Belg. J. Zool. — Volume 125 (1995) — issue 1 — pages 125-134 — Brussels 1995

FINE STRUCTURE OF THE PODIA IN THREE SPECIES OF PAXILLOSID ASTEROIDS OF THE GENUS *LUIDIA* (ECHINODERMATA)

by

PATRICK FLAMMANG *
Laboratoire de Biologie Marine, Université de Mons-Hainaut,
19 av. Maistriau, B-7000 Mons, Belgium

SUMMARY

Individuals of the paxillosid asteroid genus *Luidia* use their podia in locomotion and burrowing. Each podium in the three considered species consists of a stem with a pointed knob at its tip. The knob consists of four tissue layers that are, from the inside to the outside, a mesothelium, a connective tissue layer, a nerve plexus, and an epidermis. The latter is made up of four cell categories : secretory cells, neurosecretory cells, non-secretory ciliated cells, and support cells. The epidermal cells of the podial knob are presumably functioning as a duo-gland adhesive system in which adhesive secretions would be produced by secretory cells while de-adhesion, on the other hand, would be due to neurosecretory cell secretions. Although the podia of the three considered species of *Luidia* share numerous similarities, there are nevertheless several important morphological differences between, on the one hand, the podia of *L. ciliaris* and *L. maculata*, and, on the other hand, the podia of *L. penangensis*. These differences stress that carefulness is required when generalizations, drawn from the morphology of a single species, are applied to related species having the same life style ; but also that the genus *Luidia* needs to be re-examined from a taxonomic point of view.

Keywords : ultrastructure, podia, adhesive organs, Asteroidea, Echinodermata.

INTRODUCTION

The podia, *i.e.* the external appendages of the ambulacral system of echinoderms, are multifunctional organs. Indeed, according to the considered class, they can take part in locomotion, feeding, burrowing, respiration or sensory perception. In asteroids, the main function of the podia is locomotion (LAWRENCE, 1987). Most species possess podia which end with a flat disk allowing the podium to adhere to the substratum. Yet species of the order Paxillosida, which are soft sub-

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* Senior Research Assistant, National Fund for Scientific Research, Belgium.

strate dwellers, possess podia ending with a pointed knob. In these asteroids, the podia take part in both locomotion and burrowing.

Whereas asteroid disk-ending podia have been the subject of a number of ultrastructural investigations (Chaet, 1965 ; Harrison and Philpott, 1966 ; Souza Santos and Silva Sasso, 1968 ; Flammang *et al.*, 1994), the available data about knob-ending podia are scarce. In fact, there is only a single paper on these podia concerning one species of the genus *Astropecten* (Engster and Brown, 1972).

The aim of the present work is to describe the ultrastructure of the podia of three asteroid species of the paxillosid genus *Luidia* and to consider whether their burrowing behaviour and the shape of their podia correspond with a different epidermal organization compared to podia of other asteroid species.

MATERIAL AND METHODS

Individuals of three species of the paxillosid asteroid genus *Luidia* were investigated. They were all collected by dredging either in the bay of Morlaix, Brittany, France (*Luidia ciliaris* (Philippi, 1837)) in August 1994, or off the east coast of Singapore mainland (*Luidia maculata* Müller and Troschel, 1842 and *Luidia penangensis* de Loriol, 1891) in March and September 1994.

For scanning electron microscopy (SEM), podia were fixed in Bouin's fluid for 24 h. They were dehydrated in graded ethanol, dried by the critical point method (with CO_2 as transition fluid), mounted on aluminum stubs, coated with gold in a sputter coater and observed with a JEOL JSM-6100 scanning electron microscope.

For transmission electron microscopy (TEM), podia were fixed in 3 % glutaraldehyde in cacodylate buffer (0.1M, pH 7.8) for 3 h at 4° C, rinsed in cacodylate buffer, and post-fixed for 1 h in 1 % osmium tetroxide in the same buffer. After a final wash in buffer, they were dehydrated in graded ethanol and embedded in Spurr. Semithin sections (1µm in thickness) were cut on a Reichert Om U2 ultramicrotome equipped with a glass knife. They were stained with a 1:1 mixture of 1 % methylene blue in 1 % natrium tetraborate and 1 % azur II. Ultrathin sections (40-70 nm) were cut with an LKB III ultramicrotome equipped with a diamond knife, stained with uranyl acetate and lead citrate, and observed with a Zeiss EM 10 transmission electron microscope.

RESULTS

Outer aspect of the podia

In the three species, the podia are arranged in two rows on the oral surface of the arms. Mean size podia measure about 1,25 mm in diameter and 15 mm in length. They consist of an extensible cylindrical stem topped by a knob (Figs 1-3). In both *L. ciliaris* and *L. maculata*, the knob is ovoid, somewhat pointed, measuring about 800 µm in diameter and 1.2 mm in height (Figs 1, 2). In *L. penangensis*, the knob has the shape of an hemispherical cap about 300 µm high and 800 µm in diameter (Fig. 3).

Whatever the species, the knob surface is covered with cilia and pores (Figs 4, 7). The cilia are about 1 µm long (Figs 5, 8); the pores measure from 450 nm to 600 nm in diameter (Figs 6, 9). In *L. ciliaris* and *L. maculata*, the cilia and the pores are mixed and uniformly distributed over the knob (Fig. 4). In *L. penangensis*, they are always separated, the cilia being clustered in islets (Fig. 7). These islets range from 5 to 20 µm in diameter and enclose from 5 to 50 cilia which arise from cuticular bulges about 0.5 µm high and 0.5 µm in diameter (Figs 7, 8).

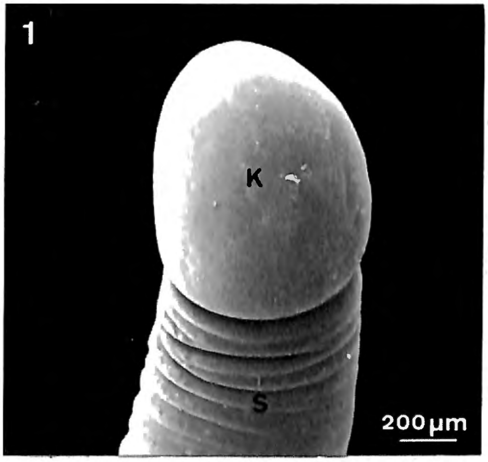
Histology and cytology of the podial knob

The knob always consists of four tissue layers that are, from the inside to the outside, a mesothelium, a connective tissue layer, a nerve plexus, and an epidermis covered by a cuticle.

The inner tissues (Fig. 10). The mesothelium, the most internal layer, surrounds the ambulacral lumen. It is a pseudostratified epithelium comprising adluminal cells and myoepithelial cells. The latter together form the retractor muscle of the podium which anchors distally to the connective tissue layer of the knob. In both *L. ciliaris* and *L. maculata*, this layer is thick (about 300 µm and 150µm in thickness, respectively) and is made up of loose connective tissue. In *L. penagensis*, the layer is thinner (about 25 µm) and consists of dense connective tissue. At the base of the knob, the nerve plexus of the stem is thickened to form the nerve ring. In both *L. ciliaris* and *L. maculata*, the nerve ring gives off radial branches, the nerve strands, that extend over the surface of the connective tissue layer, and join at the pointed apex of the knob. On the other hand, in *L. penangensis*, the nerve ring gives off a nerve plexus, thicker than the one of the stem, that extends over the whole knob surface (compare Fig. 10 A and B).

The epidermis. The epidermis of the knob is thickened compared to the stem epidermis (Fig. 10). It is about 150 µm thick in *L. ciliaris* and *L. maculata*, 100 µm in *L. penangensis*. In the three species, it is made up of four cell categories : secretory cells, neurosecretory cells, non-secretory ciliated cells, and support cells (Figs 11-14). In both *L. ciliaris* and *L. maculata*, the secretory cells are of two types (S1 and S2 cells ; Figs 11, 13, 15) whereas in *L. penangensis*, there is only one type of secretory cells (S1 cells ; Figs 12, 14). In *L. ciliaris* and *L. maculata*, cells of the four categories are mixed in the epidermis. In *L. penangensis*, they are segregated : non-secretory ciliated cells are gathered in islets surrounded by areas enclosing secretory and neurosecretory cells. Support cells occur in both the islets and the secretory areas.

Secretory cells (S1 and S2 cells) are flask-shaped. Their enlarged cell bodies are located basally, and each one sends out an apical process that reaches the surface of the epidermis (Figs 11, 12). The cytoplasm of both the cell body and the apical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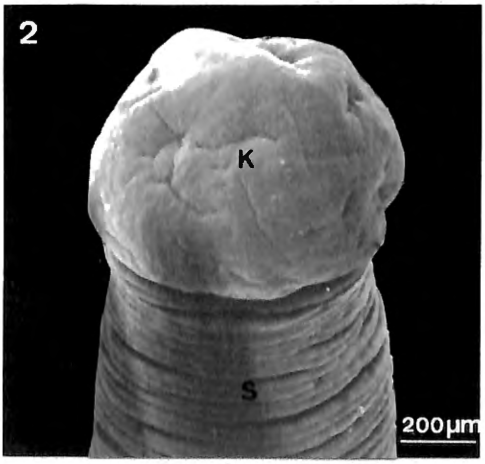
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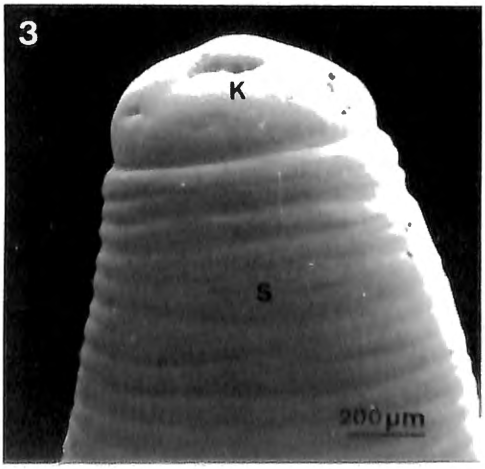
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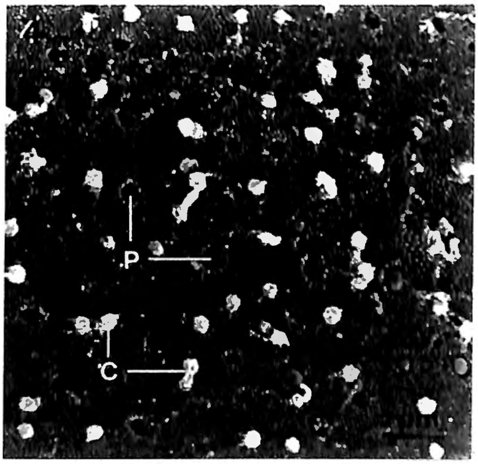
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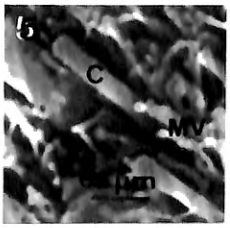
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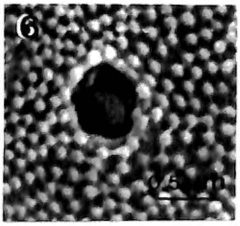
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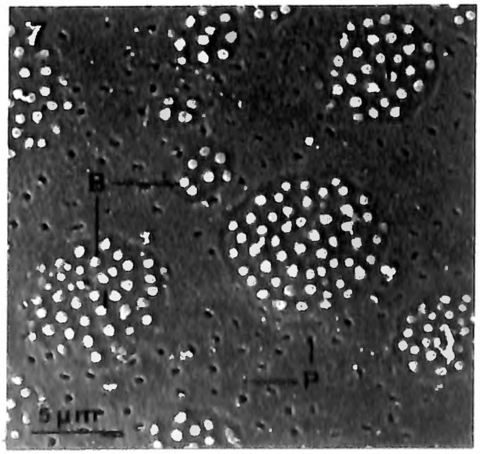
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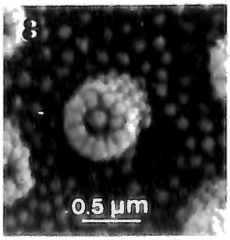
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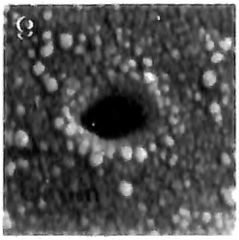
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process is filled with densely packed membrane-bound secretory granules. The cytoplasm of the cell body also contains a well-developped Golgi apparatus and an extensive rough endoplasmic reticulum (RER) (Fig. 13). At the end of the apical processes of the secretory cells, the granules are extruded through a duct delimited by a ring of microvilli and opening onto the disk surface as a cuticular pore (Figs 11, 12). These pores correspond to those observed on SEM pictures of the knob surface (Figs 6, 9).

In the three species, S1 cell secretory granules are ellipsoids about 1,5 × 1 μm (Figs 11, 12, 14, 15). They consist of a large central core made of parallelly oriented fibrils, surrounded by a thin clear ring. In *L. ciliaris* and *L. maculata*, S2 cell secretory granules are spherical, about 1 μm in diameter (Figs 11, 13, 15). They enclose an electron-dense granular material limited by very electron-dense dots and surrounded by a belt of electron luscent granular material.

Neurosecretory cells are narrow and have a centrally located nucleus (Fig. 16). Apically, they end with a bulge just beneath the cuticle (Figs 12, 14, 15). The entire cytoplasm of the cell is filled with spherical membrane-bound secretory granules, ranging from 200 to 350 nm in diameter (Figs 15, 16). They contain an electron-dense homogeneous material surrounded by a thin clear belt. The cytoplasm also contains a Golgi apparatus, numerous mitochondria and RER cisternae (Fig. 16).

Non-secretory ciliated cells have the same shape and size as neurosecretory cells. Their characteristic feature is a single short cilium (about 3 μm long) whose apex protrudes into the outer medium (Fig. 17). These cilia are visible on SEM pictures of the knob surface (Figs 5, 8).

Support cells have a centrally to apically located nucleus. One longitudinal bundle of intermediate filaments traverses the cell and joins its apical and basal membranes (Figs. 11, 17).

DISCUSSION

The three species of *Luidia* considered in this work live on soft substrata, in which they can burrow, and feed on mollusks and other echinoderms (Jangoux, 1982 ; Lawrence, 1987). The main functions of their podia are locomotion and burrowing ; the knob-shaped apical part of the podia is indeed well adapted to dig in the sediment.

Figs 1-9. — Outer aspect of the podia – 1. Protracted podium of *L. maculata* – 2. Retracted podium of *L. ciliaris* – 3. Protracted podium of *L. penangensis* – 4. Knob surface (*L. maculata*) – 5. Detailed view of cilia (the cuticle has been removed) (*L. ciliaris*) – 6. Detailed view of a pore (*L. maculata*) – 7. Knob surface (*L. penangensis*) – 8. Detailed view of a cilium arising from a cuticular bulge (*L. penangensis*) – 9. Detailed view of a pore (*L. penangensis*).
B cuticular bulge ; C cilium ; I islet of cilia ; K knob ; MV microvilli ; P pore ; S stem.

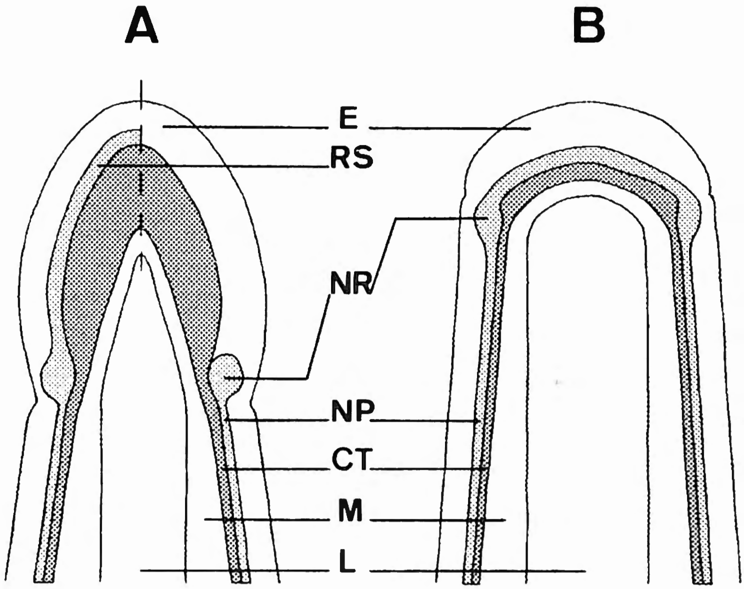


Fig. 10. — Schematic representation of two longitudinal sections through the podial knob of *Luidia ciliaris* and *Luidia maculata* (A), and *Luidia penangensis* (B).
CT connective tissue layer ; E epidermis ; L ambulacral lumen ; M mesothelium ; NP nerve plexus ; NR nerve ring ; RS radial nerve strand.

The podial knob has the classical tissular stratification of echinoderm podia, that is, from the inside to the outside : a mesothelium, a connective tissue layer, a nerve plexus, and an epidermis (KAWAGUTI, 1964 ; FLOREY and CAHILL, 1977 ; CAVEY and WOOD, 1991). In *L. ciliaris* and *L. maculata*, the connective tissue layer is thick and the nerve tissue is arranged in radial strands. This structure is similar to the one found in the disk of forcipulatid asteroid podia (FLAMMANG *et al.*, 1994). In *L. penagensis*, on the other hand, the connective tissue layer is thinner and the nerve tissue is arranged in a thick plexus. This structure is more reminiscent of the one observed in the podial knob of the paxillosid genus *Astropecten* (ENGSTER and BROWN, 1972).

In the three species, the epidermis of the knob encloses the same cell categories : secretory cells, neurosecretory cells, non-secretory ciliated cells, and support cells. These four cell categories occur in the podial adhesive areas of all other asteroid species so far examined.

In *L. ciliaris* and *L. maculata*, the secretory cells are of two types (S1 and S2 cells) whereas in *L. penangensis*, all secretory cells are S1 cells. The podial disk epidermis of forcipulatid asteroids also encloses both S1 and S2 cells (CHAET, 1965 ; FLAMMANG *et al.*, 1994). On the contrary, the podial knob epidermis of *Astropecten* only contains one type of secretory cell which, according to the size of its secretory granules and the aspect of their content, could be S2 cells. In all species so far studied, secretory cells (both S1 and S2 cells) have always been considered to be adhesive in function.

S1 secretory granules are much less complex in *Luidia* spp than in valvatid and forcipulatid asteroids in which they are made up of highly ordered parallel rods (CHAET, 1965 ; HARRISON and PHILPOTT, 1966 ; SOUZA SANTOS and SILVA SASSO, 1968 ; FLAMMANG *et al.*, 1994). ENGSTER and BROWN (1972) pointed out a possible relationship between the internal organization of the secretory granules of adhesive cells and the possible adhesive strength of the podia : asteroids confined to hard rocky substratum have complex granules enclosing a highly organized core whereas soft substratum dwelling species have granules of considerably simpler ultrastructure. Similarly, the adhesive enclosed in the granules of the secretory cells of *Luidia*'s podia is presumably adapted to the functions the podia fulfil, *e.g.* particle handling or burrow wall consolidation. As for S2 secretory granules, they are similar in all asteroid species (CHAET, 1965 ; ENGSTER and BROWN, 1972 ; FLAMMANG *et al.*, 1994).

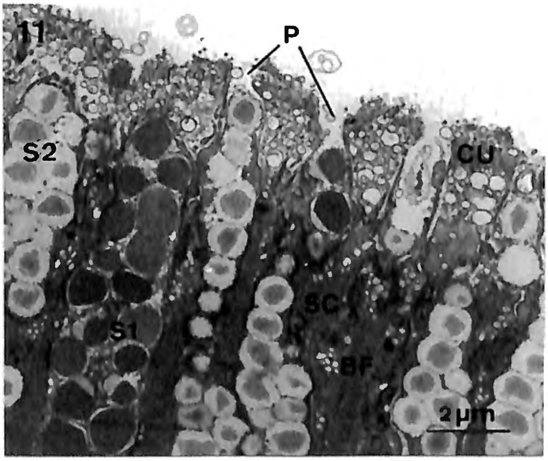
Neurosecretory cells in the podia of *Luidia* are almost identical to those in the podia of *Astropecten* and of forcipulatid asteroids (ENGSTER and BROWN, 1972 ; FLAMMANG *et al.*, 1994). However, in the latter, the neurosecretory cells possess a subcuticular cilium (FLAMMANG *et al.*, 1994). These cells are thought to be de-adhesive in function.

Non-secretory ciliated cells are sensory and are almost identical in the podia of all asteroid species studied so far (ENGSTER and BROWN, 1972 ; FLAMMANG *et al.*, 1994).

As in other asteroids, *Luidia*'s podia would thus enclose a duo-gland adhesive system in which S1 and S2 cells would be adhesive and neurosecretory cells would be de-adhesive in function (HERMANS, 1983 ; THOMAS and HERMANS, 1985 ; FLAMMANG *et al.*, 1994).

Like forcipulatid asteroid podia, the podia of *L. ciliaris* and *L. maculata* enclose two types of adhesive cells. However, the podia of *L. penangensis*, a species living in the same habitat and presumably having the same behaviour, only enclose one type of adhesive cell, as do the podia of *Astropecten*. The significance of having two types of adhesive cells in the knob epidermis of both *L. ciliaris* and *L. maculata* therefore remains obscure.

Secretory, neurosecretory and non-secretory ciliated cells are always closely associated in echinoderm podial adhesive areas but, generally, they are differently arranged according to whether the podia are handling or locomotory (FLAMMANG and JANGOUX, 1992). In the epidermis of handling podia, these cells join together to form sensory-secretory complexes (wherein the three cell types are present). Conversely, in the epidermis of locomotory podia, these three cell types form an homogeneous cellular layer together with support cells. This last arrangement is the one found in the knob epidermis of the podia of *L. ciliaris* and *L. maculata*. On the other hand, in the knob epidermis of the podia of *L. penangensis*, non-secretory ciliated cells are gathered in islets surrounded by areas enclosing secretory and neurosecretory cells, support cells occurring in both the islets and the secretory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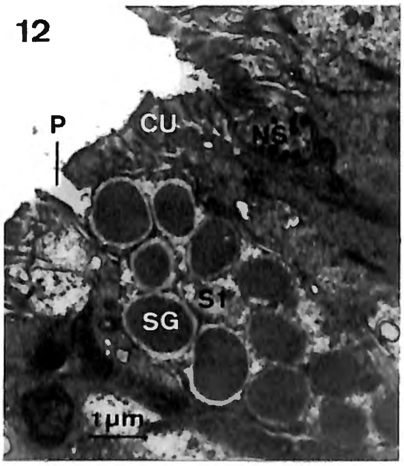
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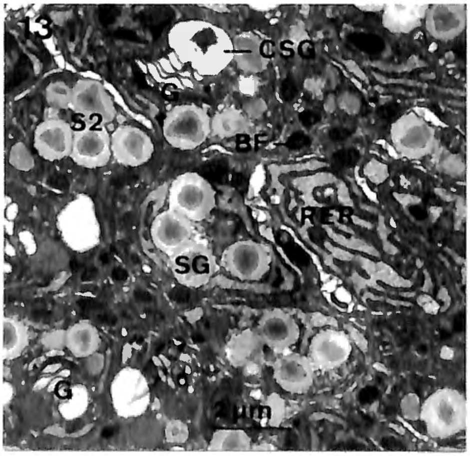
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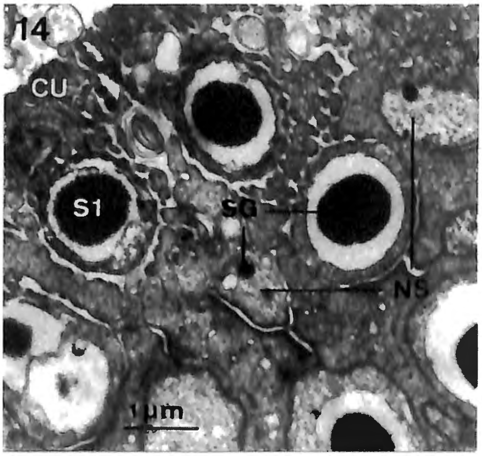
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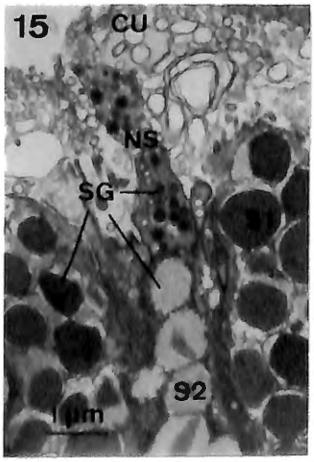
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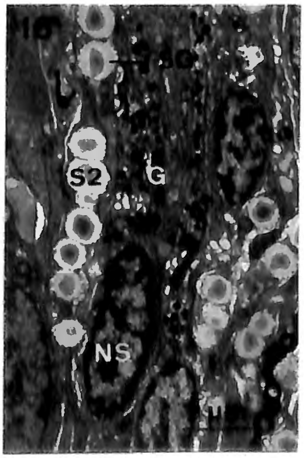
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areas. So far, this kind of cell organization has never been observed in any echinoderm podia.

This work has enlighted morphological differences between, on the one hand, the podia of *L. ciliaris* and *L. maculata*, and, on the other hand, the podia of *L. penangensis*. These differences concern the shape of their terminal knob, its tissular organization, the number of cell types enclosed in the knob epidermis and the way they are arranged. Yet the three species belong to the same genus and have the same life style. These marked differences stress that carefulness is required when generalizations, drawn from the morphology of a single species, are applied to related species having the same life style; but also that the genus *Luidia*, which is generally considered as homogeneous (it is the only genus of the family Luidiidae), needs to be re-examined from a taxonomic point of view (see also Döderlein, 1920 and Fell, 1963).

ACKNOWLEDGEMENTS

I thank Prof. M. Jangoux for his comments on the manuscript; Prof. J.A. Heuson-Stiennon for the use of the transmission electron microscope; P. Postiau for technical assistance. Work supported by EC contract n° CL1-CT91-0909 (NUS-UMH) and FRFC contract n° 2.4549.91. Contribution of the « Centre Interuniversitaire de Biologie Marine » (CIBIM).

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Figs 11-17. — Fine structure of the epidermis of podial knob – 11, 12. Longitudinal sections through the apex of the epidermis (*L. ciliaris* and *L. penangensis*, respectively) – 13. Transverse section through the base of the epidermis (*L. ciliaris*) – 14. Oblique section through the apex of the epidermis (*L. penangensis*) – 15, 16. Neurosecretory cell (apical and basal parts, respectively) (*L. ciliaris*) – 17. Non-secretory ciliated cell (*L. penangensis*).
BB basal body; BF bundle of filaments; C cilium; CSG condensing secretory granule; CU cuticle; G Golgi zone; NS neurosecretory cell; NSC non-secretory ciliated cell; P pore; RER rough endoplasmic reticulum; S1 type 1 secretory cell; S2 type 2 secretory cell; SC support cell; SG secretory granule.

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