (arrow heads), which have been fractured off, are separated from each other by a narrow space occupied by microvilli (arrow). Each cirrus is composed of 5 to 7 cilia which are in register with those of the internal row (open arrow). Both rows of cirri are separated by an unciliated space (asterisk) bearing microvilli. Stars indicate partially fractured cirri from the internal row of the outer preoral band.
Figure 22. Partially fractured cirri of the outer preoral band showing the arrangement of the double row. Each cirrus of the external row (arrow heads) is in register with those of the internal row (stars). OP2, unfractured cirri belonging to the external row of the outer preoral band.
Scale bars: **19.** 2 μm, **20.** 1 μm, **21.** 0.5 μm, **22.** 2 μm.

contracting, the siphonal blood sinuses are filled up and induce the extension of the siphons. Usually, when the foot is extended, the siphons are retracted.

With siphon differentiation, the juveniles have acquired most features of adults but are not definitely settled. When they are reared in an upward current system, they frequently stick to the wall of the sieve because of the secretion of their byssal gland. Long byssal threads may also stick to the shells of the postlarvae which may become entangled and die.

Large variations in the development and growth schedule are observed, between batches and even within the same batch, so that 15 day-old individuals may be from 200 μm-long with only two gill filaments or up to 450 μm-long with 6 gill filaments. The juve-
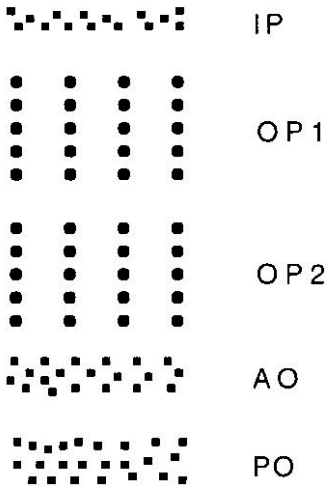
Figure 23. Disposition of cilia bases on velum of veligers of *Anomalocardia brasiliana*. The inner pre-oral band (IP) is composed of short cilia. Cirri of the internal and the external row of the outer preoral band (respectively OP1 and OP2), which are both composed of about 5 cilia, are in register with each other. The adoral and postoral bands (respectively AO and PO) are composed of short cilia apparently randomly distributed.

nile shell is clearly differentiated into prodissoconch I and II, and dissoconch, but is still roundish and very different from the adult shape (Fig. 32).

From the seventh week of development, the shell has acquired the triangular shape and pointed posterior end as well as brown zig-zag stripes, typical of *A. brasiliana*, but the individual sizes remain very variable in the same batches (Fig. 33).

DISCUSSION

The lack of efficient artificial stimuli for inducing spawning still hinders the controlled development of numerous bivalves. D'Asaro (1967) described the larval development of a Caribbean venerid clam, *Chione cancellata* from fertilization of stripped oocytes, but did not succeed in inducing spawning. This technique, which provides a small proportion of developing eggs (Loosanoff & Davis, 1962), was used also with success for *Chione stutchburyi* (Stephenson & Chanley, 1979). However, it is likely to produce a large proportion of abnormal larvae. Temperature fluctuation successfully induced spawning from some venerid clams (for review see Stephenson & Chanley, 1979), but is not efficient for most

species, even in the same family (D'Asaro, 1967) and not for *Anomalocardia brasiliana*. On a few occasions, we obtained spawning through serotonin following the method described by Matsutani and Nomura (1982); but this method which is a very powerful one for other species (Gibbons & Castagna 1984; Frenkiel & Mouëza, 1988) gives very irregular results for *A. brasiliana*. Osmotic stress was a more effective stimulus, which seems to be consistent with the natural conditions of mass spawning induced by osmotic stress due to heavy rain as observed by Monti *et al.* (1991). However, the best results were obtained by an appropriate feeding which induces the emission of gametes without additional stimulus. The fact that few females spawn simultaneously and their poor fecundity are consistent with analyses of gametogenesis, in Brazil (Narchi, 1976; Grotta & Lunetta, 1980, 1982) as well as in Guadeloupe, (Mouëza, Frenkiel & Monti, 1988) which have shown that in *A. brasiliana*, maturation is asynchronous not only inside a population but even in one and the same gonad. Our attempts to synchronize maturation were unsuccessful. Therefore, we were unable to increase the yield of larvae from each batch of adults.

The eggs of venerid species have a medium size and a limited vitellus supply typical of planktotrophic development (Ockelmann, 1965). The egg of *A. brasiliana* (60 μm) appears smaller than the eggs of *Mercenaria mercenaria* (73 μm) described by Loosanoff & Davis (1950) and *C. cancellata* (75 μm) described by D'Asaro (1967) but in the same range. The egg envelopes appear as most variable in the family Veneridae; *C. cancellata* and *A. brasiliana* have no jelly coat unlike the egg of *M. mercenaria* that is surrounded by a jelly coat which disappears generally between the blastula and the trochophore stages (Loosanoff & Davis, 1950). In *Venus striatula*, a similar gelatinous coat surrounding the egg is shed at the trochophore stage which becomes free swimming (Ansell, 1961). The features of the egg are generally not described for most species, but even when present, the jelly coat of venerid clams disappears at an early stage of development and is never retained up to the veliger stage as it is in the Tellinoidea, *Scrobicularia plana* (Frenkiel & Mouëza, 1979), in the lucinids, *Codakia orbicularis* (Alatalo *et al.*, 1984; Gros *et al.*, 1997), *Linga pensylvanica, Lucina pectinata, Lucinoma aequizonata* (personal observations), and probably in most, if not all, species of Lucinidae.
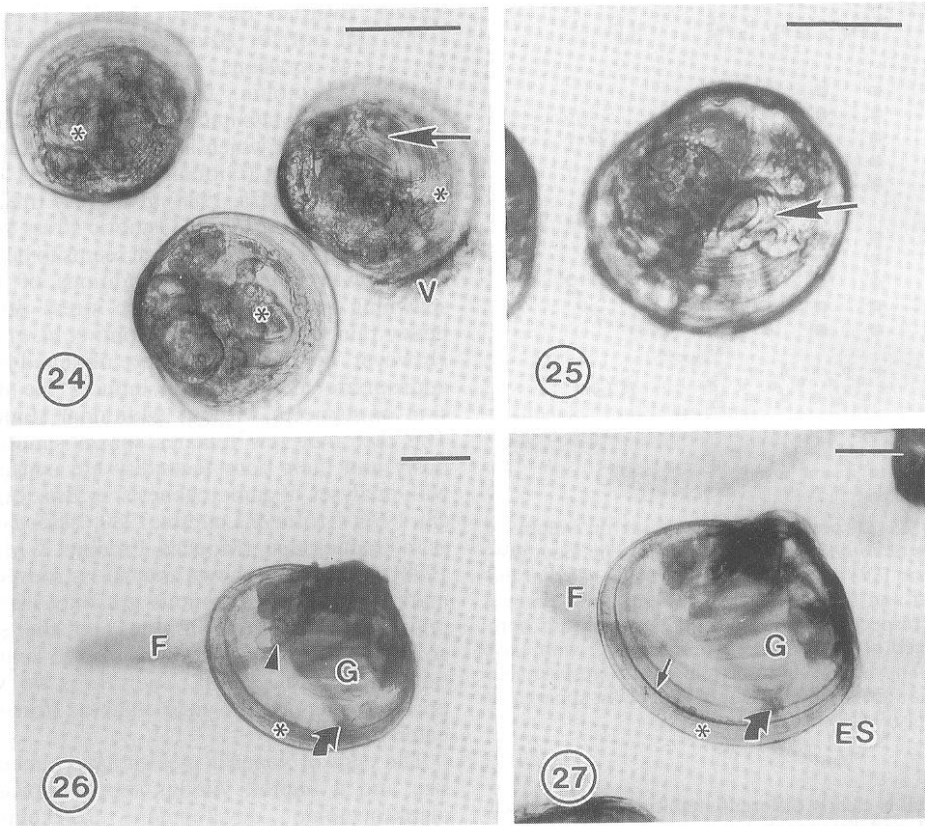
Figs 24–27. From pediveliger to early juvenile; foot, gill and siphon differentiation (Light microscopy).

Figure 24. 10-day old pediveligers with foot rudiment (asterisk) inside the shell and early gill crypts indicated by an arrow. V: velum.
Figure 25. 10-day old pediveliger with gill crypts (arrow) looking as a m-figure in the posterior part.
Figure 26. Early juvenile with extended foot (F) but no siphon; the gills have acquired several filaments (G) and their posterior parts are anchored symetrically on the siphonal septum (curved arrow). The statocyst is indicated by an arrow head and the dissoconch by an asterisk.
Figure 27. Older juvenile with foot (F) and exhalant siphon (ES) partly extended; most often only one or the others extended. There is a clear demarcation (arrow) between the prodissoconch II and the dissoconch (asterisk). G: gill filaments; curved arrow: siphonal septum.
Scale bars: **24–27.** 100 μm.

The early stages of embryogenesis are fairly rapid and result in a ciliated blastula, 3 hours after fertilization: in the same conditions, this stage is attained only after 9 hours in *C. orbicularis* (Gros *et al.*, 1997); this difference is probably related to the low vitelline supply of *A. brasiliana* when compared with the large yolky egg of the lucinid clam. During gastrulation, two major events occur, which are the appearance of the shell field and the formation of the prototroch. Authors do not agree on the order of appearance of these structures (for review see Waller, 1981). A sequential sampling at hourly intervals shows that the invagination of the shell field begins before the differentiation of the prototroch, in *A. brasiliana*, as well as in the lucinid clam *C. orbicularis* (Gros *et al.*, 1997). The succession of morphogenetic sequences may be quite variable and the shell field may appear later in some species as descried by Eyster & Morse (1984), for *Spisula solidissima*. In the trochophore of *A. brasiliana*, the shell material evaginates as a transverse pad recessed below the surrounding surface of the larva. Further, the transverse pad which becomes dumbbell-shaped is quite similar in

oysters (Waller, 1981), Lucinidae or Veneridae. It extends forming a saddle-shape as described by Eyster & Morse (1984) and gradually covers the soft body of the larva.

In the straight-hinge stage of veliger larvae, the cilia of the velum are well differentiated and there is an inner preoral band made of single short cilia similar to that described in *Ostrea edulis* (Waller, 1981), in *Chlamys hastata* (Hodgson & Burke, 1988) and *Pecten maximus* (Cragg, 1989), but which is lacking in *C. orbicularis* (Gros *et al.*, 1997). Waller (1981) hypothesized that it could transfer food particles to the mouth as the adoral band does; but he did not exclude the possibility that this band may constitute an upcurrent tactile receptor. However, there is no obvious reason to suggest a tactile receptor, rather than a chemoreceptive function. Observations of the velum of *A. brasiliana* shows that the more conspicuous ciliary band is the double outer preoral band made up of cirri which have less cilia each than it is reported for other species (for review see Cragg, 1996). The postoral band consists of randomly arranged short single cilia. Observations of the stage immediately before the straight-hinge veliger showed that the postoral tuft does exist before the postoral band. Some cilia situated between the mouth and the postoral tuft represent the first hint of the postoral band to be.

The apical flagellum, made up of nonmotile long cilia, which is conspicuous from the trochophore up to the veliger stage is a common structure in numerous bivalve species. It has been considered as a sense organ, but its ultrastructure was unknown until recently (for review, see Tardy & Dongard, 1993). It is neither characteristic of the family Veneridae as supposed by Ansell (1962) nor of Heterodonta as supposed by D'Asaro (1967). It appears as a stiff structure in trochophores as well as in live veligers, but with SEM the contact between cilia appears limited to their basal region and the cilia are free for most of their length unlike the laterofrontal cirri of the gills as well in *A. brasiliana* as in *Ruditapes phillipinarum* (Tardy & Dongard, 1993) or in *C. orbicularis* (Gros *et al.*, 1997). When alive, they may be embedded in some mucus component possibly dissolved by the critical point drying chemicals. Tardy & Dongard (1993) described, in *R. philippinarum*, a central unit which is considered to have a mechanoreceptor and perhaps a chemoreceptor function, and a peripheral unit made up of closed crypts with cilia inside, oriented head down, with still

unknown sensory function. The hypothesis of a photoreceptor function has not been considered. However, most bivalve veliger larvae have a positive photolactic behaviour combined with a negative geotropic behaviour as demonstrated experimentally for *Mytilus edulis* by Bayne (1964) which is replaced by a negative phototactic behaviour in plantigrades which have lost the apical sense organ. It will be necessary to check the presence and structure of the peripheral unit.

A punctate-stellate pattern has been described on the prodissoconch I of Ostreidae (Carriker & Palmer, 1979; Waller, 1981). The punctate portion of this pattern is considered to represent initial mineralization of conchiolin (Carriker & Palmer 1979) in agreement with Eyster & Morse (1984) who emphasize that, in *S. solidissima*, the calcification of the shell is initiated at the trochophore stage. Nevertheless, it is difficult to consider that the irregular wrinkles observed on the early shell of *A. brasiliana* represent the punctate-stellate pattern which is a more regularly organized structure. Similar wrinkles have been observed as well on *S. solidissima* (Eyster & Morse, 1984), on *P. maximus* (Cragg 1989) and on *C. orbicularis* (Gros *et al.*, 1997). However, it is most likely that these wrinkles represent artifacts of preparation for SEM which is known to induce a shrinkage up to 30% of soft tissues that may be responsible for irregular wrinkles. In *A. brasiliana*, neither the punctate-stellate pattern nor radial lines or fine commarginal striae were observed on the surface of prodissoconch I, the surface pattern of which is made of shallow pits up to the edge which is bordered with pointed arrow-heads similar to those described by Carriker & Palmer (1979). Such a different pattern may suggest a different calcification process which ought to be clarified, possibly by using X-ray microanalysis.

Gill primordia have been described in *Pandora inaequivalvis* (Allen, 1961), *C. cancellata* (D'Asaro, 1967), *C. orbidularis* (Alatalo, Berg & D'Asaro, 1984) and described as gill filaments during their evolution from pediveliger to plantigrade larvae. We suggest the name of ctenidial crypts for these arched structures which are very similar to that observed in pediveligers and plantigrades of *A. brasiliana* to discriminate it from functional gill filaments. Therefore, the term gill *anlage* may be retained for the tissue which is plicated at the inner face of the mantle to constitute the ctenidial crypts, and gill buds, for the posterior pillar of the primordial ctenidial crypts from which successive
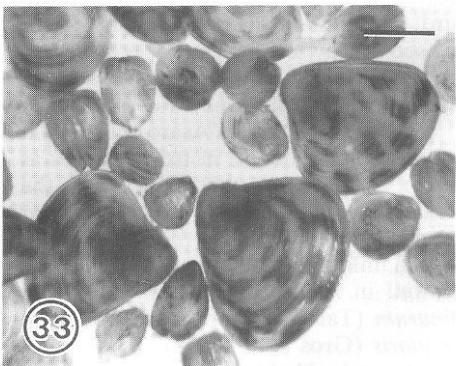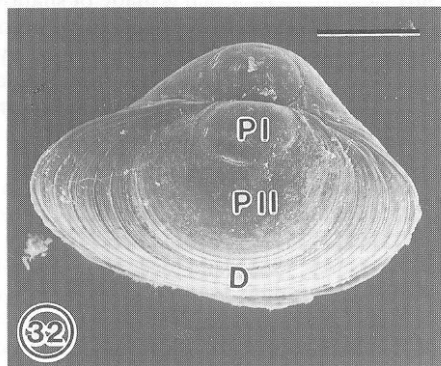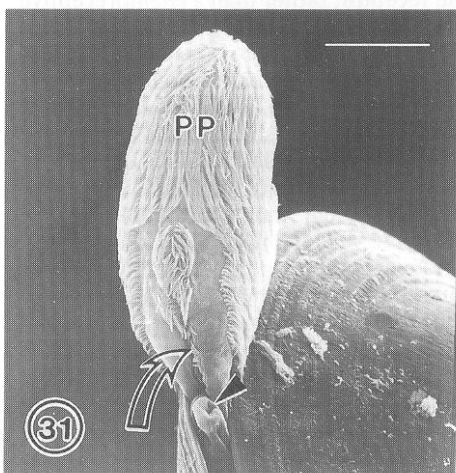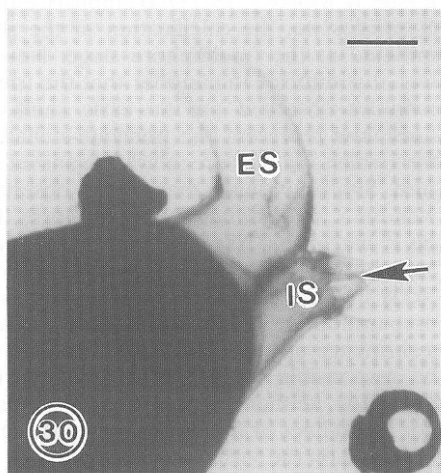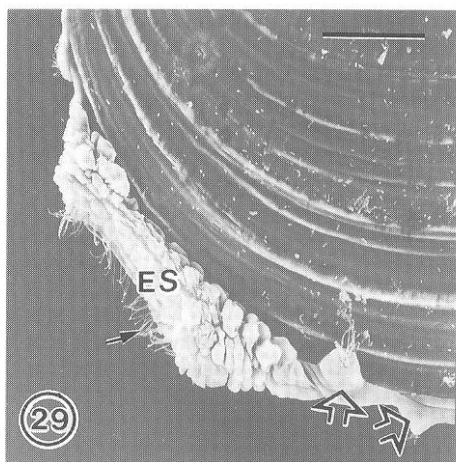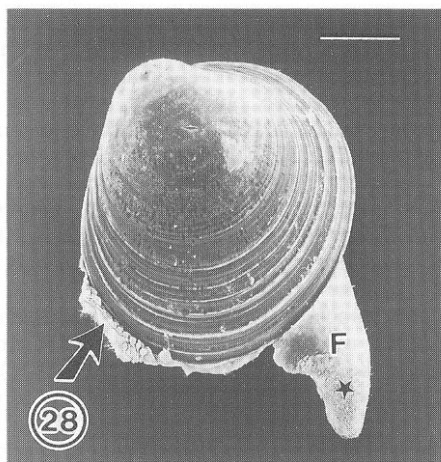
Figs 28–33. Postlarval development (SEM except 30 & 33, light microscopy).

Figure 28. 15-day old juvenile stage with exhalant siphon (arrow) merging from the shell gap together with foot (F). The extended foot is covered by cilia (star) from the tip to the heel.

Figure 29. Detail of exhalant and inhalant siphons. The exhalant siphon (ES) is fringed with cilia (arrow) whereas the inhalant siphon is only represented by two small tentacles (empty arrows) identified ventrally to the exhalant siphon.

Figure 30. Exhalant dorsal membranaceous siphon without tentacles (ES) and inhalant siphon (IS) fringed with single tentacles (arrow). Both are coalescent from the tentacular fringe of the inhalant one. The primary exhalant siphon, differentiated before the inhalant one, is free.

Figure 31. Detail of the foot to show the byssal gland canal (arrow head) and the pedal ventral groove (curved arrow) up to the ciliated propodium (PP).

Figure 32. Dorsal view of shell showing the prodissoconch I (PI), the prodissoconch II (PII), and the dissoconch (D).

Figure 33. Typical shape and pattern of 5-week old juveniles. Notice the large size range within a single batch. Scale bars: **28.** 50 μm, **29.** 20 μm, **30.** 200 μm, **31.** 10 μm, **32.** 50 μm, **33.** 400 μm.

new filaments are budding off during the second period of gill setting. The term gill filament will be used only to denominate the elongated functional filaments. These definitions are designed to avoid the confusing use of the term gill filament for any larval structure related to the ontogeny of the gills.

Quayle (1952) and Ansell (1962) described, in pediveliger larvae of two venerid species, *Venerupis pullastra* and *Venus striatula*, four elongated gill filaments which are different from the gill filaments described by Allen (1961), D'Asaro (1967) and Alatalo *et al.* (1984), the latter being earlier typical ctenidial crypts. Such a precocious development of the gills associated with the posterior mantle fusion which constitutes the siphonal septum has not been observed in *A. brasiliana* which follows a similar sequence of events as in *C. cancellata* (D'Asaro, 1967). In both species, the ctenidial crypts are differentiated in the pediveliger stage, well before the siphonal septum, with symmetrical ctenidial crypts on each inner face of the mantle. The general rotation of the body, due to the disappearance of the velum and to differential growth, puts these structures in a posterior position in the plantigrade, and the subsequent fusion of the siphonal septum in the same area leads to close proximity between the gill buds and the siphonal septum such as described by Quayle (1952) and Ansell (1962). Paradoxically, the general development of *V. pullastra* and *V. striatula* is slower than the development of *C. cancellata* and *A. brasiliana* which become plantigrades before the differentiation of gill filaments. We should conclude that, even in the family Veneridae, the sequence of organogenesis is not so similar in different species as it has been postulated.

Similar ctenidial crypts have been observed in species of the families Veneridae (D'Asaro,

1967), Semelidae (Frenkiel & Mouëza, 1979), and Lucinidae (Alatalo *et al.*, 1984; Gros *et al.*, 1997). Conversely, gill differentiation appears quite different in the pectinid *P. maximus* with a primordial gill bud in a posterior position already present in the pediveliger (Beninger *et al.*, 1994) and no structures similar to the crypts, which appear as typical of eulamellibranchia. However, the gill basket described as second stage of gill differentiation in the fillibranch *P. maximus* seems to be the counterpart of the ctenidial crypt-stage of Eulamellibranchia and in both cases such structures cannot fulfil the nutritional functions of gill filaments.

The differentiation of gill filaments successively shed from the posterior gill buds is a necessary condition for metamorphosis. In some species such as *A. brasiliana*, this change goes on without interruption, but in other species, an environmental cue is necessary to trigger the transition from crypt stage to functional gill filament stage. Such a delay of metamorphosis has been described most frequently between the pediveliger and the plantigrade stage (Bayne, 1976; Frenkiel & Mouëza, 1979) but may occur only after the plantigrade stage, as in the lucinid clam *C. orbicularis* (Gros *et al.*, 1997). Therefore the crucial step of metamorphosis appears not to be the regression of the velum but the differentiation of functional gill filaments, which is the condition of a new type of nutrition, only possible after the multiplication, elongation and cell differentiation of gill filaments.

The differentiation of the siphons is the last step of metamorphosis, typical of burrowing clams. In the venerid, *V. pullastra*, Quayle (1952) described the first tentacular ring of the inhalant siphon as constituted by the inner mantle fold, whereas a common outer ring of

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