Comparative Histological and Immunohistochemical Study of Sea Star Tube Feet (Echinodermata, Asteroidea)

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ABSTRACT Adhesion in sea stars is the function of specialized structures, the tube feet or podia, which are the external appendages of the water-vascular system. Adhesive secretions allow asteroid tube feet to perform multiple functions. Indeed, according to the sea star species considered, the tube feet may be involved in locomotion, fixation, or burrowing. Different tube foot shapes usually correspond to this variety of function. In this study, we investigated the variability of the morphology of sea star tube feet as well as the variability of the composition of their adhesive secretions. This second aspect was addressed by a comparative immunohistochemical study using antibodies raised against the adhesive material of the forcipulatid *Asterias rubens*. The tube feet from 14 sea star species representing five orders and 10 families of the Class Asteroidea were examined. The histological study revealed three main tube foot morphotypes, i.e., knob-ending, simple disc-ending, and reinforced disc-ending. Analysis of the results suggests that tube foot morphology is influenced by species habitat, but within limits imposed by the evolutionary lineage. In immunohistochemistry, on the other hand, the results were very homogeneous. In every species investigated there was a very strong immunolabeling of the adhesive cells, independently of the taxon considered, of the tube foot morphotype or function, or of the species habitat. This indicates that the adhesives in all the species considered are closely related, probably sharing many identical molecules or, at least, many identical epitopes on their constituents. J. Morphol. 263: 259–269, 2005. © 2004 Wiley-Liss, Inc.

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The importance of the tube feet (or podia), i.e., the external appendages of the water-vascular system, in sea star biology has been emphasized by several workers (Smith, 1937; Nichols, 1966; Lawrence, 1987; Flammang, 1996). According to the species considered, the tube feet may be involved in one or several of the following functions: locomotion, fixation to the substratum, feeding, and burrowing. These different functions all rely on the mobility of the tube foot stem as well as on the secretion of an adhesive material by the tip of the tube foot (Flammang, 1996).

Asteroid tube feet are traditionally subdivided into two categories: those in which the tip is pointed, the knob-ending tube feet, and those in which the tip is flattened, the disc-ending tube feet (Hyman, 1955; Lawrence, 1987; Clark and Downey, 1992). The former are present in the species of the order Paxillosida; the latter occur in all other species. Yet recent studies have shown that there is considerable variation in tube foot morphology within each category (Flammang, 1995; Vickery and McClintock, 2000). According to Vickery and McClintock (2000), a consistent relationship would exist between tube foot morphotype and asteroid classification at the ordinal level. Blake (1990), on the other hand, argued that tube foot morphology is an adaptation to habitat, more precisely to the type of substratum on which the sea star dwells.

Whatever their external morphology, however, the histological structure of asteroid tube feet is remarkably constant. They consist of four tissue layers: an inner myomesothelium surrounding the water-vascular lumen, a connective tissue layer, a nerve plexus, and an outer epidermis covered by a cuticle (see Flammang, 1996, for review). At the level of the tube foot tip, these tissue layers are specialized for adhesion and sensory perception: the connective tissue layer and the nerve plexus are thickened, and the epidermis is differentiated into a well-developed sensory-secretory epithelium. The latter functions as a duo-gland adhesive system comprising two types of secretory cells: cells releasing an adhesive secretion and cells releasing a de-

TABLE 1. List of the asteroid species used in this study with taxonomic classification, preferred habitat, and place of collection

Species[1]	Habitat[2]	Place of collection
PAXILLOSIDA		
Astropectinidae		
Astropecten aranciacus (Linnaeus, 1758)	On sandy substrata in which they may completely bury themselves	Banyuls-sur-mer, France
Astropecten polyacanthus Müller & Troschel, 1842	On sandy substrata in which they may completely bury themselves	Toliara, Madagascar
Luidiidae		
Luidia savignyi (Audouin, 1826)	On sandy substrata in which they may completely bury themselves	Toliara, Madagascar
VALVATIDA		
Acanthasteridae		
Acanthaster planci (Linnaeus, 1758)	On hard substrata of living corals, either in the lagoons or on the slopes of coral reefs	Toliara, Madagascar
Archasteridae		
Archaster typicus Müller & Troschel, 1840	On sandy substrata in which they may completely bury themselves	Pulau Semakau, Singapore
Asterinidae		
Asterina gibbosa (Pennant, 1777)	Under boulders or in crevices on rocky shores	Roscoff, France
Ophidiasteridae		
Linckia laevigata (Linnaeus, 1758)	In sheltered areas of coral reefs	Toliara, Madagascar
Oreasteridae		
Culcita schmideliana (Retzius, 1805)	On coral rubbles in sheltered areas of coral reefs	Toliara, Madagascar
Protoreaster lincki (de Blainville, 1830)	On soft substrata in sheltered areas of coral reefs	Toliara, Madagascar
Pentaceraster mammillatus (Audouin, 1826)	On soft substrata in sheltered areas of coral reefs	Toliara, Madagascar
VELATIDA		
Solasteridae		
Crossaster papposus (Linnaeus, 1776)	In semi-exposed rocky or bouldery sites	Oban, Scotland
SPINULOSIDA		
Echinasteridae		
Echinaster sepositus (Retzius, 1783)	On rocky substrata in both sheltered and moderately exposed conditions	Banyuls-sur-mer, France
FORCIPULATIDA		
Asteriidae		
Asterias rubens Linnaeus, 1758	Very wide range of habitats (from sheltered soft sediment sites to fully exposed rockfaces)	Audresselles, France
Marthasterias glacialis (Linnaeus, 1758)	Very wide range of habitats (from sheltered soft sediment sites to fully exposed rockfaces)	Banyuls-sur-mer, France

[1]Classification from Clark and Downey, 1992.
[2]Data from field observations and from Jangoux, 1986; Picton, 1993; Gosliner et al., 1996; Gibson et al., 2001.

adhesive secretion (Hermans, 1983; Thomas and Hermans, 1985; Flammang, 1996; Flammang et al., 1998). According to the tube foot function, the adhesive secretion allows the tube feet to attach firmly to the substratum during locomotion and fixation, to capture and handle food items, or to reinforce burrow walls to prevent collapse. This diversity of functions suggests that the composition of asteroid adhesive material could vary within the class, from one group to another. Such a variability has already been postulated based on differences in the ultrastructure of the adhesive cell secretory granules in different sea star taxa (Engster and Brown, 1972; Flammang, 1995, 1996).

The present study had two main goals: 1) to examine the diversity of tube foot morphologies in sea stars and find out if morphology is related to taxonomic position, habitat, or both; and 2) to shed some light on the variability of the composition of asteroid adhesive substances. This second aspect was addressed by a comparative immunohistochemical study. Indeed, the exact nature and composition of the adhesive material is not known for any sea star species, but polyclonal antibodies are available that specifically recognize this material in the forcipulatid asteroid *Asterias rubens* (see Flammang et al., 1998). Therefore, these antibodies were used to evaluate the differences in the composition of the contents of the tube foot adhesive cells by looking for antibody cross-reactivity on histological sections made from the tube feet of several asteroid species. These species were chosen on the basis of their diversity in terms of taxonomic position within the class, habitat, and tube foot function.

MATERIALS AND METHODS

The tube feet from 14 sea star species representing 10 families of the Class Asteroidea were examined in the present study. Asteroids were collected from a variety of geographical regions (Table 1) and their tube feet fixed directly on the site of collection.

Histology

In every species considered, tube feet were dissected from mid-arm portions of the ambulacra and were fixed for periods of time ranging between 1–8 h at 4°C in Bouin's fluid without acetic

acid.* They were then dehydrated in a sequence of graded ethanol and embedded in paraffin wax (Paraplast, Sigma, St. Louis, MO) using a routine method. The tube feet were sectioned longitudinally at a thickness of 7 μm with a Microm HM 340E microtome and the sections were collected on clean glass slides. They were stained with Masson's trichrome or with azocarmine coupled with aniline blue and orange G (Gabe, 1968). The sections were then observed and photographed with a Leitz Orthoplan light microscope equipped with a Leica DC 300F digital camera.

Immunohistochemistry

Sea star tube feet fixed, embedded, and sectioned as described for routine histology were subjected to immunohistochemistry using an indirect immunofluorescence method. After deparaffinization, the sections were permeabilized in phosphate-buffered saline (PBS) containing 0.25% Triton-X-100 for 1 h and preincubated for 30 min in 10% normal swine serum (DAKO, Glostrup, Denmark) in PBS to block nonspecific antigenic sites. Four antisera were used that had been obtained by immunizing two rabbits with the adhesive material of the forcipulatid asteroid *Asterias rubens* (Flammang et al., 1998). These antisera, diluted 1:100 in PBS containing 1% Tween 20 and 3% BSA (PBS-Tween-BSA), were applied on the sections for 1 h at room temperature. After several washes in PBS, the sections were incubated for 1 h in FITC-conjugated swine antirabbit immunoglobulins (DAKO) diluted 1:50 in PBS-Tween-BSA. After a final wash in PBS, they were mounted in Vectashield mounting medium containing propidium iodide (Vector, Burlingame, CA) and observed with a Leica TCS 4D confocal laser scanning microscope. The four antisera were tested on the different species and gave identical results in each case.

In each experiment, a section from a tube foot of *Asterias rubens* was included as a positive control. Two types of negative control were also carried out: 1) substitution of the primary antiserum with PBS-Tween-BSA, and 2) substitution of the primary antiserum with the corresponding preimmune serum diluted 1:100 in PBS-Tween-BSA.

RESULTS

Based on their external and internal morphology, asteroid tube feet can be divided into three main morphotypes: knob-ending tube feet, simple disc-ending tube feet, and reinforced disc-ending tube feet. Tube feet from all three morphotypes, however, possess the same histological organization. They consist of four tissue layers that are, from the inside to the outside: a myomesothelium surrounding the water-vascular lumen, a connective tissue layer, a basiepidermal nerve plexus, and an epidermis covered by a cuticle.

Knob-Ending Tube Feet

Among the asteroid species investigated in this work, knob-ending tube feet occurred exclusively in paxillosids (Table 2), in the genus *Astropecten* as well as in the genus *Luidia*. Knob-ending tube feet are made up of a basal cylindrical stem ending distally with a pointed knob (Figs. 1A, 2). The water-vascular lumen extends into the knob where it tapers off to a point. Except for the myomesothelium, all tissue layers are thicker in the knob than in the stem (Fig. 2). The adhesive epidermis is a tall columnar epithelium consisting mostly of granule-filled secretory cells. The nervous tissue consists of a nerve ring on the proximal side of the knob and a thick basiepithelial nerve plexus underlying the adhesive epidermis. Both layers lie on a thick layer of loose connective tissue.

The knob-ending tube feet of the three species considered were strongly and specifically labeled with the different antisera (Table 2). Immunolabeling was the strongest in the knob epidermis due to the presence of many granule-containing adhesive cells whose secretory granules were very immunoreactive (Figs. 8, 9). A moderate immunolabeling was also observed in the stem epidermis, where it was restricted to the apex of all the epidermal cells, and at the level of the cuticle (Fig. 8). In addition to this epidermal immunoreactivity, we observed a weak labeling at the level of the nerve plexus (Fig. 8).

Simple Disc-Ending Tube Feet

Simple disc-ending tube feet have been observed in most species of the order Valvatida (Table 2). They consist of a basal cylindrical stem with an apical extremity that is enlarged and flattened to form the so-called disc (Figs. 1B, 3). The disc has the same basic structure as the knob of knob-ending tube feet: it encloses the distal extremity of the water-vascular lumen and its epidermis, nerve plexus, and connective tissue layer are thickened compared to their equivalents in the stem. In the ophidiasterid *Linckia laevigata,* the disc is only slightly larger than the stem and looks like a flattened knob. In the other species investigated, the disc is much larger than the stem, giving the tube foot a flared shape (Figs. 1B, 3). In these species, the water-vascular lumen extends radi-

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*In a previous study, we demonstrated that immunolabeling of the adhesive secretions on sections of tube feet in *Asterias rubens* was fixation-dependent, and that tube feet fixed in paraformaldehyde retained a good immunoreactivity in light microscopy (Flammang et al., 1998). However, the need to prepare the paraformaldehyde solution freshly (Hayat, 2000) made this fixative inconvenient to use in the different locations where sea stars were collected for this study. Bouin's fluid was therefore selected as the fixative because it had already been used successfully for immunohistochemical studies on echinoderm tissues (see, e.g., Moore and Thorndyke, 1993; Newman et al., 1995). Different fixation conditions were tried in order to obtain the strongest immunolabeling of the tube feet of *A. rubens.* Two parameters were modified: the fact that the fixative contained or did not contain acetic acid, and the duration of fixation. Both parameters influenced the immunoreactivity of the tissues, the labeling decreasing with the addition of acetic acid and with increasing fixation time. The addition of acetic acid radically affected the immunolabeling. Although tube feet fixed for 1 h in this fixative presented a moderate immunolabeling in the disc epidermis, those that were fixed for longer periods of time showed no labeling at all. When using Bouin's fluid without acetic acid, we noticed no difference in the immunoreactivity of tube feet fixed for periods of time ranging from 1–8 h. Only after 24 h of fixation did the immunoreactivity decrease. Therefore, the best results were obtained with tube feet that had been fixed for a period of time ranging between 1–8 h in Bouin's fluid without acetic acid, and we used these conditions to fix the tube feet of the other species.

TABLE 2. Variability of asteroid tube feet in terms of morphology, function, and biochemical composition of the epidermal secretions

Species	Type of tube foot	Function of the tube feet	Immunoreactivity of the epidermal layer		
			Adhesive epidermis	Nonadhesive epidermis	Cuticle
PAXILLOSIDA					
Astropectinidae					
Astropecten aranciacus	Knob-ending	Locomotion, burrowing	++	+ (apex of all epidermal cells)	+
Astropecten polyacanthus	Knob-ending	Locomotion, burrowing	++	+ (apex of all epidermal cells)	+
Luidiidae					
Luidia savignyi	Knob-ending	Locomotion, burrowing	++	+ (apex of all epidermal cells)	+
VALVATIDA					
Acanthasteridae					
Acanthaster planci	Simple disc-ending	Locomotion, fixation	++	+ (scattered secretory cells)	+
Archasteridae					
Archaster typicus	Simple disc-ending	Locomotion, burrowing	nt	nt	nt
Asterinidae					
Asterina gibbosa	Reinforced disc-ending	Locomotion, fixation	++	+ (scattered secretory cells)	+
Ophidiasteridae					
Linckia laevigata	Simple disc-ending	Locomotion, fixation	++	+ (apex of all epidermal cells)	+
Oreasteridae					
Culcita schmideliana	Simple disc-ending	Locomotion	++	+ (scattered secretory cells) ++ (glandular cells facing the skeleton)	+
Protoreaster lincki	Simple disc-ending	Locomotion	++	+ (scattered secretory cells) ++ (glandular cells facing the skeleton)	+
Pentaceraster mammillatus	Simple disc-ending	Locomotion	++	+ (scattered secretory cells) ++ (glandular cells facing the skeleton)	+
VELATIDA					
Solasteridae					
Crossaster papposus	Reinforced disc-ending	Locomotion, fixation	++	+ (scattered secretory cells)	+
SPINULOSIDA					
Echinasteridae					
Echinaster sepositus	Reinforced disc-ending	Locomotion, fixation	++	+ (scattered secretory cells)	+
FORCIPULATIDA					
Asteriidae					
Asterias rubens	Reinforced disc-ending	Locomotion, fixation	++	+ (scattered secretory cells)	+
Marthasterias glacialis	Reinforced disc-ending	Locomotion, fixation	++	+ (scattered secretory cells)	+

+, weak to moderate immunolabeling; ++, strong immunolabeling; nt, not tested.

ally into the disc margin and usually has a scalloped circumference. In *Archaster typicus,* the whole peripheral area of the disc lumen is compartmentalized by radial connective tissue septa (Fig. 4) and conspicuous muscle fibers occur within the mesothelium lining the proximal and distal surfaces of the disc lumen as well as within the one lining the septa. In the oreasterid species *Culcita schmideliana, Pentaceraster mammillatus*, and *Protoreaster lincki,* the large disc is supported by a skeleton (Fig. 5) made up of numerous calcareous ossicles and spicules located within the connective tissue layer, on the proximal side of the disc lumen. The epidermis of the proximal side of the disc (i.e., the epidermis facing the skeleton) encloses large spumous glandular cells (Fig. 5) that were not observed in the other valvatid species. These glandular cells are abundant in *P. lincki,* less numerous in *P. mammillatus*, and scarce in *C. schmideliana.*

In the five species considered, the simple disc-ending tube feet were specifically immunolabeled with
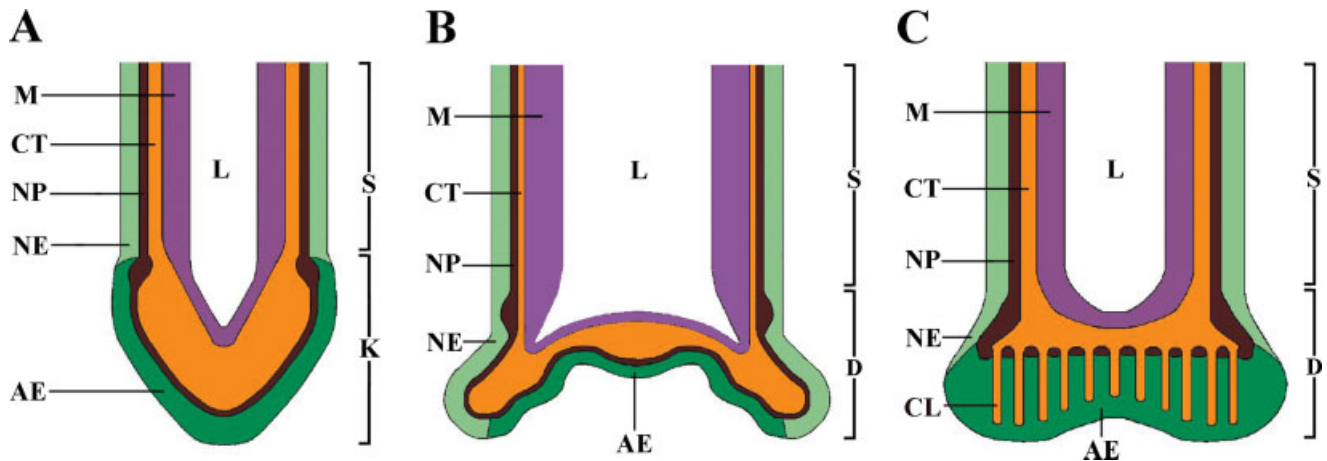
Fig. 1. Schematic drawings of the three tube foot morphotypes observed in asteroids (not to scale). **A:** Knob-ending tube foot. **B:** Simple disc-ending tube foot. **C:** Reinforced disc-ending tube foot. AE, adhesive epidermis; CL, connective tissue radial lamellae; CT, connective tissue layer; D, disc-shaped tube foot tip; K, knob-shaped tube foot tip; L, water-vascular lumen; M, myomesothelium; NE, nonadhesive epidermis; NP, nerve plexus; S, tube foot stem.

the different antisera (Table 2). The immunoreactivity was almost entirely restricted to the epidermis, with the exception of a weak labeling of the nerve plexus in the different species and a weak labeling of the connective tissue surrounding the skeleton in the three oreasterid species. Both the disc and stem epidermis were immunolabeled, although the labeling was much stronger in the former (Fig. 10). At this level an intense labeling may be observed in the secretory granules of the cells of the adhesive epidermis (Fig. 11). The pattern of immunoreactivity in the stem epidermis differed from one species to another. In *Linckia laevigata,* the apex of all epidermal cells was labeled, in a way similar to what was observed in knob-ending tube feet. On the other hand, in all the other species the immunolabeling was restricted to scattered granule-containing secretory cells (Fig. 10). In addition, in the three oreasterid species the large spumous glandular cells associated with the skeleton were always intensely labeled (Fig. 10). Finally, in all the species investigated the cuticle was strongly immunoreactive.

Reinforced Disc-Ending Tube Feet

Reinforced disc-ending tube feet occurred in five of the considered species: in the forcipulatids *Asterias rubens* and *Marthasterias glacialis,* in the spinulosid *Echinaster sepositus,* in the valvatid *Asterina gibbosa,* and in the velatid *Crossaster papposus* (Table 2). Reinforced disc-ending tube feet have more or less the same external morphology as simple disc-ending tube feet: they comprise a basal cylindrical stem topped by a flattened disc (Figs. 1C, 6). However, the diameter of their disc never greatly exceeds that of the stem. The histological organization of the tube feet of *E. sepositus* and *C. papposus* is close to that of simple disc-ending tube feet except that their lumen does not extend into the disc margin and that

their disc connective tissue layer sends numerous bundles of collagen fibers distally that insinuate themselves within the adhesive epidermis (Fig. 7). The internal organization of the tube feet of the three other species differs much more from that of simple disc-ending tube feet by the fact that most of the volume of the disc is occupied by a specialized arrangement of the tissue layers. The lumen tapers off to a point, ending just below the center of the disc. The connective tissue layer is particularly developed, consisting mainly of a dense circular structure—the terminal plate—that underlies and supports the whole disc. Distally, the surface of the terminal plate is drawn out into a series of radial lamellae that thrust into the disc epidermis (Figs. 1C, 6). This epidermis is particularly thickened compared to the stem epidermis.

The reinforced disc-ending tube feet were strongly and specifically labeled with the different antisera (Table 2). Immunolabeling was once more restricted to the epidermal layer and was much stronger in the disc than in the stem. In the former, it was localized in the secretory granules of adhesive cells, whereas in the latter it was present in scattered secretory cells (Figs. 12, 13). The cuticle covering both the disc and the stem was also strongly labeled in all the species possessing reinforced disc-ending tube feet (Fig. 12).

DISCUSSION
Variability of the Morphology of Sea Star Tube Feet

Our comparative morphological study of sea star tube feet allowed us to recognize three main morphotypes: knob-ending tube feet, simple disc-ending tube feet, and reinforced disc-ending tube feet. This goes beyond the traditional subdivision of knob-ending tube feet vs. disc-ending tube feet (Lawrence,
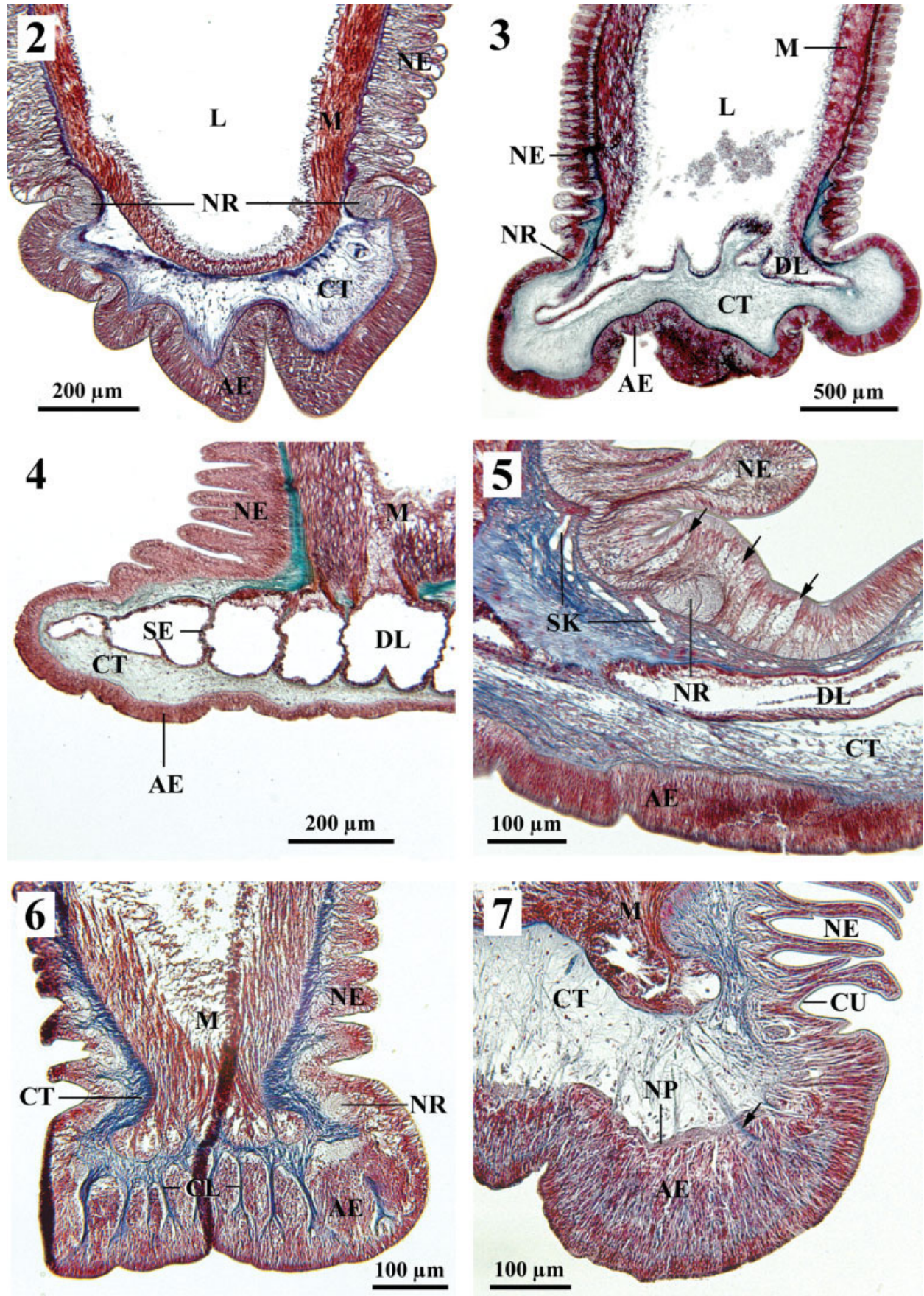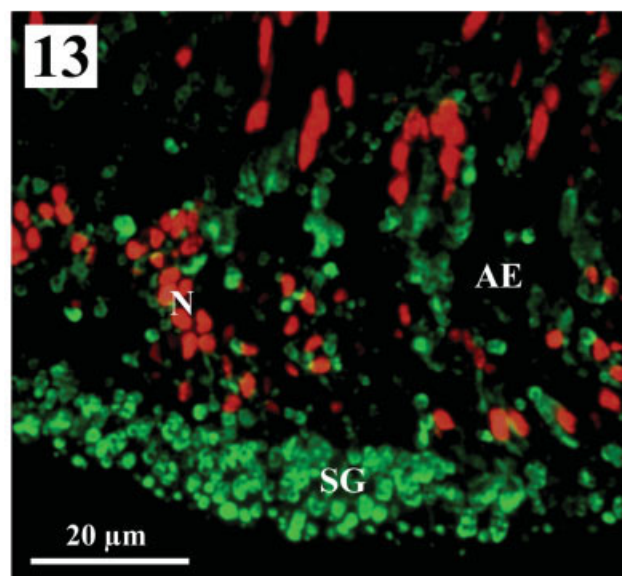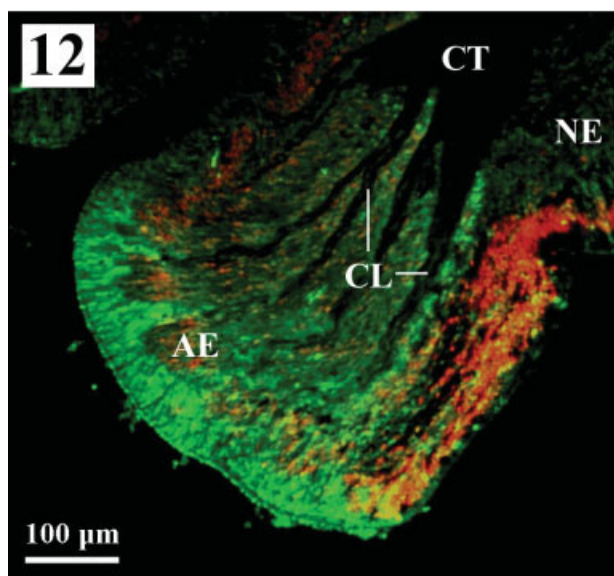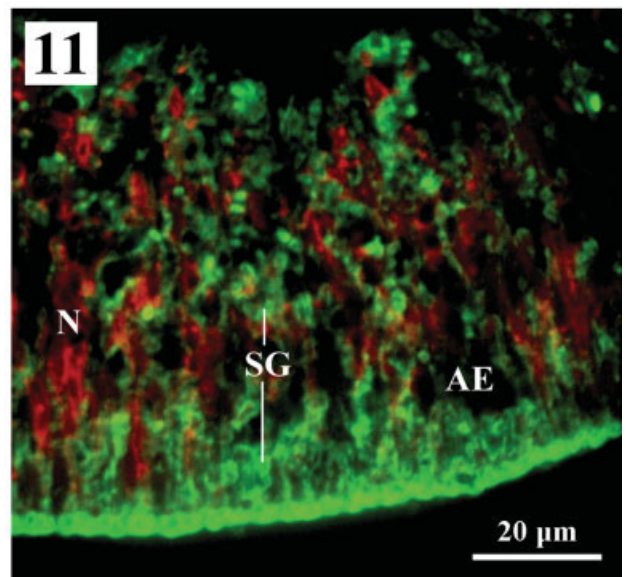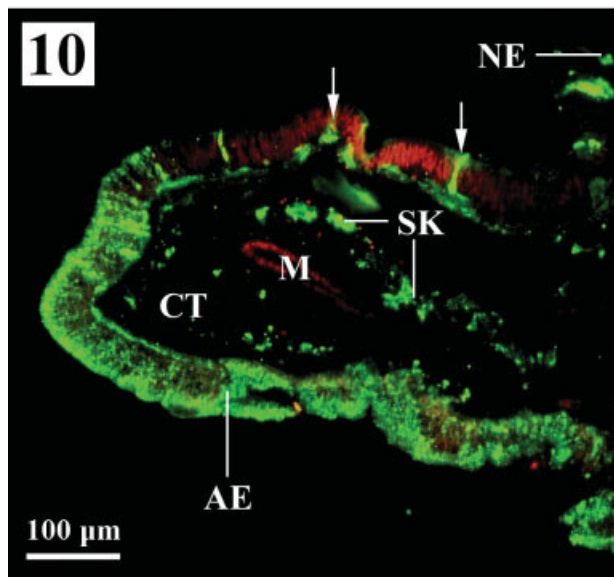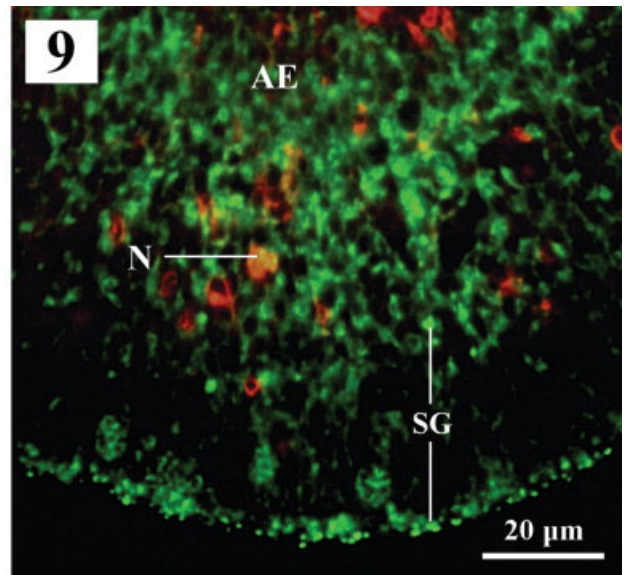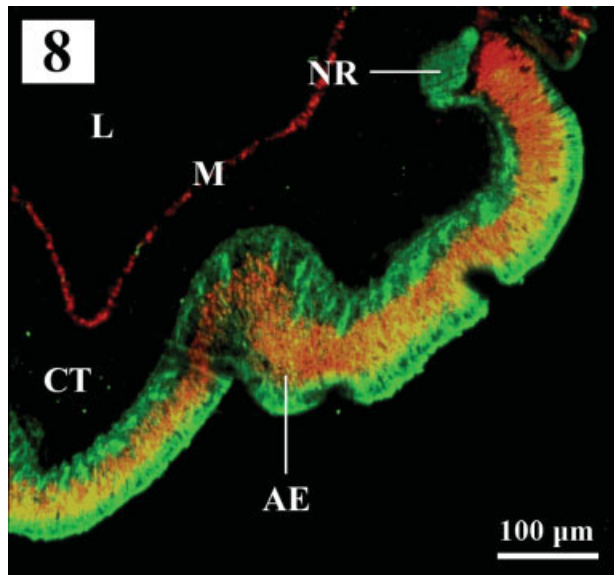
Figures 2–7

1987; Flammang, 1996), but is in accordance with the recent report of Vickery and McClintock (2000), who also described three tube foot morphotypes in asteroids. According to their descriptions, their three tube foot morphotypes, i.e., pointed nonsuckered, flat-tipped nonsuckered, and flat-tipped suckered, would approximately correspond to knob-ending, simple disc-ending, and reinforced disc-ending tube feet, respectively. In this study, we used a different terminology because the names used by Vickery and McClintock (2000) imply a suction cup-like functioning of the tube foot for which there is no consensus (see Flammang, 1996, for review).

Although easily distinguishable, the three tube foot morphotypes described in this study are clearly variations around a common design. From a purely morphological point of view (i.e., without any evolutionary aim), both knob-ending tube feet and reinforced disc-ending tube feet can be derived from simple disc-ending tube feet like the ones observed in *Linckia laevigata*. Knob-ending tube feet are constructed by reducing the diameter of the tube foot tip and giving it a convex apex. The rounded or pointed knob formed this way is probably an adaptation to dig in soft substrata. Indeed, knob-ending tube feet were observed in paxillosids which all have the ability to completely bury themselves (Lawrence, 1987; Blake, 1990). They dig vertically by thrusting their tube feet into the sediment and then bending them in the abradial direction, thus pushing the sediment particles laterally from beneath the body (Heddle, 1967). The knob-shaped apex of the tube feet would therefore facilitate the thrusting movement. Except for paxillosids, only a very limited number of asteroid species are known to completely bury themselves (Blake, 1989). The valvatid *Archaster typicus* is one of them (Jangoux, 1986). Contrary to what occurs in paxillosids, the tube feet in this species end with a simple disc. The structure of the disc, however, is peculiar by the fact that the lumen is divided radially by collagenous septa lined by a myomesothe-

lium. Interestingly, a similar morphological organization is found in the disc of the penicillate tube feet of spatangoid echinoids, which are also sand burrowers, and is thought to confer great mobility and plasticity to the tube foot disc (Flammang et al., 1990). In *A. typicus,* it would enable modifications of the disc shape and orientation to allow digging in the sediments. In addition to *A. typicus,* simple disc-ending tube feet were found in most valvatid species investigated which use them mainly for locomotion. The tissular organization of the disc in simple disc-ending tube feet provides the tube foot with a large flat distal surface that can be used in locomotion on soft substrata (e.g., in *Pentaceraster mammillatus* and *Protoreaster lincki)* as well as on hard substrata (e.g., in *Acanthaster planci* or in *Culcita schmideliana).* Simple disc-ending tube feet are also used for attachment to the substratum in a few valvatid species (e.g., in *A. planci* or in *Linckia laevigata).* Reinforced disc-ending tube feet differ from simple disc-ending tube feet by the fact that their disc connective tissue layer sends bundles of collagen fibers distally that spray throughout the epidermis in an arborescent manner, insinuating themselves between the epidermal cells and reaching up to the cuticle. These fibers presumably function as tension-bearing structures, transferring the stresses due to adhesion from the distal cuticle to the connective tissue of the stem. Reinforced disc-ending tube feet are therefore presumably better designed for strong adhesion than simple disc-ending tube feet. Contrary to the other types of tube feet that were restricted to species from a single order of Asteroidea, reinforced disc-ending tube feet were observed in species from the orders Forcipulatida, Spinulosida, Valvatida, and Velatida. Morphological differences occur, however, between the reinforced disc-ending tube feet of these species and there is a gradient of complexity from the tube feet of the velatid *Crossaster papposus* which have few uniformly distributed bundles of collagen fibers to the tube feet of the forcipulatid *Asterias rubens* which possess a complex array of radial collagenous laminae.

For Blake (1990), morphological diversity in the Asteroidea is a result of adaptation to habitat and, specifically, tube foot morphology is an adaptation to varying types of substrata. On the other hand, according to Vickery and McClintock (2000), tube foot morphotype would be related to the order of sea star considered (for example, paxillosids would possess only knob-ending tube feet, valvatids only simple disc-ending tube feet, and forcipulatids only reinforced-disc-ending tube feet) and only small morphological variations within each category of tube foot would reflect adaptations to variable environments. Our results suggest that neither of these hypotheses is completely true or false. Knob-ending tube feet are adapted for digging in soft substrata and are found exclusively in paxillosids that have the ability of self-burial. Blake (1989) reported that

Figs. 2–7. Histology of asteroid tube feet. Fig. 2. Longitudinal section through a knob-ending tube foot of *Luidia savignyi.* Fig. 3. Longitudinal section through a simple disc-ending tube foot of *Acanthaster planci.* Fig. 4. Longitudinal section through the disc of a simple disc-ending tube foot of *Archaster typicus.* The section goes through the margin of the disc to show the radial septa. Fig. 5. Detail of a longitudinal section through the disc of a simple disc-ending tube foot of *Protoreaster lincki* (arrows indicate the spumous glandular cells). Fig. 6. Longitudinal section through a reinforced disc-ending tube foot of *Asterina gibbosa.* The section goes through the margin of the disc to show the connective tissue radial lamellae. Fig. 7. Detail of a longitudinal section through the disc of a reinforced disc-ending tube foot of *Crossaster papposus* (arrow indicates a bundle of collagen fibers penetrating the adhesive epidermis). AE, adhesive epidermis; CL, connective tissue radial lamellae; CT, connective tissue layer; CU, cuticle; DL, disc lumen; L, water-vascular lumen; M, myomesothelium; NE, nonadhesive epidermis; NP, nerve plexus; NR, nerve ring; SE, septum; SK, skeleton.

Figures 8–13

the valvatid *Archaster typicus,* which also has the capacity to bury itself completely, is homeomorph with paxillosid species of the genus *Astropecten.* This homeomorphy, however, does not extend to the tube feet. In this case, therefore, the taxonomic position of the species seems to supplant habitat constraints. On the other hand, the valvatid *Asterina gibbosa,* which lives in the rocky intertidal, possesses reinforced disc-ending tube feet, whereas all other valvatid species investigated possess simple disc-ending tube feet. This stresses that, contrary to the assumption of Vickery and McClintock (2000), tube foot morphology cannot be used as a taxonomic character at the ordinal level. Like *A. gibbosa,* most species inhabiting turbulent environments, where strong attachment to the substratum is required, possess reinforced disc-ending tube feet regardless of their taxonomic position. So, this time, adaptation to habitat overrides belonging to a particular order. The present study thus suggests that tube foot morphology is influenced by species habitat, but within limits imposed by the evolutionary lineage. It is not surprising, therefore, that the most important tube foot diversity in terms of structure and function is observed within the order Valvatida, which is the largest and the most heterogeneous asteroid order (Blake, 1990; Clark and Downey, 1992).

Variability of the Composition of the Adhesive in Sea Star Tube Feet

Whatever their morphology and function, all asteroid tube feet possess a well-developed sensory-secretory epidermis (i.e., the adhesive epidermis) at the level of their distal tip. In every species investigated so far, this epidermis encloses a duo-gland adhesive system that always comprises two types of cells: adhesive cells containing large heterogeneous secretory granules and de-adhesive cells full of small homogeneous granules (Flammang, 1996). However, the exact nature and composition of the adhesive material is not known for any asteroid species, but polyclonal antibodies are available that specifically recognize this material in *Asterias rubens* (Flam-

Figs. 8–13. Immunofluorescent labeling of asteroid tube feet with antibodies raised against the adhesive material of *Asterias rubens* (immunoreactive structures are labeled in green while nuclei appear in red). Fig. 8. Knob-ending tube foot of *Astropecten polyacanthus.* Fig. 9. Knob epidermis a tube foot of *Luidia savignyi.* Fig. 10. Simple disc-ending tube foot of *Culcita schmideliana* (arrows indicate the spumous glandular cells). Fig. 11. Apical disc epidermis of a simple disc-ending tube foot of *Linckia laevigata.* Fig. 12. Reinforced disc-ending tube foot of *Marthasterias glacialis.* Fig. 13. Apical disc epidermis a reinforced disc-ending tube foot of *Crossaster papposus.* AE, adhesive epidermis; CL, connective tissue radial lamellae; CT, connective tissue layer; L, water-vascular lumen; M, myomesothelium; NE, nonadhesive epidermis; N, nuclei; NP, nerve plexus; NR, nerve ring; SG, secretory granules; SK, skeleton.

mang et al., 1998). These antibodies were used to investigate the variability of the adhesive secretions in the different asteroid families by looking for cross-reactivity in immunohistochemistry. The results are very homogeneous: in the 13 species considered, there was a strong immunolabeling of the adhesive epidermis as well as a weak to moderate immunolabeling of the nonadhesive epidermis and of the cuticle. This immunoreaction pattern corresponds to the one that was originally described for the tube feet of *A. rubens* (Flammang et al., 1998) and has been confirmed in this study using a different fixation.

In every species investigated, the strongest immunoreactivity was observed in the tube foot adhesive epidermis where secretory granules of the adhesive cells were clearly labeled (a previous TEM study showed that the secretory granules of de-adhesive cells are never immunolabeled; Flammang et al., 1998). This important immunoreactivity seems to be independent of the taxon considered (order, family, etc.), of the type of tube foot (reinforced disc-ending, simple disc-ending, or knob-ending), or of the function of the tube feet (attachment, locomotion, burrowing). This indicates that the adhesives in all these species are closely related, probably sharing many identical molecules or, at least, many identical epitopes on their constituents. This relatedness was unexpected. Indeed, at the ultrastructural level, interspecific differences occur regarding the number of adhesive cell types present in the sensory-secretory epidermis as well as the aspect of their secretory granules. Two types of adhesive cells co-occur in the tube foot epidermis of some species (Chaet and Philpott, 1964; Chaet, 1965; Flammang et al., 1994; Flammang, 1995), their secretions being released simultaneously and mixing to form the adhesive material (Flammang et al., 1998); while in other species only one type of adhesive cells is present (Engster and Brown, 1972; Flammang, 1995). The secretory granules of the adhesive cells are made up of at least two materials of different electron density which gives them a complex heterogeneous ultrastructure (Chaet and Philpott, 1964; Harrison and Philpott, 1966; Engster and Brown, 1972; Flammang et al., 1994; Flammang, 1995). Flammang (1996) reviewed the ultrastructure of these granules and distinguished two broad categories: 1) dense-cored granules consisting of an electron-dense core surrounded by less-dense material, and 2) granules with a central filamentous bundle resembling granules of the previous category but in which the core is made up of a parallel arrangement of fibrils and/or rods. The significance of these ultrastructural differences between different asteroid taxa is unknown at present. However, Engster and Brown (1972) pointed out a relationship between the internal organization of adhesive cell secretory granules and species habitat: 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. They suggested that the different substructure of the adhesive cell granules would depend on the nature and composition of their contents that, in turn, could be related to the possible adhesive strength of the tube feet. Our immunohistochemical results do not support this hypothesis but, on the contrary, indicate great similarities in the composition of the various adhesives of sea stars. Such a similarity suggests comparable adhesive capabilities for every tube foot morphotype and would explain the observation that both *Astropecten* and *Luidia,* although they are primarily burrowers in sand, can also locomote on hard surfaces and climb the sides of an aquarium tank (Romanes and Ewart, 1881; pers. obs.). However, our results do not prove that the adhesive granule contents of the three tube foot morphotypes of asteroids are identical either. Differences in the adhesive secretion composition may exist that are not detected by the antisera we used and that could account for the differences observed in the structure and function of asteroid tube feet.

In all the asteroid species studied, in addition to the adhesive epidermis, the cuticle and the nonadhesive epidermis were also immunolabeled, although much less strongly. In *Asterias rubens,* Flammang et al. (1998) have shown that the footprint material, against which the antibodies were raised, included mostly the contents of the adhesive cells of the disc epidermis but also some constituents of the cuticle. It is not surprising, therefore, that this structure is reactive to the antibodies in the other sea star species, the constant labeling suggesting a similarity of composition within the class. As far as the epidermal cells of the nonadhesive epidermis are concerned, it was proposed that these cells could enclose cuticular precursors that are recognized by the antibody as being cuticular material or could secrete a general-purpose mucus that could occasionally be incorporated into the adhesive footprints (Souza Santos and Silva Sasso, 1968; Flammang et al., 1998). Whatever the explanation for the immunolabeling of the nonadhesive epidermis, it is at this level that clear differences occur between different asteroid species. In the different species of paxillosids as well as in the valvatid *Linckia laevigata,* the apex of all nonadhesive epidermal cells was labeled, while in all the other species investigated the immunolabeling was restricted to scattered granule-containing secretory cells. The functional and evolutionary significance of these different immunoreaction patterns, however, remains enigmatic. Also enigmatic is the function of the spumous glandular cells that were observed in the oreasterid species and whose contents cross-reacted strongly with the different antisera.

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