

Post-settlement metamorphosis of
Megabalanus californicus (Cirripedia, Sessilia,
Balanidae) documented by means of scanning
electron microscopy and video recording

Klemens Gusenbauer

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Begutachter: Univ. Prof. Dr. Waltraud Klepal

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Abstract

Post-settlement metamorphosis and early juvenile development of an advanced balanid was studied on the example of *Megabalanus californicus* (Pilsbry). A twelve hour period starting with the moult of the cyprid carapace was observed in detail. Developmental changes of living specimens were studied by means of dissecting microscopes and recorded on video tape for further analysis. The successive ontogenetic stages were fixed and studied with scanning electron microscopy.

When shedding the cyprid carapace the juvenile is composed of a „capitulum“ with primordial carina and primordial opercular plates as well as a vestigial peduncle, both being about the same size. The peduncle vestige differs from the „capitulum“ in the structure of its surface and the lack of cuticular hairs. The „capitulum“ consists of the developing wall, which is surrounded by two circles of cuticular hairs, and the operculum holding two additional rows of such hairs. In scanning electron microscopic images the five primordial plates (scuta, terga and carina) are distinguished by their dented appearance.

In the course of development the shape of the juvenile barnacle is changing rapidly. As the body is getting flatter and broader the surface of the rudimentary peduncle approaches the substratum. About four hours after ecdysis the juvenile has achieved the typical cone shape of adult barnacles. The peduncle vestige has become the basis and is getting fixed to the ground by juvenile cement. Cuticular knobs are densely covering most of the surface of the wall and the operculum. The beginning deposition of calcite hardens their plates. About 12 hours after ecdysis the basis is completely fixed to the substratum.

Introduction

During ontogeny sessile barnacles go through dramatic changes in their morphology and their way of life. The last of the six nauplius stages is transformed through the presettlement metamorphosis into a non-feeding cyprid larva, whose purpose is to find a suitable substratum for settlement. First the cyprid attaches with cement, which it excretes from the antennulae. Then there seems to follow a stage of non activity, when observed from outside. In earlier publications this was also called „pupal stage“ (e.g. Darwin 1854:13,102ff, Runnström 1925). This stage comes to an end with another metamorphosis¹. During this post-settlement metamorphosis the cyprid sheds its bivalved carapace together with the compound eyes, which are unique to the cyprid larva. The still delicate juvenile now forms a broad basis, on which its body, taking on more and more the shape of a cone, resides. The body of the juvenile gets encircled by a ring of shell plates, which, by means of calcification, form a solid wall. Apically there is the opening to the mantle cavity, being protected by opercular plates, which get calcified as well.

As Anderson (1994:267) points out, only few papers have been published on post-larval development and the topic has been virtually ignored since the end of the sixties of the past century. It has been only in the last decade that this topic has been picked up again. On the one hand by Newman (1989), who described juvenile stages of the most primitive still

¹ Most authors used the term „metamorphosis“ to describe the transition of the cyprid larva to the juvenile organism. Walley (1969) enhanced the meaning of the term in the sense that it also includes the previous transformation from the nauplius-VI stage to the cyprid. Very clear is the terminology used by Anderson (1994). He calls those two transformations „presettlement“ and „post-settlement metamorphosis“. Newman (1989) also used the term „metamorphosis“ to describe the sudden transition from a pedunculate to a sessile stage in juveniles of the most primitive extant sessile barnacle (cf. Discussion).

living sessile barnacle, on the other hand by Glenner & Høeg (1993) and Shalaeva (1996, 1997), who both did comparative studies on postlarval development of barnacles using scanning electron microscopy. Three examples of classical works on the topic are the morphological study on the ontogeny of *Semibalanus balanoides* by Runnström (1925), the description of shell development of *Balanus improvisus* by Costlow (1956) and a comprising study of the larval development and metamorphosis of *S. balanoides* by Walley (1969).

From the phylogenetic point of view, there is a general consensus, that sessile barnacles have evolved from pedunculate ones. Therefore it can be assumed that in the ontogenesis of sessile species we can also find evidence of this origin, e.g. a vestigial peduncle. For instance Newman (1989) showed that the most primitive still living sessile barnacle, *Neoverruca brachylepadoformis*, goes through several pedunculate stages. Though already Darwin (1854:130) mentions a transitory pedunculate stage of *Semibalanus balanoides*, there is little evidence to be found in other literature on higher evolved Sessilia. Only more recently Glenner & Høeg (1993) and Shalaeva (1997) describe peduncle rudiments. One goal of this paper is to find out if a vestigial peduncle can be found in a higher developed species.

As object of research I use *Megabalanus californicus* (Pilsbry), a member of the family Balanidae, which has, as is indicated already by its name, its distribution from Northern California to the northwest coast of Baja California (Henry & McLaughlin 1986). Up to now there exists only one paper on the ontogenesis of this species, which is a description of its presettlement development (Miller & Roughgarden 1994). In my work I want to trace and describe the processes during the post-settlement metamorphosis as accurately as possible. For this purpose observations were made on living specimens by means of light microscopy and recorded on videotape. To show the morphological details the different developmental stages were studied with scanning electron microscopy.

Material and methods

The individuals used to examine post-settlement metamorphosis in *Megabalanus californicus* (Pilsbry) were collected in La Jolla (USA, Southern California) between September and November 1993. For this purpose plastic plates were used as substrate for settlement of cyprid larvae. The plates were fixed to the pillars at the far end of the pier at the Scripps Institution of Oceanography, a place where numerous adult individuals of *M. californicus* settle. They measured 75 mm in height and 115 mm in width, were non-transparent with the surface roughened and fixed about 1.5 m below the average water line.

They were checked daily and plates with attached cyprid larvae were transferred to the laboratory and reared in aquaria in flowing seawater pumped in directly from the pier. During research the water temperature was between 19° and 21° Celsius. Recording the exact location of the specimens on the plate allowed to follow the development of each individual. Development of living specimens was followed with dissecting microscopes, from the moment of attachment to about 12 hours after ecdysis, which was also documented on videotape.

Specimens in different stages of development were removed from the substratum and fixed, half of them in 70% ethanol, the other half in 2.5% glutaraldehyde respectively. Unfortunately those fixed in glutaraldehyde could not be used due to a technical problem. Therefore electron microscopy had to be carried out with specimens fixed in ethanol. They were dehydrated in a graded series of ethanol and critical point dried. With double-sided tape the specimens were mounted on stubs and coated with gold. A JEOL JSM-35CF operated at 15 kV was used for scanning electron microscopic (SEM) examination.

In order to acquire a preparation of a cross section of the cuticular knobs ethanol fixed specimens were returned to an aqueous solution, fixed in 2.5% glutaraldehyde and postfixed in 1% OsO₄. For embedding Epon resin was used. The thin sections were stained with uranyl acetate and examined in a EM9-S2 (Zeiss) transmission electron microscope (TEM).

Results

This chapter is structured chronologically following the development in *Megabalanus californicus* as observed on living specimens and documented on video tape. A diagram (Fig. 1) and SEM (scanning electron microscopic) images (Figs. 2 to 14) illustrate the successive developmental stages. This can also be watched „live“ in the enclosed video.

About 16 to 18 hours after attachment the cyprid finishes post-settlement metamorphosis by shedding its carapace. This is initiated by numerous contractions, approximately every ten seconds in the last minutes before the moult. When using light microscopy, the body of the metamorphosing barnacle is visible through the transparent cyprid carapace. At this stage it has already detached from the carapace at the anterior and posterior ends. The body appears to be divided into two zones. The anterior half of the body shows small, elongated, whitish spots on a darker, translucent ground. The posterior half appears lighter showing large white spots. Two pairs of these are well marked and can be identified as the primordial opercular valves, the others form a loose belt, which might be the incipient wall plates (Fig. 1). So it can be assumed that the posterior region is homologous with the capitulum of the Pedunculata, whereas the anterior area could be addressed as the peduncle vestige.

The actual moult starts with a 90° rolling movement, that takes four to five minutes and brings the cyprid carapace into an upright position (as can be seen in the enclosed video). Now the shed compound eyes become visible ventrally outside the carapace (Figs. 2 & 3). The body is stretching and contracting constantly and trying to squeeze itself out of the carapace. About three minutes later ecdysis has completed and the carapace, including the attached compound eyes, is drifting away. Now the juvenile gets entirely visible and it is quite obvious that it has a vestigial peduncle. Immediately it starts a rhythmic pumping activity of the thorax, interrupted approximately every ten beats by lateral nodding movements,

first to one side, then to the other. For a short period the peduncle rudiment is actually a functional peduncle, providing the juvenile barnacle with limited mobility. But the vestigial peduncle is shortening rapidly. In this stage the barnacle can be divided into three sections of roughly equal height: the operculum, the developing wall and the peduncle rudiment. The operculum is easily identified by its well marked primordia of scuta and terga. They are characterized by a surface that appears in SEM images conspicuously dented, a feature they have in common with the primordial carina (Fig. 4). At this stage the ocelli show through the body rostromedian at the basal verge of the scuta.

Mantle tissue is protruding from the apical edge of the operculum from the very beginning and does not disappear during the observation period. As SEM reveals, it is decorated distally with a dense field of cuticular processes (Fig. 5).

Four rows of cuticular hairs can be distinguished on the juvenile. Two of them encircle the developing wall parallel to the substratum. These hairs are about 100 μm long. A third row is located at the basal edge of the opercular primordia. The fourth row - consisting of much smaller cuticular hairs - is situated at the apical edge of the operculum, nestling against the protruding mantle „crest“.

Taking a closer look on the structure of the surface a clear distinction can be made between the developing wall and the peduncle rudiment. Whereas the wall is coarsely furrowed, the cuticle of the peduncle rudiment appears finely wrinkled (Fig. 6).

About one hour after ecdysis the juveniles shape has flattened (Fig. 7) and the basal margin of the developing wall is getting closer to the substratum. This process is going on until the peduncle vestige is oriented completely towards the substratum and becomes the basis of the barnacle. Throughout this only the shape of the juvenile alters while its volume stays constant. About four hours after ecdysis, the typical conical shape is achieved (Fig. 8). Meanwhile the wall is getting stiffer and less furrowed and the boundaries of the wall plates get visible (Figs. 7 & 9). It is now becoming obvious, that the wall as well as the operculum - except the five primordial plates - are covered all over with small cuticular knobs of roughly 3 μm in diameter (Fig. 9). The above mentioned concave dents of the primordia are of about the same size as the convex knobs (Fig. 10). The primordial carina is conspicuously shaped like a sickle (Fig. 11).

On the scutum a smooth area is perceptible. It might reflect the scutal adductor scar (Fig. 12). Cross sections of the cuticular knobs, viewed by transmission electron microscopy, show that they are not the result of a bulged cuticle but are rather „hollow“ (Fig. 13).

Growth of the wall is becoming noticeable. About six hours after ecdysis the carino-lateral plates can be distinguished, easily in the living specimen when using light microscopy, more difficult in SEM images (Fig. 10).

The cirri are beating quickly and continuously, interrupted only occasionally by retractions of the operculum. Here we can see that the wall is already forming a stiff unit by now, with exception of a flexible area that joins the carina with the sickle-shaped primordium (cf. enclosed video). This shows that the process of calcite deposition has started. Further evidence is given by the fact, that the wall gets fractured when specimens are removed from the substratum at this stage (Fig. 12). The retractions of the operculum also show that it is not until 12 hours after ecdysis, that the basis gets fixed to the substratum completely. Up to this point the wall is moving slightly towards the ground when the depressor muscles contract. SEM images of the basis of individuals that were removed from the substratum a few hours after ecdysis show that excretion of juvenile cement starts at around the center of the basis, where the cyprid originally had attached itself with cyprid cement. The cyprid cement in the center, the already excreted juvenile cement around it and the not yet attached basis can be discerned clearly. The surface structure of the latter is still as finely wrinkled as before (Fig. 14).

An interesting aspect is revealed when the rostrum is observed through a dissection microscope. Median the whitish spots are absent. This forms a vertical dark „line“. SEM-images of that area show less knobs that are somewhat drawn-out and could also be described as folds (Fig. 13).

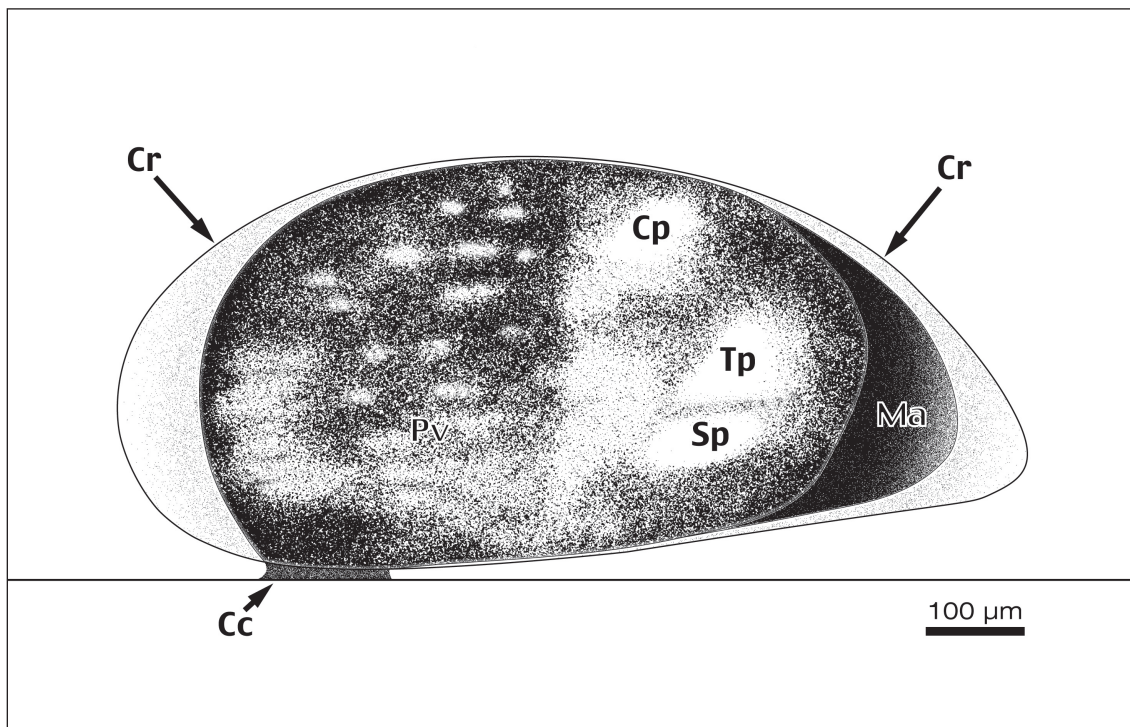


Fig. 1 Metamorphosing barnacle at the beginning of the moult, left lateral view. The anterior and posterior ends have already detached from the cyprid carapace (Cr). The anterior half (left) of the enclosed juvenile is the peduncle vestige (Pv) and can be clearly distinguished from the posterior half, the „capitulum“ (right). The primordia of scutum (Sp), tergum (Tp) and carina (Cp) appear as white areas.

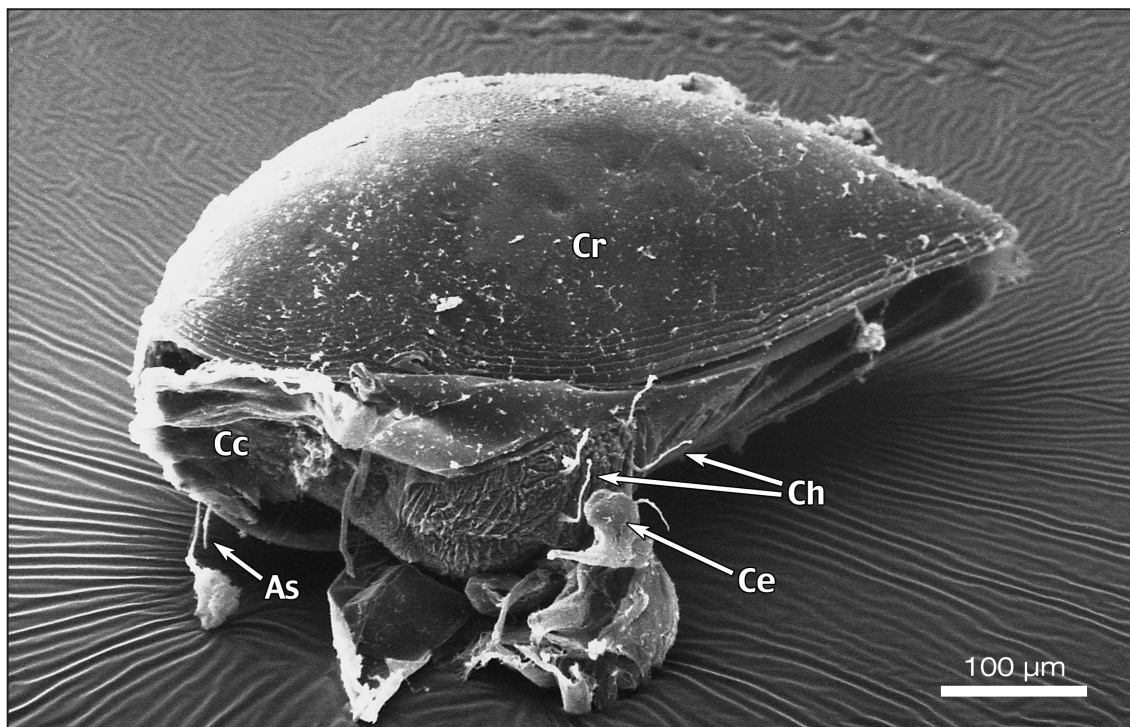


Fig. 2 Specimen fixed at the moment of shedding the cyprid carapace (Cr), ventro-lateral view. This specimen, like all others, was removed from the substratum, where it was attached by its cyprid cement (Cc). Part of the antennular setae (As) are projecting from behind the cement. The compound eyes (Ce) have been shed but remain connected to the carapace.

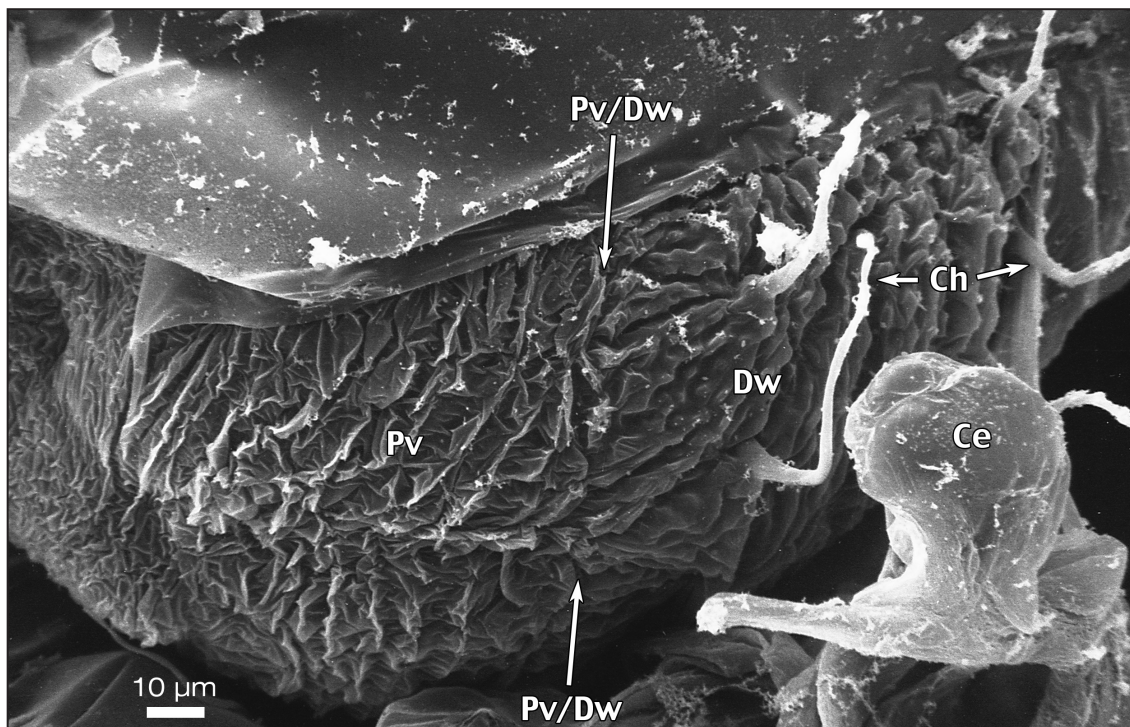


Fig. 3 Detailed view of the specimen shown in Fig. 2. The difference in the texture of the cuticular surface between the peduncle vestige (Pv) and the developing wall (Dw) can be noticed. Whereas the vestigial peduncle is finely wrinkled the developing wall appears coarsely furrowed (cf. Fig. 6). The long arrows (Pv/Dw) point out the boundary between them.

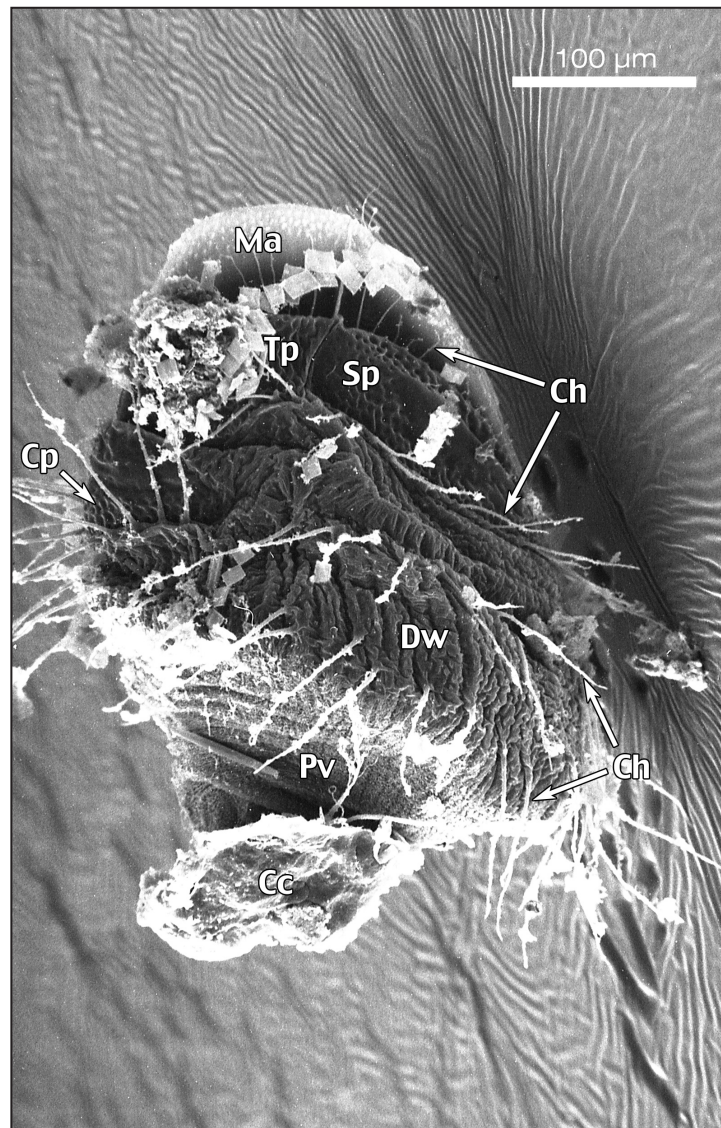


Fig. 4 Juvenile after shedding the cyprid carapace, left lateral view. Vestigial peduncle (Pv), developing wall (Dw) and operculum are of roughly the same height. The primordia of the opercular plates scutum (Sp) and tergum (Tp), as well as the primordial carina (Cp) are already identifiable by their dented appearance. The body is surrounded by cuticular hairs (Ch). The protruding mantle (Ma) and the tergum are contaminated, most likely by algae.

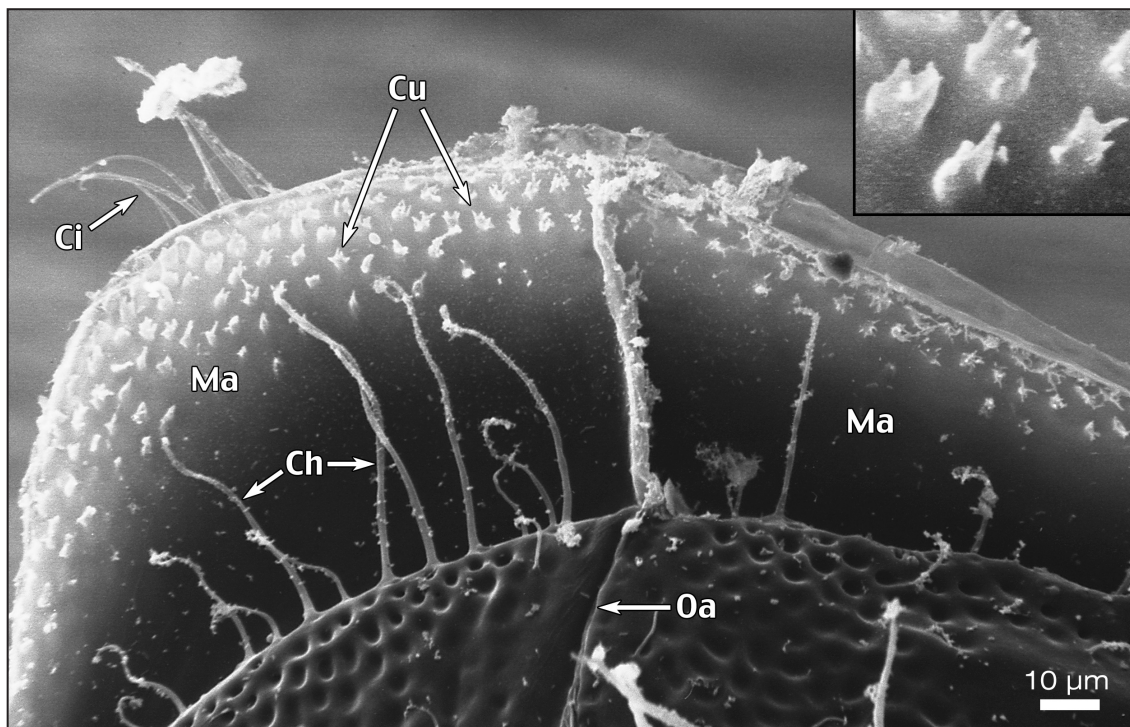


Fig. 5 Detailed view (in a later stage) of the protruding mantle (Ma), left lateral view. Note the cuticular processes (Cu) which are shown enlarged in the inset. Tips of cirri (Ci) are protruding from behind the mantle. Cuticular hairs (Ch), varying in length, originate from the edge of the operculum and nestle against the mantle crest. The opercular articulation (Oa) is continued in the mantle tissue.

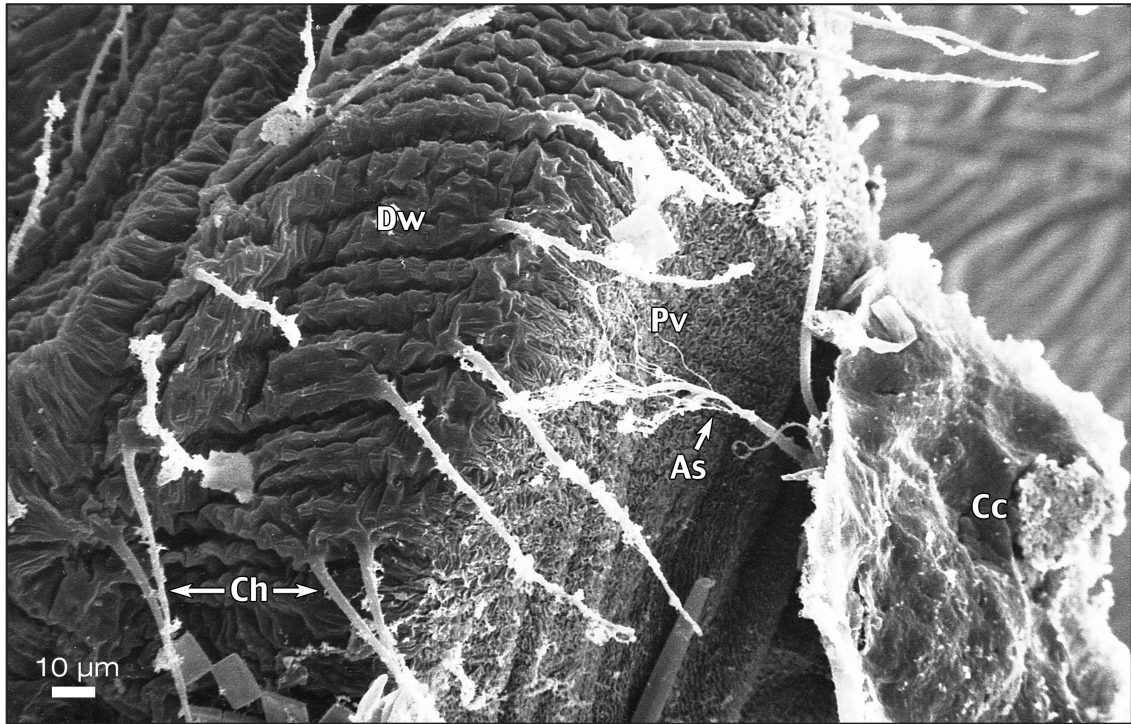


Fig. 6 Same specimen as in Fig. 4, left lateral view, detail. Note the difference in the surface structure of the developing wall (Dw) and the peduncle vestige (Pv). Cuticular hairs (Ch) emerge from the developing wall, but are absent on the rudimentary peduncle. This image also allows a closer view of the antennular setae (As).

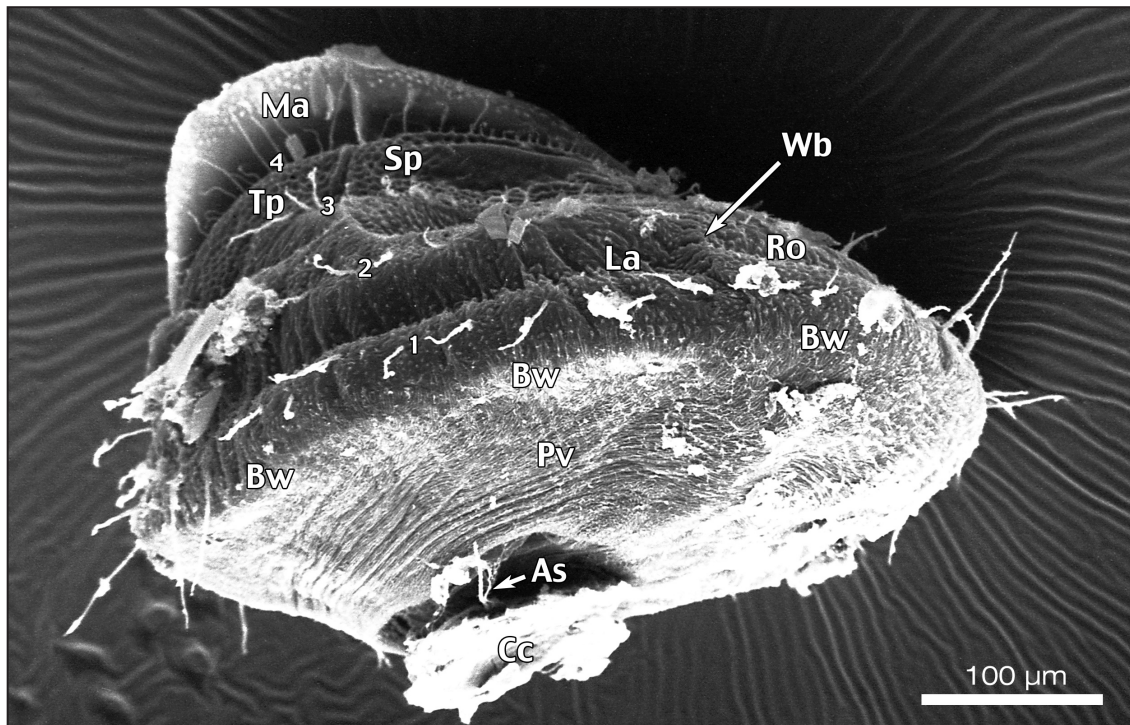


Fig. 7 Specimen about one hour after ecdysis, left lateral view. The juvenile is approaching the typical cone-shape as the body is increasing in diameter, thus making the boundary between the vestigial peduncle (Pv) and the developing wall more pronounced (Bw). The rudimentary peduncle is getting oriented towards the substratum. The cuticular hairs are arranged in two rows on the developing wall and in two rows on the operculum (Sp, Tp), indicated by the numbers 1 to 4. On this specimen the evolving wall boundary (Wb) separating rostrum (Ro) and lateral (La) can be identified.

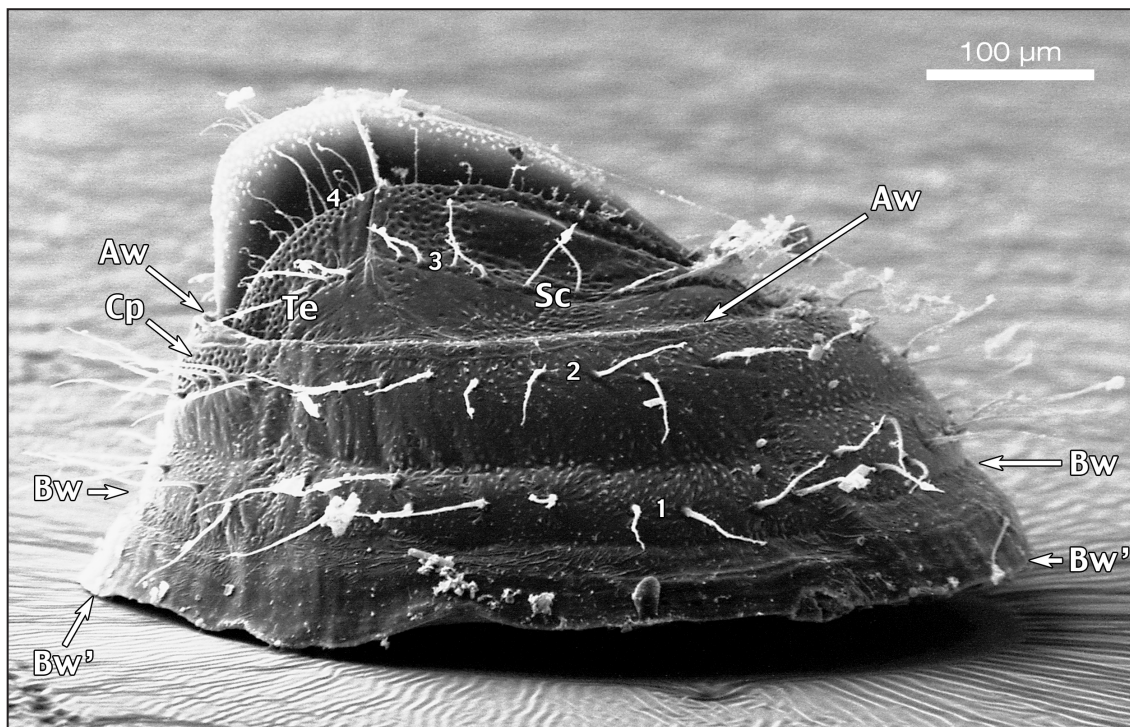


Fig. 8 Juvenile several hours after ecdysis, left lateral view. The typical cone shape has been achieved. The upper part of the wall is clearly delimited from the operculum by an apical boundary (Aw) forming the orifice. The basal boundary (Bw') is now in contact with the substratum as the rudimentary peduncle has become the basis. Since the wall has started its growth in height (Bw—Bw') by basal accretion, the original boundary (Bw) between the peduncle vestige and the wall has risen. The operculum (scutum Sc, tergum Te) is still in a highly exposed position. Note the primordial carina (Cp), which can be seen in detail in Fig. 11.

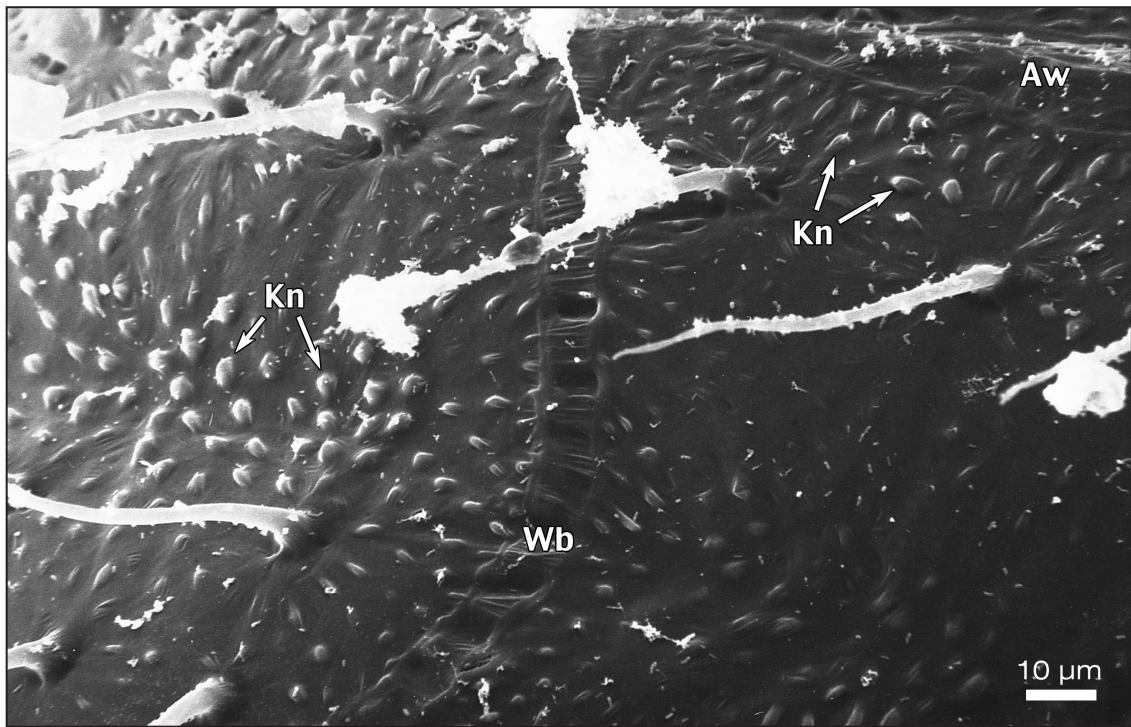


Fig. 9 Same specimen as in Fig.8, right lateral view, detail. The boundary (Wb) between rostrum (left) and lateral (right) causes a mark on the surface of the cuticle. Note the knobs (Kn) covering the wall.

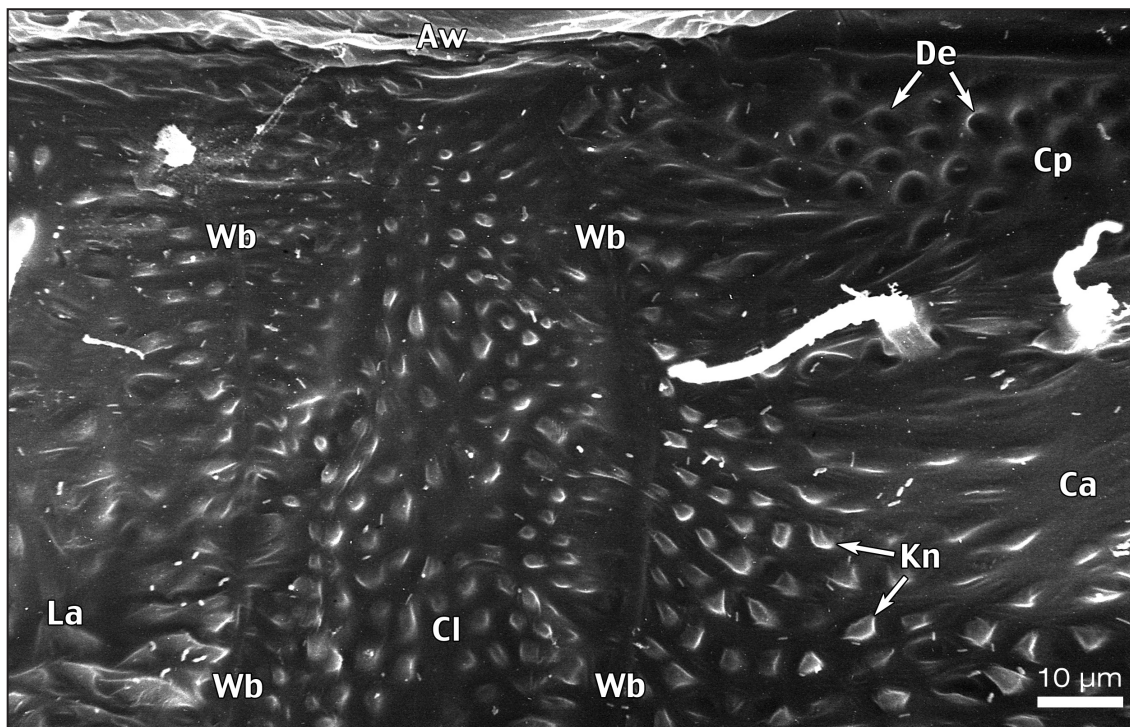


Fig. 10 Specimen several hours after ecdysis, detail of the boundaries between carina (Ca), carinolateral (Cl) and lateral (La). Note the convex knobs (Kn) contrasting with the concave dents (De) on the primordial part (Cp) of the carina.

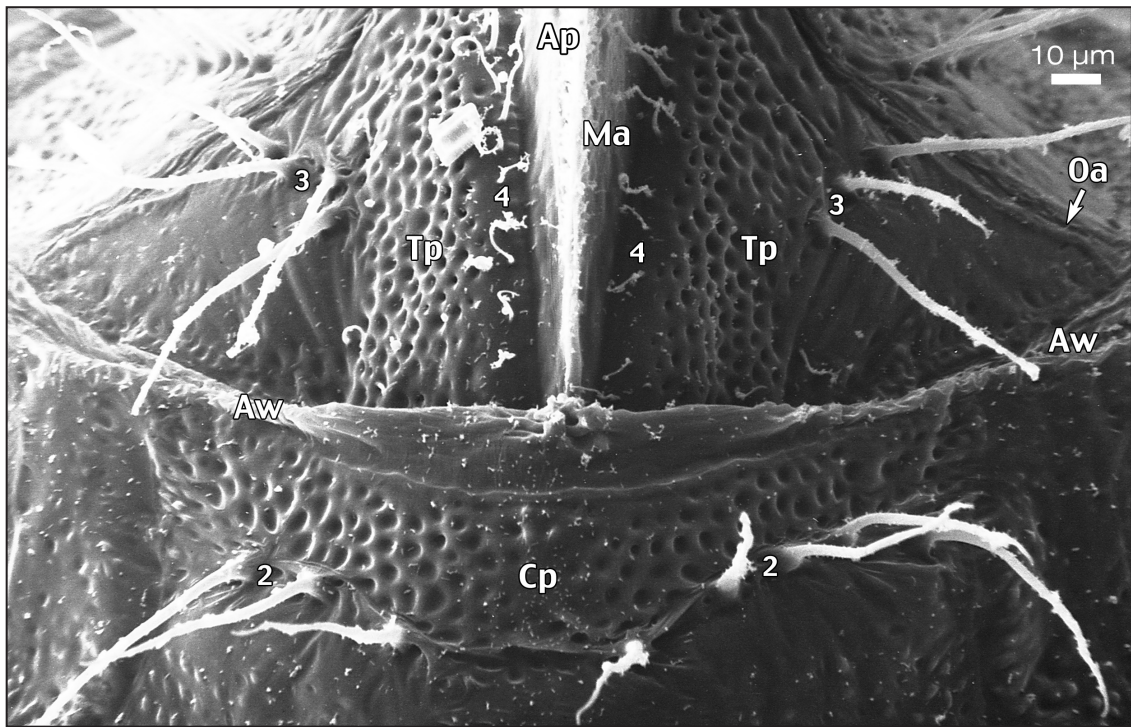


Fig. 11 Same specimen as in Fig. 8, plain view of the sickle-shaped primordial carina (Cp). Note the tergal primordia (Tp) which are dented similarly. Cuticular hair rows (2, 3) mark the basal limits of the primordial plates. Between the mantle flaps (Ma) the aperture (Ap) opens, appearing white in this image.

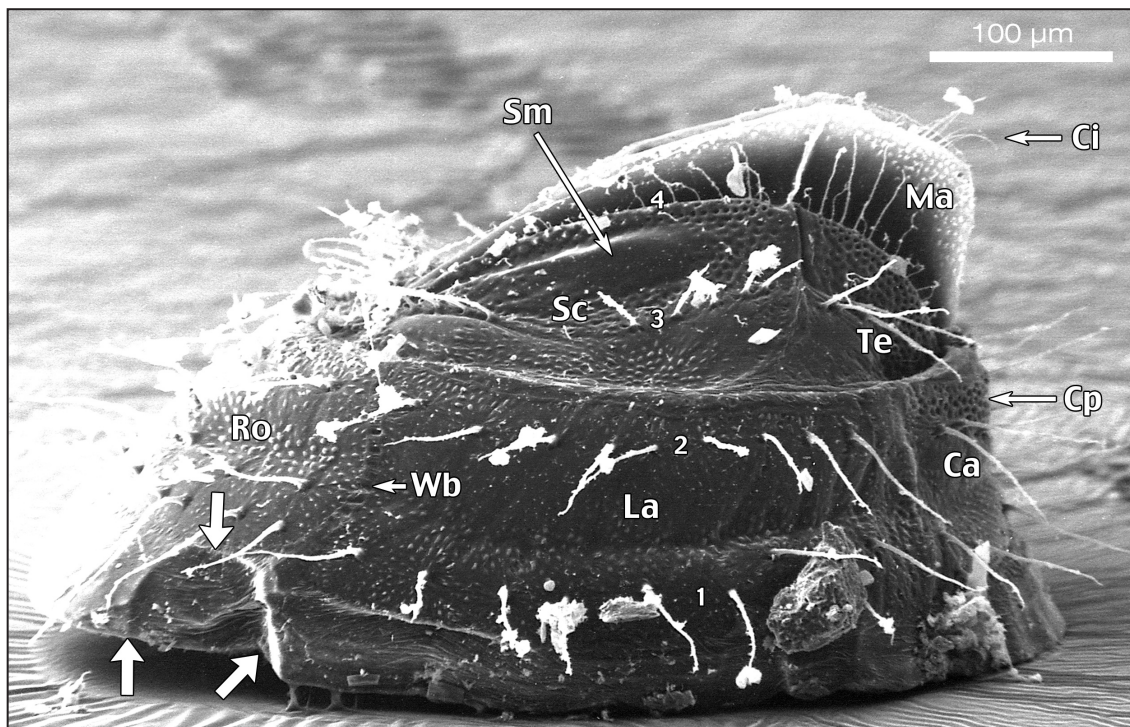


Fig. 12 Same specimen as before, right lateral view. Note the smooth area (Sm) on the part of the scutum, where internally the adductor muscle attaches. The wall has got fractured (thick arrows) when removed from the substrate, which gives evidence that the process of calcite deposition has started.

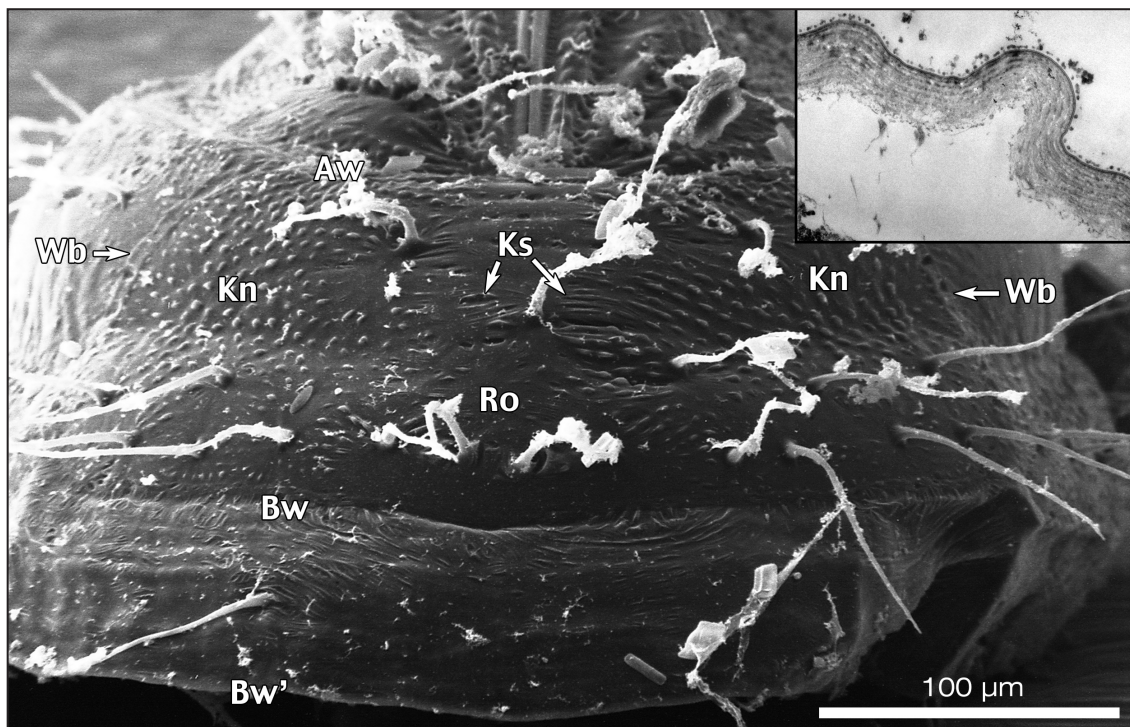


Fig. 13 Same specimen as before, rostral view. Note that on the median area of the rostrum there are not as many knobs as on the rest of the wall. Some of the knobs appear to be stretched out horizontally (Ks). Bw—Bw' indicate the shells increase in height. The inset shows a cross section of a cuticular knob. This transmission electron microscopic image reveals that the knob is „hollow“.

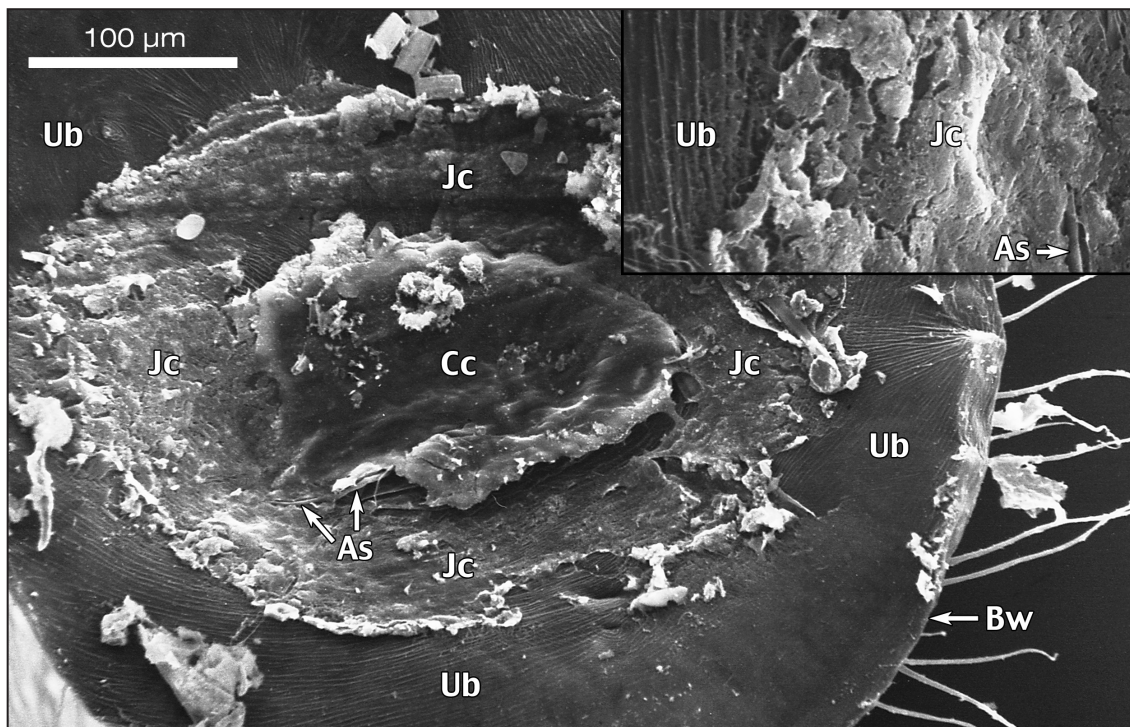


Fig. 14 Same specimen as in Fig.10, basal view. In the center the cyprid cement (Cc) can be seen. There the cyprid larva had originally attached. It is surrounded by juvenile cement (Jc) that fixes the basis to the substratum. On the periphery the part of the basis that is not yet attached (Ub) can be identified. The inset shows a detailed view of the juvenile cement and the still fine wrinkled surface of the not yet attached basis. Parts of the antennulae (As) that get embedded into the juvenile cement can be discerned.

Discussion

This is the first study dealing with post-settlement development of *Megabalanus californicus*. Like Glenner & Høeg (1993) and more recently Shalaeva (1996, 1997) metamorphosis and post-larval development was studied using scanning electron microscopy as well as observation of living specimens. At large the ontogenetical processes occurring in *M. californicus* resemble those described for *Balanus improvisus* and *Balanus amphitrite* by the authors quoted above. In this section the differences are discussed that become obvious when looked upon in detail. Some of the results presented in this paper confirm those of other authors, others raise issues to be questioned and some of them lead to conclusions concerning the phylogenesis of Sessilia.

SEM appearance of the primordial plates

The five chitinous primordial plates scuta, terga (both occurring in pairs) and carina appear in the ontogeny of most thoracicans. Due to their dented appearance they immediately catch the eye in SEM (scanning electron microscopic) images of *Megabalanus californicus* as soon as it has shed its carapace. In her comparative studies Shalaeva (1996, 1997) describes their structure as „honeycomb primordia“. Though they are not referred to explicitly in the text of Glenner & Høeg (1993) the SEM pictures of *Balanus improvisus* and *B. amphitrite* also show the dented appearance of their primordial plates. In the same paper *Semibalanus balanoides*, on the contrary, is shown covered evenly with knobs and displays a longitudinal fold on each scutum. Shalaeva (1997) states that the plates develop from plicae of the external cover and/or from „honeycomb primordia“.

The vestigial peduncle

Newman & Hessler (1989) describe the most primitive form of extant sessile barnacles, an inhabitant of abyssal hydrothermal vents. Newman (1989) presents a description of its unique juvenile ontogeny. *Neoverruca brachylepadoformis* goes through several pedunculate juvenile stages until it suddenly metamorphoses into a sessile form. This astonishing discovery of a peduncle homology in Sessilia raises the question if vestiges of a peduncle can be found also in higher evolved sessile barnacles. Already Darwin (1854:130) describes a transitory pedunculate stage in *Semibalanus balanoides*. In Runnström (1925) a description of a vestigial peduncle cannot be found in the text, but some details in the illustrations could be interpreted as such. In this sense he gets cited in Anderson (1994:270) and Yamaguchi & Newman (1990). In her notable study on *S. balanoides* Walley (1969) doesn't mention a peduncle rudiment.

In *Balanus improvisus* Glenner & Høeg (1993) describe a convex bead, that develops around the basal circumference of the body, and call it rudimentary peduncle. As a reason for their assumption they state that on this „bead“ there would be cuticular hairs that resemble those found on the stalk of the juvenile pedunculate *Scalpellum scalpellum*. In my opinion this cannot be seen as evidence since the less advanced sessile *S. balanoides* does not show similar cuticular hairs. Numerous such hairs, on the contrary, can clearly be found on the developing wall of the object of this study, the more advanced *Megabalanus californicus* (Fig. 8). For this reason the occurrence of cuticular hairs on this bead can hardly be seen as a plesiomorphic attribute and therefore it is questionable to use it as an argument for homologizing the bead with a peduncle.

Glenner & Høeg (1993) themselves question their interpretation of the bead as a vestigial peduncle due to their observation that the boundaries of the plates get visible on it. This shows clearly that the bead is part of the developing wall and that would mean that the basal part of the future wall is homologous with the peduncle whereas the apical part, together with the developing operculum, would be homologous with the capitulum in Pedunculata. In my opinion the clear partition into capitulum and peduncle through the capitular/peduncular growth zone (Anderson 1994:247ff) stands against that interpretation. In *M. californicus*

this partition corresponds most likely to the boundary between the developing wall and the developing basis (cf. also Shalaeva (1996, 1997) as referred to later on).

M. californicus also shows such a „bead“ which holds the most basal row of cuticular hairs. A close look on the fine structure of the surface doesn't show any difference between the bead and the other part of the developing wall. Both are coarsely furrowed in the same way. The surface structure of the developing basis, on the contrary, is clearly different, namely finely wrinkled. This and the fact that this part actually acts functionally like a peduncle for a short period makes it rather plausible to homologize the developing basis with the stalk of the Pedunculata.

Shalaeva (1996) was able to show a difference in the cuticular structure between capitulum and peduncle in the stage before ecdysis in a number of pedunculate species. For that she removed the cyprid carapace. She also found this differentiation in the development of sessile species (Shalaeva 1997). From that she infers as well, that the developing basis is the vestigial peduncle and therefore homologous to the stalk of the Pedunculata.

The video recordings of the metamorphosing cyprid, that were made for this study, show a clear partition into a developing „capitulum“ with the primordial plates and a peduncle vestige about the same size, even when the carapace is not removed. Just as the peduncle in cyprids of Pedunculata is formed already before ecdysis², the vestigial peduncle in *M. californicus* already exists in the cyprid, but transforms into the basis within a few hours after ecdysis. Yet Glenner & Høeg (1993), as mentioned above, interpret the basal part of the wall in *Balanus improvisus* as a peduncle. But this „bead“ is formed in a later stage, where the developing basis is already orientated totally towards the substratum. That would mean, that the peduncle vestige suddenly appears when the juvenile barnacle turns sessile, which seems to be quite improbable.

A recently described juvenile („Jensen's x-juvenile“, Høeg & Newman 1997) which most likely belongs to the primitive sessile *Imbricaverruca yamaguchii*, a neoverrucid, has a capitulum as well as a peduncle about the same size in the stage before ecdysis (Newman 2000). Thus it can be assumed that during post-larval metamorphosis all Sessilia have a

² Glenner & Høeg (1993) themselves show that in *Scalpellum scalpellum*.

pedunculate phase in common. This can vary from several stalked stages as found in *Neoverruca* to a transient peduncular stage in advanced species like the object of this study.

Newman (1987, — & Hessler 1989, Yamaguchi & — 1990, — & Yamaguchi 1995) assumes Balanomorpha being phylogenetically descendent from Scalpelloidea via Brachylepadomorpha, whereas Anderson (1994:255ff), based on the growth pattern of the capitular and peduncular plates in Pollicipedinae, argues that the Balanomorpha have directly evolved from a scalpelloid ancestor. In this the peduncle would have gotten gradually shorter and broader and the basal disc would have transformed into the balanomorph basis. But if the post-larval metamorphosis in pedunculates and balanomorphs is compared with special emphasis on the surface structure of the cuticle it appears more likely that the whole peduncle rather than solely the basal disc is homologous to the basis. That stands against Andersons assumption.

Fixation of the basis on the substrate

Although the vestigial peduncle of *Megabalanus californicus* orients itself towards the substratum and thus „disappears“ already four hours after ecdysis it lasts about 12 hours more until it gets totally attached. With this „functional“ basis the transition to sessility is completed. Yule & Walker (1987) show in *Semibalanus balanoides* that in the first days after attachment it exudes juvenile cement and secretion of adult cement starts more than a month later. The basis of *M. californicus* gets attached with juvenile cement too, but contrary to the archaeobalanid *Semibalanus* with its membranous basis it gets calcified. This process apparently begins immediately after the fixation of the basis. At least even in this early stage it could be observed that quite often the calcareous basis remained on the substratum when the specimens were removed from it.

The cuticular hairs

Cuticular hairs have been mentioned in earliest juvenile stages of Balanidae already decades ago (e.g. Doochin 1951, Costlow 1956:403, Bernard & Lane 1962³). But due to their delicacy it was not before the availability of scanning electron microscopic methods that their exact allocation and structure could be documented. Glenner & Høeg (1993) and Shalaeva (1996, 1997) state that the length and allocation of the cuticular hairs can be used as diagnostic attributes to determine the species of juvenile barnacles.

In this study the arrangement of such hairs in *Megabalanus californicus* is shown. The developing wall holds two well defined rows of cuticular hairs, but different from *Balanus improvisus* and *B. amphitrite* an area without hairs can't be found on the rostrum. Basal to the dented primordial plates on both tergum and scutum (not only on the tergum as in *B. improvisus*) the operculum shows a well developed row of cuticular hairs. On the apical edge of the opercular plates we see a further row of hairs, but finer and shorter.

Glenner & Høeg (1993) propose the theory that the long cuticular hairs on the developing wall act, like „guy ropes“, as an additional means of attachment of the freshly metamorphosed „mobile“ sessile juveniles since they are otherwise fixed to the substratum only by cyprid cement. Nevertheless they could not find any adhesive qualities in the tips of the hairs. The observations on living individuals made for this study didn't show such adhesive properties in *M. californicus* either. To the contrary they led to the assumption that these cuticular hairs have supporting function during the crucial phase immediately after ecdysis, when the still pedunculate juvenile is stretching and moving heavily. On the living object those hairs appeared to be standing off straight when it was in an upright position. When it made nodding movements the tips of the hairs touched the substratum, which gave the impression that they are rather of supportive nature. High resolution light microscopic video documentation of these processes could clarify the issue.

³ It must be mentioned that one important result of the Bernard & Lane study, the existence of a „decorticated stage“ immediately after ecdysis in *B. amphitrite*, has been clearly falsified by Glenner & Høeg (1993).

The cuticular knobs

Quite interestingly, in the work of Glenner & Høeg (1993) a comparably extensive distribution of cuticular knobs like in *Megabalanus californicus* is documented in the archaeobalanid *Semibalanus balanoides*, but not in the balanids *Balanus improvisus* and *B. amphitrite*. Accordingly, it is improbable that these structures are homologous.

The folds situated median on the rostrum seem to be drawn-out knobs and suggest that the knobs are not just thickenings of the cuticle but blisters in the sense that the cuticle is „hollow“ underneath. Transmission electron microscopic cross section images confirmed that assumption (Fig. 13). Immediately after ecdysis the juvenile cuticle is still folded heavily, which provides the freshly metamorphosed barnacle with the flexibility it needs to stretch and develop its typical conical shape. When the juvenile „unfolds“ the knobs get visible. They might give the body some room for limited growth and stretching while the cuticle hardens and gains robustness. The folds that are interpreted as drawn-out knobs could be relics of such activity.

A comment on the rostrum

Runnström (1925) describes in his ontogenetic study the rostrum of *Semibalanus balanoides* as developing through fusion of two rostrilaterals which would have impact on the evaluation of the evolution of Balanoidea (Newman & Ross 1976:22). Yet thereafter, latest being Glenner & Høeg (1993), no evidence could be found of a compound rostrum. Also in *M. californicus* the rostrum develops as one plate. Nevertheless it should be mentioned that when observing the living metamorphosing juvenile one can clearly see a vertical blurred dark line median on the rostrum. This impression seems to result from a gap between the little whitish non-transparent spots which are otherwise dispersed over the developing wall in a regular pattern. In light microscopy the whitish spots seem to be light reflecting structures just below the surface while the vertical dark line supposedly results from a lack of them. Anyways it is questionable that this has something to do with wall plate development. Maybe Runnström observed something similar in *S. balanoides* and interpreted it as fusing plates.

Benefits and limitations of SEM methods

In scanning electron microscopic images of *Megabalanus californicus* it is difficult to see the boundaries of the wall plates. Only the boundaries between rostrum and laterals can be recognized quite easily on a closer look, especially in individuals that already have entered growth in height. They look a little like a ladder: two lines connected by „rungs“. Those might be the incipient radii of the rostrum when circumferential growth starts.

But closer examination also reveals the boundaries between laterals, carinolaterals and carina. It is much easier to discern the wall plates using light microscopy. This also shows the limits of SEM technique that visualizes only the very surface and can't look beneath it. In order to get as close as possible to „reality“ it is therefore very important to combine both methods, light and electron microscopy in a suitable way.

Already Glenner & Høeg (1993) and Shalaeva (1996, 1997) emphasize that SEM is also useful as a taxonomic means to identify the species of juvenile barnacles. Besides the general shape and size of the juvenile the distribution of its cuticular hairs and its cuticular knobs, the shape of its primordial plates and its protruding mantle can be used as criteria. In *M. californicus* the latter is characteristically ornamented with cuticular processes and is already developed at the time when the cyprid carapace gets shed. In *Balanus improvisus* on the contrary the mantle protrudes during the first hour after ecdysis (Glenner & Høeg 1993).

Post-settlement development - summary

It took the cyprids of *Megabalanus californicus* 16 to 18 hours from attachment to ecdysis, giving a water temperature of 19 to 21°C. Before ecdysis the cyprid develops a vestigial peduncle that measures about half the size of the larva. The post-settlement moult takes only seven to eight minutes, from the start of the rolling elevation to the point where the carapace is completely shed. Right after ecdysis the juvenile enters a stalked phase with limited mobility similar to pedunculates. The capitulum shows the five primordial plates scuta, terga and carina as well as numerous cuticular hairs and the thorax starts

rhythmically pumping movements.

Within the next four hours the vestigial peduncle flattens and orients itself towards the substratum and so becomes the basis. In that the circumference of the juvenile increases and its apicobasal extent decreases at the same time. That marks the start of sessility. The surface of the now „unfolded“ juvenile is densely covered with cuticular knobs.

Within the next two hours calcification starts and growth in height begins. Rostrum, laterals, carina and finally also carinolaterals can be differentiated. At that point of time the basis is not yet attached entirely to the substratum, until total fixation another six hours elapse.

Phylogenetic conclusions - summary

Megabalanus californicus, although being an advanced balanid, proved to possess a rudimentary peduncle in its early juvenile ontogeny. This confirms the pedunculate origin of Sessilia.

Anderson assumes that balanomorphs are directly descent from a scalpelloid ancestor (diphyletic origin of the sessile taxa Brachylepadomorpha/Verrucomorpha and Balanomorphs). That would imply that the basis evolved from the basal disc, whereas my findings show that the peduncle vestige as a whole becomes the basis of the now sessile organism. From this perspective Newmans theory of Balanomorphs being descent from brachylepadomorphs (monophyletic origin of Sessilia) seems to be more plausible.

Zusammenfassung

Sessile Seepocken haben sich aus gestielten Pedunkulaten entwickelt - diese Meinung ist allgemein akzeptiert. Man kann daher in der Ontogenese sessiler Arten Hinweise auf die pedunkulate Herkunft vermuten. Stielrudimente wurden bei ursprünglichen und weiter entwickelten Seepocken beschrieben. Ein Ziel der vorliegenden Arbeit war herauszufinden, ob auch bei höher entwickelten Balanoidea ein rudimentärer Pedunkel nachweisbar ist. Zu diesem Zweck wurde die Metamorphose der Cyprislarve zum Juveniltier (post-settlement metamorphosis) und die frühe Juvenilentwicklung am Beispiel von *Megabalanus californicus* (Pilsbry) studiert. Um eine möglichst genaue Darstellung der Vorgänge zu erreichen wurden einerseits lichtmikroskopische Lebendbeobachtungen vorgenommen und mittels Video dokumentiert, andererseits wurden die aufeinanderfolgenden Entwicklungsstadien für das Rasterelektronenmikroskop (REM) präpariert.

Bei Beginn der Häutung des Cypriscarapax lassen sich in diesem frühesten Juvenilstadium zwei Abschnitte vergleichbarer Größe unterscheiden: das „Kapitulum“ mit den primordialen Operkularplatten und primordialer Carina, sowie dem Pedunkelrudiment. Dieser Stiel unterscheidet sich vom „Kapitulum“ durch seine Oberflächenstruktur und dem Fehlen von Kutikularhaaren. Das „Kapitulum“ besteht aus der sich entwickelnden Mauerkrone, die von zwei Kränzen kutikularer Haare umgeben ist und dem Operkulum, welches zwei zusätzliche Reihen solcher Haare trägt. Im REM-Bild fallen die fünf Primordialplatten von Scuta, Terga (jeweils paarig) und Carina durch ihr gedelltes Erscheinungsbild auf.

Die äußere Gestalt der juvenilen Seepocke ist einer sehr raschen Änderung durch Anpassung an die sessile Lebensweise unterzogen. Der Körper wird flacher und breiter wobei sich die Oberfläche des Stielrudiments immer mehr an das Substrat annähert. Etwa vier Stunden nach der Cyprishäutung wird der typisch kegelförmige Seepockenhabitus erreicht und der rudimentäre Pedunkel wird zur Basis. Diese wird durch Ausscheidung von Juvenilzement am Substrat befestigt. Mauerkrone und Operkulum sind fast flächendeckend mit kutikularen Noppen bedeckt. Die beginnende Kalkeinlagerung festigt die Schalenplatten. Am Ende des 12-stündigen Beobachtungszeitraums ist die Basis vollständig am Substrat fixiert.

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Abbreviations of figures

1, 2, 3, 4	cuticular hair rows
Ap	aperture
As	antennular setae
Aw	apical boundary of wall
Bw	basal boundary of wall
Ca	carina
Cc	cyprid cement
Ce	shed compound eyes
Ch	cuticular hair
Ci	cirri
Cl	carinolateral
Cp	primordial carina
Cr	cyprid carapace
Cu	cuticular processes
De	dents
Dw	developing wall
Jc	juvenile cement
Kn	knobs
Ks	stretched knobs
La	lateral
Ma	protruding mantle
Oa	opercular articulation
Pv	peduncle vestige
Ro	rostrum
Sc	scutum
Sm	smooth area on scutum
Sp	primordial scutum
Te	tergum
Tp	primordial tergum
Ub	unattached basis
Wb	boundary of wall plates

Lebenslauf

Klemens Gusenbauer

1070 Wien | Zieglergasse 67/3/7

01 - 522 18 74 | 0699 - 12698935

klemens.gusenbauer@telering.at

Geboren am 24. 12. 1968 in Luftenberg/Donau, Oberösterreich

- 1975 - 1979 Volksschule in St.Georgen/Gusen
- 1979 Eintritt in das Akademische Gymnasium in Linz
- 1986 - 1987 Einjähriger Auslandsaufenthalt in San Luis Potosí, Mexiko im Rahmen eines Schüleraustauschprogrammes
- 1988 Matura mit gutem Erfolg am ORG der Diözese Linz
Beginn des Studiums der Biologie an der Universität Wien
- 1993 1. Diplomprüfung, Studium der Zoologie
Viermonatiger Forschungsaufenthalt an der Scripps Institution of Oceanography in La Jolla, San Diego, USA
- 1996 Zivildienst bei der Lebenshilfe Wien
- seit 1997 Angestellter der Lebenshilfe Wien