*Biofouling*, 2006; 22(3): 187 – 200

Taylor & Francis
Taylor & Francis Group

# Morphology and tenacity of the tube foot disc of three common European sea urchin species: a comparative study

ROMANA SANTOS* & PATRICK FLAMMANG

*Université de Mons-Hainaut, Académie universitaire Wallonie-Bruxelles, Laboratoire de Biologie marine, Mons, Belgium*

*(Received 17 October 2005; accepted 6 April 2006)*

**Abstract**
The variation in tenacity of single tube feet from three sea urchin species with contrasted habitats was assessed and correlated with the ultrastructure of their adhesive secretory granules. The tube feet of *Arbacia lixula* and *Sphaerechinus granularis* have larger discs and more complex adhesive granules than those of *Paracentrotus lividus*, but *A. lixula* attaches to glass with significantly lower tenacity (0.05 – 0.09 MPa) than the other two species (0.10 – 0.20 and 0.11 – 0.29 MPa, respectively). However, the estimated maximal attachment force one tube foot can produce is similar for all three species investigated. No clear relationship between tube foot size, tenacity, adhesive secretory granule ultrastructure and species habitat can therefore be established. For *P. lividus* the tenacity of single tube foot discs on four different smooth substrata was also compared, which showed that both the total surface energy and the ratio of polar to non-polar forces at the surface influence tube foot attachment strength. This influence of the surface characteristics of the substratum appears to affect the cohesiveness of the adhesive secretion more than its adhesiveness.

**Keywords:** Arbacia, Paracentrotus, Sphaerechinus, *adhesion, cohesion, secretory granule ultrastructure, surface energy*

## Introduction

Regular sea urchins possess hundreds of small, fleshy mobile appendages, the tube feet. Among them, those directed towards the bottom, the adoral tube feet, are involved in strong attachment to the substratum. They consist of a proximal extensible cylinder, the stem, which is topped apically by an enlarged and flattened structure, the disc (Flammang, 1996). The stem and the disc form together a functional unit, the stem allowing the tube foot to lengthen, flex and retract whereas the disc makes contact with and adheres to the substratum. Although tube feet can adhere very strongly to the substratum, they are also able to detach easily and voluntarily from it, before reinitiating another attachment-detachment cycle (Thomas & Hermans, 1985; Flammang, 1996). This is due to the presence of a duo-glandular adhesive system in the epidermis of the disc, which comprises two types of secretory cells that release separately, adhesive and de-adhesive secretions (Hermans, 1983; Flammang & Jangoux, 1993; Flammang, 1996). Adhesive secretions are delivered through the disc cuticle onto the surface where they form a thin film that binds the tube foot disc to the substratum (Flammang et al. 2005). De-adhesive secretions are released within the cuticle, where they might function as enzymes, causing the discarding of its outermost layer, the so-called fuzzy coat. Thus, after detachment, most of the adhesive material remains strongly attached to the substratum as a footprint (Flammang & Jangoux, 1993; Flammang, 1996; Flammang et al. 1998a).

In adhesion studies, the physico-chemical properties of the adhesive and the surface properties of the adherends (i.e. the surfaces to be joined together) both influence the strength of adhesion (Waite, 1987). Therefore, measurement of the adhesion strength of sea urchin tube feet and of its variation under different conditions may give clues to how their temporary adhesive functions. Reports on the attachment strength of echinoderm adhesive organs, including tube feet, are scarce (Paine, 1926; Flammang & Walker, 1997; Haesaerts et al. 2005; Santos et al. 2005). At present, only one study, done

Correspondence: Romana Santos or Patrick Flammang, Université de Mons-Hainaut, Laboratoire de Biologie marine, Pentagone 2B 6 Avenue du Champ de Mars, B-7000 Mons, Belgium. E-mail: romana_santos@yahoo.com or Patrick.Flammang@umh.ac.be
*Present address: Instituto de Tecnologia Química e Biológica, Mass Spectrometry Laboratory, Avenida da República (EAN), Apartado 127, 2781-901 Oeiras, Portugal.

ISSN 0892-7014 print/ISSN 1029-2454 online © 2006 Taylor & Francis
DOI: 10.1080/08927010600743449

on asteroids, has evaluated single tube foot tenacity on substrata with different chemical characteristics (Flammang & Walker, 1997). These authors reported that tube foot tenacity was proportional to the polarity (estimated by water drop contact angle) of the substratum; i.e. tube feet adhere more strongly to polar than to non-polar substrata. However, the substrata used also presented different roughnesses, rendering comparisons non-valid. Indeed, it was recently demonstrated that substratum roughness influence the adhesion strength of echinoderm tube feet (Santos et al. 2005) as it does for many other organisms, e.g. Granhag et al. (2004).

The chemical composition of sea urchin tube foot adhesive and its variation among species has not been reported. A link has been suggested, however, between the ultrastructure of the secretory granules of adhesive cells and the nature of their contents (Flammang, 1996). In asteroids for example, Engster and Brown (1972) pointed out a relationship between the internal organization of adhesive cell secretory granules and species habitat: sea stars confined to a 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.

The aim of the present study was to measure the adhesion strength of single sea urchin tube feet, and its variation according to the species and substrata under consideration. Adhesion strength was measured as critical removal force and as tenacity, the latter representing the adhesion force per unit surface area, which is therefore a size-independent parameter. Three echinoid species (*Arbacia lixula, Paracentrotus lividus* and *Sphaerechinus granularis*) that belong to three different echinoid orders (Smith, 1992), were chosen in view of their contrasting habitats. In order to search for possible correlations between tube foot tenacity and morphology, a detailed comparative analysis of the morphology of the discs of the adoral tube feet from the three species was performed, with special emphasis on the ultrastructure of the adhesive cells. Moreover, for one of the species, the tenacity of single tube foot discs was also evaluated and compared on four different smooth substrata.

## Material and methods

### *Study sites and specimens collection*

Sea urchins of the three species were collected in the Mediterranean Sea (Banyuls-sur-mer, France), in a subtidal rocky area with sandy bottoms. The arbacioid *Arbacia lixula* (Linné, 1758) and the echinoid *Paracentrotus lividus* (Lamarck, 1816) were collected on vertical rocky boulders of considerable size, in a shallow area (1 – 3 m) exposed to wave action. Although the populations of the two species overlap, individuals of *A. lixula* were usually observed deeper than individuals of *P. lividus*. The temnopleuroid *Sphaerechinus granularis* (Lamarck, 1816) was found in deeper areas (10 m) with sandy bottoms and low hydrodynamism. After collection, the animals were kept in re-circulating aquaria at 14 – 15°C and 35‰ where they were placed in netting bags to prevent attachment to the aquarium walls. Individuals of *P. lividus* were also obtained from an aquaculture facility located in the north of France (Luc-sur-Mer, Normandy). These specimens were used to measure adhesion strength on different substrata.

### *Morphology and ultrastructure of tube foot discs*

For external morphometric measurements ten tube feet were dissected from five randomly chosen sea urchins of each species. These freshly-cut tube feet were measured with a Leica MZ8 binocular equipped with a graduated eyepiece.

For light microscopy (LM), unattached tube feet were dissected from individuals of the three echinoid species and fixed in Bouin's fluid for 24 h. They were then dehydrated in a sequence of graded ethanol and embedded in paraffin wax (Paraplast; Sigma, Steinhem, Germany) using a routine method (Gabe, 1968). The tube feet were sectioned longitudinally at a thickness of 7$\mu$m with a Microm HM 340E microtome and the sections were collected on clean glass slides. These sections were stained with Masson's trichrome (Gabe, 1968) and photographed with a Leitz Orthoplan light microscope equipped with a Leica DC 300F digital camera.

For scanning electron microscopy (SEM), tube feet fixed in Bouin's fluid as described above were dried by the critical point method (using $CO_2$ as transition fluid), mounted on aluminium stubs and coated with gold in a sputter coater. Tube foot disc skeletal elements were obtained by incubating dissected discs in 10% (v/v) common bleach. The cleaned ossicles and spicules were rinsed in distilled water, air-dried, mounted on aluminium stubs and coated with gold. Both tube feet and disc skeletal elements were observed with a JEOL JSM-6100 scanning electron microscope.

For transmission electron microscopy (TEM), tube feet were fixed in 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.8; adjusted to 1030 mOsm with NaCl) for 3 h at 4°C. Then they were rinsed in cacodylate buffer and post-fixed for 1 h in 1% $OsO_4$ in the same buffer. After a final buffer

wash, they were decalcified with a solution of ascorbic acid according to the method of Dietrich and Fontaine (1975), dehydrated in graded ethanols, and embedded in Spurr's resin. Longitudinal ultrathin sections (about 80 nm in thickness) were cut with a Leica UCT ultramicrotome equipped with a diamond knife, collected on copper grids, and stained with uranyl acetate and lead citrate before observation with a Zeiss LEO 906E transmission electron microscope.

*Tenacity of single tube feet*

Adhesion force measurements of single tube feet were performed with an electronic dynamometer (AFG 10 N; Mecmesin, Horsham, UK) attached to a Mecmesin-Versa Test motorised stand. This dynamometer measures forces up to 10 N with a precision of 0.002 N. Experiments were performed with sea urchins totally immersed in containers filled with seawater. Specimens were put upside-down (to induce tube foot attachment) and a 1 $cm^2$ piece of glass substratum, connected to the dynamometer by a surgical thread, was presented to the tube feet. When a single tube foot remained attached to the substratum for at least 10 sec, the dynamometer was moved upwards at a constant speed of 15 mm $min^{-1}$ in order to apply a force normal to the disc. After tube foot detachment, the maximum adhesive force, or critical removal force, was recorded (Flammang & Walker, 1997; Santos et al. 2005). The piece of substratum was then immediately immersed for 1 min in a 0.05% aqueous solution of the cationic dye crystal violet to stain the footprint left by the tube foot after it had become detached (Flammang et al. 1994; Santos et al. 2005). The footprint diameter was measured with a graduated eyepiece mounted on a Leica Laborlux light microscope, and used to calculate the maximal surface area of the whole circular footprint ($S_{max}$). Then, footprints were photographed and their digitised images analysed with Semaphore® software (Jeol, Tokyo, Japan) to measure their stained surface area ($S_{stain}$) (Santos et al. 2005). A minimum and a maximum values of tenacity ($T_{min}$ and $T_{max}$; expressed in N $m^{-2}$ or Pascal) were then calculated by dividing the measured attachment force (F; expressed in N) by the corresponding footprint maximal and stained surface area, respectively ($S_{max}$ and $S_{stain}$; expressed in $m^2$).

$$T_{min} = F/S_{max} \quad (1)$$

$$T_{max} = F/S_{stain} \quad (2)$$

Tenacity measurements were carried out on about 30 tube feet from at least 5 randomly chosen sea urchins of each species.

The influence of the type of substratum on the tenacity of single tube feet was also investigated in one of the species, *P. lividus*. For this purpose, tube foot tenacity was measured as described above, but in addition to pieces of glass substratum, tenacity was also measured on the smooth surface of three polymer substrata, viz. poly(methylmetacrylate) (PMMA), polypropylene (PP) and polystyrene (PS) (see Santos et al. 2005, for details). After each measurement, the piece of substratum was either replaced by a new piece from the same batch, or carefully cleaned.

Results were analysed with the Statistica® software (Statsoft Inc., Tulsa, OK, USA) in order to reveal intra- and interspecific differences in tube foot tenacity. When necessary, data were arcsin- or log-transformed before comparison by multi-factorial analysis of variance (ANOVA) and Tukey tests for multiple comparisons.

## Results

*External morphology and ultrastructure of tube feet*

In the three species considered, adoral tube feet consisted of an extensible cylinder, the stem, topped by a flattened extremity, the disc (Figure 1A–C). In all the species considered, the tube foot discs (0.7–1.2 mm in diameter) were always larger than their stems (0.5–0.6 mm in diameter). The tube foot discs of *A. lixula* and *S. granularis* were significantly larger than those of *P. lividus*, both in terms of diameter and surface area ($p_{Tukey} < 0.001$) (see Table I).

SEM observations of the disc revealed the presence of a large central circular area separated by a groove from a narrow peripheral area, the latter being continuous with the stem. In the three species considered, both the central and peripheral areas presented cilia (Figure 1, D–I). The central area was covered with uniformly distributed, single cilia ranging from 1–3 $\mu$m in length, whereas the peripheral area presented clusters of cilia 4–5 $\mu$m long. In *S. granularis* these peripheral ciliary clusters were arranged in radial rows around the central area, each row standing on a bulge of the peripheral area (Figure 1F). Common to the three echinoids was the presence, in non-attached tube feet, of a central depression where the single cilia were particularly abundant (Figure 1, G–I).

Internally, the tube foot disc of sea urchins is supported by a calcified skeleton composed of two superposed structures, viz. a distal rosette and a proximal frame. Both structures are ring-shaped and disposed in a circle around the ambulacral lumen. In the three species considered, the rosette consisted of four or five large ossicles with several finger-like projections, which extended towards the apical

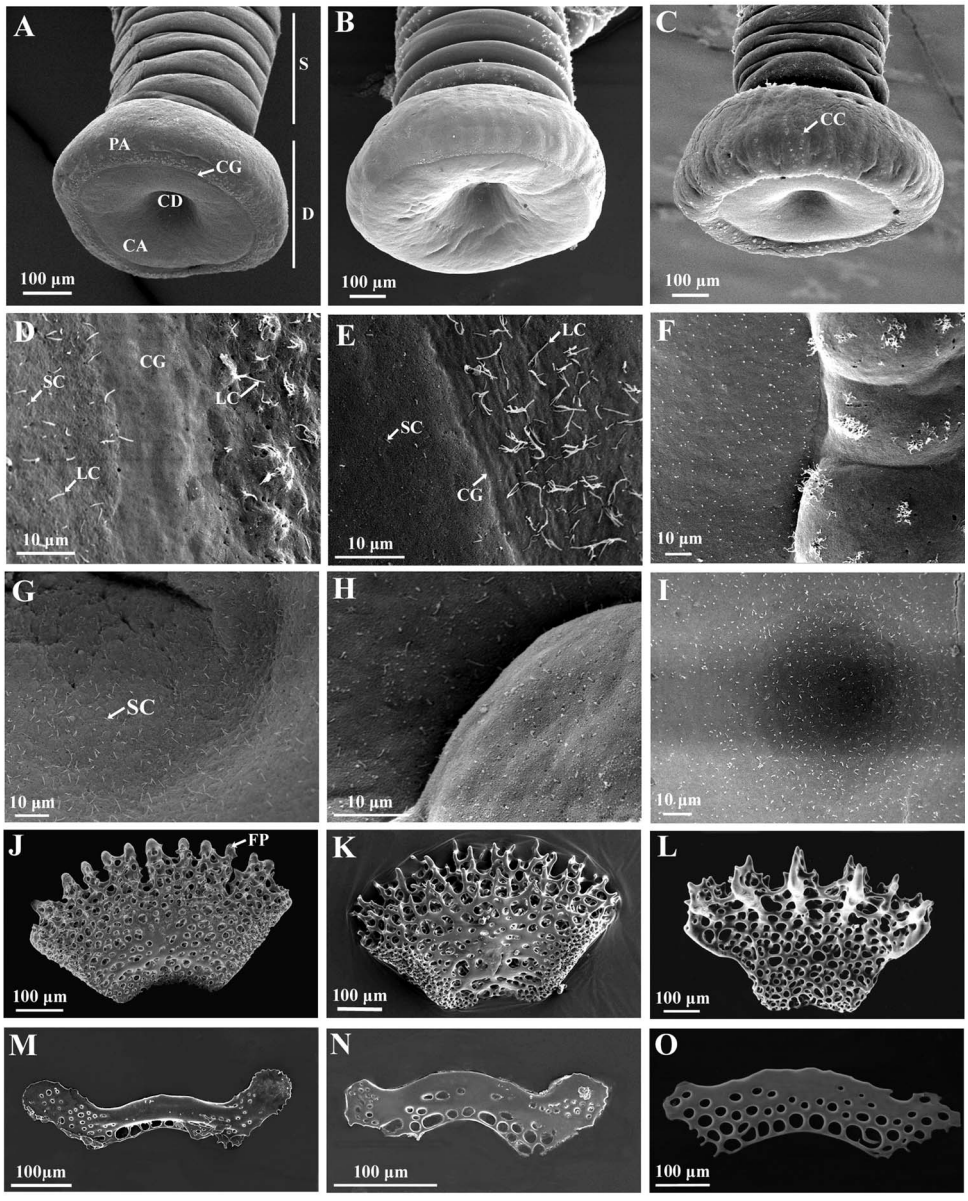


Figure 1. External morphology of non-attached adoral tube feet and their ossicles in *A. lixula* (A,D,G,J,M), *P. lividus* (B,E,H,K,N) and *S. granularis* (C,F,I,L,O). General view of adoral tube feet (A–C); detailed view of the peripheral (D–F) and central (G–I) areas of the disc; ossicle of the rosette (J–L); spicule of the frame (M–O). CA, disc central area; CC, ciliary clusters; CD, disc central depression; CG, circular groove; D, disc; FP, finger-like projection; LC, long cilia; PA, disc peripheral area; S, stem; SC, short cilia.

Table I. Morphometric measurements (mean ± SD, $n = 5$) in the three echinoid species considered.

| | Species | | | 1-factor ANOVA | |
|---|---|---|---|---|---|
| | *A. lixula* | *P. lividus* | *S. granularis* | $F_{2,12}$ | $p$ |
| Morphometric measurement | | | | | |
| A. Test | | | | | |
| Diameter (mm) | $43.46 \pm 7.75^{a}$ | $44.58 \pm 4.04^{a}$ | $87.22 \pm 6.59^{b}$ | 78.0 | $<0.001$ |
| Height (mm) | $21.13 \pm 5.03^{a}$ | $22.83 \pm 4.48^{a}$ | $53.17 \pm 5.50^{b}$ | 64.5 | $<0.001$ |
| B. Fresh tube foot discs | | | | | |
| Diameter (mm) | $1.160 \pm 0.158^{b}$ | $0.666 \pm 0.061^{a}$ | $1.017 \pm 0.041^{b}$ | 43.0 | $<0.001$ |
| Surface area ($mm^2$) | $1.072 \pm 0.291^{b}$ | $0.350 \pm 0.062^{a}$ | $0.814 \pm 0.065^{b}$ | 43.0 | $<0.001$ |

Significant differences between means for a given parameter are indicated by letters in superscripts; means sharing at least one letter are not significantly different ($p_{Tukey} \geq 0.05$).

surface of the disc (Figure 1, J–L). The frame was made up of numerous small arc-shaped spicules (Figure 1, M–O). Although the tube feet of the three species possessed similar skeletal elements, those from *A. lixula* and *P. lividus* were comparatively more robust and dense than those from *S. granularis*.

In terms of histology, the structure of the tube feet is constant in all echinoid species. Their tissue stratification consists of four layers, viz. an inner myomesothelium surrounding the water-vascular lumen, a connective tissue layer, a nerve plexus and an outer epidermis covered externally by a cuticle (Figure 2).

The myomesothelium is composed of peritoneocytes that line the ambulacral lumen, and of myocytes that are arranged to form the retractor and levator tube foot muscle systems. The retractor muscle comes from the stem and anchors apically to the connective tissue of the disc at the level of the ossicles of the rosette (Figure 2B). The levator muscle also anchors to the connective tissue, at the level of the spicules of the frame on one end, and in the middle of the central area of the disc (diaphragm, see below) on the other end (Figures 2 and 3B). The connective tissue encloses collagen fibres, fibrocytes and various other types of mesenchymal cells (e.g. macrophages and spherulocytes), and the skeletal elements. It forms a circular structure, the terminal plate, which supports the whole disc. The centre of the terminal plate, called the diaphragm, is very much thinner than its margin, and caps the ambulacral lumen (Figures 2B, 3C). The distal surface of the terminal plate sends off numerous branching connective tissue protrusions, rich in collagen fibrils, which invade the epidermis up to the apex of the disc (Figure 3A, 3D, 3E). The thinnest, distal branches of these protrusions attach apically to the support cells of the epidermis (Figure 3D). Nerve tissue is present at the base of the disc as a nerve ring resulting from the thickening of the stem nerve plexus (Figure 2). The nerve ring gives rise to several radial branches that extend over the proximal surface of the terminal plate, where they run between the connective tissue protrusions. These radial nerve strands are made up of neurites that run mainly in a plane parallel to the apical surface of the tube foot disc. Just above the nerve plexus lies the epidermis, coated by a well-developed, multilayered glycocalyx, the so-called cuticle (Figure 3F). Two different epidermal organisations can be distinguished in the disc on the basis of the cell types they enclose, viz. a central adhesive epidermis extending over the central area of the disc and a peripheral sensory epidermis extending over the peripheral area of the disc (Figure 2). Only the central of these two areas is involved in tube foot attachment to the substratum. It encloses four types of cells, viz. support cells, sensory cells, adhesive secretory cells and de-adhesive secretory cells. All epidermal cells are flask-shaped, with an enlarged nucleus-containing basal part located on the distal surface of the connective tissue terminal plate, and a long, narrow apical neck extending up to the disc surface. The epidermal cells usually occur in clusters where the four types of cells are represented, the clusters being separated by connective tissue protrusions (Figure 3A–E). Support cells are the most abundant cells of the disc and possess a characteristic enlarged apical neck filled with intermediate filaments and bearing numerous microvilli (Figures 3D and 4C). Sensory cells have a single short apical cilium that traverses the cuticle. These cilia correspond to those observed on SEM pictures of the central area of the disc (Figure 1G–I). Adhesive secretory cells contain large spherical secretory granules in their cytoplasm. The adhesive granules in *A. lixula* varied from 400–700 nm in diameter and presented a highly organised cylindrical core consisting of 4–8 electron-dense parallel plates stacked one on the other, and surrounded by an electron-lucent material (Figure 4B). *S. granularis* on the other hand presented smaller adhesive granules (300–400 nm in diameter) with 2–3 electron dense parallel plates surrounded by a material of medium electron-density (Figure 4E). The disc epidermis of *P. lividus* presented two types of adhesive secretory cells. Those restricted to a small zone in the middle

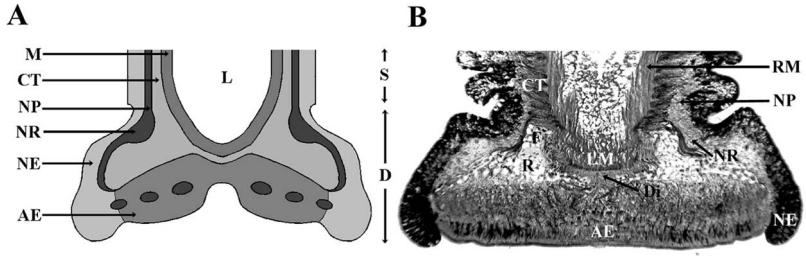


Figure 2. Histological structure of adoral tube foot from regular sea urchins. (A) Schematic drawing and (B) longitudinal section through an adoral tube foot of *A. lixula*. AE, adhesive epidermis; CT, connective tissue; Di, diaphragm; F, frame; LM, levator muscle; M, myomesothelium; NE, non-adhesive epidermis; NP, nerve plexus; NR, nerve ring; R, rosette; RM, retractor muscle.

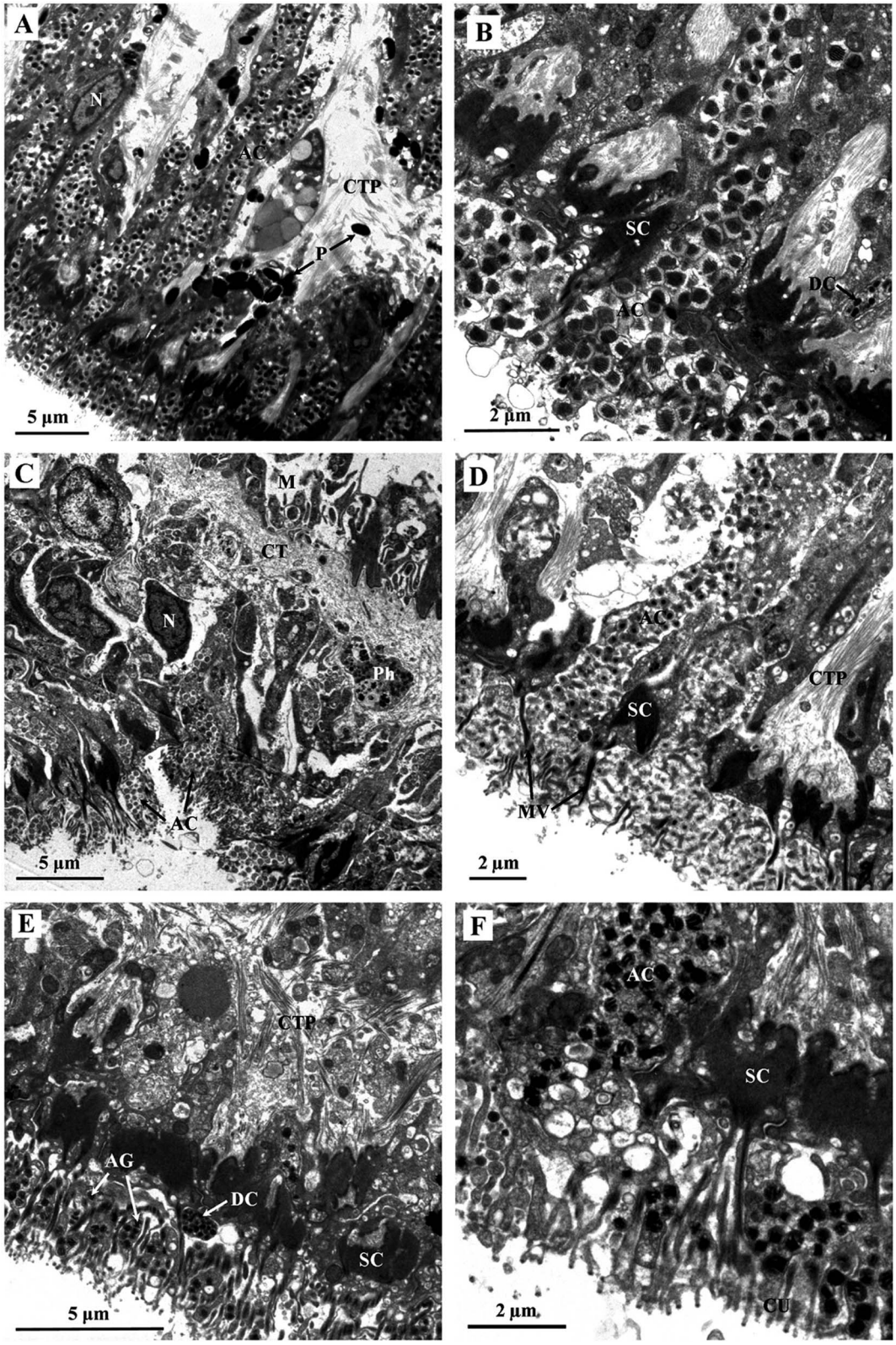


Figure 3. Ultrastructure of the disc of adoral tube feet of *A. lixula* (A,B), *P. lividus* (C,D) and *S. granularis* (E,F). General view of longitudinal sections through the tube foot disc (A,C,E); detail of secretory cells containing adhesive (B,D,F) and de-adhesive granules (B). AC, adhesive secretory cell; CT, connective tissue layer; CTP, connective tissue protrusion; CU, cuticle; DC, de-adhesive secretory cell; M, myomesothelium layer; MV, microvillar-like cell projection; N, nucleus; P, pigment cell; Ph, phagocyte; SC, support cell.

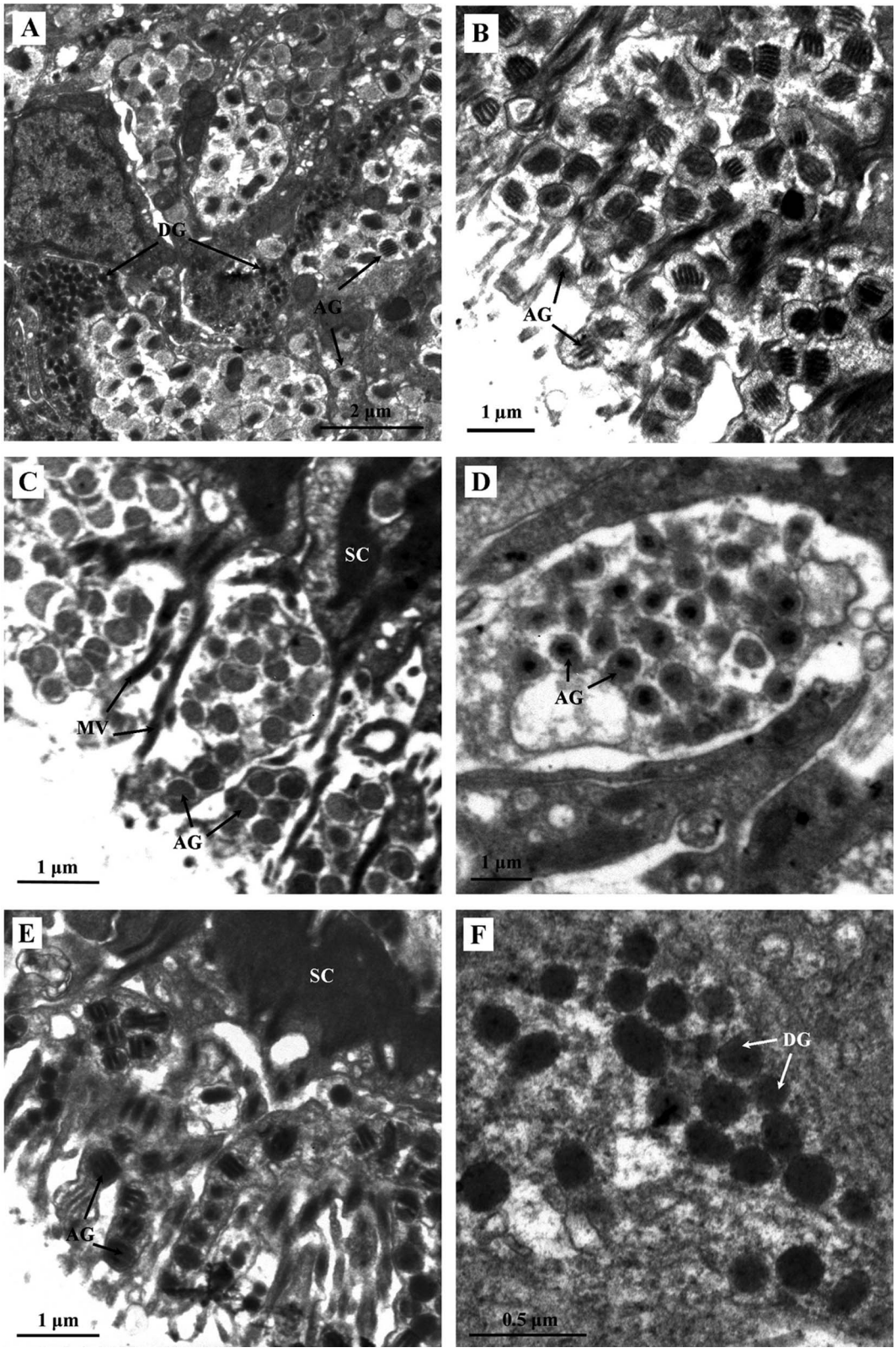


Figure 4. Ultrastructure of the secretory granules of the disc of adoral tube feet of *A. lixula* (A,B), *P. lividus* (C,D) and *S. granularis* (E,F). Detail of the aspect of adhesive (B–E) and de-adhesive granules (A,F); different aspect of adhesive granules in the central depression (C) and central area (D) of the disc of *P. lividus*. AG, adhesive granule; DG, de-adhesive granule; MV, microvillar-like cell projection; SC, support cell.

of the central area of the disc had granules 500–700 nm in diameter with a homogenous core of medium electron density, surrounded by a thin clear space (Figure 4C), whereas those distributed in all the rest of the central area enclosed smaller granules (300–500 nm in diameter) with a very electron dense small core, surrounded by a large electron-lucent rim (Figure 4D). Common to all three species was the presence of only one type of de-adhesive cells filled with small, membrane-bound elliptic secretory granules of 150–200 × 200–300 nm in size (Figure 4A and 4F), with a characteristic small subcuticular cilium in the apical part of the cell.

*Single tube foot tenacity*

Microscopic observation of adhesive footprints left on the substratum after tube foot detachment shows that they can be subdivided into complete footprints, which are circular and evenly stained (Figure 5A, 5B), and incomplete footprints, which may or may not be circular and present large areas devoid of stained material (Figure 5C, 5D). The latter represents on average 63, 50 and 16% of the total footprints in *P. lividus*, *S. granularis* and *A. lixula*, respectively. Among incomplete footprints, some clearly correspond to complete footprints in which part of the adhesive material has been torn off the substratum during detachment (adhesive mode of failure; Figure 5C). On the other hand, other incomplete footprints come from tube feet that have adhered with only a fraction of their disc surface (Figure 5D). In many cases, however, microscopic observation did not allow discrimination between both types of incomplete footprints. Consequently, the surface area of the footprints was evaluated in two different ways. It was either calculated from the diameter of the footprint ($S_{max}$) or measured on basis of the stained surface area of adhesive material ($S_{stain}$) (Figure 6). The maximal surface area is more accurate for footprints in which adhesive failure has occurred, but it overestimates the footprint surface area when the tube foot has adhered by only part of its disc surface. This last case is better described by the stained surface area which, on the contrary, underestimates the surface area in case of adhesive failure. The actual adhesion surface area thus probably lies between these two values. It is noteworthy that the largest difference between the two footprint surface areas was observed in *P. lividus*, with a ratio $S_{stain}/S_{max}$ of about 0.4, compared to the other two species in which this ratio was about 0.6 (Figure 6).

When footprint surface areas were compared to disc surface areas (Figure 6), it was found that,

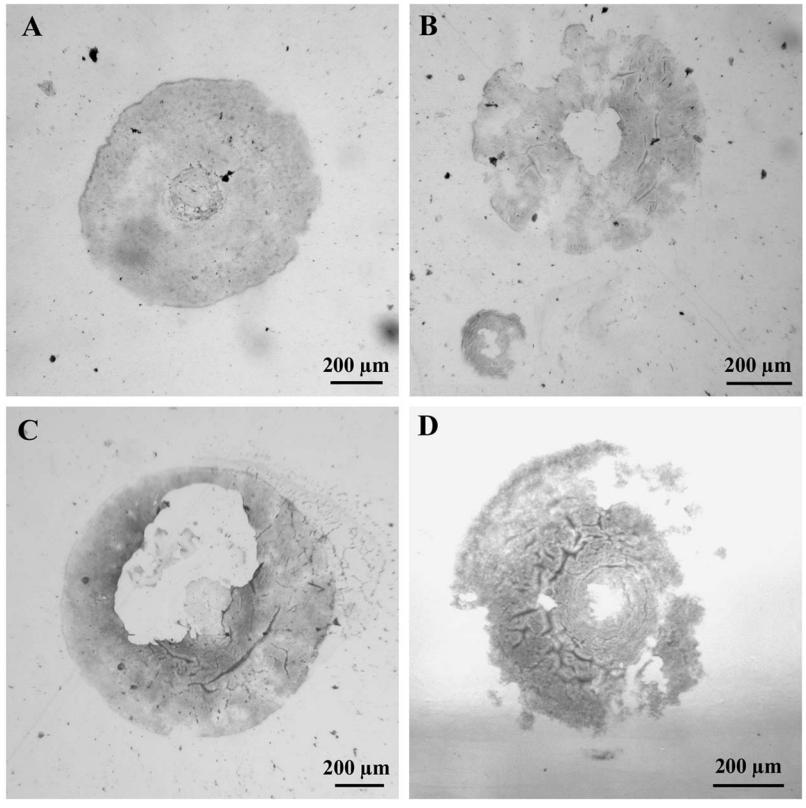


Figure 5. Microscopic aspect of footprints left on the substratum after tube foot detachment and staining with crystal violet. (A) Complete footprint left by one tube foot of *S. granularis* on glass. (B) The two types of complete footprints left by the tube feet of *A. lixula* on glass. (C,D) Incomplete footprints left by the tube feet of P. lividus on glass and poly(methyl methacrylate), respectively, and resulting from partial adhesive failure (C) or attachment by only one part of the disc (D).

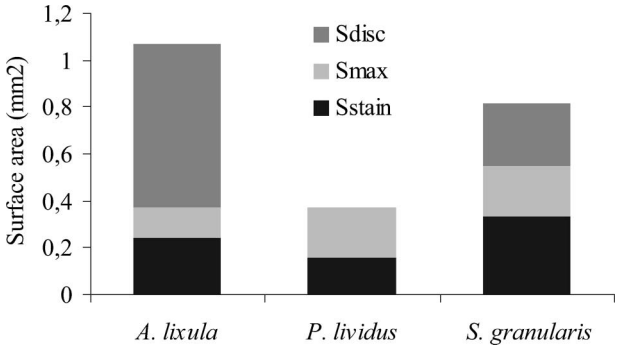


Figure 6. Mean values of the surface area of whole tube foot discs ($S_{disc}$) and of the surface area of footprints deposited on glass calculated from their diameter ($S_{max}$) or from stained adhesive material ($S_{stain}$) in *A. lixula, P. lividus* and *S. granularis*.

although *A. lixula* possesses the largest tube foot discs, this was the species which produced the smallest footprints, using on average only 22% ($S_{stain}$) to 34% ($S_{max}$) of its disc to attach. This result can be correlated with the reluctance of this species to attach to the glass substratum during the experiments. In fact, *A. lixula* often attached with the middle sensory zone of the disc central area, indicating that the smaller footprints produced by this species correspond to the contact of this middle zone during substratum exploration (small footprint in Figure 5B). In contrast, *P. lividus* usually attached to the glass substratum with the entire surface area of its disc ($S_{max} = S_{disc}$; Figure 6). *S. granularis* presented an intermediate situation, using on average 41 ($S_{stain}$) to 67% ($S_{max}$) of its disc adhesive surface to attach (Figure 6).

Both $S_{stain}$ and $S_{max}$ were used to calculate a maximum and a minimum tenacity, respectively. Tenacity measurements were first analysed in order to search for significant differences between the different individuals tested for each species. One-factor analyses of variance did not detect any significant inter-individual difference in either the minimum or the maximum tenacity (Table II). Therefore, data were pooled within each species.

In the three species, the measurement of single tube foot tenacity was made difficult by the presence of several hundreds of adoral tube feet and, in almost half of the measurements, more than just one tube foot attached to the experimental substratum. However, only measurements with less than ten tube feet were taken into account and these were divided into three groups (those measured with one single tube foot, with a group of 2–5 tube feet, or with a group of 6–10 tube feet). Two-factor ANOVAs were then used to test the respective influence of species and number of tube feet involved on tenacity (Table III). These tests revealed that both maximum and minimum tenacity varied significantly between species.

Table II. Results of 1-factor ANOVAs testing the inter-individual variation of the minimum and maximum tube foot tenacity in the three species considered.

| | d.f. | *F* | *P* |
|---|---|---|---|
| *A. lixula* | | | |
| Minimum tenacity | 4,21 | 1.189 | 0.345 |
| Maximum tenacity | 4,21 | 0.997 | 0.431 |
| *P. lividus* | | | |
| Minimum tenacity | 5,25 | 0.637 | 0.673 |
| Maximum tenacity | 5,25 | 1.582 | 0.203 |
| *S. granularis* | | | |
| Minimum tenacity | 6,22 | 0.610 | 0.720 |
| Maximum tenacity | 6,22 | 0.695 | 0.656 |

Table III. Summary of 2-factor ANOVAs examining the effect of species (*A. lixula, P. lividus, S. granularis*) and number of attached tube feet (1, 2–5, 6–10) on the minimum and maximum tenacity.

| | Source of variation | d.f. | MS | *F* | *p* |
|---|---|---|---|---|---|
| Minimum tenacity | Species (SP) | 2 | 0.667 | 11.174 | <0.001 |
| | Number of tube feet (TF) | 2 | 0.375 | 6.291 | 0.003 |
| | SP × TF | 4 | 0.151 | 2.536 | 0.047 |
| | Error | 76 | 0.060 | | |
| Maximum tenacity | Species (SP) | 2 | 1.381 | 16.376 | <0.001 |
| | Number of tube feet (TF) | 2 | 0.201 | 2.384 | 0.099 |
| | SP × TF | 4 | 0.156 | 1.847 | 0.128 |
| | Error | 76 | 0.084 | | |

On the other hand, only minimum tenacity varies according to the number of tube feet attached, single tube feet always producing a significantly higher tenacity than groups of tube feet ($p_{Tukey} < 0.01$). This is presumably because, when several tube feet are attached, their respective time of attachment (see Materials and methods) may be different and, moreover, they may not all detach at exactly the same moment. The critical detachment force measured, and hence the tenacity, is therefore underestimated.

In terms of maximum tenacity, *P. lividus* attached with a strength (0.29 MPa) one and a half times higher than *S. granularis* (0.20 MPa), and three times higher than *A. lixula* (0.09 MPa) (Figure 7), each value being significantly different from the others ($p_{Tukey} < 0.02$). In terms of minimum tenacity, on the other hand, the species ranking remained the same, but the values of tenacity for the tube feet of *P. lividus* (0.11 MPa) and *S. granularis* (0.10 MPa) were no longer different, both remaining significantly higher than those measured in *A. lixula* (0.05 MPa) ($p_{Tukey} < 0.01$). The ratio $T_{min}/T_{max}$ is lower in *P. lividus* (about 0.4) than in the other two species (about 0.5), reflecting the higher proportion of incomplete footprints in the former.

For each species considered, the maximum attachment force one single tube foot would be able to produce can be estimated by multiplying each measurement of maximum tenacity by the mean surface area of the complete footprints observed (only the largest in *A. lixula*). Interspecific comparison of the means of this maximum attachment force (0.10, 0.11 and 0.13 N for *A. lixula*, *P. lividus* and *S. granularis*, respectively) shows that there is no more differences between the three species for this parameter (one-factor ANOVA, $F_{2,82} = 0.60$, $p = 0.55$).

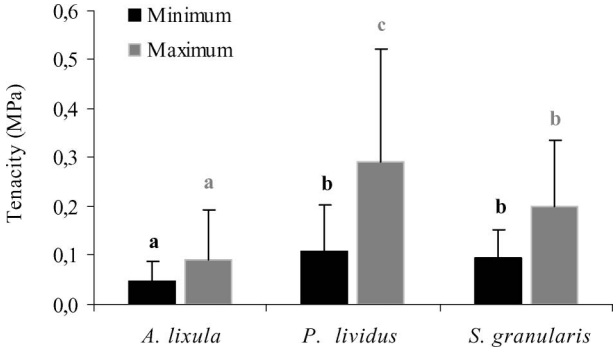


Figure 7. Mean values (+SD) of minimum and maximum tenacity measured on glass substratum for the tube feet of *A. lixula*, *P. lividus* and *S. granularis*. Significant differences between the means are indicated by letters; means sharing at least one letter are not significantly different ($p_{Tukey} \geq 0.05$).

To evaluate the influence of the type of substratum on tenacity, the attachment of the tube feet of *P. lividus* was also tested on three smooth polymers (PMMA, PP and PS), in addition to the glass substratum. This time, only measurements with single tube feet were used. One-factor ANOVAs demonstrated that the nature of the substratum significantly influenced tube foot tenacity (Table IV). Both maximum and minimum tenacity varied similarly with substratum, the highest values being measured on PMMA and the lowest on PP (Figure 8). The observed differences come mostly from significant differences in critical detachment forces, the surface areas calculated with footprint diameter ($S_{max}$) being identical on each substratum (Table IV). However, the surface area of stained adhesive material ($S_{stain}$) varied with substratum, being significantly larger

Table IV. Adhesion measurements (mean ± SD) of single tube feet from *P. lividus* attached to four different smooth substrata.

| | Substrata | | | | 1-factor ANOVA | |
|---|---|---|---|---|---|---|
| | Glass ($n = 21$) | PMMA ($n = 33$) | PP ($n = 18$) | PS ($n = 18$) | $F_{3,86}$ | $p$ |
| Attachment force (N) | $0.05 \pm 0.02^{b}$ | $0.12 \pm 0.06^{c}$ | $0.04 \pm 0.02^{a}$ | $0.06 \pm 0.02^{b}$ | 28.144 | $< 0.001$ |
| Adhesive surface area ($mm^2$) | | | | | | |
| Circular footprint ($S_{max}$) | $0.53 \pm 0.15^{a}$ | $0.60 \pm 0.14^{a}$ | $0.54 \pm 0.13^{a}$ | $0.50 \pm 0.17^{a}$ | 2.184 | 0.096 |
| Stained footprint ($S_{stain}$) | $0.23 \pm 0.14^{a}$ | $0.42 \pm 0.19^{b}$ | $0.35 \pm 0.18^{ab}$ | $0.24 \pm 0.14^{a}$ | 7.774 | $< 0.001$ |
| $S_{stain}/S_{max}$ (%) | $45 \pm 25^{a}$ | $71 \pm 28^{b}$ | $67 \pm 33^{ab}$ | $49 \pm 22^{a}$ | 5.023 | 0.003 |
| Tenacity (MPa) | | | | | | |
| Minimum | $0.15 \pm 0.21^{ab}$ | $0.22 \pm 0.13^{b}$ | $0.07 \pm 0.04^{a}$ | $0.12 \pm 0.05^{ab}$ | 5.000 | 0.003 |
| Maximum | $0.31 \pm 0.18^{ab}$ | $0.34 \pm 0.20^{b}$ | $0.17 \pm 0.18^{a}$ | $0.29 \pm 0.13^{ab}$ | 3.804 | 0.013 |

Significant differences between means for a given parameter are indicated by letters in superscripts; means sharing at least one letter are not significantly different ($p_{Tukey} \geq 0.05$).

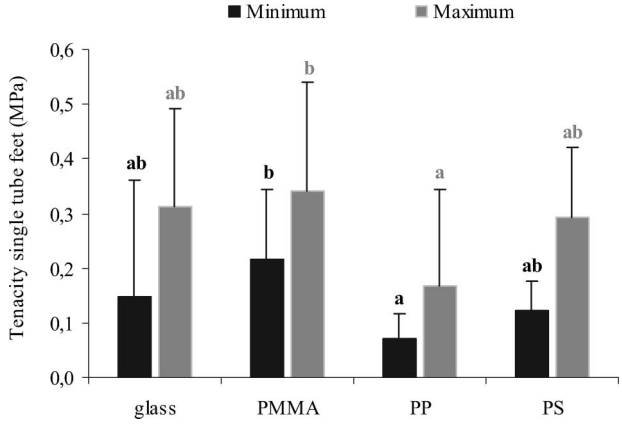


Figure 8. Mean values (+SD) of minimum and maximum tenacity of single tube feet from *P. lividus* measured on glass, poly(methyl methacrylate) (PMMA), polypropylene (PP), and polystyrene (PS). Significant differences between the means are indicated by letters; means sharing at least one letter are not significantly different ($p_{Tukey} \geq 0.05$).

on PMMA than on glass and PS ($p_{Tukey} < 0.002$; Table IV). The ratio $S_{stain}/S_{max}$ varied similarly (Table IV).

## Discussion

Among regular sea urchins, adoral tube feet can present different degrees of development, a fact frequently interpreted as an adaptation to life in different habitats. From this standpoint, Smith (1978) categorised coronal tube feet as different types according to the size of their disc, viz. 300–600 $\mu$m in diameter as type 2 tube feet, 700–900 $\mu$m as type 3 tube feet; and 1000–1200 $\mu$m as type 4 tube feet. According to this author, the degree of development of the tube feet would be directly related with the maximum environmental energy a species can withstand. Consequently, species possessing type 4 adoral tube feet were reported to have possibly the highest tenacity, inhabiting steep rocky bottoms or coral reefs in high-energy environments, whereas species with type 2 tube feet were described as presumably possessing the lowest tenacity, thus being restricted to soft or firm sediment bottoms in low to moderate energy environments. Species with type 3 tube feet would have intermediate tenacity, being present on rocky bottoms in moderately exposed environments, but being restricted to burrows or crevices when exposed in high-energy environments. In the Mediterranean Sea, the three phylogenetically unrelated species *A. lixula, P. lividus* and *S. granularis* live in different habitats. The first two species co-occur in areas of hard substrata and high hydrodynamism, whereas *S. granularis* usually inhabits deeper areas with soft substrata and low hydrodynamism (Régis, 1979; Chelazzi et al. 1997; Bulleri et al. 1999; Santos & Flammang, 2005). According to Smith (1978), *A. lixula, P. lividus* and *S. granularis* possess type 4, type 3 and type 2 tube feet, respectively. For the three populations investigated in this study, *A. lixula* and *S. granularis* had tube feet with significantly larger discs ($>1000$ $\mu$m) than *P. lividus* ($\sim 670$ $\mu$m), disagreeing with the classification of Smith (1978). During adhesion measurements, the tube feet of *P. lividus* and *S. granularis* promptly attached to the pieces of glass substratum and often with the entire adhesive area of the disc. On the contrary, *A. lixula* was quite reluctant to attach, touching the glass substratum repeatedly with the central protrusion of the disc (during tube foot extension, the central part of the disc forms a conical projection due to the increased hydrostatic pressure exerted by the ambulacral fluid; Flammang & Jangoux, 1993). The tube feet of *A. lixula* detached very often just after contact of the central protrusion with the substratum leaving small footprints, and much more rarely did the tube feet attach with the whole disc central area. Comparatively, therefore, *A. lixula* used only 34% of its disc to attach in contrast with *P. lividus* and *S. granularis*, which attached, respectively, with 100 and 67% of their disc adhesive area (surface measurements based on footprint diameter [$S_{max}$]).

Tenacity is an adhesion measurement taking into account the adhesive surface area used. It therefore allows comparisons between the three species considered, even though they present different morphologies and behaviours. Its accurate estimation, however, is made difficult by the fact that, in some cases, detached tube feet leave incomplete footprints. These footprints may result either from partial adhesive failure (some of the adhesive material has been removed from the substratum at detachment) or from incomplete adhesion (with only a fraction of the disc surface area), and it is often not possible to distinguish between these two origins. These two opposite footprint origins are considered in terms of the minimum and maximum tenacity reported in this study. Although there is a factor of two between them, both tenacity measurements usually lead to the same results when inter- or intraspecific comparisons on attachment strength are made. For instance, the mean tenacities of the tube feet of *A. lixula* (0.05 and 0.09 MPa) were always significantly lower than those measured for the tube feet of *P. lividus* and *S. granularis* (0.11 and 0.29, and 0.10 and 0.20 MPa, respectively). However, the two last species differed in terms of maximum tenacity but not in terms of minimum tenacity. Santos and Flammang (2006) reported tenacity measurements for the same species, but for whole sea urchins attached to a glass substratum. In their study, individuals of *A. lixula* were found to attach with the significantly lowest tenacity (0.12 MPa), followed by *S. granularis* (0.18 MPa) and finally by *P. lividus* (0.37 MPa). These values are closer to the maximum tenacity measured for single tube foot than to minimum tenacity, suggesting that the former may be the best estimation of the actual tenacity. It is interesting to note that, for the three species considered, tenacity and size of the tube foot disc counterbalance each other, the result being that the estimated maximal attachment force one tube foot can produce is similar in the three species. Hence, there is no clear relationship between tube foot size, tenacity, and species distribution in the field. Similarly, no relationship was found between the mechanical properties of the tube foot stems and habitat in the same species (Santos & Flammang, 2005).

The morphology of the tube foot disc was also very similar in the three species studied. Only two types of structures appear to differ from one species to the other, viz. the skeletal ossicles and spicules, and the

secretory granules of the adhesive cells. The observation of the skeletal elements that support the disc revealed that both the ossicles of the rosette and the spicules of the frame were much denser and more robust in *A. lixula* and *P. lividus* than in *S. granularis*. This result might be related to the distinct habitat of these species. In fact, the disc skeletal elements reinforce the terminal plate, a supporting structure that gives the disc its rigidity (Flammang & Jangoux, 1993; Santos et al. 2005). These skeletal elements presumably also function in distributing wave-generated stresses from the tube foot stem to the whole surface of the disc, and hence to the adhesive layer. Therefore, it can be hypothesised that species inhabiting hard substrata in areas with high hydrodynamic forces possess more robust discs (with denser skeletal elements) than species typical of soft substrata in less exposed zones, and this independently of the tube foot tenacity of the species under consideration.

In terms of the ultrastructure of the disc epidermis, the three echinoid species possessed four types of epidermal cells, viz. support cells, sensory cells, adhesive secretory cells and de-adhesive secretory cells (Coleman, 1969; Burke, 1980; Flammang & Jangoux, 1993). The cytoplasm (cell body, apical process and microvillar-like projections) of adhesive cells was filled with densely packed, spherical secretory granules. At this level, some interspecific differences were found in terms of the internal organisation of these adhesive secretory granules. In *A. lixula*, the adhesive granules were the most complex, with diameters from 400 – 700 nm. Their core was highly organised with a stack of 4 – 8 electron dense parallel plates surrounded by an electron-lucent material. Comparatively, *S. granularis* presented smaller adhesive granules (300 – 400 nm in diameter) with a less complex internal structure, the core enclosing 2 – 3 electron dense parallel plates surrounded by a material of medium electron density. In the disc epidermis of *P. lividus*, two types of adhesive cells could be distinguished. In the middle of the central area of the disc, the adhesive secretory cells were filled with granules 500 – 700 nm in diameter with an homogenous core of medium electron density, surrounded by a thin clear space. In the rest of the central area, the adhesive cells contained smaller secretory granules (300 – 500 nm in diameter), with a very electron dense small core, surrounded by a large belt of material of medium electron density. The presence of two types of epidermal adhesive secretory granules had already been demonstrated in the primary tube feet of *P. lividus* larvae as well as on the adoral tube feet of adults (Flammang et al. 1998b). Some authors speculate that these ultrastructural differences in the internal organisation of the adhesive secretory granules reflect the adhesive power of the tube foot and thus are related with species habitat. Engster and Brown (1972) pointed out that asteroids confined to hard rocky substrata have more complex granules, with a highly organised core, whereas soft substratum dwelling species have granules of considerably simpler ultrastructure. However, Flammang (1995) identified the presence of granules with a complex central fibrillar bundle in the adhesive epidermis of three soft substratum dwelling asteroids of the genus *Luidia*. As for echinoids, the rock dweller *Arbacia punctulata* was reported to possess highly structured secretory granules (Harrison, 1966 cited in Engster & Brown, 1972) whereas *Diadema antillarum,* typical of coral sands, possesses simpler dense cored homogeneous granules (Coleman, 1969). In the present study, *A. lixula*, a species confined to exposed rocky habitats, had unequivocally the most complex adhesive granules, but it is the species presenting the lowest tenacity. As for the other two species, the ultrastructural observations indicate that the internal organisation of the adhesive granules of *P. lividus* is simpler relatively to *S. granularis*, although the former presents the highest tenacity. Once again, therefore, no relationship could be established between tube foot tenacity, adhesive secretory granule ultrastructure, and species habitat.

In *P. lividus*, the adhesion strength of single tube feet on different types of substrata was also tested. It was observed that tube foot tenacity varied significantly with the substratum used, the highest values being recorded on PMMA and the lowest values on PP. These variations in tenacity resulted mostly from significant differences in the critical detachment forces of tube feet, the surface area of the disc adhesive footprints remaining relatively constant. The influence of substratum surface characteristics on tenacity has been investigated in several other marine organisms also known for adhering strongly to the substratum, such as barnacles, limpets and mussels (see e.g. Grenon & Walker, 1981; Crisp et al. 1985; Yule & Walker, 1987). As a general rule, there is a positive correlation between tenacity and the substratum critical surface energy. The tenacity of *P. lividus* tube feet attached to PS is intermediate between those measured on PMMA and PP though the critical surface energy of PS (33 mJ $m^{-2}$) is much closer to that of PP (32 mJ $m^{-2}$) than to that of PMMA (39 mJ $m^{-2}$) (Wu, 1982). PS, however, is much more polar than PP, and in this respect more similar to PMMA (Wu, 1982). Similar results were reported in barnacle cyprids, for which a substratum with a higher polar free energy content promoted stronger temporary adhesion than a substratum with an equivalent total surface free energy but a higher contribution from non-polar forces (0.09 MPa on 3-HEPS [glass coated with perfluorinated silane; polar free energy of 16 mJ $m^{-2}$, non-polar free

energy of 3 mJ $m^{-2}$ and total surface free energy of 19 mJ $m^{-2}$] and 0.06 MPa in DCDMS [glass coated with dichlorodimethylsilane; polar free energy of 4 mJ $m^{-2}$, non-polar free energy of 22 mJ $m^{-2}$ and total surface free energy of 26 mJ $m^{-2}$]). This indicates that the ratio of polar to non-polar forces at the surface is more important in determining attachment strength than the total surface energy (Yule & Walker, 1987). The higher tenacity of marine organisms on high-energy, polar surfaces is usually interpreted as the result of a better adhesive spreading and greater molecular forces on these surfaces, which is in accordance with the richness in charged and polar residues of the adhesive secretions of marine organisms (Grenon & Walker, 1981; Waite, 1987; Callow et al. 2005; Aldred et al. 2006). Neither of these explanations appears to hold true for echinoid tube foot attachment. Indeed, footprint diameter was identical on all tested surfaces, indicating that footprint size is defined mostly by disc size and not by surface wettability. Moreover, the fact that footprints are retained by all surfaces suggests that the failure is cohesive in nature between the tube foot and the secreted adhesive rather than between the adhesive and the surface. Nevertheless, substratum surface characteristics significantly influence tube foot critical detachment force and tenacity. An identical phenomenon has been demonstrated in adult barnacles in which the removal of animals attached to low-energy surfaces was due in a large part to cohesive failure of the barnacle adhesive plaque (Sun et al. 2004). In these organisms, it was shown that the adhesive laid on low-energy surfaces was thicker, softer (lower Young's modulus) and more hydrated than adhesive laid on high-energy surfaces (Berglin & Gatenholm, 2003; Wiegemann & Watermann, 2003; Sun et al. 2004). Similar observations have been made with adsorbed protein films constituted of mussel foot protein-1 (Mefp-1; see Berglin et al. 2005, for review). This protein forms an elongated, flexible film with substantial amounts of hydrodynamically coupled water on non-polar surfaces, whereas it forms a rigidly attached adlayer with little hydrodynamically coupled water on polar surfaces. Therefore, substratum properties influence not only the spreading and molecular adhesion of marine bioadhesives, but also their bulk properties. It is possible that in echinoids too, adhesive footprints deposited by tube feet on non-polar surfaces would be softer, being thus more prone to cohesive failure and leading to a decreased tenacity.

## Acknowledgements

We thank the Director and staff of the Observatoire Océanologique Arago at Banyuls-sur-mer (France) for providing facilities, and P. Postiau for technical assistance. R.S. benefitted from a doctoral grant of the Foundation for Science and Technology of Portugal (SFRH/BD/4832/2001). P.F. is Research Associate of the National Fund for Scientific Research of Belgium (FNRS). This study is a contribution from the "Centre Interuniversitaire de Biologie Marine" (CIBIM; http://www.ulb.ac.be/sciences/biomar/html/adherence.html).

## References

Aldred N, Ista LK, Callow ME, Callow JA, Lopez GP, Clare AS. 2006. Mussel (*Mytilus edulis*) byssus deposition in response to variations in surface wettability. J R Soc Interface 3:37–43.

Berglin M, Gatenholm P. 2003. The barnacle adhesive plaque: morphological and chemical differences as a response to substrate properties. Colloids Surf B: Biointerfaces 28:107–117.

Berglin M, Hedlund J, Fant C, Elwing H. 2005. Use of surface-sensitive methods for the study of adsorption and cross-linking of marine bioadhesives. J Adhesion 81:805–822.

Bulleri F, Benedetti-Cecchi L, Cinelli F. 1999. Grazing by the sea urchins *Arbacia lixula* L. and *Paracentrotus lividus* Lam. in the Northwest Mediterranean. J Exp Mar Biol Ecol 241:81–95.

Burke RD. 1980. Podial sensory receptors and the induction of metamorphosis in echinoids. J Exp Mar Biol Ecol 47:223–234.

Callow JA, Ista MA, Lopez LK, Chaudhury MK. 2005. The influence of surface energy on the wetting behaviour of the spore adhesive of the marine alga *Ulva linza* (synonym *Enteromorpha linza*). J R Soc Interface 2:319–325.

Chelazzi G, Serra G, Bucciarelli G. 1997. Zonal recovery after experimental displacement in two sea urchins co-occurring in the Mediterranean. J Exp Mar Biol Ecol 212:1–7.

Coleman R. 1969. Ultrastructure of the tube foot sucker of a regular echinoid, *Diadema antillarum* Philippi, with special reference to secretory cells. Z Zellforsch Mikrosk Anat 96:151–161.

Crisp DJ, Walker G, Young GA, Yule B. 1985. Adhesion and substrate choice in mussels and barnacles. J Colloid Interface Sci 104:40–50.

Dietrich HF, Fontaine AR. 1975. A decalcification method for ultrastructure of echinoderm tissues. Stain Technol 50:351–354.

Engster MS, Brown SC. 1972. Histology and ultrastructure of the tube foot epithelium in the phanerozonian starfish, *Astropecten*. Tissue Cell 4:503–518.

Flammang P. 1995. Fine structure of the podia in three species of paxillosid asteroids of the genus *Luidia* (Echinodermata). Belg J Zool 125:125–134.

Flammang P. 1996. Adhesion in echinoderms. In: Jangoux M, Lawrence JM, editors. Echinoderm studies. Vol. 5. Rotterdam: Balkema. pp 1–60.

Flammang P, Jangoux M. 1993. Functional morphology of coronal and peristomeal podia in *Sphaerechinus granularis* (Echinodermata Echinoida). Zoomorphology 113:47–60.

Flammang P, Walker G. 1997. Measurement of the adhesion of the podia in the asteroid *Asterias rubens* (Echinodermata). J Mar Biol Assoc UK 77:1251–1254.

Flammang P, Demeulenaere S, Jangoux M. 1994. The role of podial secretions in adhesion in two species of sea stars (Echinodermata). Biol Bull 187:35–47.

Flammang P, Gosselin P, Jangoux M. 1998a. The podia, organs of adhesion and sensory perception in larvae and post-metamorphic stages of the echinoid *Paracentrotus lividus* (Echinodermata). Biofouling 12:161–171.

Flammang P, Santos R, Haesaerts D. 2005. Echinoderm adhesive secretions: From experimental characterization to biotechnological applications. In: Matranga V, editor. Progress in molecular and subcellular biology, marine molecular biotechnology. Echinodermata. Berlin: Springer-Verlag. pp 199–218.

Flammang P, Van Cauwenberge A, Alexandre H, Jangoux M. 1998b. A study of the temporary adhesion of the podia in the sea star *Asterias rubens* (Echinodermata, Asteroidea) through their footprints. J Exp Biol 201:2383–2395.

Gabe M. 1968. Techniques histologiques. Paris: Masson.

Granhag LM, Finlay JA, Johnson PR, Callow JA, Callow ME. 2004. Roughness-dependent removal of settled spores of the green alga *Ulva* (syn. *Enteromorpha*) exposed to hydrodynamic forces from a water jet. Biofouling 20:117–122.

Grenon JF, Walker G. 1981. The tenacity of the limpet, *Patella vulgata* L. an experimental approach. J Exp Mar Biol Ecol 54:277–308.

Haesaerts D, Finlay JA, Callow ME, Callow JA, Grosjean Ph, Jangoux M, Flammang P. 2005. Evaluation of the attachment strength of individuals of *Asterina gibbosa* (Asteroidea, Echinodermata) during the perimetamorphic period. Biofouling 21:229–235.

Hermans CO. 1983. The duo-gland adhesive system. Oceanogr Mar Biol Ann Rev 21:281–339.

Paine VL. 1926. Adhesion of the tube feet in starfishes. J Exp Zool 45:361–366.

Régis MB. 1979. Particularités microstructurales du squelette de *Paracentrotus lividus* et *Arbacia lixula*: rapports avec l'écologie et l'éthologie de ces échinides. Mar Biol 54:373–382.

Santos R, Flammang P. 2005. Morphometry and mechanical design of tube foot stems in sea urchins: a comparative study. J Exp Mar Biol Ecol 315:211–223.

Santos R, Flammang P. 2006. Intra- and interspecific variation of the attachment strength in sea urchins. Mar Ecol Prog Ser (In press).

Santos R, Gorb S, Jamar V, Flammang P. 2005. Adhesion of echinoderm tube feet to rough surfaces. J Exp Biol 208:2555–2567.

Smith AB. 1978. A functional classification of the coronal pores of echinoids. Palaeontology 21:759–789.

Smith AB. 1992. Echinoderm phylogeny: morphology and molecules approach accord. Trends Ecol Evol 7:224–229.

Sun Y, Guo S, Walker GC, Kavanagh CJ, Swain GW. 2004. Surface elastic modulus of barnacle adhesive and release characteristics from silicone surfaces. Biofouling 20:279–289.

Thomas LA, Hermans CO. 1985. Adhesive interactions between the tube feet of a starfish, *Leptasterias hexactis*, and substrata. Biol Bull 169:675–688.

Waite JH. 1987. Nature's underwater adhesive specialist. Int J Adhesion Adhesives 7:9–14.

Wiegemann M, Watermann B. 2003. Peculiarities of barnacle adhesive cured on non-stick surfaces. J Adhesion Sci Technol 17:1957–1977.

Wu S. 1982. Polymer interface and adhesion. New York: Marcel Dekker.

Yule AB, Walker G. 1987. Adhesion in barnacles. In: Southward AJ, editor. Crustacean issues. Vol. 5. Biology of barnacles. Rotterdam: Balkema. pp 389–402.