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The fine structure of epidermal lines on arms and head of postembryonic *Sepia officinalis* and *Loligo vulgaris* (Mollusca, Cephalopoda)

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Summary. The structure and cell types of the epidermal lines that are found on the surface of the head and arms of *Sepia officinalis* and *Loligo vulgaris*, were investigated by light- and transmission electron microscopy. The lines consist of ciliated cells and non-ciliated, accessory cells. The axon of the ciliated cell and the ultrastructure of the latter indicate that this element is sensory; therefore, the epidermal lines of cephalopods may be regarded as a type of sensory organ.

Key words. Epidermal lines – Ciliated cells – *Sepia officinalis* – *Loligo vulgaris* – Cephalopoda

In the epidermis of embryos of different cephalopod species four to five pairs of fine, longitudinally directed lines appear in the late stages of development. These lines are bilaterally symmetrical and run from the exterior arm surface or from the base of the arm over the head; they persist after hatching.

Naef (1928) described these lines for embryos of the following deca- and octopod species: *Loligo vulgaris*, a nondetermined Oegopsid, *Sepia officinalis, Sepiola ligulata, Sepietta owenia*, and *Octopus vulgaris*. He considered them as lines of glandular cells (= *Drüsenlinien*). A light-microscopic study (Sundermann-Meister 1978) showed that these lines contain ciliated cells. This observation was confirmed by Arnold and Williams-Arnold (1980), who found in a scanning-microscopical study corresponding lines of single-filed ciliated cells running along the head and ventral arms of *Loligo pealei*. The present study describes the structure of the epidermal lines and the ultrastructure of the corresponding ciliated cells.

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Materials and methods

Specimens of late embryonic stages, postembryonic stages and juveniles (about two weeks after hatching) of *Sepia officinalis* as well as late embryonic and postembryonic stages of *Loligo vulgaris* were investigated. The animals of both species originated from the western Mediterranean sea (Banyuls-sur-mer, France). Tissue was fixed in 2% OsO_4 dissolved in 0.5% $K_2Cr_2O_7$ in 70% seawater, pH 7.2–7.4, embedded in Durcupan (Fluka) and cut on an LKB-Ultrotome III. The ultrathin sections were stained with 1% lead citrate and examined with a Siemens Elmiskop 101 electron microscope.

The spatial orientation follows the swimming position of the adult (= physiological orientation): arms = rostral or in front; mantle top = caudal or behind; funnel = ventral or below.

Results

The following observations pertain in general to postembryonic animals; it is noted specifically when they refer to late embryonic stages.

In the skin of dead animals, which are in a good state of preservation, the epidermal lines are visible on arms and head (Figs. 1, 6). Two pairs of lines are developed in both species on the dorsal surface and one pair on the dorsolateral surface of the head. One pair is formed on the ventrolateral head in *Sepia* and *Loligo*, and an additional pair on the ventral head only in *Loligo*.

The middle dorsal pair L1 leads rectilinearly from the dorsal arms toward the mantle edge. The second dorsal pair L2 starts at the base of the dorsolateral arms and is situated between L1 and L3. The latter, L3, originates in the epidermis of the ventrolateral arms and courses to the upper margin of the eye, behind which it leads straight to the back. The ventrolateral pair L4 begins at the base of the ventrolateral arms and runs beneath the eye up to its caudal edge. In *Loligo* the pair L5 runs from the lower half of the ventral arms for a short distance backward in the head epidermis. These are the shortest lines in *Loligo*.

Light microscopy

Sepia officinalis. The skin of the late embryonic stages and even of the postembryonic animals is composed, essentially, of large goblet cells. In this epidermis the cells of the lines are conspicuous due to their dense cytoplasm (Figs. 2–4). The lines do not form a continuous row of ciliated cells and accessory cells; single-standing ciliated cells surrounded by accessory cells alternate with groups of ciliated cells and regions without any of these cell types. Accessory cells are more numerous than ciliated cells.

The accessory cell is a slender elongated element with a relatively dense cytoplasm. The free surface bears a border of microvilli. The small ellipsoid nucleus stains darker than that of the ciliated cell. Its position depends on the spatial conditions of the epidermis, but usually the nuclei are seen above the nuclear region of the ciliated cell.

The ciliated cells of the lines (Figs. 3–5) differ from ciliated cells in other regions of the postembryonic epidermis. They are very voluminous (height ~ 30 µm, width ~ 15 µm) with a large-sized axon (diameter up to

10 μm), which originates at the base of the cell and extends into the connective tissue. The free surface is slightly bent outward and bordered with microvilli and cilia. The nucleus is always round and centrally positioned; its diameter is about 10 μm. The basal cytoplasm shows a conspicuous pattern of light and dark areas, which continues into the axoplasm. In the arms the axons of several ciliated cells converge in the connective tissue and run together into the axial nerve cord. The site of entry into the central musculature, which surrounds the axial nerve cord, is close to the para-axial nerve cord. Due to technical factors it was not possible to follow the course of the axons in the head.

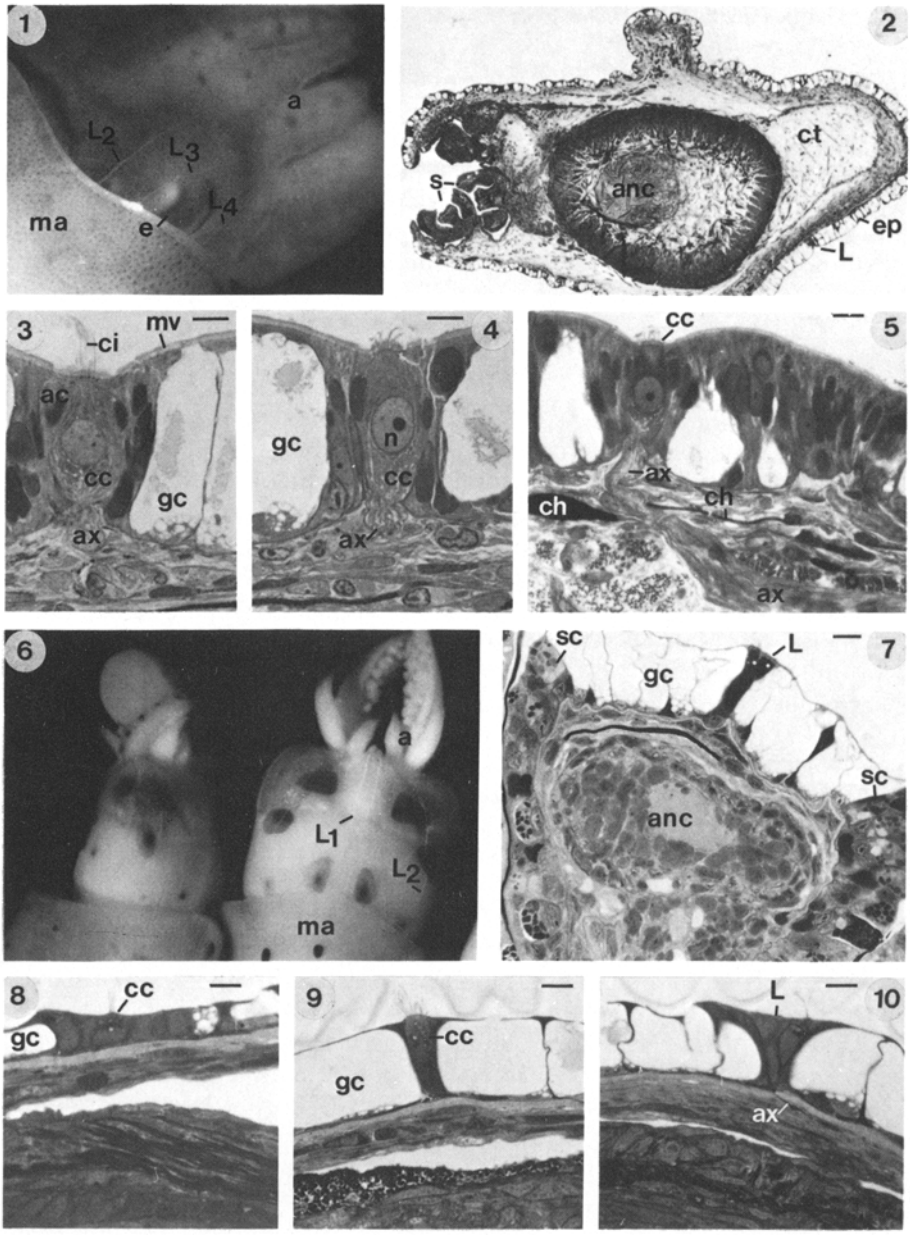
Loligo vulgaris. The comparison of postembryonic stages between *Loligo* and *Sepia* shows some differences in the structure of the epidermal lines. In *Loligo* the cells that build up the lines (as well as the other epidermal cells) are smaller, with an average height of about 15–20 μm and a diameter of about 5–7 μm. The lines contain a smaller number of accessory cells (Figs. 7, 9). The accessory cells are slender and possess a dark-staining cytoplasm and nucleus.

Ciliated cells exist in two slightly different types. The first type is a flask-shaped cell with a small depressed surface, which bears few long cilia (Fig. 8). The second type resembles more closely the ciliated cell in *Sepia* (Fig. 9). However, neither type shows the "mottled" appearance of the basal cytoplasm as seen in *Sepia*. The axons are much smaller and difficult to detect (Fig. 10).

Electron microscopy

Sepia officinalis. The cytoplasm of the accessory cells resembles that of the common epidermal cell in the adult animal. The apical end of this type of cell bears a microvillous border. The microvilli are invested by a surface coat consisting of fine filamentous material. The Golgi apparatus and numerous small membrane-bounded vesicles are visible in the distal cytoplasm. The rough endoplasmic reticulum is mainly developed in the basal perinuclear region. Longitudinally arranged fibrous bundles are often seen in the basal portion of the cell. Mitochondria of the tubular type are dispersed throughout the entire cytoplasm. Neighboring cells display deep interdigitations.

The ciliated cell has an extended contact area with the basal lamina (Fig. 11). On the free surface the cilia are located between microvilli; they show the typical 9 + 2 pattern. The ciliary base projects about 0.5 μm above the cell surface; the ciliary rootlets are slender, short and bifurcated (Fig. 13). Abundant granules of ribosomal size give a moderate density to the cytoplasm. The entire cytoplasm is rich in mitochondria. In the peri- and supranuclear region, vesicles with diameters up to 1 μm are found. Their content is heterogeneous, partly electron dense. Many small vesicles with average diameters of 70–1000 nm are also present in the distal cytoplasm. The inhomogeneous appearance of the basal cytoplasm is due to



Figs. 1–5. *Sepia officinalis*

Fig. 1. Head of a juvenile animal, dorsolateral view. The epidermal lines *L2–L4* are seen as thin white structures; *a* arms; *e* eye; *ma* mantle

Fig. 2. Cross section through the arm of a juvenile animal, showing the line (*L*) in the epidermis (*ep*), which is mainly made up of goblet cells; *anc* axial nerve cord; *ct* connective tissue; *s* sucker. Durcupan-embedded material

Figs. 3, 4. Sections through the line of the arm. The axons (*ax*) leaving the ciliated cells (*cc*) are shown in cross section; *ac* accessory cell; *ci* cilia; *gc* goblet cell; *mv* microvilli; *n* nucleus

the alternation of electron-dense regions with electron-translucent ones (Figs. 11, 12, 14). The dense regions display endoplasmic reticulum, numerous granules of ribosomal size, small vesicles with diameters somewhat larger than that of the granules and many small mitochondria. These mitochondria differ from those that have hitherto been described in *Sepia* as smaller and more electron-dense elements. The electron-translucent regions of the cell do not display organelles but only filamentous material embedded in a light matrix.

The heterogeneous electron density of the cytoplasm may be an artefact due to fixation, but neither the neighboring cells nor the other types of ciliated cell in the epidermis show this appearance. It must be considered, therefore, that a specific cytoplasmic condition in the ciliated cells of the epidermal lines causes this peculiar distribution of organelles. In the proximal portion of the cell globular, fibrous clusters are frequently observed (Fig. 12). The basal cytoplasm continues into the axon without conspicuous structural change. The axon traverses the basal lamina immediately and extends among the chromatophores into the connective tissue of the cutis. The axonal cytoplasm is characterized by scattered small mitochondria, some loosely arranged filaments, ribosome-like granules and small vesicles of various diameters, occasionally possessing a dense core. Profiles of the endoplasmic reticulum are rarely seen. The axon is ensheathed by glial cells.

In the late embryo the ciliated cells are slender, approximately 2–7 μm in diameter. The distribution of organelles in the basal cytoplasm is less heterogeneous, and the axon is smaller, with an average diameter of 2–3 μm.

Loligo vulgaris. The accessory cell, like in *Sepia*, resembles the epidermal cell of the adult with regard to the density of the cytoplasm and the distribution of the organelles. The microvilli are very short (approximately 0.1 μm) and not numerous.

The first type of ciliated cell (Fig. 17) is a short, flask-like element. The diameter is largest in the basal portion of the cell containing the nucleus.

Fig. 5. Section through the line of the head. Note the large axon (*ax*) leaving the ciliated cell (*cs*) and running between two chromatophores (*ch*)

Figs. 6–10. *Loligo vulgaris*

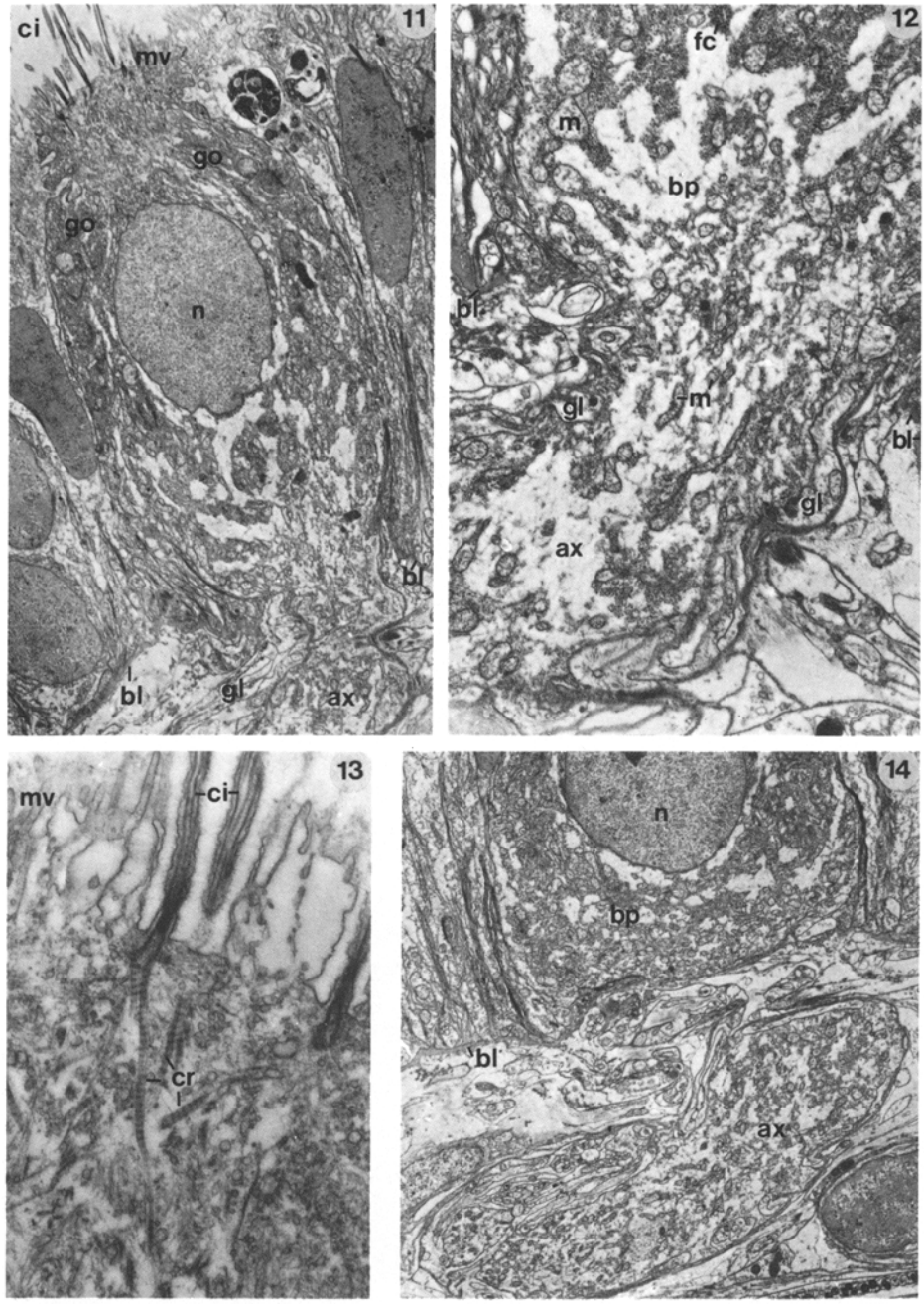
Fig. 6. Dorsal view of the head of a hatched animal. Lines 1 and 2 (*L1, L2*); *a* arms; *ma* mantle

Fig. 7. Cross section through the arm with an epidermal line (*L*) between goblet cells (*gc*). At the lateral aspect the epidermis consists of secretory cells (*sc*); *anc* axial nerve cord

Fig. 8. Section through a line in the head epidermis showing a ciliated cell (*cc*) of type 1; *gc* goblet cell

Fig. 9. Section as in Fig. 8 showing a ciliated cell (*cc*) of type 2

Fig. 10. Section through a line in the head epidermis with an axon (*ax*) leaving the line (*L*). Scale bar = 10 μm



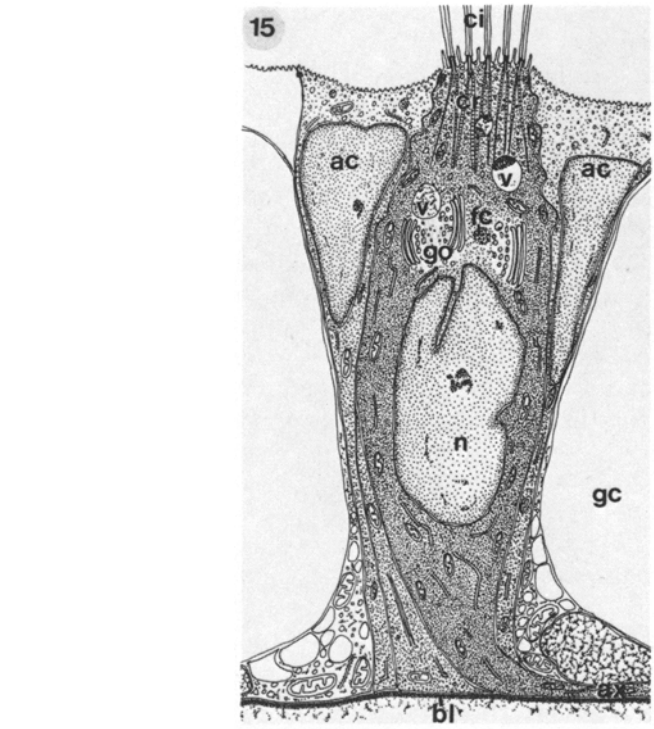
Figs. 11–14. *Sepia officinalis*

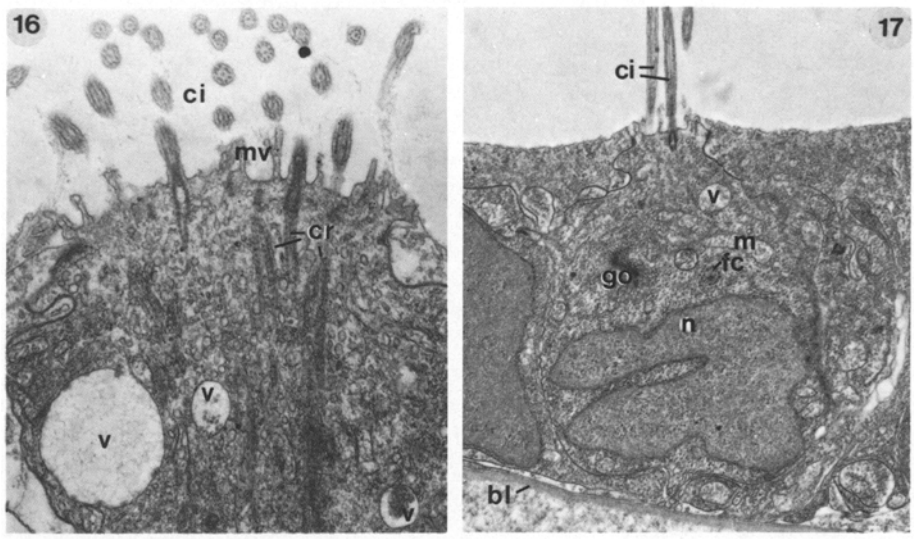
Fig. 11. Electron micrograph of a ciliated cell in an epidermal line of the head. Note the axon (*ax*) penetrating the basal lamina (*bl*); *ci* cilia; *gl* glia; *go* Golgi complex; *mv* microvilli; *n* nucleus. × 3500

Fig. 12. Electron micrograph of the basal region of a ciliated cell. The heterogeneous appearance of the basal cytoplasm (*bp*) continues into the axon (*ax*); *bl* basal lamina; *fc* fibrous cluster; *gl* glia; *m* mitochondrion. × 6500

Fig. 13. Electron micrograph of the apical region of a ciliated cell, showing the ciliary rootlets (*cr*); *ci* cilia; *mv* microvilli. × 13000

Fig. 14. Electron micrograph of an obliquely cut axon (*ax*) in the head region; *bl* basal lamina; *bp* basal part of the ciliated cell; *n* nucleus. × 3500





Figs. 15–17. *Loligo vulgaris*

Fig. 15. Schematic representation of a longitudinally sectioned epidermal line; *ac* accessory cell; *bl* basal lamina; *ci* cilia; *cr* ciliary rootlets; *fc* fibrous cluster; *gc* goblet cell; *go* Golgi complex; *n* nucleus; *v* large vesicle

Fig. 16. Electron micrograph of the apical region of a ciliated cell of the second type. Note the large vesicles (*v*) and the sparse ciliary rootlets (*cr*); *ci* cilia; *mv* microvilli. × 11 000

Fig. 17. Electron micrograph of a type-1 ciliated cell; *m* mitochondrion; for other abbreviations see Fig. 15. × 6500

The organelles are similar in both types of cell. The second type of ciliated cell (Figs. 15, 16) displaying a height of ~15–20 μm and a diameter of ~5–7 μm, has more in common with the ciliated cell of *Sepia:* the cilia-bearing surface is slightly bent outward. The cilia are located between well-developed microvilli with a length of about 1 μm; the rootlets of the cilia are relatively poorly developed. The organelles are similar, but there are some differences: (i) the cytoplasm appears more electron-dense due to a greater amount of ribosome-like granules throughout the entire cytoplasm, and (ii) there are many small Golgi vesicles in the distal portion of the cell. The nucleus is lobed. The axon does not pass through the basal lamina immediately but runs for a distance above it. The heterogeneous distribution of organelles, typical for the ciliated cell in *Sepia*, was not observed in *Loligo*.

Discussion

The histological examination of the epidermal lines confirms that they consist of both ciliated and accessory cells. In a study on the pattern of cilia in embryos of *Loligo pealei* Arnold and Williams-Arnold (1980) described lines of single-filed cilia, which accord exactly with the course of the epidermal lines of *Loligo vulgaris* and *Sepia officinalis*.

The ultrastructure of the ciliated cell with its thick axon indicates an sensory function. The organelles and their arrangement resemble the patterns characteristic of the poorly differentiated ciliated epithelial receptors described by Emery (1975a, c) in the lip and the least differentiated type 1 of ciliated neurons in the olfactory organ of *Lolliguncula brevis*. In accordance with the assumed sensory function of the ciliated cell in *Sepia* are the small mitochondria in the basal cytoplasm and in the axon. Emery (1975b) described small mitochondria also for the interneurons in the connective tissue of the lip of *Octopus joubini*. The motility of the cilia observed by Arnold and Williams-Arnold (1980), however, does not speak in favor of a sensory function; on the other hand, the axon leaving the cell makes any other but a sensory function improbable. The epidermal lines can thus be regarded as a sense organ. Their structure as well as the ultrastructure of the ciliated cells and the accessory cells is identical over the entire epidermal line. More support for this interpretation can be found in the fact that in the arms the axons of successive ciliated cells run together to enter the axial nerve cord.

The differences between the ciliated cells of *Sepia officinalis* and *Loligo vulgaris* are probably a result of different developmental stages at hatching. *Sepia* hatches in a more adult-like state than *Loligo* (Fioroni 1977) and probably represents the more advanced type of epidermal lines. It also seems that there exist species-specific differences as, for example, the fifth pair of lines is present in *Loligo* but not in *Sepia*.

Since the epidermal lines appear to be so common for cephalopod embryos and hatching stages, they may be considered as a typical sense organ of cephalopods.

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