universität wien

DIPLOMARBEIT

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„Charakterisierung des Adhäsivsystems von *Euprymna scolopes* Berry, 1913 (Cephalopoda)“

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Zusammenfassung:

Adhäsion ist ein weitverbreiterter und (über-)lebenswichtiger Prozess in der Natur, der bei den verschiedensten Spezies von Bedeutung ist. Er spielt bei Abwehr, Beutefang und Schlüpfen, sowie bei Tarnung und Verankerung der Lebewesen eine tragende Rolle. Vier Gattungen von Cephalopoden, zu vier verschiedenen Familien gehörend (*Euprymna*, Sepiolidae; *Idiosepius*, Idiosepiidae; *Nautilus*, Nautilidae und *Sepia*, Sepiidae), produzieren biologische Klebstoffe für temporäre Anheftung von Substrat oder zur Verankerung.

Euprymna scolopes Berry, 1913 lebt in küstennahen, benthischen Gewässern des Indopazifischen Ozeans (Singley, 1982; Shears, 1988). Die Tiere sind nachtaktiv und verbringen die Tagesstunden im Meeresboden vergraben (Moynihan, 2002).

Ultrastrukturelle Untersuchungen zeigen, dass die adhäsiven Zellen bei *Euprymna scolopes* nur in der dorsalen Epidermis liegen. Das epitheliale Sekretionssystem produziert einen schleimartigen Adhäsivkleber, der es den Tintenfischen ermöglicht, während der Fortbewegung Sand und anderes Bodenmaterial an ihre dorsale Körperoberfläche zu heften. Diese tarnende Schutzschicht kann bei Gefahr blitzschnell abgestoßen werden und soll als sinkender “Köder” nahende Räuber ablenken (Shears, 1988; Norman, 2000).

In der dorsalen Adhäsivregion finden sich vier verschiedene Arten von Drüsenzellen, ventral hingegen nur zwei. Alle diese Zelltypen erstrecken sich über die gesamte Breite des Epithels und setzen Sekretionsmaterial an der Oberfläche frei. Das Mantelepithel misst zwischen 34 und 60 µm und wird von einer 0.3 µm dicken Basalmembran abgegrenzt.

Singley (1982) vermutete, dass aus “goblet” Zellen (Becherzellen) sekretierte Mukopolysaccharide verantwortlich für die Adhäsion seien, während aus “ovate” (ovalen) Zellen stammende, saure Mukoproteine einen gegenteiligen Effekt auslösen. Neue histochemische Beobachtungen belegen, dass das Sekretionsmaterial von “ovate” Zellen keine starke Reaktion bei Tests für saure Gruppen zeigen.

Unter dem Drüsenpithel von *Euprymna scolopes* befinden sich verschieden orientierte Dermal- und Mantelmuskelschichten, Dies deutet darauf hin, dass das Abwerfen der körperbedeckenden Sandschicht mechanisch induziert wird- und nicht chemisch, wie von Singley (1982) vorgeschlagen.

Abstract

Bio-adhesion is a common and crucial process in nature and is used by several different species for camouflage, prey capture, hatching or to avoid drifting. Four genera of cephalopods belonging to four different families (*Euprymna*, Sepiolidae; *Idiosepius*, Idiosepiidae; *Nautilus*, Nautilidae and *Sepia*, Sepiidae) produce glue for temporary attachment (von Byern and Klepal, 2006).

Euprymna live in near-shore benthal habitats of the Indo-Pacific Ocean (Singley, 1982; Shears, 1988). They are noctual active and bury into the seafloor during the day (Moynihan, 2002). The animals secrete glue to coat themselves totally with sand. In case of danger they release the adhesive glue as a sinking decoy instantaneously to deflect predators (Shears, 1988; Norman, 2000).

Ultrastructural observations show that the adhesive structures of *Euprymna scolopes* are located in the dorsal epidermis. The epithelial secretory system produces a mucous coat and gives the squid the ability to fix sand and other bottom material to the dorsal

surface of it´s body while moving. Presumably the coat acts as camouflage over the matching substrate.

Four types of gland cells occur in the dorsal adhesive region, whereby only two of them are present ventrally. All cell types span the full thickness of the epithelium and release secretory material to the surface. The thickness of the mantle epidermis ranges dorsally from 34 to 60 µm and is delimited by a 0.3 µm thick basement membrane.

Singley (1982) assumed that neutral mucopolysaccharides, secreted from the goblet cells, were responsible for adhesion, whereas de-adhesion was caused by acidic mucoproteins released from the ovate cells. New histochemical observations showed that the secretory material of ovate cells does not display a strong reaction to tests for acidic groups.

Beneath the epithelium variously oriented dermal and mantle muscle layers were found. This makes a mechanically related release of sand particles more likely than chemically induced de-adhesion, as suggested earlier (Singley, 1982).

Introduction

Euprymna scolopes is a very small species, endemic to the shallow waters of the Hawaiian Islands, with a mantle length of approximately 3.5cm and a weight of up to 2.7g. Juveniles behave seemingly identical to adults: they remain buried in the sand during the day and hunt for prey at night. Light intensity appears to be decisive for successful feeding: lower light enhances feeding, whereas bright light seems to retard it (Hanlon et al., 1997).

Females deposit serial clutches of eggs on the underside of coral ledges or other hard substrates (in the environment), but do not tend the eggs, as characteristic for some

cephalopod species. Instead, they cover the eggs with a patina of sand, and the embryos develop independent of parental care. The embryonic period depends on the temperature and ranges from 18 to 26 days (Arnold et al., 1972). Like other cephalopods, *E. scolopes* does not have a true larval stage, the juvenile animal hatches as a miniature adult. This quick development is inevitable because of their short lifetime (3-10 months). As in many marine animals, hatching is inhibited by light; consequently juveniles that are going to hatch on a particular day typically do so at the onset of darkness (Hanlon et al., 1997).

Euprymna scolopes Berry, 1913, is a member of the family Sepiolidae in the order Sepioidea (according to the nomenclature of Voss, 1977), in which cuttlefish are included. The term “squid” is commonly applied to some sepioids, although true squids are members of the order Teuthoidea. The genus *Euprymna* was first defined by Steenstrup in 1887. As it currently stands, twelve nominal species have been characterized:

- *E. morsei* (Verrill, 1881) from Japan,
- *E. albatrossae* (Voss, 1963) from the Philippines,
- *E. berryi* (Sasaki, 1929) from Japan, China and the Gulf of Tonkin,
- *E. hoylei* (Adam, 1986) from the Sulu Archipelago, Phillippines
- *E. hyllebergi (*Nateewathana, 1997) from the Andaman Sea,
- *E. penares* (Gray, 1849) from Singapore,
- *E. phenax* (Voss, 1963) from the Philippines,
- *E. scolopes* (Berry, 1913) from the Hawaiian Islands,
- *E. stenodactyla* (Grant, 1833) from the western Indo Pacific (Mascarene Islands to Queensland and Polynesia),
- *E. tasmanica* (Pfeffer, 1884) from the Bass Strait, Australia,

- *E.bursa* (Pfeffer, 1884) from Hong Kong and
- *E.similis* (Sasaki, 1913) from Japan)

Most species of the genus *Euprymna* are only distinguished by the number and position of enlarged suckers in mature males. No diagnostic characters are available to identify females or immature male specimens. At least two additional unresolved taxa exist in Australian waters. Preliminary DNA analysis has demonstrated a distinction to locally occurring species, but no morphological characters have yet been found to distinguish the several taxa from each other. (Norman & Lu, 1997)

Bioadhesion is a common and crucial process in the plant and animal kingdom and is used by several different species for camouflage, prey capture, hatching or to avoid drifting. The variety of the shown techniques is of great diversity and importance, which becomes apparent in the different ways of attachment. Carnivorous plants, for example, use adhesive traps for prey capture, while sticky shoots are widespread in plants and serve to protect especially the flowers and seeds. Insects employ adhesives for tarsal attachment during locomotion, mating, egg anchorage, phoresy and parasitism, among others, while the glue, produced by frogs to adhere males to females during amplexus, was even used industrially for mending crockery and glassware.

Especially marine species developed adhesive systems to either attach themselves onto other living organisms, rocks or man-made materials *via* cement, as observed in Barnacles; entangle predators by ejecting sticky tubules as documented in sea cucumbers; bond prey organisms tight onto tentacles despite strong movements of the victims as used by Ctenophores or build up composite mineralized tubes for shelter like the Sandcastle worm *Phragmatopoma californica* (all examples taken from: von Byern &

Grunwald, 2010).

In cephalopods four genera belonging to four different families (*Nautilus* sp., Nautilidae; *Sepia* sp., Sepiidae; *Euprymna* sp., Sepiolidae and *Idiosepius* sp., Idiosepiidae) are known to produce glue in an adhesive area within the mantle or on tentacles (Nesis, 1982; Norman, 2003). Still, the adhesive substances of these animals are used in different ways and studies about the several mechanisms allow a comparison in the various genera. Morphological studies have shown that *E. scolopes* possesses multiple adhesive glands in the dorsal epidermis by which it affixes sand to it´s body surface (Singley, 1982).

Since *E. scolopes* spends much of its life buried in the sand, it developed a special technique to adhere sand grains to its dorsal mantle and head, using the second arm pair to rake sand over, to form a “sand coat” (Singley, 1982; Shears, 1988). This sand coat remains attached to the squids when flushed from the substrate during daylight hours, but not at night. Presumably the coat acts as camouflage over the matching substrate during daylight hours. The animals are capable of instantaneously shedding their coat (Singley, 1982). Earlier observations indicated a duo-gland adhesive system (Hermans, 1983) to be responsible for adhesion and de-adhesion, but the present study suggests a chemical induced bonding and a release by muscles.

Earlier Studies and Gland Morphology

The association between *Euprymna scolopes* and the luminous bacterium *Vibrio fischeri* has been studied for more than 15 years as a model for the establishment, development and maintenance of horizontally transmitted symbioses (McFall-Ngai & Ruby, 1991; McFall-Ngai & Ruby, 1998; Nyholm & McFall-Ngai, 2004; McFall-Ngai, 2002). The squid maintains the bacteria extracellularly in a ventral tissue complex, the light organ, and

feeds a sugar and amino acid solution to its symbiont. *Vibrio fischeri* produces light that hides the squid's silhouette when viewed from above, which means camouflage and protection from predators (Singley, 1983; McFall-Ngai & Montgomery, 1990; Claes & Dunlap, 2000).

Within the genus *Euprymna* the majority of the species is well-defined according their morphological characteristics. However, only in *Euprymna scolopes* extensive studies were conducted, concerning gene activity (Foster et al. 2000; Nyholm et al. 2002; Small & McFall-Ngai, 1999), structure and function of the hatching gland (Hoyle organ) (Arnold, 1972; Arnold et al. 1990), as well as structure and histochemistry of the adhesive region (Singley, 1982; Singley, 1983).

The adhesive structures of *E. scolopes* are located in the dorsal epidermis. The epithelial secretory system produces a mucous coat and gives the squid the ability to fix sand and other bottom materials to the dorsal surface of its body while moving (Singley, 1982). In the adhesive region four types of gland cells occur, ventrally only two of them exist. All cell types present span the full thickness of the epithelium and release secretory material to the surface.

Singley (1982) was the first and, until now, sole author to investigate ultrastructurally and histochemically the epidermal constitution of *E. scolopes*. He describes the epidermis as a pseudostratified columnar epithelium, composed of three morphologically distinct cell types and delimited by a thick basement membrane. The basal region of the epithelium is often undulated and the surface layer is comprised in part of a pile of microvilli.

As the first gland cell type Singley (1982) describes the polymorphic **interstitial cell.** The cells are the most abundant cell type in the dorsal epidermis and less frequently observed in the ventral epidermis. On the apical surface there is a layer of microvilli. The

rounded nucleus is found near the epithelial surface. The cytoplasm is typically filled with thick (10nm to 15nm) and thin (5nm to 8nm) filaments, which are especially prominent in the basal region. The cells possess small vesicles containing material of varying electron density. No conclusive evidence of exocytosis has been observed by Singley (1982).

Secondly Singley (1982) characterizes **ovate cells**, which he observed in all regions of the epidermis with the exception of the distal surfaces of the fins and arms. The ovate cell is the largest of all cell types and ovate to sac-like in shape. It contains a fine granular secretory material of uniform appearance, which flattens the nucleus against the basal surface. Various amounts of rough endoplasmatic reticulum occur within the cytoplasm surrounding the nucleus, but only few Golgi bodies exist. Within the peripheral cytoplasm Singley found a reticulum of filaments (5nm to 8nm) that becomes rearranged and tightly packed during secretory activity.

Additionally, these filaments appear to produce deep folds in the plasma membrane, beginning near the apical end of the cells and progressing towards the basal end.

The third cell type described is the **goblet cell**, which occurs only in the dorsal epidermis and is especially abundant in the head and dorsal mantle. Goblet cells are rounded at their base, taper inward toward their apical end and have rounded cross sectional profiles. The cells are infolded laterally toward their apices displaying a branching profile in longitudinal section. The surface area of the goblet cells is much smaller in comparison with the interstitial cells and the microvilli are shorter.

Near the bases of the goblet cells a large rough endoplasmatic reticulum (rER)-Golgi complex can be found. However the Golgi apparatus is generally more massive than the rER.

Especially in actively secreting cells numerous microtubules are oriented along the longitudinal axes of goblet cells and frequently appear in close proximity to the secretory granules. These secretory granules are characterized as highly electron-dense, spherical and with a considerable variation in size. The density is uniform and each granule is surrounded by a single unit membrane. They are distributed from the region of the elliptical nucleus near the basal end up to the apical surface. During active secretion, the granules appear to break up into smaller units as they approach the apical surface.

Results

Recent ultrastructural and histochemical investigations of the epithelium of *Euprymna scolopes* provide new details of the adhesive region and additional information can be added to Singleys (1982) observations. The thickness of the mantle epidermis in an average sized animal ranges dorsally from 34 to 60µm (Singley, 1982: 35 – 50µm), at the ventral side from 33 to 43µm and at the fins from 13 to 25µm. The basal lamina is about 0.3µm thick. Beneath the epithelium are variously orientated muscle layers. Contrary to Singley´s (1982) observations, four different cell types can be distinguished.

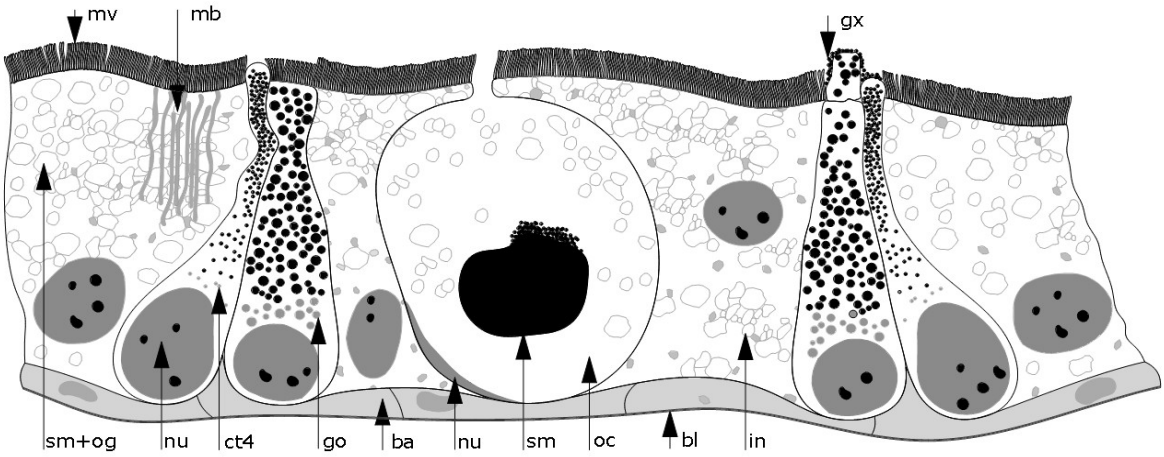


Fig.1: Schematic drawing of the adhesive epithelium in *Euprymna scolopes*. Cross section of dorsal epithelium showing the different cell types (ovate, goblet, interstitial and cell type 4).

Abbreviations:

ba = basal cells
bl = basal lamina
ct 4 = cell type 4
gx = glycocalix
go = goblet cell
in = interstitial cells
mb = membranes
mv = microvilli
nu = nucleus
oc = ovate cell
og = organelles
sm = secretory material

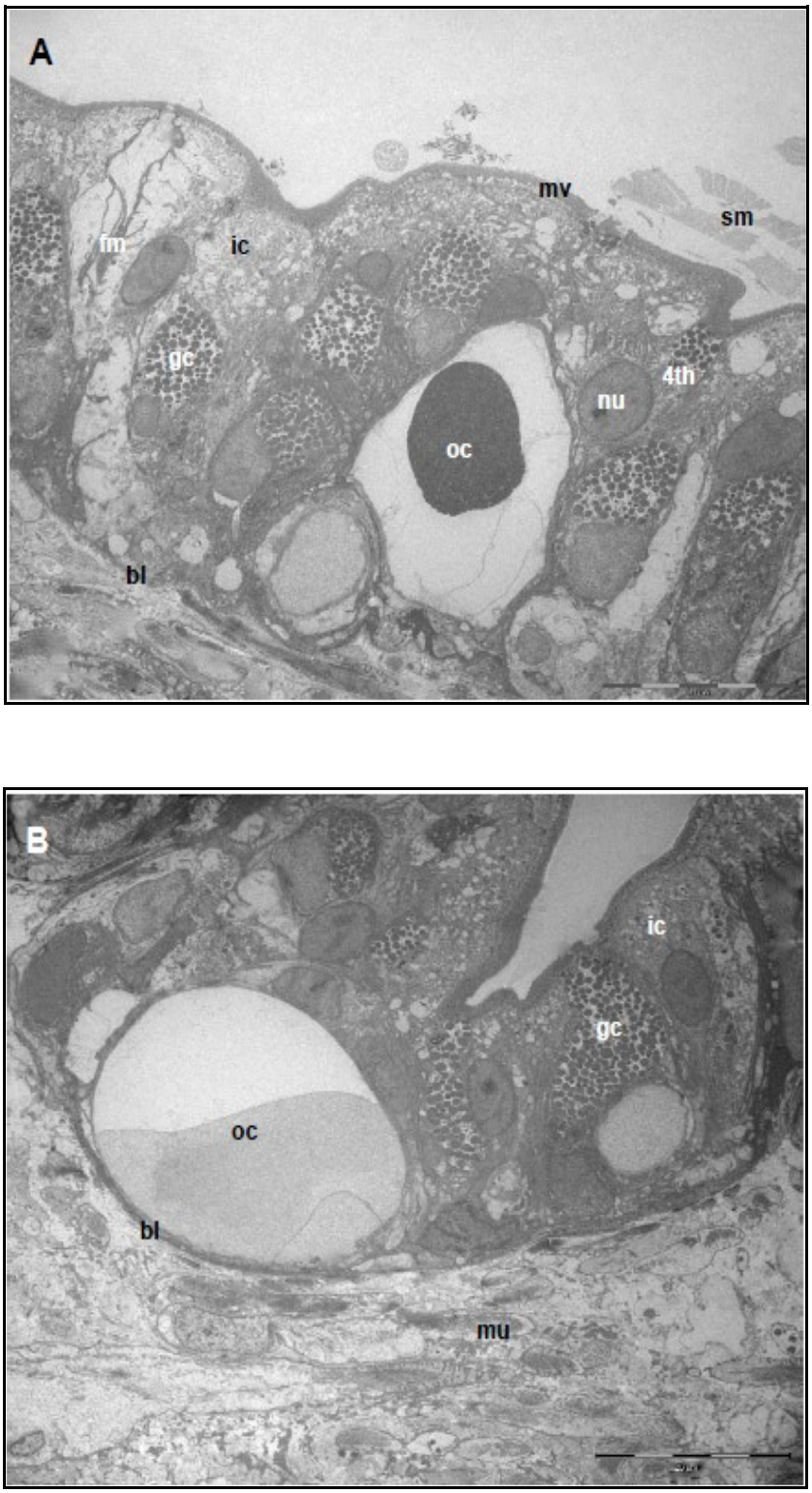
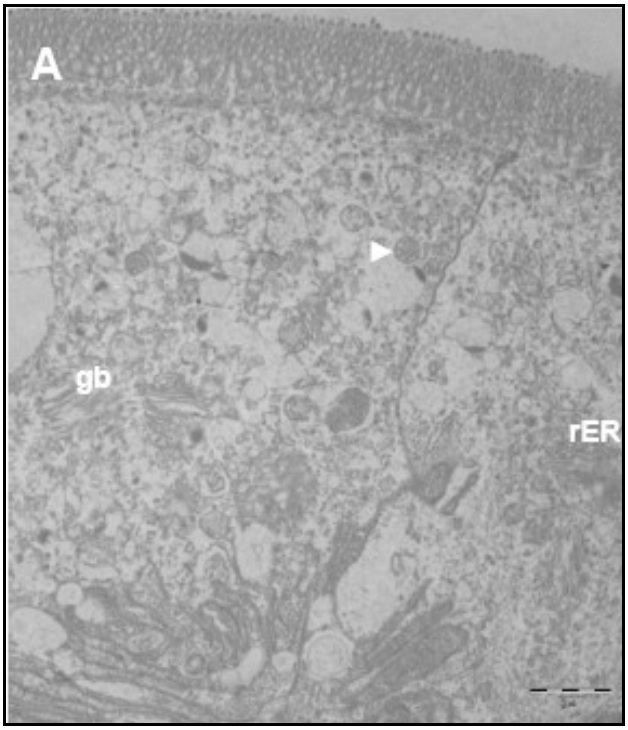
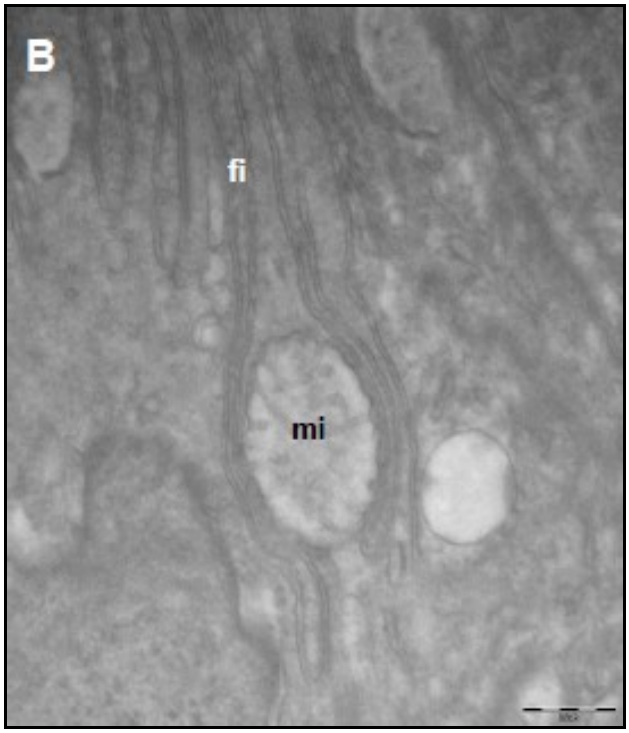


Fig.2: Epithelium. (A) Overview of the adhesive region of *E.scolopes.* All four cell types span the full thickness of the epithelium; (B) Cross section of dorsal epithelium showing the different cell types, basal lamina and variously orientated muscle layers beneath. Scale bars in (A) = 10µm, (B) = 20µm.

Interstitial cells occur in the entire mantle epithelium. Typically, interstitial cells appear among the gland cells and show very variable shape and size. The cells separate ovate from goblet cells in the adhesive region and are dominant in the normal mantle tissue. At the apical surface of the cells is a dense layer of microvilli 1.3 to 1.5µm long (Singley, 1982: 0.5 – 0.6µm). Nuclei were found either at the basal or at the apical end of the cell and are usually rounded to ovate. In the cells are numerous organelles, especially mitochondria, but also rER and Golgi bodies, evenly distributed. In-between the longitudinally orientated filaments mitochondria are often omnipresent.

Singley (1982) did not demonstrate any secretion of these cells, but our observations show evidence of (rare) exocytosis. This function might be essential to form the glycocalix on the apical pale of the cells between and on top of the microvilli.





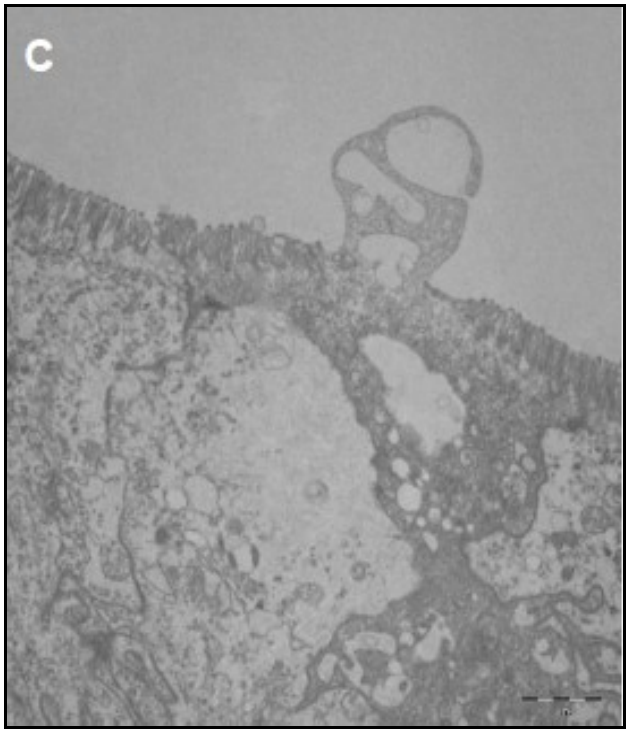


Fig.3: Interstitial Cell. (A) Terminal web (arrowhead) through which vesicles transit to and from the apical membrane; (B) Longitudinal running filaments are often interspersed with mitochondria; (C) Secretion process. Scale bars in (A and C) = 2µm; (B) = 0.5µm

Ovate cells are not only found in the adhesive region of *Euprymna scolopes*, but also in the regular mantle epithelium at the ventral side of the animal and on the basis of the fins. Contrary to Singley (1982), who described a lack of a microvilli layer at the surface of the cells, microvilli, 0.8 to 2µm long, were detected.

Ovate cells have been observed in different phases of activity. Some cells have very dense secretory material, which accumulates centrally in the cell. Others contain loose secretory material evenly distributed over the entire cell area, and also completely empty cells are found. The latter seem to represent a stage, when secretory material is completely released. Further investigation is needed to determine whether ovate cells "refill" themselves or induce apoptosis.

Ultrastructural observations of the secretory process indicate a direct release of (secretion) material through an opening in the cell's surface.

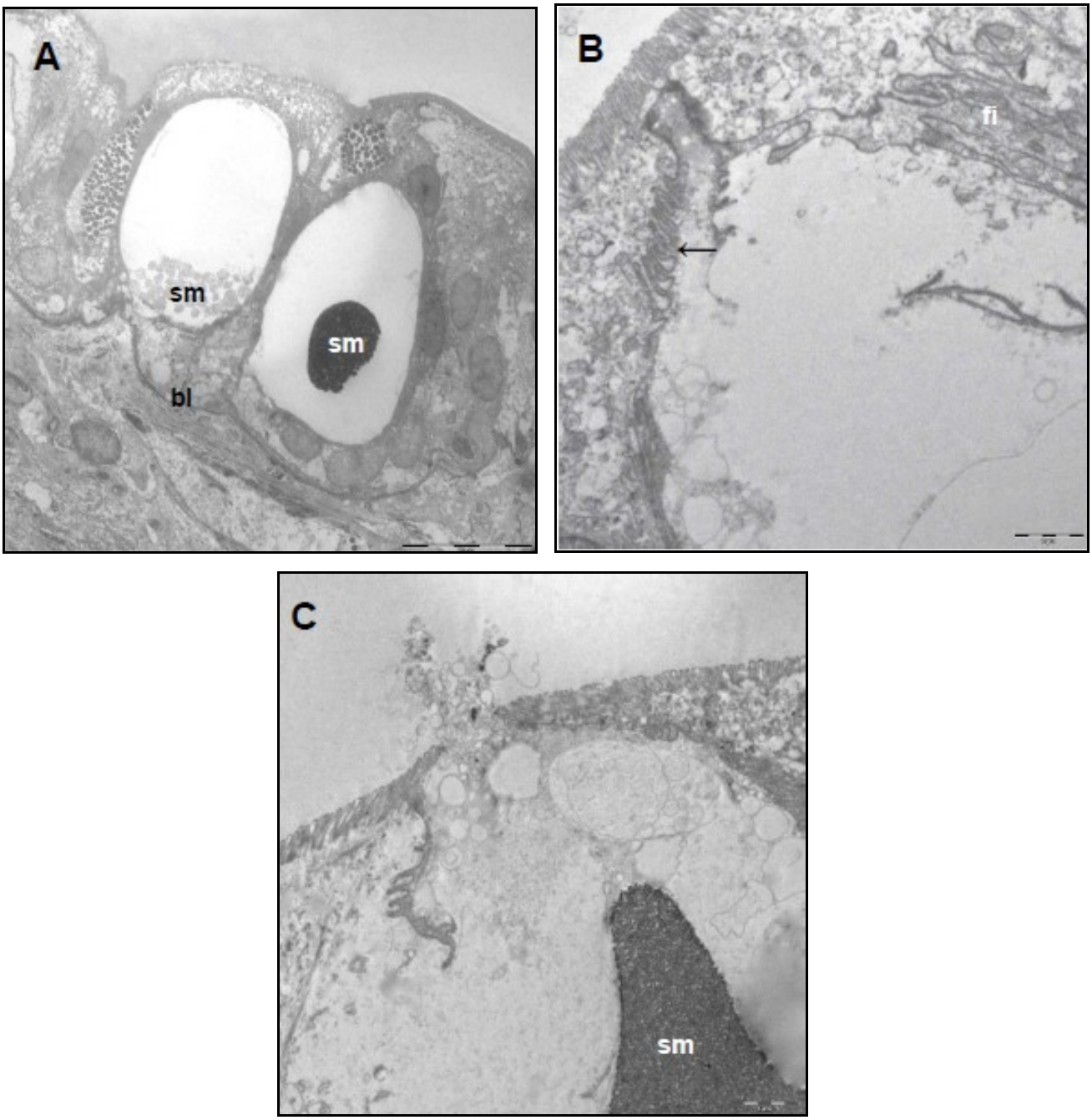
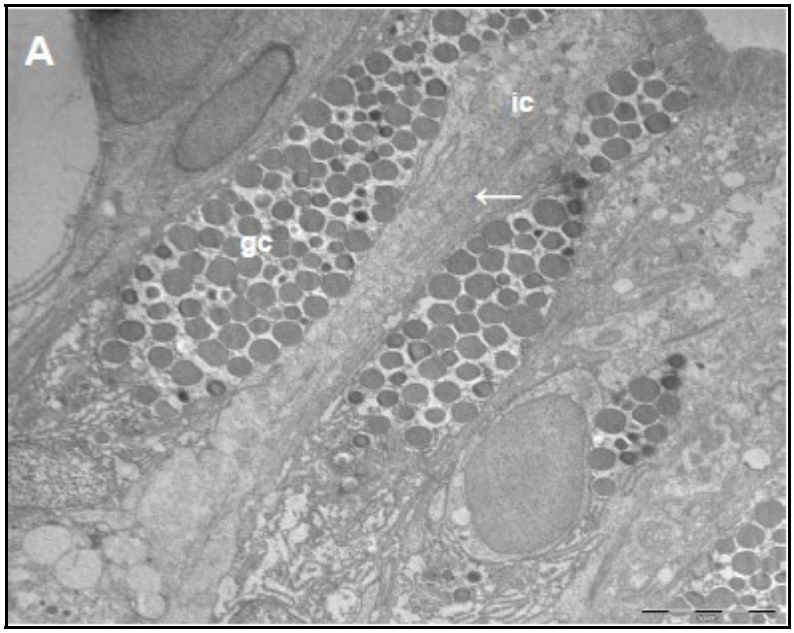


Fig.4: Ovate cell. (A) Fine granular secretory material in different density stages, (B) Membranes produce deep folds beginning near the apical end of the cells and progressing toward the basal end; (C) Secretion process. Scale bars in (A) = 20µm; (B and C) = 2µm

The third cell type, the **goblet cell**, occurs in the dorsal part of the mantle and at the fin base. Their microvilli are approximately 0.7µm long and thus shorter than the microvilli of the interstitial cells. Organelles, especially rER and Golgi apparatus and few mitochondria, can be found in the area around the nucleus. Vesicles, released from the Golgi apparatus, are electron lucent, but they become more electron dense the nearer they get to the apical end of the cell. They are always characterized by a uniform appearance, showing a homogenous density and shape. The vesicles have a diameter of 0.8 to 1µm (Singley, 1982: 0.5 – 0.7µm), are spherical and membrane bound.

Goblet cells differ in their way of secretion to the other cell types. Instead of releasing their secretory material trough an opening in their surface, as it happens in the interstitial and ovate cells, they pinch off the most apical part of the cell content, which includes some cytoplasm and secretory vesicles. It is likely that the cells reproduce the released part instantaneously.

Additionally an atypical appearance of the goblet cell was detected. It shows a frequent condensation of vesicles and therefore an increased appearance of secretory material. Possibly a dysfunction of the secretion process causes an accumulation of the secretory vesicles; also an overproduction of those could be the reason.



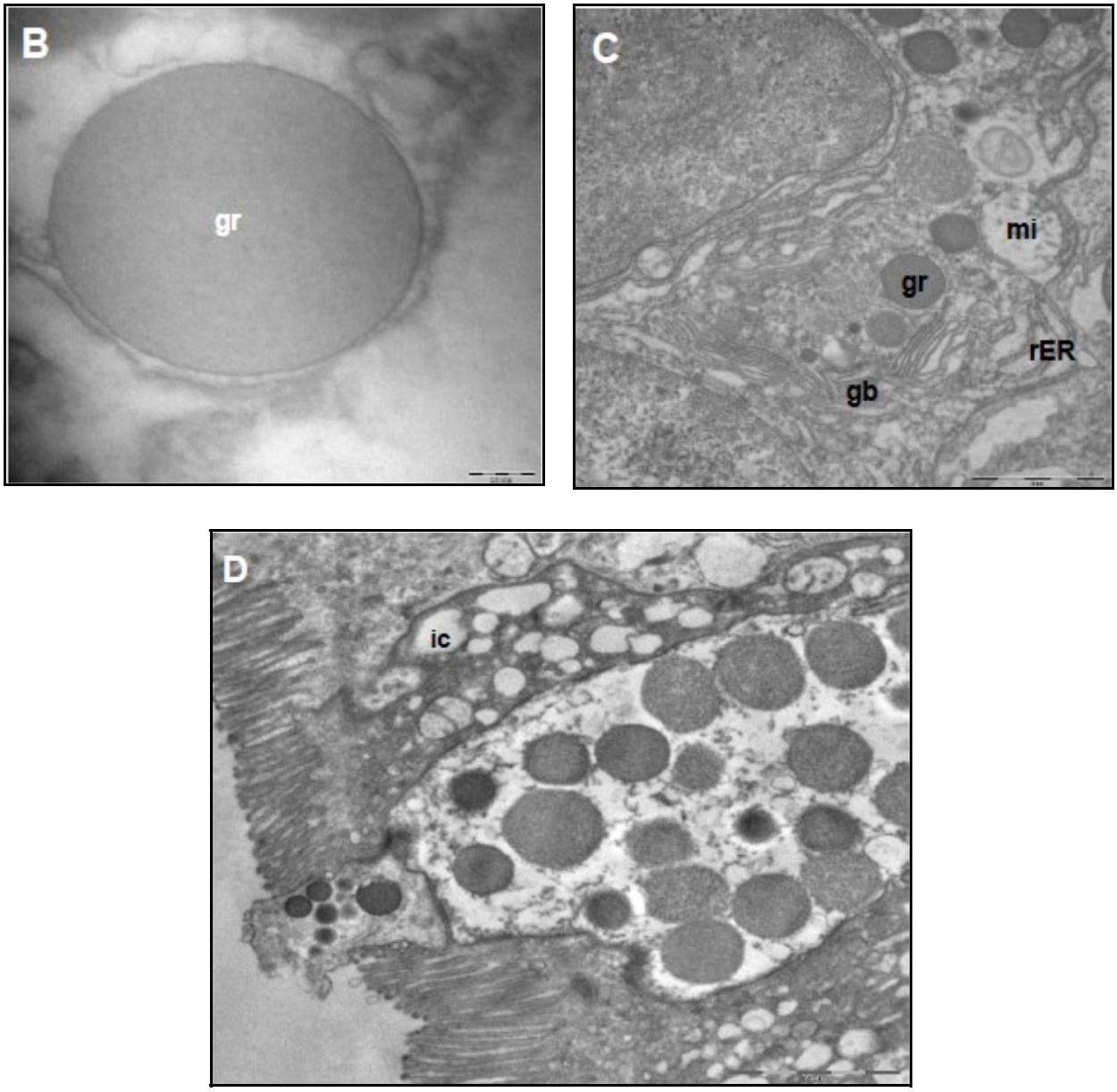


Fig.5: Goblet cell. (A) often appears grouped, but always interspersed among interstitial cells. Note intracellular filaments in the interstitial cells (arrow); (B) Spherical and membrane bound vesicle; (C) Golgi apparatus, rER and mitochondrium. Vesicles released from the Golgi apparatus appear electron lucent, while the granules are more electron dense. (D) Secretion process. Scale bars in (A) = 5µm; (B) = 200nm (C) = 2µm; (D) = 0.5µm

Beside the aforementioned cell types described by Singley (1982), a **fourth cell type** was identified. Similar to the goblet cells this cell type contains spherical, membrane-bound and very electron dense granules, but they are much smaller (0.2-0.4µm) and evenly distributed over the cell.

The fourth cell type always occurs in the immediate vicinity of a goblet cell and can only be found in the adhesive region of *Euprymna scolopes*. It differs from the other epithelium cells, this cell type does not possess a microvilli layer. The cell is elongated with a rounded base and is more slender than the goblet cell. Organelles (mitochondria, rER and Golgi apparatus) are found at the basal end of the cell near the elongated nucleus. The cell shows, different to the goblet cell, a release of secretory material through a small opening on the surface.

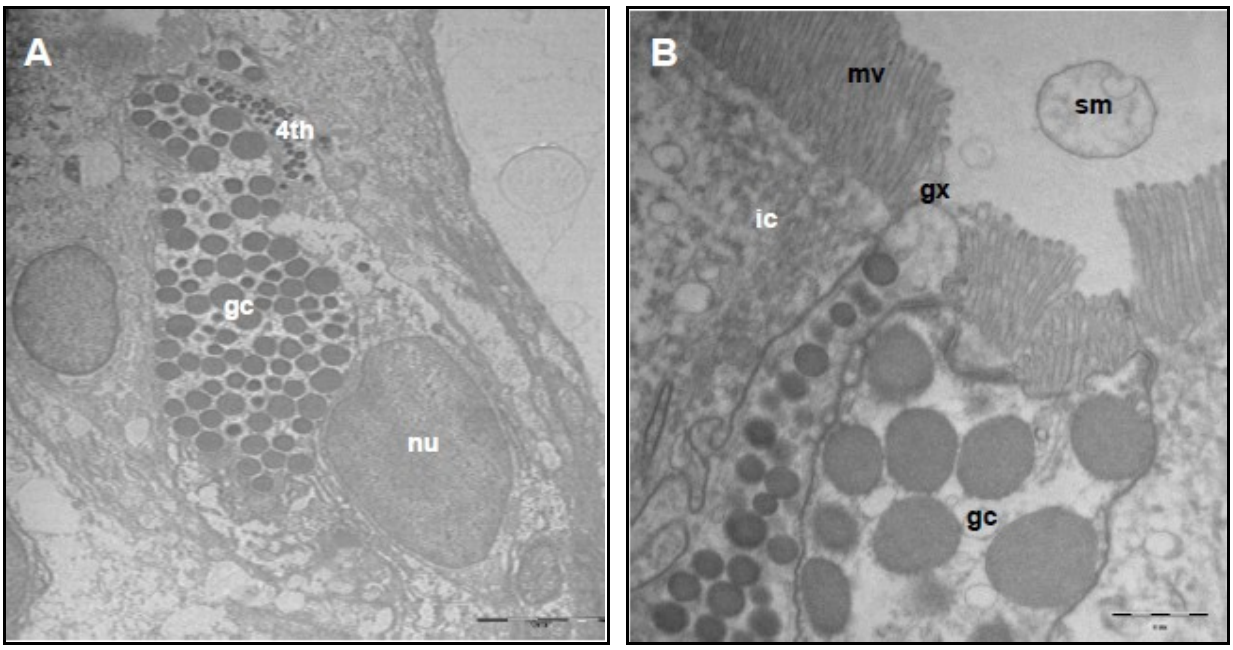


Fig.6: Cell type 4. (A) occurs in immediate vicinity of a goblet cell. It's elongated nucleus is located at the basal end; (B) The granules are transported by filaments to the cell surface. The surface area is covered with glycocalix, but no microvilli as on the goblet cells. Scale bars in (A) = 5µm; (B) = 0.5µm

Histochemistry

Histochemical observations of Singley (1982) showed that **ovate cells** secrete carbohydrate-rich substances. The secretory material of the ovate cells appeared to be a highly sulphated protein-polysaccharide complex. This material is periodate-unreactive and strongly reactive to basic proteins (histological staining methods see Singley 1982). Their negative reactivity to tests for polysaccharides and acidic groups by the majority of ovate cells, contrasted with the very strong staining of this material in **evacuating ovate cells**. Singley (1982) therefore suggested that acidic groups are present but masked in the unsecreted material.

Evacuating ovate cells were, like ordinary ovate cells, periodate unreactive. The marked reactivity to tests for acidic groups of evacuating ovate cells suggests that these are sulphate groups.

Concerning the **goblet cells** Singley (1982) characterized the secretory material as neutral sugars. Because of the lack of staining for the specific tests, observed in goblet cell granules, the absence of strongly acidic groups was suggested.

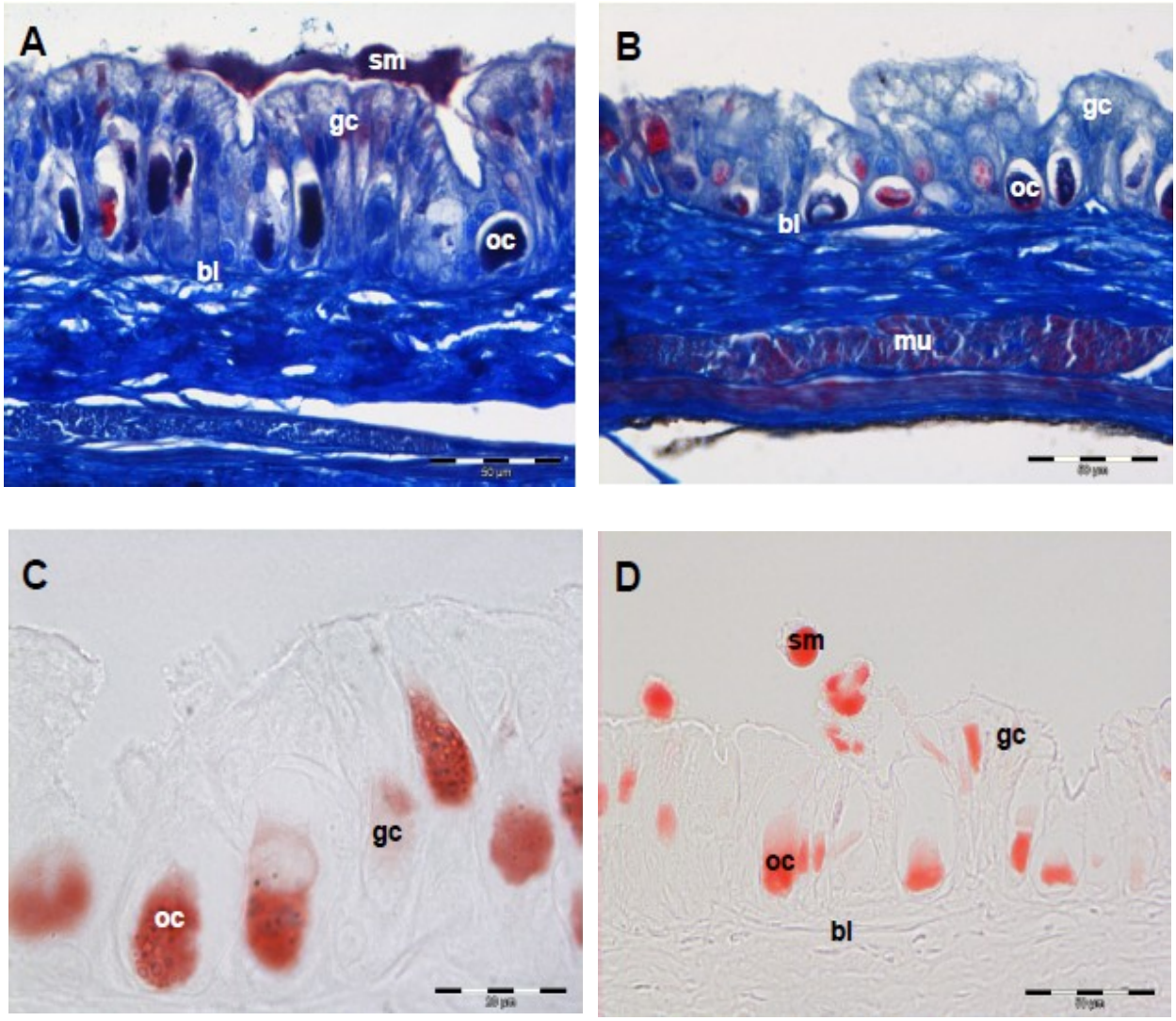
Recent histochemical studies carried out by Dr. Dipl.-Biol. Janek von Byern in 2010, show similar results when compared with those of Singley (1982). Whereas for **goblet cells** a negative reactivity with tests for basic protein was observed, there was a strong reaction with tests for mucoplysaccharides.

Ovate cells were strongly reactive with tests for basic, but showed no reaction with tests for mucopolysaccharides.

Interstitial cells and the **fourth cell type** did not show any reaction to the tests

applied so far. Further investigations are necessary to gain more information about their reactivity to the specific tests.

Variances between the findings of Singley (1982) and the new results appear with regard to the evacuating ovate cells. Tests for acidic proteins (AB pH 1.0 and AB pH 2.5) showed no positive reaction both in goblet and in ovate cells, but weak staining in the secretory material at the cell's surface was observed. There was no change in pH-value due to blending with sea water, as Singley (1982) suggested.



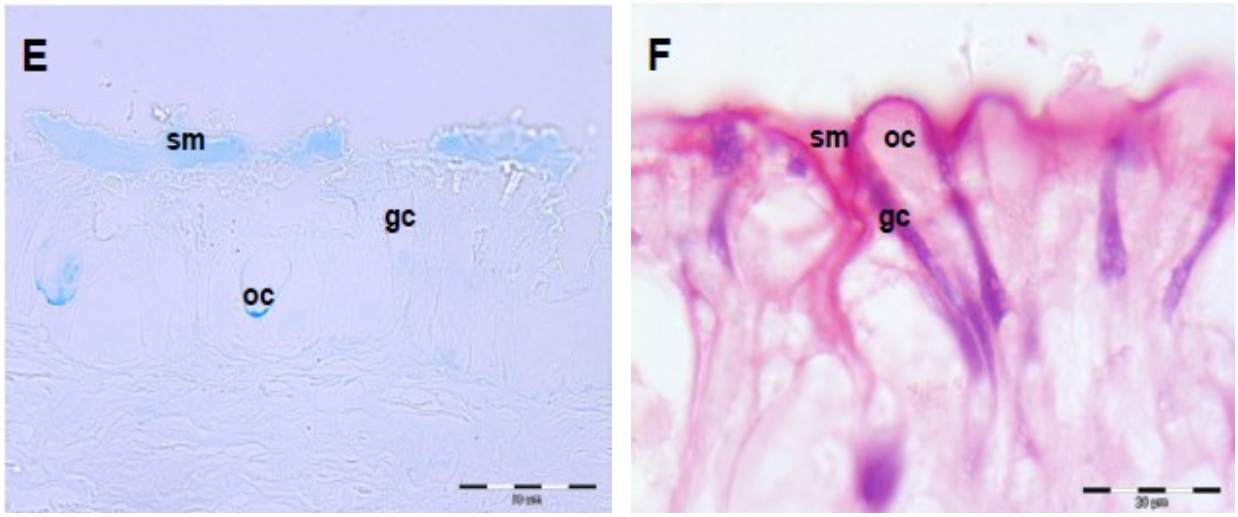


Fig.7. Histochemistry. (A) The dorsal adhesive epithelium is higher than (B) the ventral epithelium. Both contain goblet, ovate and interstitial cells. Beneath is a high dermal layer consisting of connective tissue and muscle fibers (stain AZAN), Ovate cells (C) on the ventral and (D) dorsal side bear basic proteins around pH 6.0 (in C) up to pH 8.5 (in D, E). The ovate cells are also light reactive to acid proteins (Alcian Blue pH 2.5), while the secretory material of the goblet cells consists of neutral sugars (PAS reaction). Scale bars in (A,B) and (E) = 50 µm; (C) and (F) = 20 µm; (D) = 100 µm

Discussion

Temporary adhesion is often effected by a duo-gland system, which includes two types of secretory cells, viz. cells responsible for adhesion and cells releasing a de-adhesive secretion (Hermans, 1983). Non-secretory supporting cells are located between the glandular cells. In many cases cilia are present in this adhesive system. They are either part of the glandular cells or of separate ciliary cells. The duo-gland system was predominantly described for interstitial organisms as Turbellaria (Tyler, 1976), Gastrotricha (Boaden, 1968; Teuchert, 1977) and Archiannelida (Martin, 1978). Echinoderms (Flammang et al., 1998a; Flammang, 2006; Santos et al., 2005a; Santos et al., 2005b) and gastropods (Grenon and Walker, 1980) use temporary adhesive mechanisms for

attachment and locomotion, which diverge from the duo-gland system. Singley (1982) assumed that in cephalopods neutral mucopolysaccharides, secreted from the goblet cells, were responsible for adhesion while de-adhesion was caused by acidic mucoproteins, released from the ovate cells.

Histochemical observations showed that the secretory material of ovate cells does not display a strong reaction to tests for acidic groups. Singley (1982) suggested that during segregation basic proteins transform to highly sulphated acidic proteins. However a clear distinction of the diverse secretory material of the different cell types is not detectable.

Further Singley (1982) indicated an influence of the surrounding sea water to the evacuating cells causing a reaction towards an acidification of the secretory material. This hypothesis can not be supported by the author, due to the steady pH-value of the cells, which implicates that no acidic proteins derived from the sea water are detected in the secretory process.

Assuming a duo-gland adhesive system in *Euprymna scolopes,* the here described fourth cell type is more likely to take on the function of de-adhesion, while the goblet cell remains responsible for adhesion. Since ovate and interstitial cells also occur in the normal mantle tissue, their contribution to the adhesive process seems negligible.

Nevertheless, a recent re-characterization of the epithelium of *Euprymna scolopes* speaks against a duo-gland adhesive system, as suggested by Singley (1982). Ovate cells presumably do not play a major role in the adhesion/de-adhesion process. These cells were also found in the regular mantle tissue and are likely to produce mucus, which covers the squid’s body surface. Interstitial cells most probably contribute to the production of the glycocalix, which is found at the microvilli layer on the surface of the epithelium. The fourth

cell type, which could be responsible for de-adhesion of sand particles, seems too small to produce sufficient secretory material.

Three species of *Sepia* (*S. tuberculata, S. typica* and *S. papillata*) (Sepiidae) use a mechanical mechanism (reduced pressure system) combined with chemical adhesives for attachment. Adherence is achieved by a defined area on the ventral mantle and on the posterior surface of the ventral arms (Roeleveld, 1972; von Boletzky and Roeleveld, 2000).

However, adhesion in *Euprymna* could be induced by a distinctive muscle layer, as described in *Sepia*, which represents a mechanical way of attachment. Chemical secretions released from the adhesive organ might be used to increase the strength of attachment (Von Byern & Klepal, 2006). Since several, different orientated dermal and mantle muscle layers were found beneath the epithelium of *Euprymna scolopes*, a muscle related system for de-adhesion of sand particles is more likely than a chemically induced release. Due to the distinct dermal and mantle muscle layers the animals are capable to react considerably faster, than it would be possible with a chemical induced release of secretory material. Also a better control of the de-adhesion process is given. Nevertheless, this possibility was never mentioned by Singley (1982).

Recent data suggest in all four cephalopod genera that possess an adhesive system, a cooperation of two secretory cells in terms of a two-component system. In *Euprymna*, *Nautilus* and *Sepia* a muscular de-adhesion seems to be the primary mechanism. In *Idiosepius* the absence of an explicit mantle musculature beneath the adhesive area speaks against such a release mechanism, instead an attachment to surfaces by a gel, as is known for gastropods (Smith, 1991, 2002; Smith et al., 1999) is most probable (Von Byern et al. in Von Byern & Grunwald, 2010).

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