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Muscles and Attachment of the Body to the Shell in Embryos and Adults of *Nautilus belauensis* (Cephalopoda)

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ABSTRACT

This paper summarizes observations on the position of the musculature that attaches the body to the shell in embryos and adults of *Nautilus belauensis* Saunders. In the early organogenetic stage, the cephalic retractor muscles appear as a dense mass of tissue between the developing eye primordium and the branchial heart. As the mantle expands to cover the embryonic visceral organs, the cephalic retractor muscles and the mantle fuse together and join the inside of the shell, forming bilateral, crescent-shaped areas of attachment. The first chamber appears as a space between the posterior end of the embryo and the inside of the shell in the cicatrix region. The mantle is more muscular than that in adults; it possesses numerous longitudinal muscles that extend anteriorly and ter-

minate near the mantle margin where they form a zone of muscular attachment to the shell. In adults, the body is posteriorly attached to the shell along the area of origin of the cephalic retractor muscles, the septal myoadhesive band, and the area of origin of the weakly developed longitudinal mantle muscles. Anteriorly, the mantle is mainly attached to the shell by numerous epithelial extensions, which are housed in pores in the inner layer of the shell at the apertural margin. There are two pairs of retractor muscles in *Nautilus*, the cephalic and hyponome retractors, which correspond to the two pairs of nephridia, gills, and atria, suggesting that *Nautilus* may retain some evidence of metamerism.

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INTRODUCTION

The attachment of the muscles to the shell in embryonic and adult ectocochliate cephalopods is of interest not only taxonomically but functionally and behaviorally as well. The musculature in adult specimens of *Nautilus* has been studied by several authors (e.g., Owen, 1832; Appellöf, 1893; Mutvei, 1964; Blind, 1976; Bandel and Spaeth, 1983; Doguzhaeva and Mutvei, 1986). However, early embryonic stages were not known until 1986 when the first embryos of *Nautilus belauensis* Saunders were obtained from the Waikiki Aquarium (Arnold and Carlson, 1986; Arnold, 1987, 1988). The structure of the embryonic shell of this species has been described by Arnold et al. (1987) and Landman et al. (1989). This paper focuses on the position and attachment of the major muscle groups in this species and how they develop through ontogeny.

MATERIALS AND METHODS

Adults and embryos of *Nautilus belauensis* were obtained as part of the captive breeding program at the Waikiki Aquarium. Details of the capture, handling, and breeding of adult animals, and, recently, the hatching of juveniles, are given in Carlson (1987, 1991). Two embryos are described in this paper, one at an early stage of organogenesis and one at the stage of formation of the first chamber. The exact age in days of these two embryos is unknown but they are comparable to stages 22–23 and 27, respectively, of *Loligo* according to the revised staging of Arnold (1990). After live observations of the embryos, they were fixed in 2.6% glutaraldehyde–sea water buffered to pH 7.4, rinsed in sea water, and postfixed in 1% osmium tetroxide. Dehydration was through a series of ethanols, after which the embryos were critical point dried, sputter coated with gold and palladium, and examined with a Joel 840 scanning electron microscope at 15 kV. The older embryo was subsequently embedded in paraffin wax and sectioned at 10-micrometer intervals. The sections were examined and photographed by light microscopy to confirm identification of the muscles. Living adults were carefully removed from their shells, examined and pho-

tographed while alive, and preserved in 70% ethanol. Examination of living adults allowed us to directly observe the function of various muscle groups in a state as close as possible to the natural condition.

OBSERVATIONS

EARLY ORGANOGENETIC STAGE

In the earliest organogenetic stage available to us, the shell primordium consists of an ovate flattened plate of periostracum centered in the oval-shaped mantle (fig. 1). The cicatrix appears in the clear, thin sheet of periostracum as two parallel lines of melanin located along the midline. Perpendicular to these central lines are short bands of melanin particles, which alternate with the first formed crystals of aragonite. These crystals are reflective and refractive so when viewed with the light microscope they appear as sparkling white particles on the cellular layer of the mantle. The periostracal groove is visible as a line paralleling the margin of the mantle (pg, figs. 1, 10A). The attachment site of the cephalic retractor muscles to the shell cannot be distinguished at this stage but in lateral view the primordia of these muscles are already well developed (mc, fig. 1). As the mantle grows out marginally it will cover the end of the developing muscle primordia, thus forming a future contact zone between the muscle primordia and the growing shell. The general development of the embryo at this stage is comparable to stages 22 and 23 of *Loligo*. In *Nautilus*, the gill primordia are just beginning to form at this stage but have not yet separated into gill filaments, the branchial hearts are beating (Arnold and Carlson, 1986), the mantle is growing outward but has not yet curved to cover the future visceral organs, the arms are present as buds, and the external yolk sac has developed a prominent circulatory system. At this stage of development in *Loligo*, the shell sac invagination is closed off from the mantle proper.

PRECHAMBERED STAGE

The prechambered shell is approximately 3 mm in maximum diameter and 0.17 whorls in angular length (60°). There is a prominent space between the cicatrix region of the shell

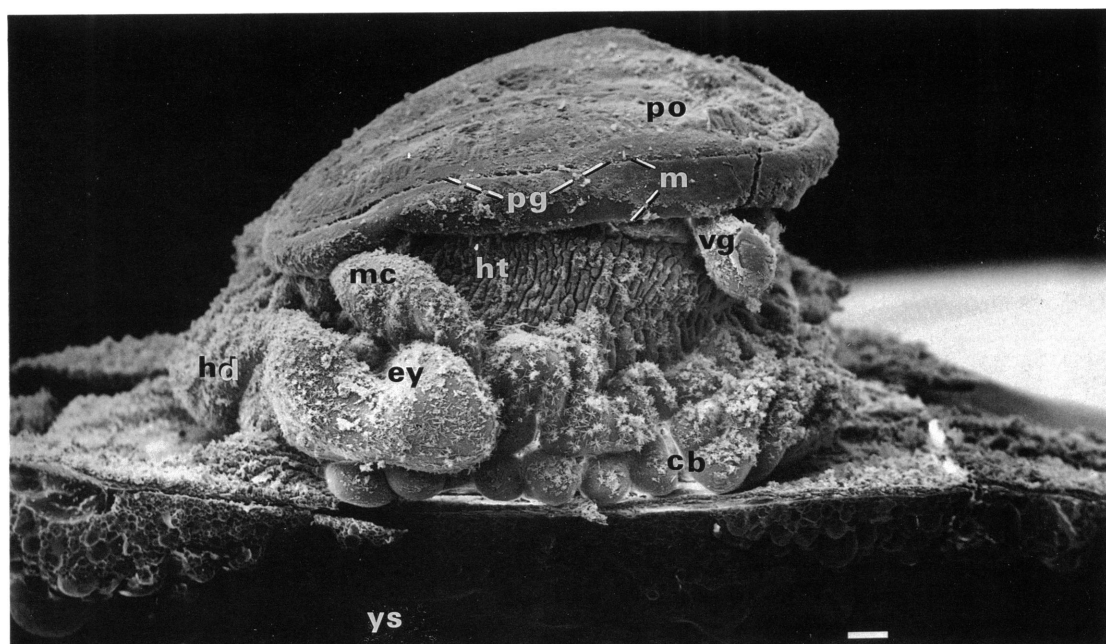


Fig. 1. Lateral view at early organogenetic stage. Scale bar = 500 μ m. cb, cirral buds; ey, eye complex; hd, hood; ht, branchial heart; m, mantle; mc, muscle complex; pg, periostracal groove; po, periostracum; vg, ventral gills; ys, yolk sac.

and the living epithelium beneath it (figs. 2–6, 10B). Neither the first septum nor the siphonal tube is present at this stage but a low elevated mound is present, which is the initiation site of the siphonal cord (si, fig. 3A, B). The posterior end of the embryo is flattened, forming a space between itself and the apex of the shell. The secretion of the first septum and the caecum has probably only just begun.

At this developmental stage the gills have developed distinct gill filaments, the arms have differentiated into the sheath and cirri, and the mantle has grown down over the visceral organs. Respiratory movements of the embryo are quite obvious because the hyponome is extended during respiration. The embryo responds to stimulation by pulling the shell down onto and over itself when it is disturbed. The whole embryo slowly rotates within the inner egg capsule and occasionally there are dramatic circular contractions of the external yolk sac a few millimeters distal of the embryonic body, which cause the embryo to rise up on a mound of yolk. These movements are further described in

Arnold and Carlson (1986, 1991). This stage is comparable in development to stage 27 of *Loligo*.

The hyponome (h), also called the funnel or siphon in the zoological literature, is highly muscular and each side continues laterally as a long muscular extension into the forming gill chamber (figs. 2, 4, 5). The distal end of the hyponome extends under the mantle (fig. 5A, B). The lateral sides of the body show large crescent-shaped areas that mark the areas of origin of the cephalic retractor muscles along the inside of the shell (rm, figs. 2, 3A, 4A, 5A, B, 10B). The exact margins of these areas are difficult to observe and therefore their precise position is unknown.

The flattened posterior portion of the body wall is thin with little musculature. Its boundary with the muscular mantle is well defined on the lateral sides of the body anterior to the area of origin of the cephalic retractors (b, figs. 2A, 10B). At this boundary and at the area of origin of the cephalic retractors, the body is attached to the shell along an annular myoadhesive epithelial zone. On the inner shell surface, this attachment zone can

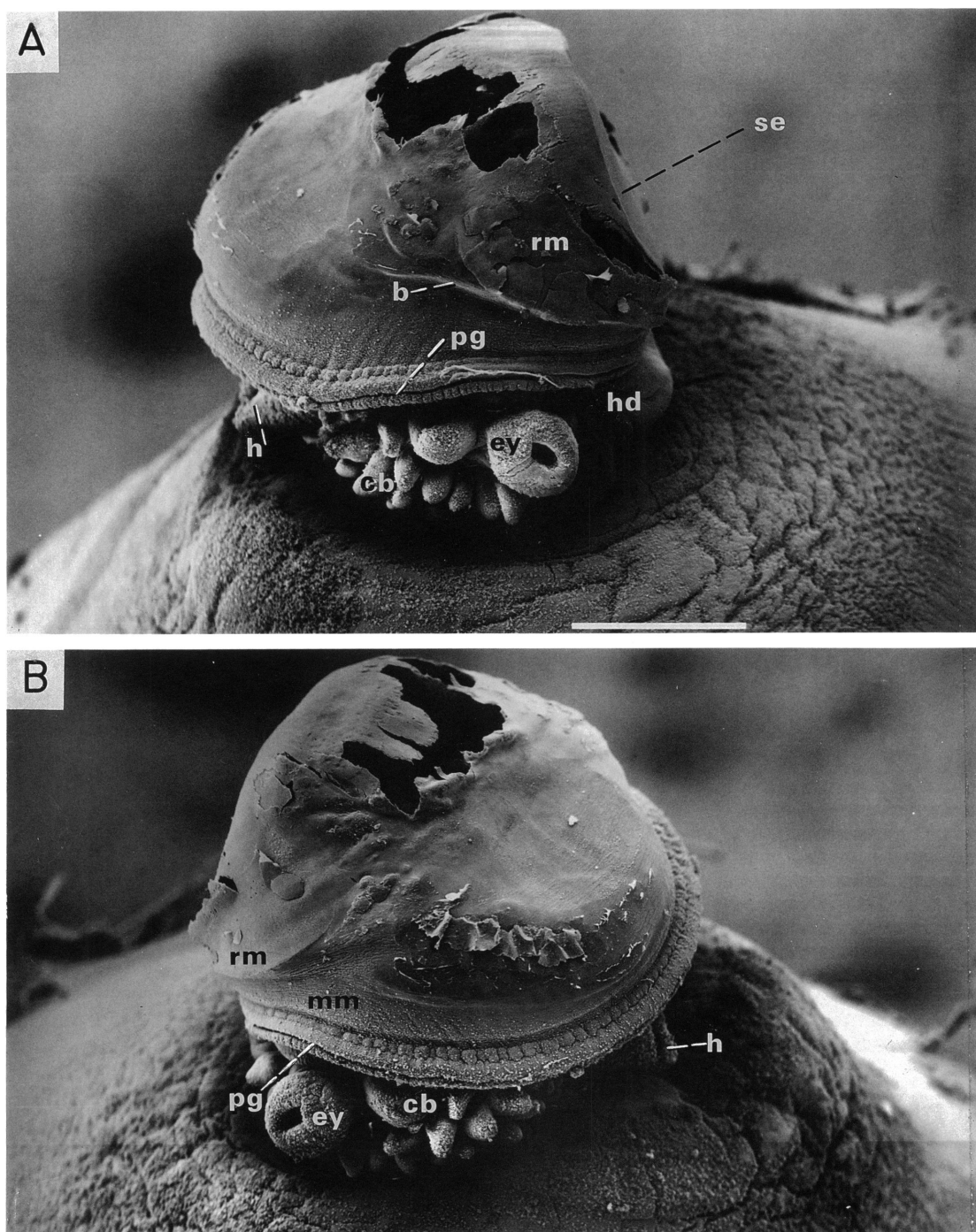


Fig. 2. A, B. Lateral and oblique posterolateral views at prechambered stage, respectively. Scale bar = 1 mm. b, boundary between muscular and nonmuscular mantle; cb, cirral buds; ey, eye complex; h, hyponome; hd, hood; mm, muscular mantle; pg, periostracal groove; rm, cephalic retractor muscle; se, septal epithelium.

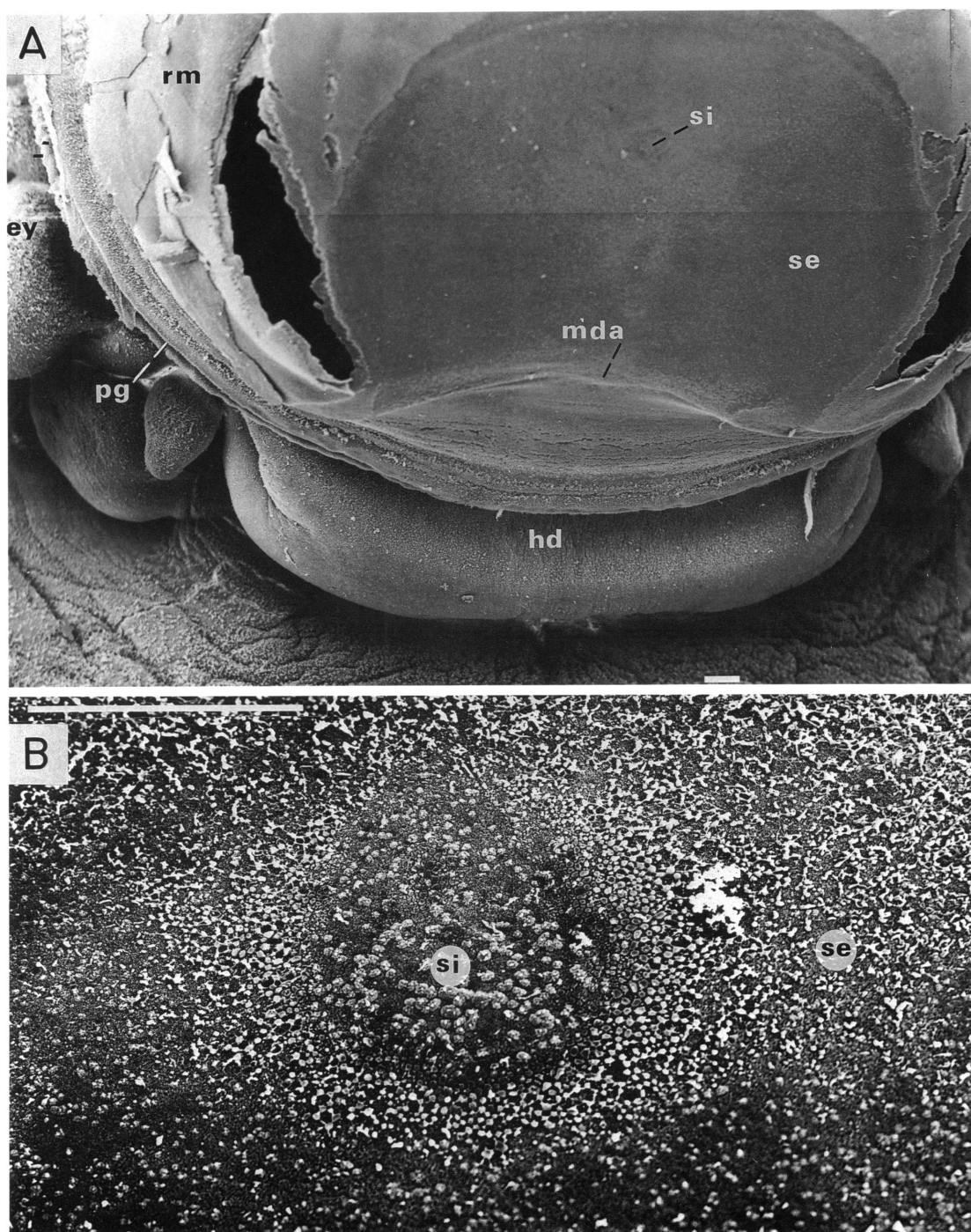


Fig. 3. **A.** Posterior view at prechambered stage. Scale bar = 200 μ m. **B.** Close-up of septal epithelium and initial portion of siphonal cord. Scale bar = 500 μ m. ey, eye complex; hd, hood; mda, middorsal attachment area; pg, periostracal groove; rm, cephalic retractor muscle; se, septal epithelium; si, initial stage of siphonal cord.

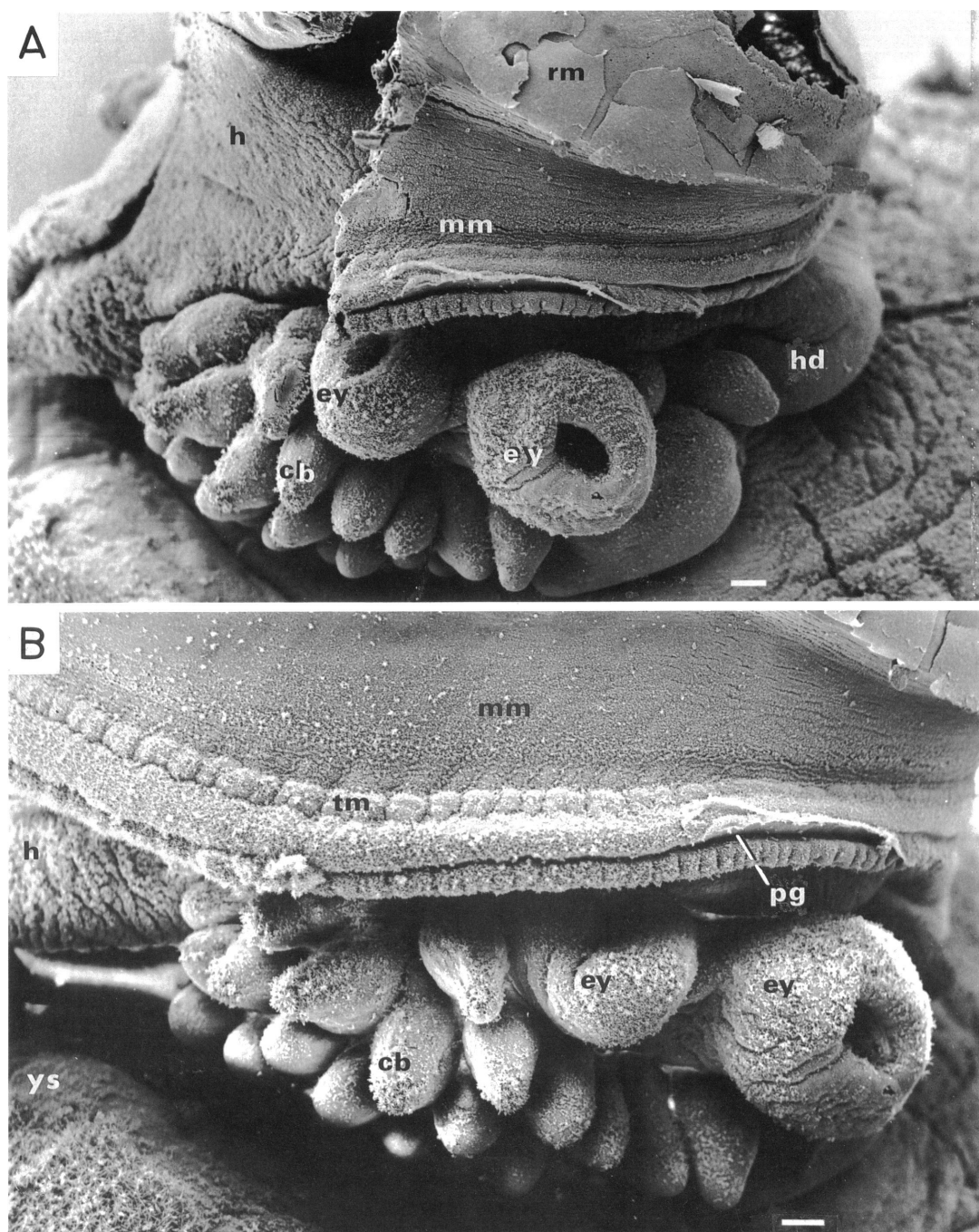


Fig. 4. **A.** Lateral view at prechambered stage. Scale bar = 200 μ m. **B.** Close-up of oblique ventrolateral view. Scale bar = 100 μ m. cb, cirral buds; ey, eye complexes; h, hyponome; hd, hood; mm, muscular mantle; rm, cephalic retractor muscle; tm, terminus of longitudinal mantle muscles; ys, yolk sac.

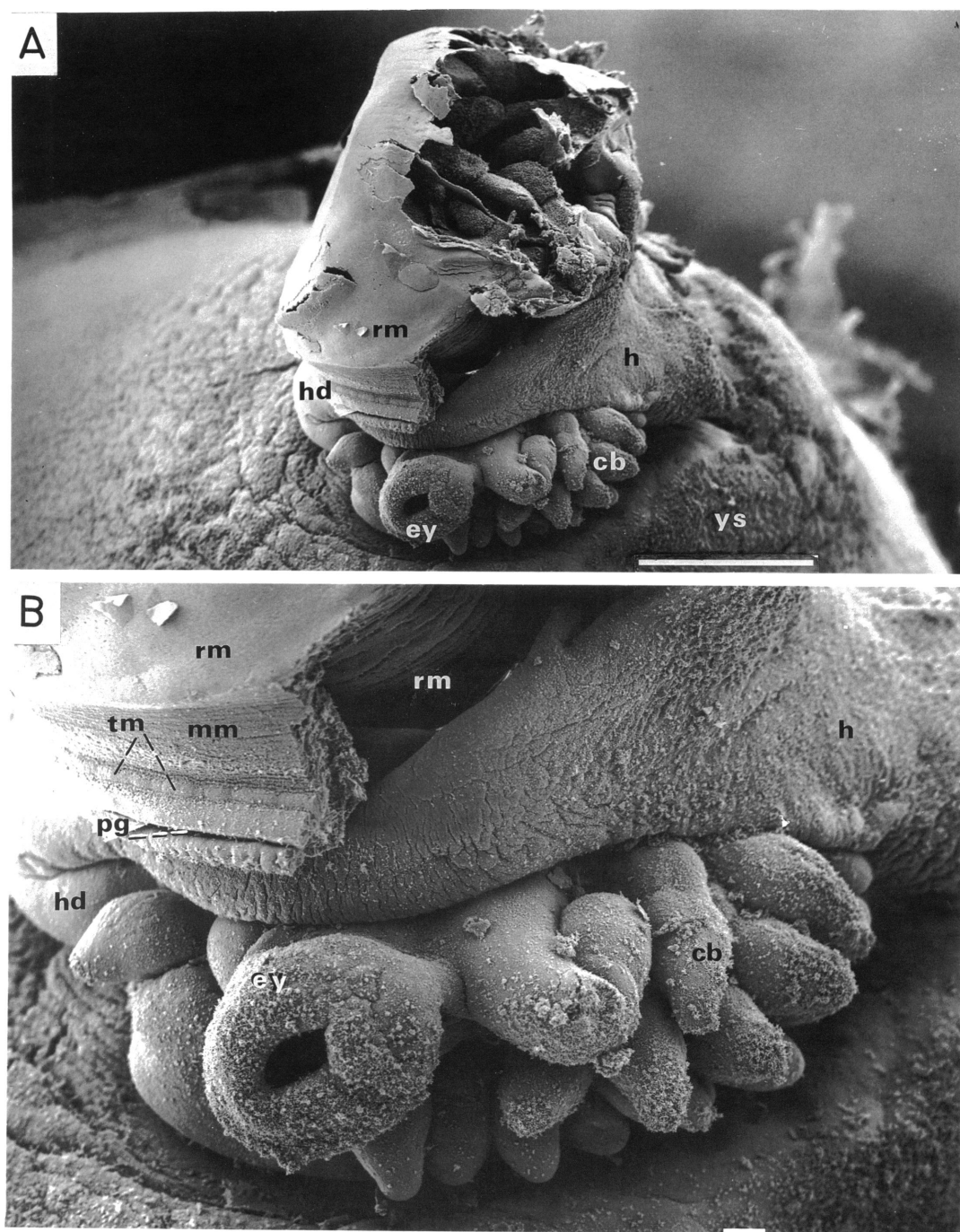


Fig. 5. A. Posterolateral view at prechambered stage, muscular mantle and posterior portion of body wall partially removed. Scale bar = 1 mm. B. Close-up of A. Scale bar = 200 μ m. cb, cirral buds; ey, eye complex; h, hyponome; hd, hood; mm, muscular mantle; pg, periostracal groove; rm, cephalic retractor muscle; tm, terminus of longitudinal mantle muscles; ys, yolk sac.

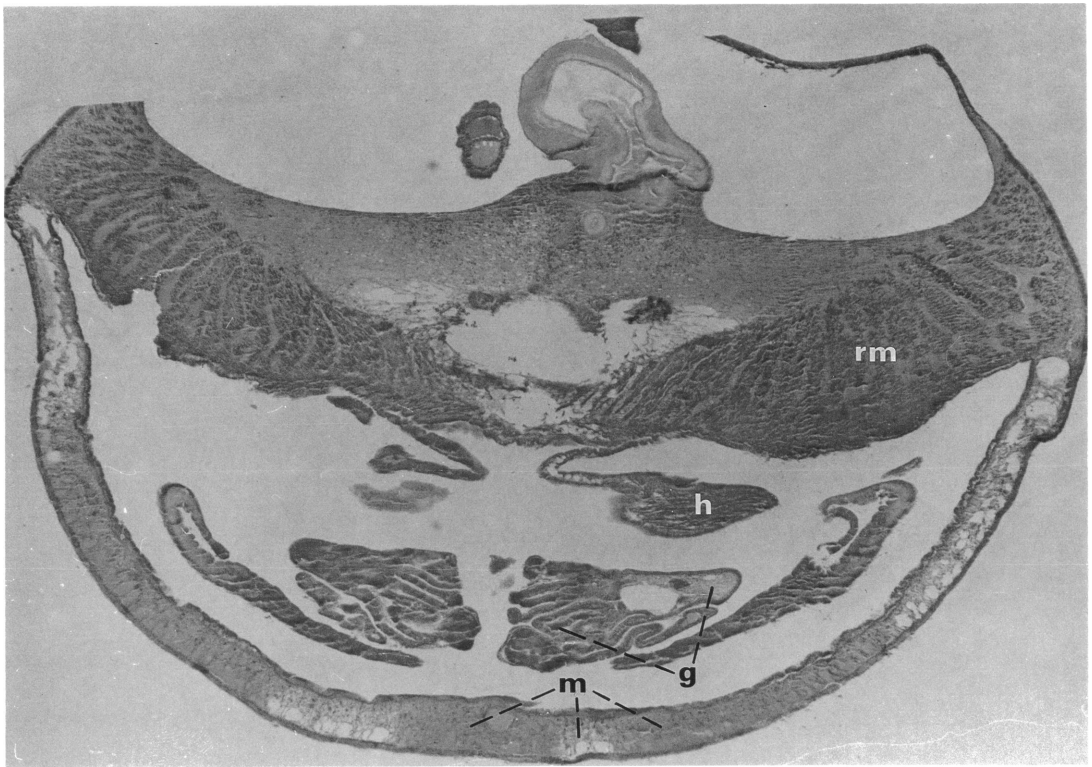


Fig. 6. Microtome section of prechambered stage. g, gill; h, hyponome; m, mantle; rm, cephalic retractor muscle. $\times 43$.

be distinguished dorsally but not laterally or ventrally (Arnold et al., 1987, fig. 9B; Landman et al., 1989, fig. 2B, C). There is a mid-dorsal scar in this attachment zone, previously described by Arnold et al. (1987, mda, figs. 9B, 10A) and Landman et al. (1989, mda, fig. 2B, C, D), which corresponds to the mid-dorsal area of the body (mda, fig. 3A). We still do not understand which embryonic structure is attached to the shell in this area, but to judge from the condition in adult animals, this scar probably represents the attachment site of the palliovisceral ligament (see below).

Numerous longitudinal muscles are visible beneath the epidermis on the lateral sides of the mantle (mm, figs. 2B, 4, 5B, 10B). These muscles extend toward the mantle margin. Anteriorly, each muscle terminates in a small semiquadrangular elevation on the mantle surface. These elevations are distinct on the ventral and lateral sides of the body, but indistinct on the dorsal side (tm, figs. 4B, 5B, 10B). They are separated by narrow inter-

spaces, and together they form a ring around the mantle, just posterior to the mantle edge. This ring is probably attached to the inner shell surface, and may correspond to the pallial line in bivalves. About 40 of these elevations appear in the specimen reported here, but the number seems to vary slightly in other specimens. The periostracal groove is prominent around the entire mantle margin (pg, figs. 2, 3A, 4B, 5B, 10B). The paraffin sections through the body confirm that the mantle (m) and hyponome (h) are strongly muscular, and that the cephalic retractor muscles (rm) are well developed (fig. 6).

ADULT STAGE

Figure 8B shows the position of the cephalic (rm) and hyponome retractor muscles (hr). The cephalic retractor muscles originate from the lateral sides of the body (fig. 10C). The hyponome retractor muscles are small and situated on the ventral surface of the cephalic retractor muscles. In contrast, in co-

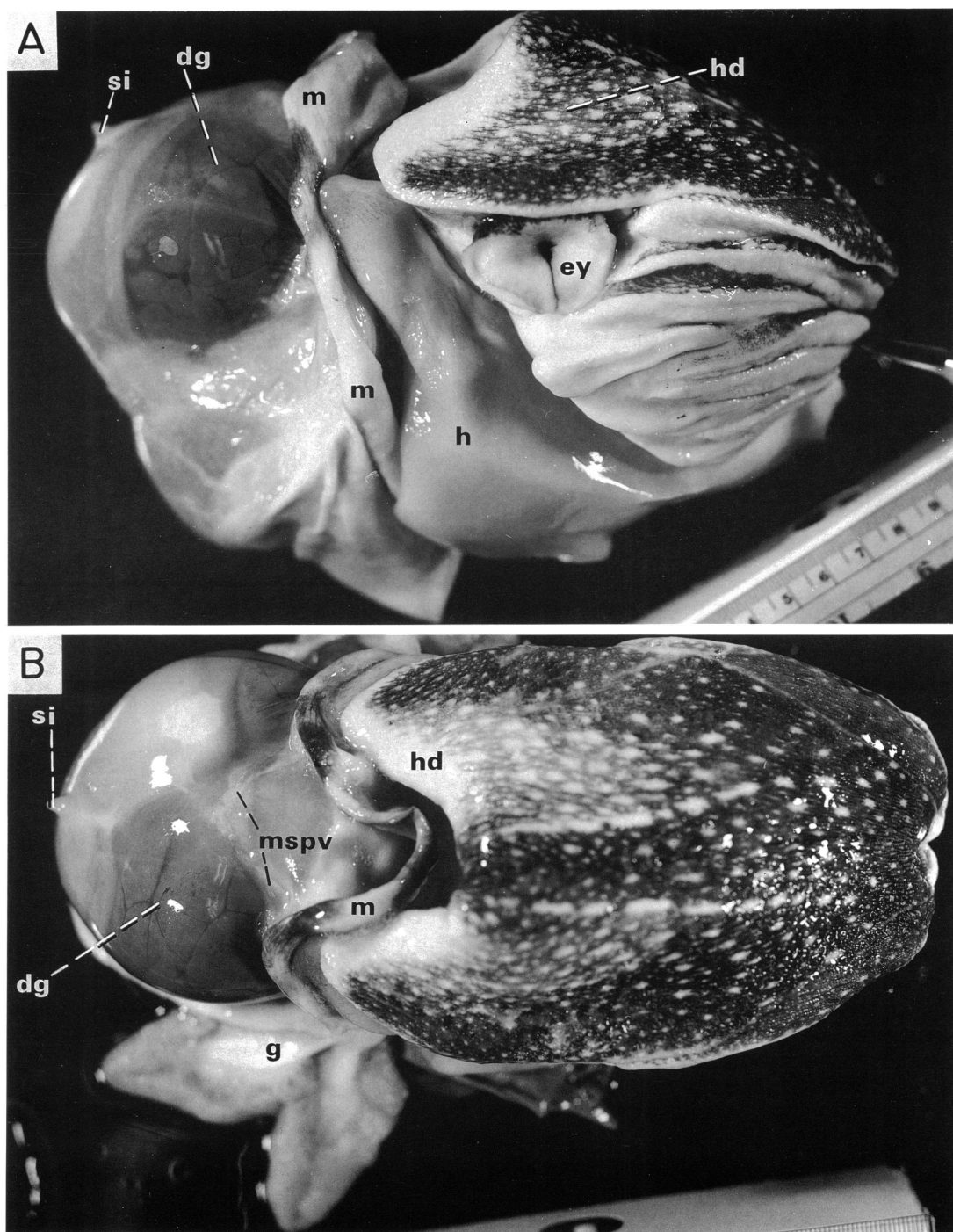


Fig. 7. **A, B.** Adult animal in lateral and dorsal view, respectively, mantle fold removed. dg, digestive gland; ey, eye complex; g, gill; h, hyponome; hd, hood; m, mantle; mspv, dorsally fused septal and mantle myoadhesive bands and palliovisceral ligament; si, siphonal cord. Irregular white splotches on figures 7–9 are from film processing.

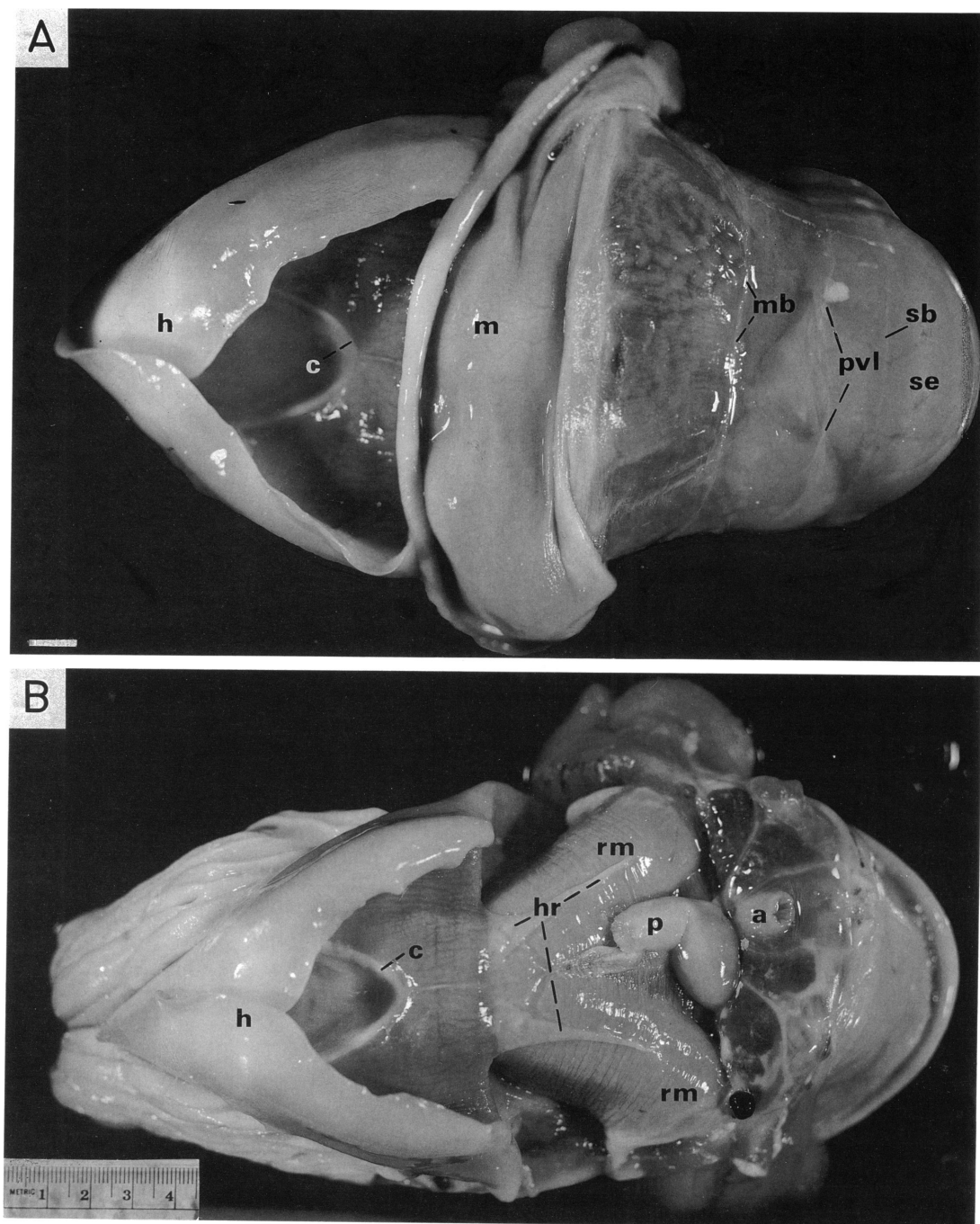


Fig. 8. **A.** Ventroposterior portion of body of adult male, mantle fold folded forward. Scale bar = 1 cm. **B.** Ventral view of adult male, mantle fold removed. a, anus; c, cartilage; h, hyponome; hr, hyponome retractor muscle; m, mantle; mb, mantle myoadhesive band; p, penis; pvl, palliovisceral ligament; rm, cephalic retractor muscle; sb, septal myoadhesive band; se, septal epithelium.

leoids, the hyponome retractor muscles are well developed and attach to the inside of the chitinous skeleton.

The following structures are visible on the ventral side of the body: (1) the longitudinal mantle muscles, seen as fine striations (lm, fig. 9B), which originate from the anterior (mantle) myoadhesive band (mb, figs. 8A, 9B, 10C); (2) the septal myoadhesive band, which is situated at the boundary with the septal epithelium (sb, figs. 8A, 9B, 10C; = "septal aponeurosis" of Willey, 1902); and (3) the palliovisceral ligament (pvl, figs. 8A, 9B, 10C), which is clearly visible between these bands through the thin transparent body wall. On the dorsal side of the body, the mantle and septal myoadhesive bands are so close together that they cannot be distinguished from each other (mspv, figs. 7B, 9A). The palliovisceral ligament on the dorsal side is also attached to the body wall at this point. This site provides the strongest support for the visceral mass.

Thus, the posterior portion of the body is firmly attached to the shell along an annular zone of myoadhesive epithelium (= "annulus" of Owen, 1832). This zone comprises the septal myoadhesive band (sb), the area of origin of the cephalic retractor muscles (rm), and the area of origin of the longitudinal mantle muscles (mb, fig. 10C).

As in embryos, adult animals have a periostracal groove (pg, fig. 10C; see Mutvei, 1964). However, the periostracal mantle/shell attachment is very weak and is easily disrupted because the periostracum is extremely thin in adult specimens of *Nautilus belauensis* as well as in *N. pompilius*.

The body is also attached to the margin of the shell along a zone at the mantle edge (see Stenzel, 1964; Doguzhaeva and Mutvei, 1986). Although the histology of this zone has not yet been studied, the inner prismatic layer of the shell at the apertural margin bears numerous pores. These pores may house fingerlike extensions of the mantle epithelium. Thus, the attachment of the mantle at the shell margin in adults is different from that in embryos. The mantle in adults is much thinner than that in embryos and attachment by means of the extremely thin periostracum is mechanically insignificant. The attachment of the mantle at the shell margin appears to

function in preventing water from entering between the mantle and shell, particularly during swimming.

The shell wall of the living chamber is structurally modified at the attachment sites of the body. At the attachment site at the apertural margin, the body epithelium secretes a thin prismatic layer termed "the mantle adhesive layer" by Doguzhaeva and Mutvei (1986, fig. 8A). This layer coats most of the inner surface of the living chamber. A second thin prismatic layer, the myostracal layer, is secreted in front of the last septum by the annulus in the posterior portion of the living chamber (Doguzhaeva and Mutvei, 1986, fig. 8A). This second layer covers the inner surface of the mantle adhesive layer. Therefore, the inner surface of the shell wall is coated with two thin prismatic layers, which have different distributions in the living chamber.

DISCUSSION

Nautilus has a heart complex with two pairs of gills, two pairs of nephridia, two pairs of atria, and two pairs of pericardial organs. Several authors have considered the repetition of the gills, nephridia, and atria as primitive features, derived from segmented ancestors (e.g., Pelseneer, 1906; Naef, 1926; Lemche and Wingstrand, 1959; Wingstrand, 1985), whereas others have considered these organs to have been secondarily duplicated (e.g., Morton and Yonge, 1964).

Wingstrand (1985) emphasized that the repetitive arrangement of eight pedal muscles, six to seven pairs of nephridia, five to six pairs of gills, two pairs of atria, and two to three pairs of gonoducts in Recent monoplacophorans (*Neopilina*, *Vema*) indicates the metameric organization of these molluscs. However, terminal proliferation of segments as in annelids and arthropods cannot occur in monoplacophorans because of the well-developed heart region in the posterior end of the body. Wingstrand (1985) therefore compared the metamerism in monoplacophorans to that in articulate (Annelida, Arthropoda) larvae and embryos where metameres tend to form more or less simultaneously.

In contrast to the occurrence of two pairs

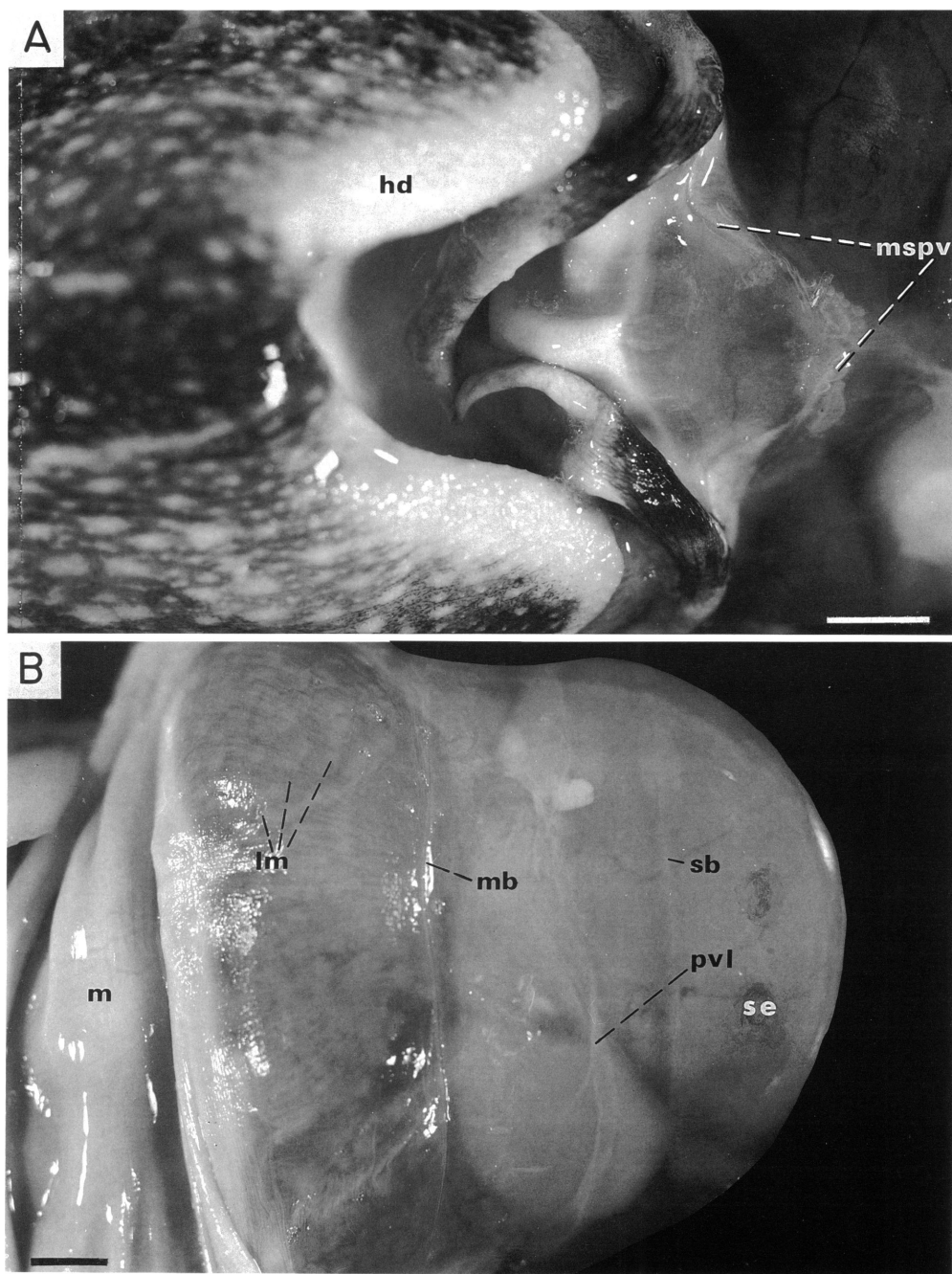


Fig. 9. **A.** Close-up of dorsal portion of body in fig. 7B. Scale bar = 1 cm. **B.** Close-up of ventral portion of body in fig. 8A. Scale bar = 1 cm. hd, hood; lm, longitudinal mantle muscles; m, mantle; mb, mantle myoadhesive band; mspv, dorsally fused septal and mantle myoadhesive bands and palliovisceral ligament; pvl, palliovisceral ligament; sb, septal myoadhesive band; se, septal epithelium.

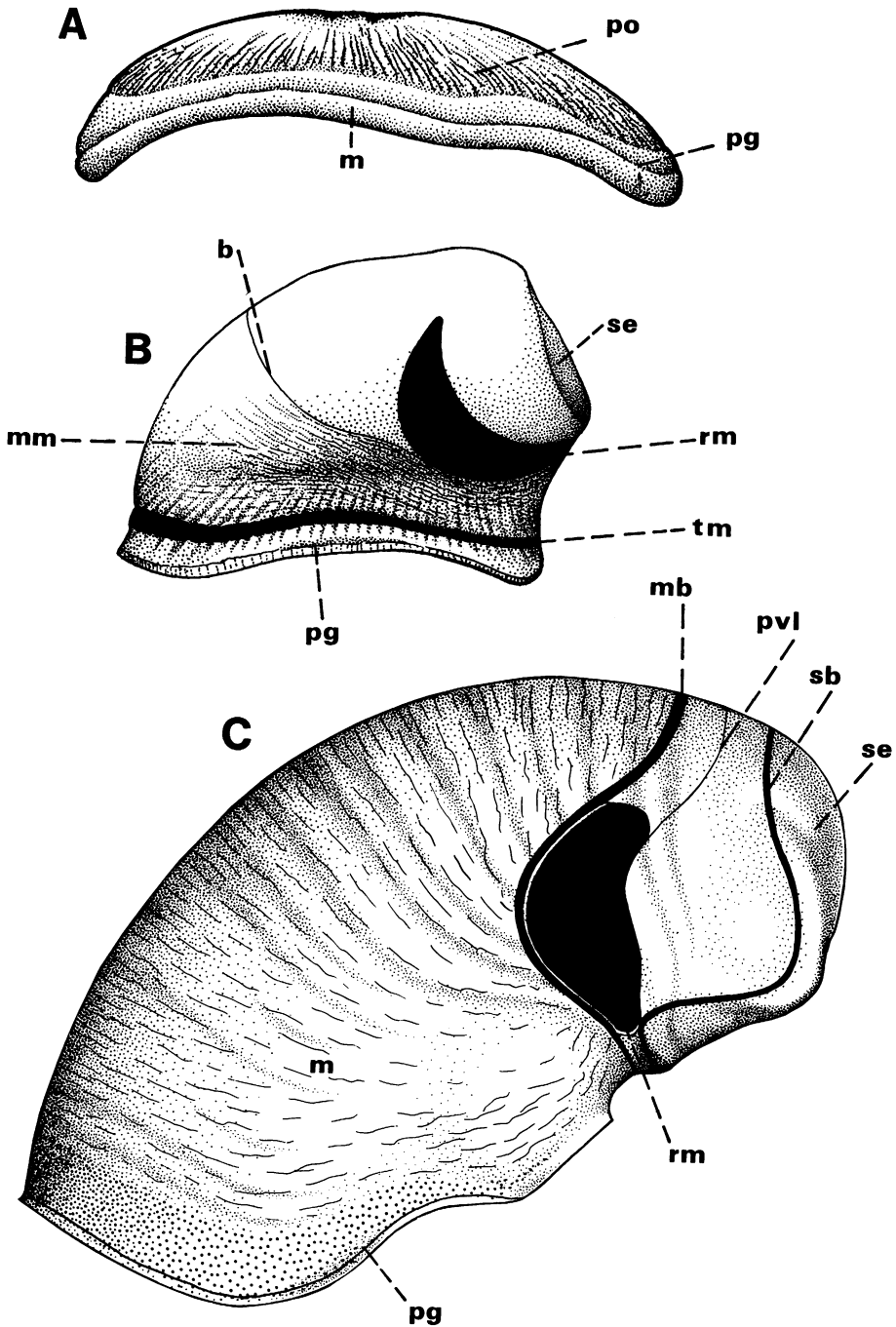


Fig. 10. A, B, C. Diagrammatic lateral views of the mantle at an early organogenetic stage, pre-chambered stage, and adult stage, respectively. b, boundary between muscular and nonmuscular mantle; m, mantle; mb, mantle myoadhesive band; mm, muscular mantle; pg, periostracal groove; po, periostracum; pvl, palliovisceral ligament; rm, cephalic retractor muscle; sb, septal myoadhesive band; se, septal epithelium; tm, terminus of longitudinal mantle muscles.

of gills, two pairs of nephridia, and two pairs of atria in *Nautilus*, there is only a single pair of cephalic retractor muscles. Wingstrand (1985: 56) pointed out that "The presence of two pairs of retractor muscles matching the two pairs of kidneys, gills and atria of *Nautilus* would certainly strengthen the homology with the heart complex of the Monoplacophora." In coleoids there is a single pair of nephridia, gills, and atria but two pairs of retractor muscles: cephalic retractors and hyponome retractors (see Wells, 1988, fig. 1). Both of these muscles extend to the head-foot region, which originates from a common organ rudiment during organogenesis and is mainly derived from the molluscan foot (Boletzky, 1987). According to Wells (1988: 289), the weakly developed hyponome retractors in *Nautilus* are probably homologous to those in coleoids. Thus, it is probable that there are two pairs of retractor (pedal) muscles in *Nautilus*, which correspond to the two pairs of nephridia, gills, and atria. Coleoids retain both pairs of retractor muscles but only a single pair of nephridia, gills, and atria, which may represent the reduced condition.

Two pairs of retractor muscles have been reported in some Ordovician nautiloids, whereas other groups of Paleozoic nautiloids, for example, the Oncoceratida and Disco-

sorida, may have had eight or more pairs of retractor muscles (Mutvei, 1957). Two pairs of lateral muscles have also been reported in the Lower Cretaceous ammonoid *Aconeceras* (see Doguzhaeva and Mutvei, 1991).

Based on the above information, it is possible that *Nautilus* retains some evidence of metamerism, but in a reduced form. This may represent a reduction from segmented molluscan ancestors, with a further reduction leading to the condition in modern coleoids.

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