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Ultrastructural characterization of the adhesive organ of *Idiosepius biserialis* and *Idiosepius pygmaeus* (Mollusca: Cephalopoda)

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Water drift and tidal rise make the use of bonding mechanisms beneficial for small benthopelagic or interstitial marine animals. Chemical adhesives for attachment are very common in molluscs; however, only a few cephalopods have glue producing organs. The family Idiosepiidae is characterized by an epithelial adhesive organ (AO) located on the posterior part of the dorsal mantle area. Previous morphological and histological studies described three non-glandular cell types (basal, interstitial and fusiform cells) and three glandular cell types (goblet, columnar and granular cells) containing protein and carbohydrate components. However, these studies provide different information about the nomenclature and characteristics of the cell types. The present ultrastructural analyses and a 3D reconstruction of the AO of Idiosepius pygmaeus *and* Idiosepius biserialis *therefore serve to investigate the cell distribution, the fine structure of the cells and possible interactions between the cells.We found that basal cells form a continuous cell layer along the basal membrane, overlapped by the other epithelial cells. Embedded in microvilli-covered interstitial cells the glandular cells are more or less evenly distributed within the AO. Goblet and granular cells are solitary glandular cells without conspicuous morphological characteristics, whereas the columnar cells are arranged in dense aggregations of 5–15 cells. Each columnar cell is enclosed by a narrow supporting interstitial cell which contains dense longitudinal filament strands. The secretory process of the cells in the aggregation is synchronized. Each columnar cell aggregate bears approximately two ciliated sensory fusiform cells. The fusiform cells are connected to a neuronal network, aligned along the epithelium base.The results suggest that the bonding system is affected by two secretory cell types (granular and columnar cells). Both are similar in content, synthesis and secretory process but columnar cells are embedded in a particular cell environment. It is unclear in what way this arrangement is associated with the function of the AO. The neurons in several parts of the AO point to a neuronal control of the bonding mechanism. Comparisons with the AO cells of other cephalopods provide no indications for a morphological relationship between the adhesive systems.*

Keywords: ultrastructure, adhesive organ, adhesion, cephalopod, glandular structure, glue, glue synthesis

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INTRODUCTION

Several cephalopod taxa possess adhesive mechanisms either for camouflage, prey capture, egg attachment or to avoid drifting. Attachment is effected by mechanical mechanisms, e.g. reduced-pressure systems in suckers on the arms and tentacles (Smith, 1976; Kier & Smith, 2002; Pennisi, 2002) or by chemical substances produced in special glandular cells. The localization and morphology of the adhesive systems vary strongly according to the species. Chemical adhesives are supposed for species of the genera *Nautilus* (Fukuda, 1980; Kier, 1987; Muntz & Wentworth, 1995), *Sepia* sp. (Roeleveld, 1972; von Boletzky & Roeleveld, 2000), *Euprymna* (Singley, 1982) and *Idiosepius* (Sasaki, 1921; Nesis, 1982; von Byern & Klepal, 2006).

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The adhesive organ (AO) of Idiosepiidae Appellöf 1898 is clearly restricted to the posterior area of the dorsal mantle surface and parts of the fins (Steenstrup, 1881). A first detailed description was carried out by Sasaki (1921), who classified two glandular cell types in the AO of *Idiosepius*: columnar cells with globular granules (Ø 1 μm) and granular cells containing closely packed polygonal granules (2–5 μm). Furthermore, cell types without secretory material are described as goblet cells, decayed columnar cells and sensory fusiform cells near the surface. Basal cells form a second cell layer aligned along the basal membrane.

Histological and histochemical studies of the AO of *Idiosepius* (von Byern *et al.*, 2008) indicate that, in addition to the granular and columnar cells, also the goblet cells possess secretory components. All three glandular cell types contain neutral polysaccharides and basic proteins but with varying amounts.

So far the sparse knowledge about the fine structure of epithelial gland structures in cephalopods, except for the few

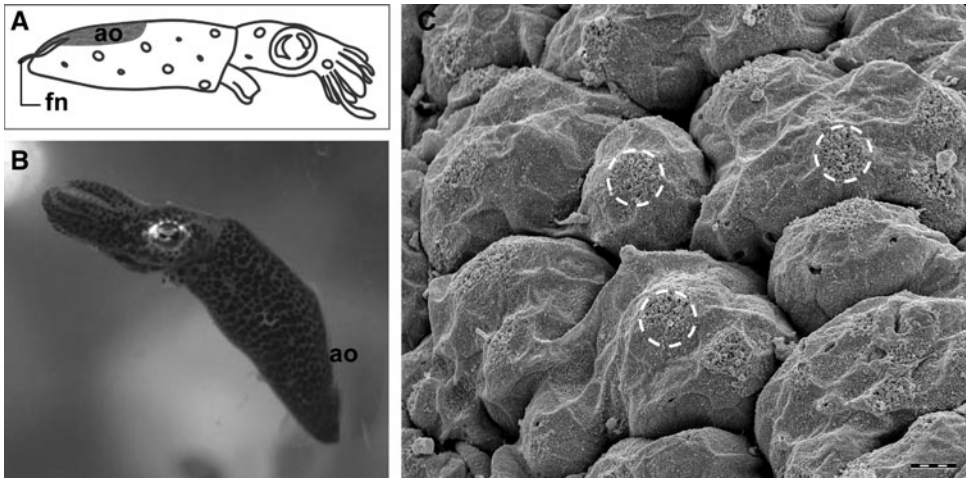

Fig. 1. *Idiosepius pygmaeus*. (A) The dorsal posterior mantle bears the adhesive organ; (B) *I. pygmaeus* attached to an aquarium glass. The contact surface is increased by the flattened dorsal mantle; (C) aggregates of columnar cells (circles) are predominantly located on the elevated parts of the surface. Scale bar: 10 μm.

species mentioned above, inhibits extensive comparison to other molluscs. In the present study ultrastructural analyses were carried out to investigate the cellular morphology of the AO of two *Idiosepius* species. The results help to understand the morphology of glandular cells and allow a more detailed comparison with chemical adhesive systems in cephalopods as well as other glue-producing marine animals.

Abbreviations used: af, actin filaments; ba, basal cell; bm; basal membrane; bv, blood vessel; ci, cilium; cl, cell lumen; cm, cell membrane; co, columnar cell; col, collagen; cs, cytoskeleton filaments; ct, connective tissue; dm, dermal muscle; ev, endocytosis vesicle; f, fusiform cell; fi, filaments; fn, fin; fs, filament strands; gc, glycocalyx; gm, granular material; go, goblet cell; gol, Golgi; gr, granular cell; gs, granules;i, interstitial cell; ime, inner mantle epithelium; lm, longitudinal muscle; ly, lysosome; mi, mitochondrion; mm, mantle musculature; mv, microvilli; nb, nucleus of basal cell; nc, nucleus of columnar cell; ne, nerve process; nf, nucleus of fusiform cell; ng, nucleus of granular cell; ngo, nucleus of goblet cell; ni, nucleus of interstitial cell; ome, outer mantle epithelium; rER, rough endoplasmic reticulum; ri, ribosomes; sc, saccular cell; sc1, secretory cell type 1; sc2, secretory cell type 2; scl, synapic cleft; si, sinus; sm, secretory material; sv, synaptic vesicle; tgn, trans-Golgi network; tpv, transport vesicle; tv, transition vesicles; tw, terminal web.

MATERIALS AND METHODS

Two species (*Idiosepius pygmaeus* and *Idiosepius biserialis*) were investigated. Collection, cultivation and preparation were carried out according to von Byern *et al.* (2008).

The mantle was pre-fixed *in toto* for 5 minutes at 29–33°C using 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.3, including 10% sucrose). Main fixation was made at same temperature for 5–8 hours. Subsequently, the samples were washed three times for 15 minutes each with 0.1 M buffer solution and stored for further processing. For post-fixation the samples were immersed for 1.5 hours in 1% osmium tetroxide with 0.1 M buffer solution and dehydrated in a graded series of ethanol.

For transmission electron microscopy the samples were embedded in Epon resin; ultrathin sections (40–80 nm) were mounted on copper slot grids coated with formvar in dioxane, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a TEM Zeiss 902.

In addition to the standard procedure the following modifications were made: (1) during the first fixation calcium chloride was added to the buffer solution until the saturation limit to improve the membrane preservation; (2) for contrast enhancement 1% potassium ferricyanide was added to the osmium tetroxide fixative; and (3) after post-fixation samples were immersed for 40 minutes in 1% tannic acid and 45 minutes in 1.5% uranyl acetate to enhance visualization of lipids and proteins.

For scanning electron microscopy the samples were washed several times in 100% acetone, immersed in HMDS (hexamethyldisilazane), dried in air over night, mounted on stubs, coated with gold in a Polaron 5800 sputter coater and viewed in the SEM Philips XL 20.

The 3D-reconstruction of the adhesive organ of *I. pygmaeus* is based on a continuous series of 60 semithin sections (1 μm), stained with toluidine blue and photographed on a light microscope. Images were edited with the software program Blender version 2.37a −2.41 using stacks of UV-textures. Measurement of cells and nuclei as well as the number of nucleoli in the distinct cell types likewise rely on this image stack.

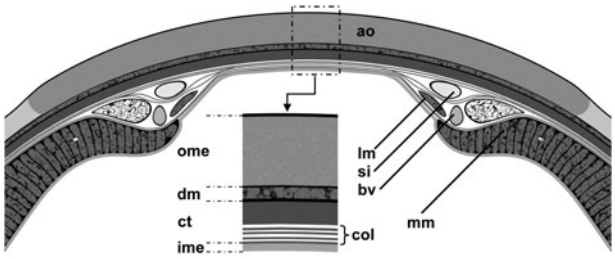

Fig. 2. Schematic drawing of the dorsal mantle tissue. The darker area of the outer mantle epithelium (above the basal membrane) marks the adhesive organ. The mantle musculature is recessed in the middle part of the dorsal mantle.

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RESULTS

While the surface of the regular mantle epithelium in *Idiosepius pygmaeus* and *I. biserialis* is smooth, the adhesive epithelium has deep furrows (Figure 1). As in *Sepia* and *Loligo* the mantle consists of the outer mantle epithelium, dermal musculature, connective tissue including chromatic elements (chromatophores, iridophores and reflector cells), collagen and an inner mantle epithelium (Figure 2). However, it differs from other cephalopods by a discontinuity of the mantle musculature in the middle part of the dorsal mantle (Figure 2). A basal membrane (0.5 μm thick) is located at the base of both the outer and inner mantle epithelium. The height of the epithelium cells within the AO (60 μm in *I. biserialis* and 80 μm in *I. pygmaeus*) are increased in relation to the regular outer mantle epithelium (30 and 40 μm, respectively). Otherwise no morphological differences can be found between the two species.

Characterization of the AO cell types

The outer mantle epithelium including the AO is generally composed of two cell layers, whereby the proximal layer exclusively consists of basal cells. In the distal cell layer three glandular (columnar, granular and goblet cells) and three non-secretory cell types (interstitial cells, saccular cells and small ciliated fusiform cells) can be distinguished morphologically (Table 1; Figures 3 & 4).

The basal cells (Figure 5) adjoin the basal membrane and form a continuous cell layer (about 5 μm high). The cells are flat ovoid to cone-shaped and mostly have a globular nucleus, lacking nucleoli. The cytoplasm is largely full of vesicles (Ø 0.5 μm), which bud by endocytosis from the membrane of the cell base (Figure 5B). They contain electron-dense particles (Figure 5C). The cells have numerous cytoskeleton elements (Figure 5C). Endoplasmic reticulum, ribosomes and mitochondria are rare.

All cell types of the distal cell layer are high-prismatic. They are isolated from the basal membrane by the basal cells. Only small extensions (Ø 2 μm) of the interstitial cells extend between the basal cells up to the basal membrane.

The columnar cells are elongate pear-shaped. From a diameter of 10 μm at their base, the cells taper to 4 μm in the middle region and remain constant in diameter up to the epithelium surface (Figure 6A). The basally located nucleus (globular to oval, Ø 5–8 μm) often contains up to 6 large nucleoli (Ø 1 μm). Around the nucleus, extensive rough endoplasmic reticulum (rER) is present, interspersed with mitochondria. Distal to the nucleus the rER releases transition vesicles, which migrate to the cis-side of the dictyosomes. On the trans-side of the Golgi cisternae, secretion-filled vesicles are released, which fuse to larger granules.

Next to the surface the columnar cells are connected to the neighbouring interstitial cells via zonulae adhaerentes (Figure 6B). Along the lateral periphery of the columnar cells are several strands of filaments. They are anchored laterally in the zonula adhaerens near the apical cell pole and are restricted to the upper third of the cell. The remaining cytoplasm is loosely filled with globular, membrane-bound

Table 1. Overview of the main characteristics of the cell types in the adhesive organ of *Idiosepius*.

	Basal	Interstitial	Fusiform	Columnar	Granular	Goblet	Saccular
Shape	Undetermined	High prismatic	Pear-shaped	Streched pear-shaped	Cylindrical	Cylindrical	Sack to balloon
Main characteristic	Vesicles with electron-dense granules	Microvilli, filaments, Golgi network	Cilia, dictyosomes numerous free ribosomes	Granules (1 μm)	Granules (2–5 μm)	Fine grained material	Without specific content
Location of nucleus	Undetermined	Central	Basal	Basal	Basal	Basal	Basal
Nucleoli	–	Several	Several	Several	Several	Several	Several
Endoplasmic reticulum	Marginal	Marginal	Marginal	Abundant rough endoplasmic reticulum (rER) next to nucleus	Abundant rER next to nucleus	Sparse rER next to nucleus	Marginal
Golgi apparatus	Marginal	Come dictyosomes and dense Golgi network	Some dictyosomes	Abundant next to nucleus	Abundant next to nucleus	Marginal	Marginal
Microvilli	–	✓	–	–	–	–	–
Secretory product	–	Glycokalyx	–	Granules	Granules	Fine grained material	–
Secretion type	–	Eccrin	–	Apocrine	Apocrine	Holocrine	–
Filaments	Cytoskeleton	Non-directional cytoskeleton and strands of actin filaments	Cytoskeleton	Filament strands in the periphery	–	–	Filament strands in the periphery
Distribution in the adhesive organ	Compose the basal cell layer	Form the basic scaffold	Always associated with the columnar cells	Regular	Regular	Quantity decrease in the middle (in cross-section)	Irregular
Presence in the normal mantle epithelium	✓	✓	–	–	–	✓	✓

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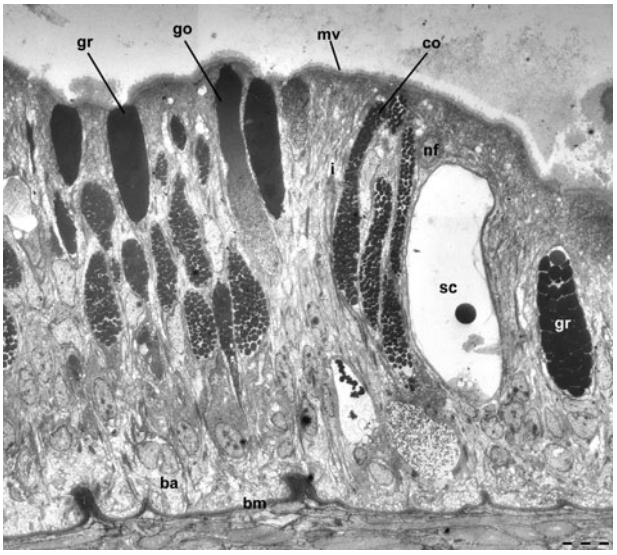

Fig. 3. Cross-section showing the cell types present in the adhesive organ of *Idiosepius*. Scale bar: 10 μm.

secretion granules (Ø 1 μm). Frequently, membrane residues are visible between the granules.

The granular cells are cylindrical, about 10 μm in diameter (Figure 6C). As in the columnar cells the nucleus (globular to oval, Ø 5–8 μm) with 3 to 6 nucleoli is located basally and surrounded by several rER-layers. The synthesis of the secretory material is similar to that found in columnar cells but the granules are larger (2–5 μm) (Figure 6D). In the loose stage they appear globular but when tightly packed they convert to a polygonal shape. Frequently, granules fuse partly and incorporate membrane residues.

The present study shows that in the columnar as well as in the granular cells only parts of the entire cell content are released during secretion. In contact with the outer medium the released granules fuse immediately to a uniform mass (Figure 6E).

Goblet cells are cylindrical or barrel-shaped with a diameter of 10–20 μm. The oval to sickle-shaped nucleus (about 6 × 3 μm) is likewise located basally. It contains up to 3 nucleoli, which are smaller than in the other glandular cells. Goblet cells are filled with homogeneous fine granular material (grain size 20–50 nm). The secretory material is either densely packed and evenly distributed or shows low density with a gradient increasing toward the apical pole of the cell. In cells with mostly loose material the rER is adjacent to the nucleus, synthesizing secretory material (Figure 7A, B).

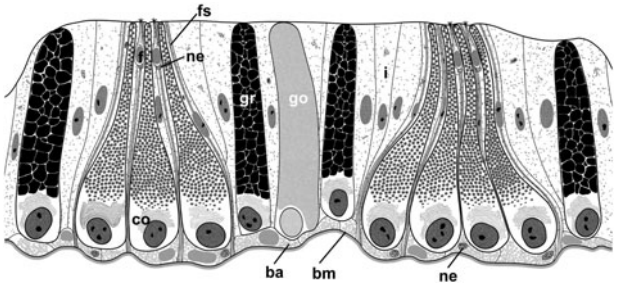

Fig. 4. Schematic drawing of the cell arrangement in the adhesive organ (adapted from Cyran *et al.*, 2005 and re-published with permission).

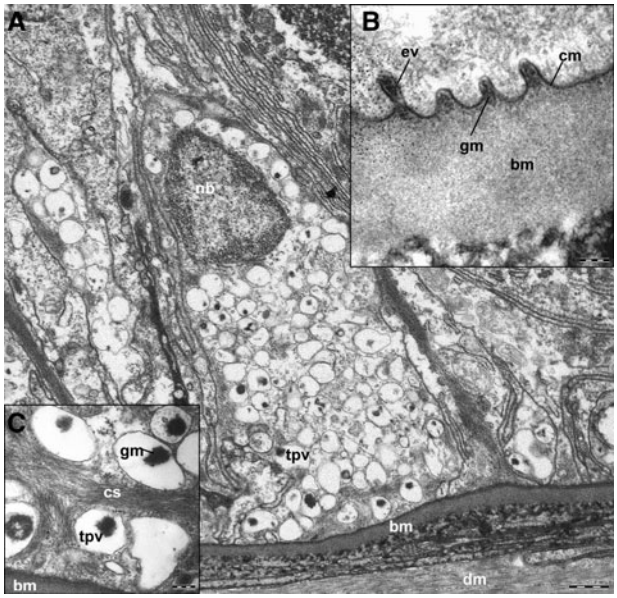

Fig. 5. Basal cell of *Idiosepius*: (A) the basal cells mainly possess transport vesicles with particles of electron-dense material; (B) endocytosis vesicles budding from the membrane of the cell base incorporating electron-dense granules; (C) cytoskeleton elements are predominantly located in the cell base. Scale bars: (A) 1 μm; (B) 100 nm; (C) 200 nm.

With increasing density the synthesis activity decreases. In uniform, densely packed goblet cells, synthesis can no longer be observed (Figure 7C, D). In contrast to the columnar and granular cells, the goblet cells release their secretory contents completely (Figure 7E).

Between the glandular cells are interstitial cells, characterized by densely arranged microvilli (0.5–1 μm in length) (Figure 8A). A glycocalyx covers the surface of the microvilli. It forms an electron-dense layer at the half length of the microvilli. The nucleus in the middle of the cell is oblong (10 × 4 μm) and contains 2 to 4 nucleoli. This cell type lacks granular secretory material. The cytoplasm contains numerous dictyosomes and a distinct trans-Golgi network with increasing abundance of cisternae towards the cell surface. Loosely distributed non-directional filaments traverse the cells.

Interstitial cells neighboured to columnar cells show some modifications: they have a significant smaller cell volume. Cell processes (Ø 2 μm) extend between the basal cells up to the basal membrane. Mitochondria and filaments are more numerous and the density of the trans-Golgi network is lower. The actin filaments, anchored in the microvilli, are not terminated in the terminal web as in the regular interstitial cells but continue inside the cell lumen (Figure 8C, D). Furthermore, the cell extensions contain filament strands, anchored in the basal cell pole and traversing the cells longitudinally (Figure 8B). The microvilli of these cells are increased in length (1.5 μm), forming a microvillous collar, which surrounds the columnar cells (Figure 8E).

The saccular cells differ morphologically from the other epithelium cells by a mostly empty central balloon-like cavity, enclosed by a membrane (Figure 9). The cavity has no specific content, but contains in several cases material from the outside medium such as granules (Figures 3 & 9) or suspended secretory components from the neighbouring cells or even protozoa. These protozoa are also found in the

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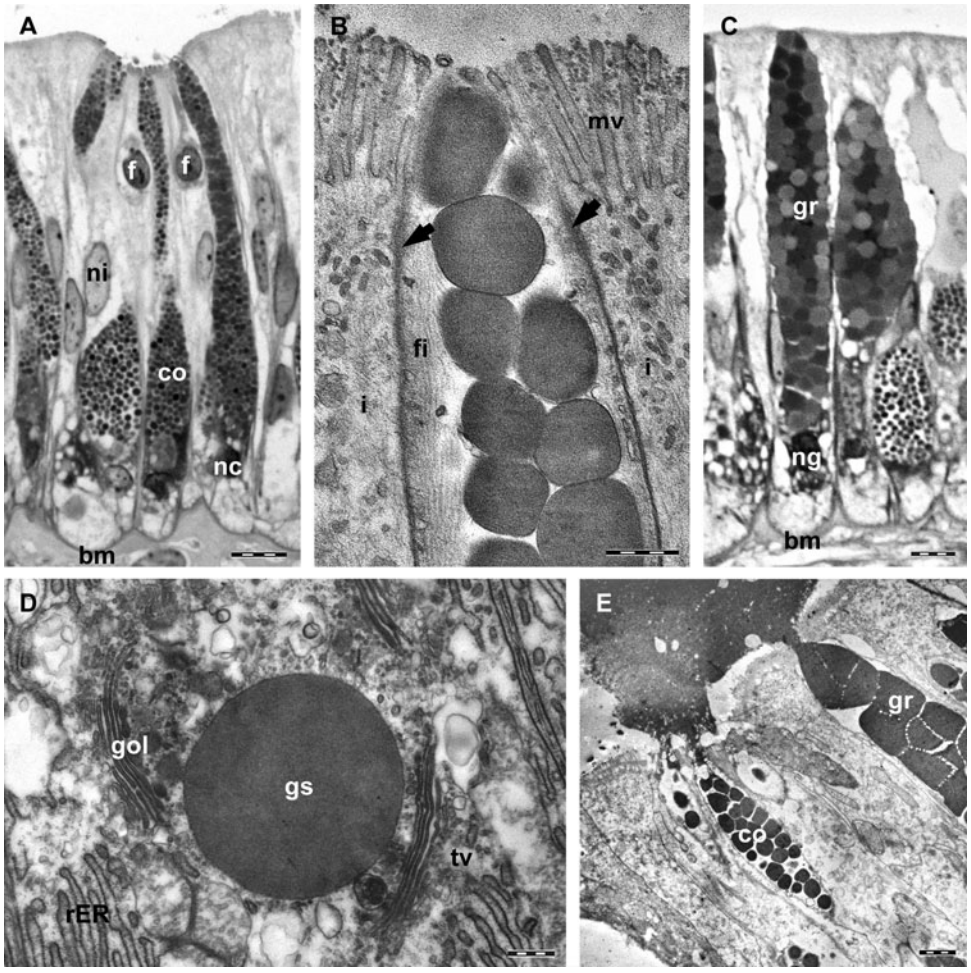

Fig. 6. Columnar and granular cell: (A) columnar cells in cross-section. Several cells form aggregations with 2 fusiform cells; (B) the actin filaments in the boundary layer of the columnar cells are anchored in zonulae adhaerentes (arrows); (C) granular cells in cross-section; (D) shows the high activity of secretory material synthesis in the granular cells; (E) the secretory process of columnar cells belonging to an aggregation always occurs simultaneously. In this section the material amalgamates with the secretion of the granular cells. Scale bars: (A & C) 10 μm; (B & D) 500 nm; (E) 2 μm.

dermal muscle cells but never in the other epithelial cells. The saccular cells mostly extend from the epithelium surface just to the half or 2/3 height of the epithelium, infrequently contacting the basal cells. The narrow (0.5 μm) cytoplasm between the cavity and the cell membrane is tightly filled with filaments (Figure 9B), some mitochondria, little rER and a narrow sickle-shaped nucleus. The filaments form continuous strands, lining the whole cell even below the nucleus.

The pear-shaped fusiform cells are about 15 μm long and always in the surface layer of the epithelium (Figure 10A). The thicker, proximal part is almost completely filled by an ovate nucleus containing several nucleoli. The cytoplasm contains many vesicles, dictyosomes, lysosomes, mitochondria and some cytoskeletal filaments (Figure 10B). In the nucleus region there is little rER but abundant free ribosomes (Figure 10E). One or more cilia (250 nm in cross-section and 2 μm long) (Figure 10C, D) are positioned on the apical pole of the cells; their centrioles are 1 μm below the cell surface. The cilia arrangement seems to be random. In some cases the distal end of the cilia appears to be widened. No ciliary root could be detected.

Bundled nerve processes are observable between the basal cells, and single neurons are joined to each fusiform cell. The basally located nerve processes, consisting of 5 to 10 dendrites, have synaptic contact to other neurons in the bundle and to compartments of epithelial cells (Figure 11).

Distribution of the cell types

The 3D-reconstruction provides an overview of the cell morphology and distribution. Columnar cells always occur in aggregates of 5–15 cells (Figures 6A & 12). Between them are narrow interstitial cells, forming a hump with their long microvilli (Figure 1C & 8E). One or more fusiform cells are positioned at the periphery of the aggregates (Figure 6A). These aggregates are regularly distributed in the AO. The granular cells are regularly distributed as well. Goblet cells are dominant at the periphery of the AO and less abundant in the middle part. The saccular cells are irregularly distributed.

Characterization of the non-adhesive mantle epithelium

The ventral mantle epithelium is composed of basal, interstitial, saccular and two secretory cell types (Figure 13). The

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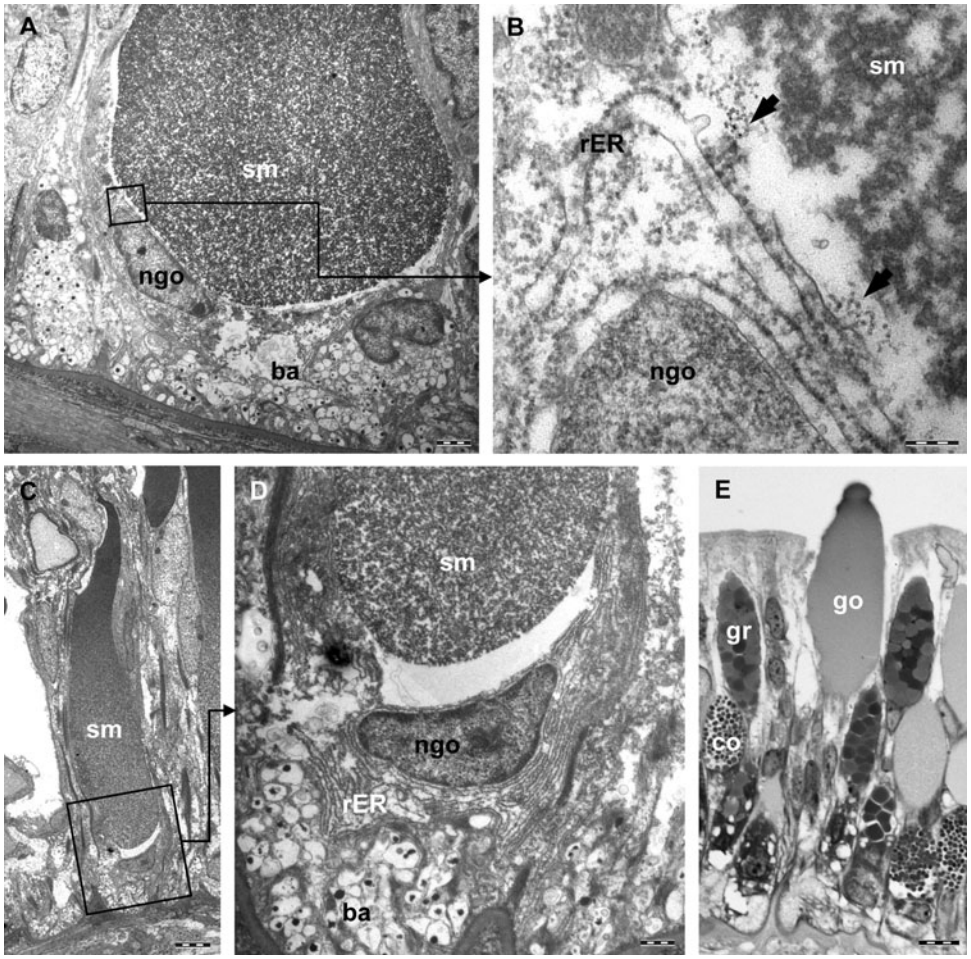

Fig. 7. Goblet cells: (A) shows a state with active secretion synthesis. The material has a uniform lower density; (B) secretory vesicles (arrows) are separated from the rER; (C) an intermediate state with beginning compression of the secretory material from the apical cell pole, resulting in a density gradient between basal and apical pole; (D) the secretory material lifts up from the nucleus. No synthesis activity is evident; (E) shows the secretion of a goblet cell. Scale bars: (A) 2 μm; (B) 200 nm; (C) 5 μm; (D) 1 μm; (E)10 μm.

interstitial, basal and saccular cells correspond structurally and in their arrangement to those in the AO. Both secretory cells are high prismatic, about 20 μm in diameter and contain a basally located, roundish–oval nucleus with several nucleoli. In the absence of a terminology they are called Type I and Type II.

Type I contains spherical to oval granules (Ø 5 μm) of dense material. In contrast to the secretory material of the granular and columnar cells, these granules are not membrane bound and frequently form a uniform mass. Type II contains fine-granular material often with increasing density toward the apical pole. The morphology of this cell type resembles the goblet cells.

Neither columnar, nor granular nor fusiform cells were detected in the non-adhesive mantle epithelium but, as in the AO, the synaptic areas of neurons lie between the basal cells.

DISCUSSION

Previous examinations about the AO of Idiosepiidae described its morphology (Sasaki, 1921) and the chemical composition of the glandular cells (von Byern *et al.*, 2008) but left a gap concerning the cell type classification. The current study aims to elucidate this question and moreover determine the distribution and the fine structure of the cells. The results help to draw conclusions and to compare the adhesive system of *Idiosepius* with those in other glue-producing animals.

Cell types: terminology and relations to other cephalopods' adhesive systems

The terminology proposed by Sasaki (1921) largely corresponds with the results of the present study, but differs in some details (Table 2).

The 'decayed columnar cells' (Sasaki, 1921) more closely resemble the interstitial cells found in *Euprymna* (Singley, 1982) than the columnar cells because: (1) the apical cell poles are part of the epithelial surface; (2) the nucleoli are located centrally as in the interstitial cells of *Euprymna* instead of basally as in the columnar cells; and (3) contrary to the examined columnar cells, which are never completely decayed, granular material is completely absent in these cells. Accordingly the proposed decayed columnar cells are not columnar cells without granules but interstitial cells as

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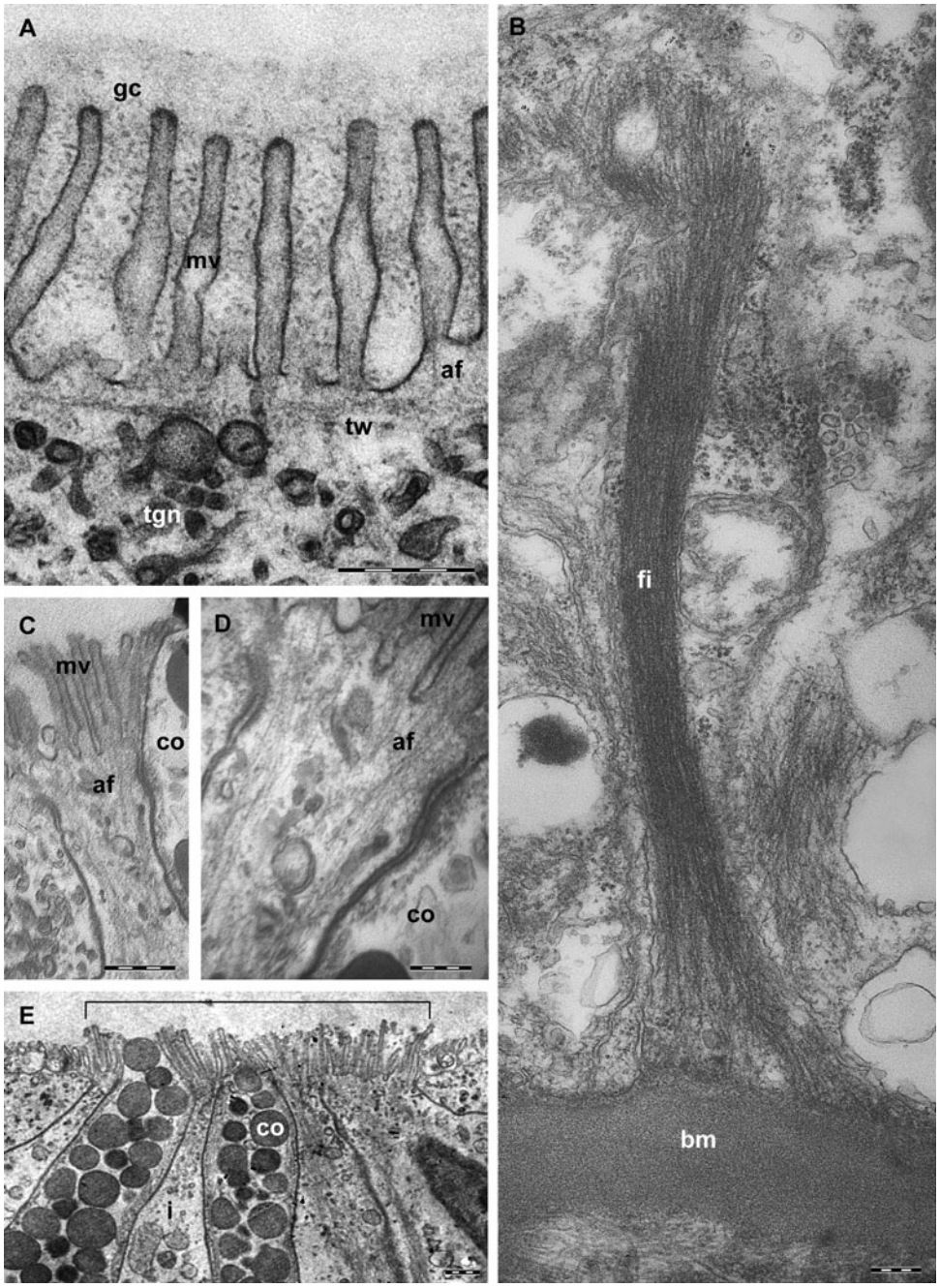

Fig. 8. Interstitial cells: (A) the surface is characterized by microvilli with glycocalyx in between; (B) interstitial cells between columnar cells are traversed by filament strands in longitudinal direction which are anchored basally; (C) the actin filaments, anchored in the microvilli, are not terminated in the terminal web (as in A); (D) instead they extend into the cell lumen; (E) the longer microvilli (marked area) indicate the columnar cells aggregation. Scale bars: (A & C) 500 nm; (B & D) 200 nm; (E) 1 μm.

in *Euprymna*. The present study points to a further cell type (saccular cell) which was not described by Sasaki (1921).

The presence of chemical adhesives in different cephalopod genera raises questions about a common origin of structures and mechanisms (Table 2). Contrary to *Idiosepius*, in *Euprymna* only two glandular cell types are present in the adhesive area (Singley, 1982). The goblet cells of *Euprymna* resemble the columnar cells of *Idiosepius* in size, alignment of granules and location of the nucleus. The goblet cells of *Euprymna*, however, are solitary cells, contrary to the columnar cell aggregations in *Idiosepius*. Whereas the shape and the nucleus of the ovate cells in *Euprymna* are similar to the saccular cells in *Idiosepius*, their content strongly matches the fine-grained substance in *Idiosepius*' goblet cells. The interstitial cells of both genera are alike. They contain strands of filaments, a central nucleus, no granular material and they are covered with microvilli.

In *Nautilus* the situation is quite different because the various cell types are separated into different areas on the digital tentacles. One glandular cell type (columnar epithelium cells) (Kier, 1987; Muntz & Wentworth, 1995) has granules equivalent to the granular cells in *Idiosepius*. The other cell type, named as mucus (Muntz & Wentworth, 1995), granular (Fukuda, 1988) or goblet cells (Kier, 1987) on the thin

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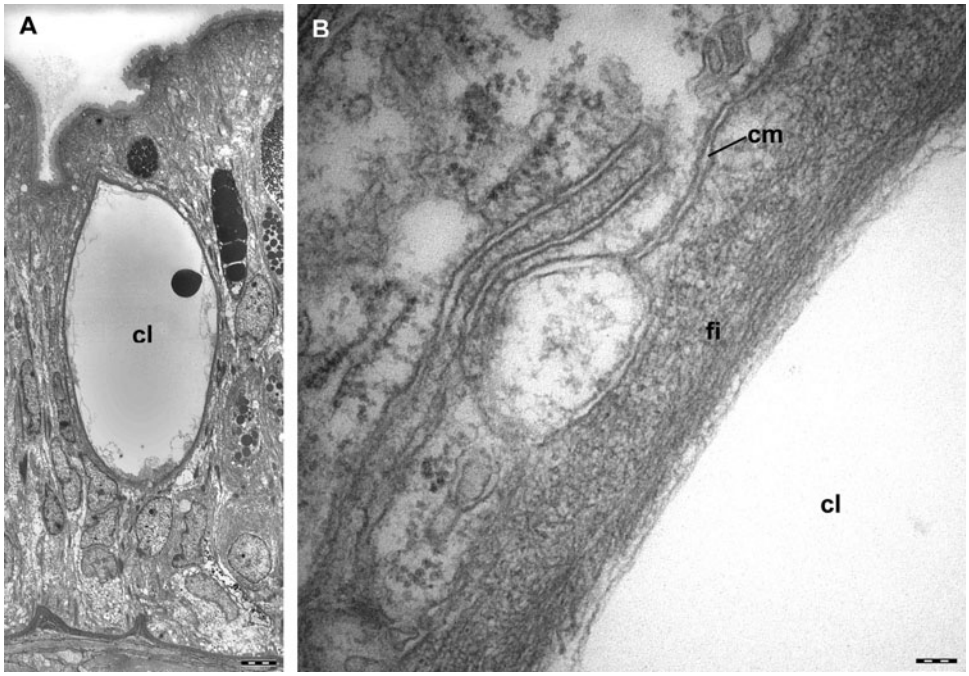

Fig. 9. Saccular cell: (A) section of a whole cell containing a single granulum inside the cell lumen; (B) the boundary cytoplasmic area is dominated by parallel filaments. Scale bars: (A) 5 μm; (B) 100 nm.

epithelium in the *Nautilus* tentacle consists of large granules (J. von Byern, unpublished results). *Nautilus* has ciliated cells with cilia like those in *Idiosepius*, but these cells are separated from the secretory cells and, furthermore, are also arranged in non-adhesive areas (Ruth *et al.*, 2002). Their direct connection with the adhesive mechanism is therefore doubtful.

As pointed out by von Byern *et al.* (2008), there is some confusion in the cell terminology, which complicates the comparison between several genera.

Some concordances in characters of the glandular cell types exist, but these do not allow a degree of relationship to be deduced. A common origin from an originally unspecific epidermis cell seems plausible only for the interstitial cells in *Euprymna* and *Idiosepius*.

The previous reports do not suggest a homology between the adhesive structures, particularly because the respective cells belong to different body parts.

Character of the adhesive organ

A predominantly mechanical adhesion or release can be excluded for *Idiosepius*. The lack of mantle musculature in the dorsal mantle area prevents contraction and thus participation in bonding and/or release from the substrate. Nevertheless, an influence of dermal musculature on detachment is conceivable, as suggested for *Euprymna* (Singley, 1982). The furrows in the epithelium may enhance the strength of attachment by increasing the ability for deformation and generating a sucker like effect (Gay & Leibler, 1999; Gay, 2002). Furthermore, the lack of mantle musculature in the middle dorsal mantle allows the animal to flatten the mantle to increase the contact area, as is obvious in Figure 1B.

While basal, interstitial, goblet and saccular cells occur also in the non-adhesive epithelium, the columnar, granular and fusiform cells are restricted to the AO of *Idiosepius*. This implies their involvement in adhesion.

The following attributes of the glandular cells support this hypothesis: both the columnar and granular cells are distributed regularly in the AO, which is not given for the goblet cells; and columnar and granular cells secrete only part of their contents as also shown by von Byern *et al.* (2008). This makes sense, because the animals can attach and detach several times per minute (Suwanmala *et al.*, 2006) and need enough glue for these bonding processes. An 'all or nothing secretion release' would require a certain time for the animal to re-synthesize and refill the cell, and provide no possibility to hide during this time under a seagrass leaf.

The cilia in the fusiform cells and the adjacent nerve process indicate a function as receptor cells. Ciliated cells comparable to the fusiform cells were also found in *Nautilus* (Muntz & Wentworth, 1995; Ruth *et al.*, 2002), whereby their association with the adhesive mechanism is not clear.

Although the basal and interstitial cells are not explicit parts of the AO, they support the functionality of the secretory cells: the migrating vesicles and the poor presence of ER, Golgi, ribosomes or nucleoli in the basal cells suggest a transport function for nutrients and other components.

In the interstitial cells adjacent to the columnar cells, the filaments ranging from the microvilli into the cell lumen without connection to the terminal web are apparently actin filaments whereas the filament strands, which are anchored at the cell base, may be actin or intermediary filaments. It is not clear so far, whether these filaments are connected in the middle region of the cells, but probably they form continuous strands. They may act as tonofilaments, bearing the tension of adhesion as indicated for the AO of Turbellaria (Tyler, 1988). Although evidence of a motor-protein is still missing, it is also conceivable, that they have a contractile function and support the secretory process by

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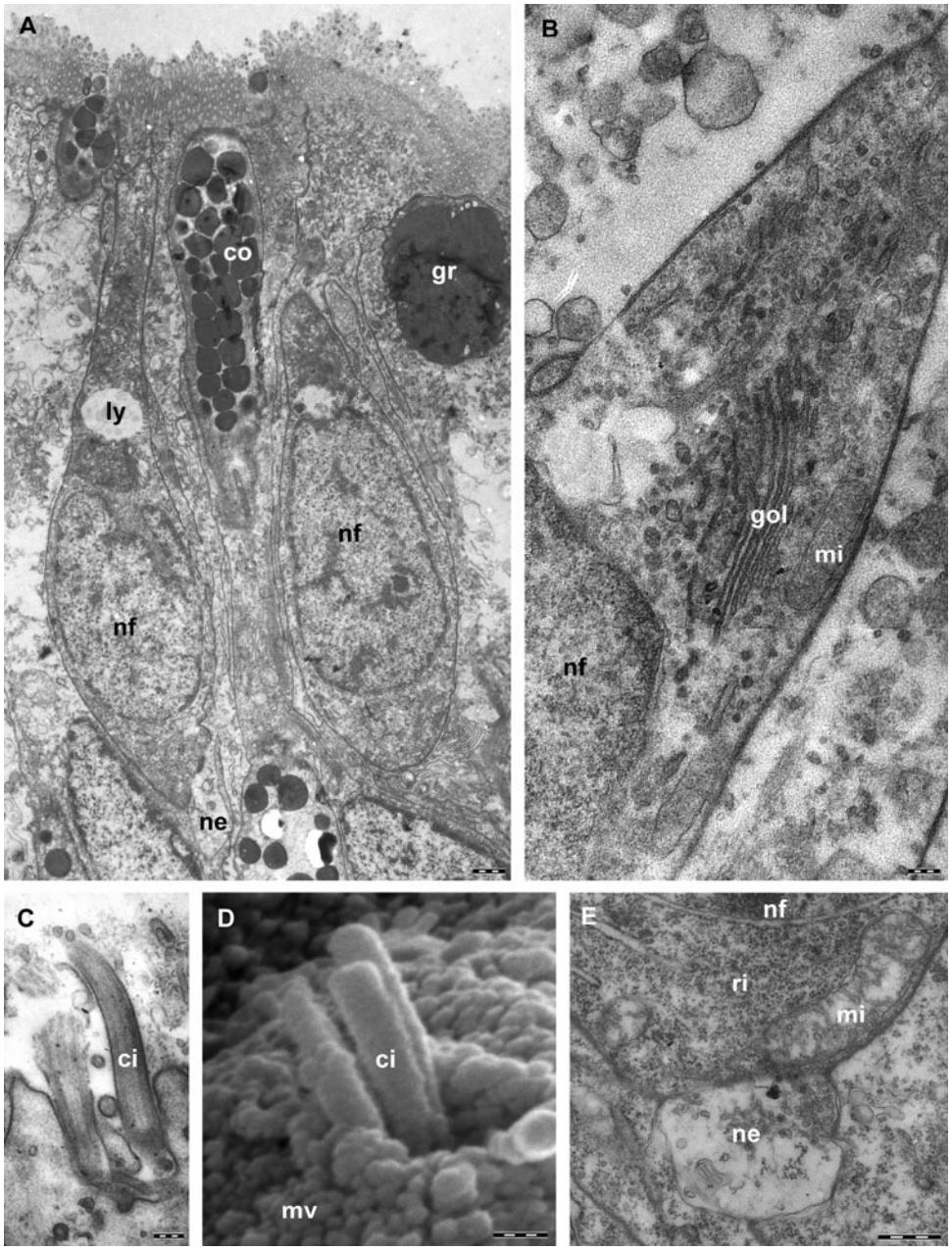

Fig. 10. Fusiform cells: (A) two fusiform cells, located near the tips of columnar cells; (B) the cytoplasm above the nucleus contains distinct Golgi apparatus, vesicles and a lysosome near the nucleus; (C) the fusiform cells bear one or more cilia; (D) scanning electron microscopy image of the cilia; (E) a nerve process is associated with the basal cell pole. Scale bars: (A) 1 μm; (B & C) 200 nm; (D & E) 500 nm.

exerting pressure on the apices of the columnar cells as supposed for the adhesive tissue of *Euprymna scolopes* (Singley, 1982).

The long microvilli on the interstitial cells form a collar around the neck of the adjacent columnar cells. The so-formed adhesive papilla is similar to the adhesive hump found in *Macrostomida* (Tyler, 1976).

As for the goblet cells also the saccular cells are distributed frequently in the normal epithelium and seem to be not involved in the adhesion process. The lack of specific contents in the cell lumen and inclusions from the outer medium was likewise observed by histological analyses (von Byern *et al.*, 2008) and provide some questions about whether the cells in principle produce secretory material or not.

Based on the present data, we could not completely exclude a possible glandular function. Secretory material such as mucus, fright- or defence-material might have been released during capture, cultivation or dissolved during fixation and affect an intake of the outer medium. However, further analyses, e.g. with cryo-fixed samples, are necessary and planned to provide new light on this question and exclude a 'human error'.

The presence of synapses in the base of the epithelium suggests a neuronal control of secretion. It remains unclear where the nerve processes traverse the basal membrane. Apparently, they are joined together to a strand and pass the basal membrane only at few sites. Synaptic contacts between neurons suggest a direct neural connection of epithelial components.

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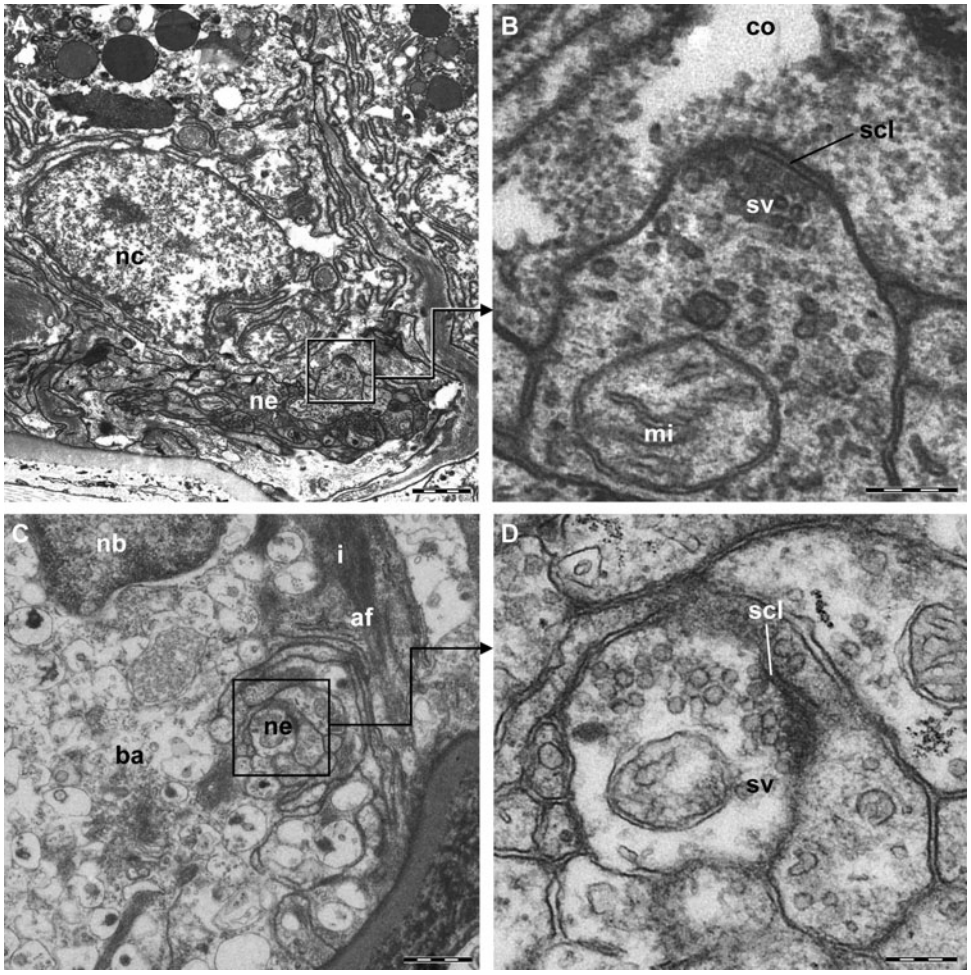

Fig. 11. Innervation of the adhesive organ: (A) nerve process adjoining a columnar cell; (B) detail with a synapse to a columnar cell; (C) nerve process next to an interstitial cell; (D) detail with a neuron to neuron synapse. Scale bars: (A) 1 μm; (B & D) 200 nm; (C) 1 μm.

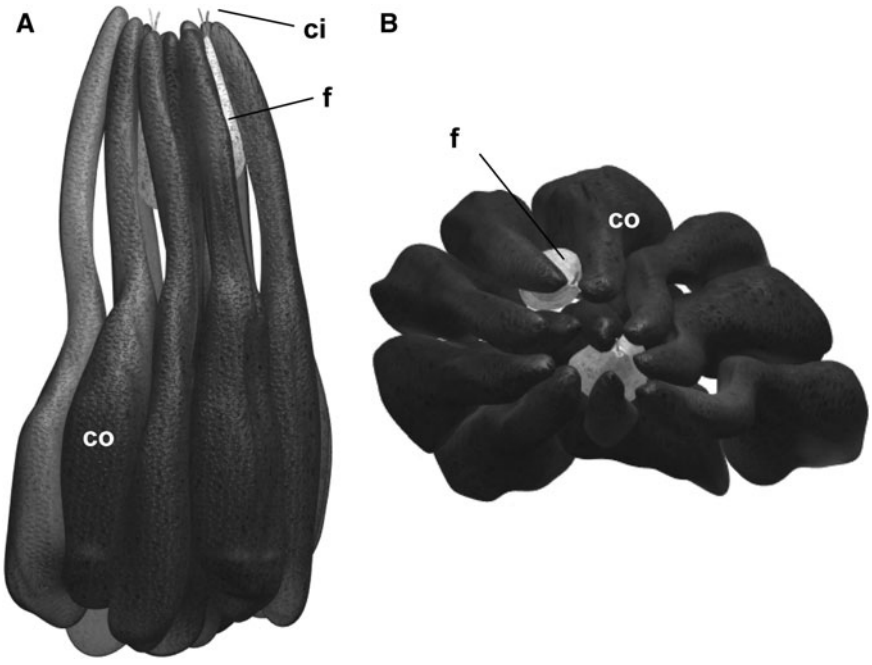

Fig. 12. A 3D-reconstruction of an aggregation of columnar cells and fusiform cells.

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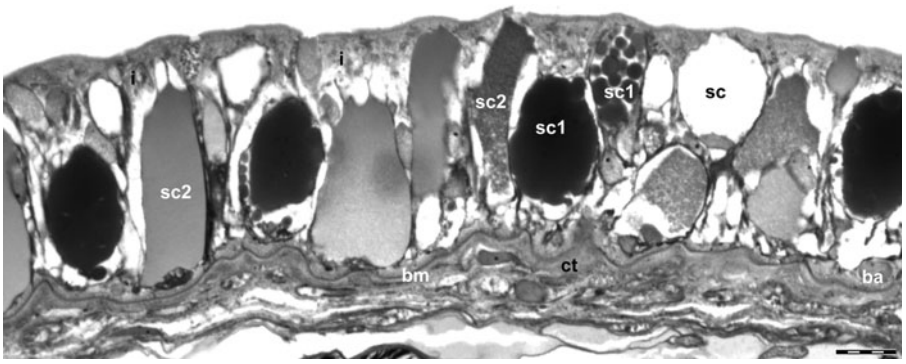

Fig. 13. The non-adhesive mantle epithelium lacks columnar and granular cells but contains two other secretory cells. Scale bar: 20 μm.

Table 2. Juxtaposition of the original cell terminology given by Sasaki (1921) and von Byern *et al.* (2008) to the present suggestion and reference to agreements in the adhesive organs of *Euprymna* (Singley, 1982) and *Nautilus* (Fukuda, 1980; Kier, 1987; Muntz & Wentworth, 1995). The terms in parentheses refer to the resembling structures.

***Idiosepius* spp.**			***Euprymna* sp.**	***Nautilus* spp.**
Present study	**Sasaki (1921)**	**von Byern *et al.* (2008)**	**Singley (1982)**	**Muntz and Wentworth (1995)**
Basal cells	Basal	Basal	Uncertain	Uncertain
Columnar cells	Columnar	Columnar	Goblet (size and shape of secretory granules, location of nucleus)	–
Granular cells	Granular	Granular	–	Columnar epithelium cells (size and shape of secretory granules)
Goblet cells	Goblet	Goblet	Ovate (fine granular material)	–
Saccular cells	–	Goblet	Ovate (shape, location of nucleus)	–
Interstitial cells	Decayed columnar	Interstitial	Interstitial (part of surface, microvilli, actin filament strands, location of nucleus, lack of granular secretory material)	–
Fusiform cells	Fusiform	–	–	Ciliated (cilia, shape, size)

Composition of the secetory products

The configuration of rER and dictyosomes in the columnar and granular cells indicates synthesis and packaging of proteins and modifying with carbohydrates. In goblet cells the proteins, produced by rER are apparently not modified by the Golgi apparatus. This is evidence for a lower level of carbohydrates in the secretory material. These findings correlate with the histochemical results (von Byern *et al.*, 2008), confirming different potein:sugar ratios (high carbohydrate rates in columnar and granular cells but a low rate in goblet cells).

Duo-gland or two components glue?

The results suggest involvement of two different secretions, assembled by columnar cells and granular cells.

Two hypotheses are possible:

(1) the cells work antagonistic as a duo-gland system, whereas one cell type is responsible for the adhesion and the other cell type produces secretory substances (mostly strong acidic) for release (Hermans, 1983). Such a system is also proposed for *Euprymna* (Singley, 1982) as well as other glue-producing animals, e.g. *Gastrotrichs* (Tyler & Rieger, 1980), *Turbellaria* (Tyler, 1976) or *Echinodermata* (Flammang, 2006); or

(2) on the other hand a two component system is conceivable, in which both glandular cells produce commonly the glue. This process of bonding could be found in *Euprymna*, *Sepia* (J. von Byern, unpublished data) as well as gastropods (Smith, 2006).

So far, we could not say definitely, which type of bonding is given for *Idiosepius*. Occasionally observed simultaneous secretory process of columnar and granular cells much speaks for a two components system. The lack of acidic components (histochemical data by von Byern *et al.*, 2008), which effect a release of the glue in duo-gland systems, confirms this observation. Biochemical analyses are necessary to solve this question finally and provide more knowledge about adhesive mechanism in cephalopods.

Finally for highly agile animals with rapid response behaviour a release from the substrate affected by body movement seems more effective and generates less expense.

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