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# ULTRASTRUCTURAL ORGANISATION OF THE EPIDERMAL GLANDS IN THE INTEGUMENT OF THE SEA SPIDERS *NYMPHON GRACILE* LEACH 1814, *ACHELIA LONGIPES* HODGES 1864, AND *PYCNOGONUM LITTORALE* (STRÖM, 1762) (CHELICERATA, PYCNOGONIDA)

by

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## SUMMARY

The present paper describes the ultrastructural organisation of the epidermal glands in three pycnogonid species : *Nymphon gracile, Achelia longipes*, and *Pycnogonum littorale*. These glands are distributed over the whole body surface and lodged in cuticular holes. They are composed of at least five cells : two secretory cells, one enveloping cell, and two (or more) accessory cells. The presence of nerve extensions in contact with the enveloping cell strongly suggests that the glands are under nervous control. They may play a defensive role.

*Keywords :* pycnogonids, integument, epidermal glands, ultrastructure.

## INTRODUCTION

Epidermal glands, also called « dermal glands », are a well-known feature of the integument in insects (NOIROT and QUENNEDEY, 1974) and crustaceans (DOUGHTIE and RAO, 1982 ; COMPÈRE, 1990), among which their morphology and precise location vary widely. Functionally, the secretions of these glands are recognised to play various roles, notably in social relationships, sexual behaviour, defence, and the formation of some cuticular layers. Epidermal glands are very numerous in the integument of sea spiders or pycnogonids, a class of marine chelicerates, where they are distributed over the whole body surface and, peculiarly, are lodged in holes within the cuticle. Although the glands are mentioned by several authors (FAGE, 1949 ;

KING, 1974 ; DAVENPORT *et al.*, 1987 ; FAHRENBACH, 1994), their ultrastructural and cellular organisation has not been described.

## MATERIAL AND METHODS

Individuals of three pycnogonid species, *Nymphon gracile, Achelia longipes*, and *Pycnogonum littorale*, were collected at the Marine Station of Wimereux (France). The tibias and femurs of the walking legs as well as the trunk were excised from living animals and fixed by immersion for 72 h at 20° C in 2.5 % glutaraldehyde buffered with diluted sea water adjusted to pH 7.4 according to MILLONIG (1976). The samples were then decalcified for 72 h at 4° C in 0.2 M EDTA at pH 8.0, rinsed in filtered sea water, and post-fixed in 1 % $OsO_4$. After washing in distilled water and dehydration through a graded ethanol series, the samples were embedded in epoxy resin according to the standard procedure.

For observations in transmission electron microscopy, ultrathin sections were cut either perpendicularly or parallel to the cuticle surface, then stained with uranyl acetate and lead citrate. They were observed in a JEOL JEM 100-SX electron microscope at 80 kV accelerating voltage.

For scanning electron microscopy, ethanol-dehydration was followed by critical point drying in $CO_2$. After coating with gold-palladium in a sputtering apparatus (BALZERS SCD 030), the samples were examined in a JEOL JSM-840A scanning electron microscope at 20 kV accelerating voltage.

## RESULTS

As shown in our various micrographs and schematised in Fig. 1, the fine structure and cellular organisation of the epidermal glands are quite similar in the three species. The glands are formed of epidermal cells lodged in large holes in the cuticle (Fig. 2). They are connected to the outer cuticular surface through a short excretory duct lined by a thin cuticle. At the cuticular surface, two symmetrical cuticle lips located in small, circular depressions form the edges of the epidermal gland openings. These openings are relatively difficult to observe owing to the presence of numerous epibiotic organisms (Fig. 3).

As a rule, each gland is composed of five cells : two secretory cells, one enveloping cell, and two (or more) accessory cells.

The secretory cells are of two types, distinguishable on the basis of the electron-density of the secretory granules filling their apical cytoplasm. One type contains large, electron-lucent granules whilst the second exhibits smaller, moderately electron-dense granules. Both types of secretory cells are connected to the excretory duct , their apices bearing a few, scarce microvilli.

The enveloping cell surrounds the secretory cells ; it isolates them from each other and from the other epidermal cells (Figs 3 and 4). Distally, between the apex of the secretory cells and the lower end of the excretory duct, it forms a sort of

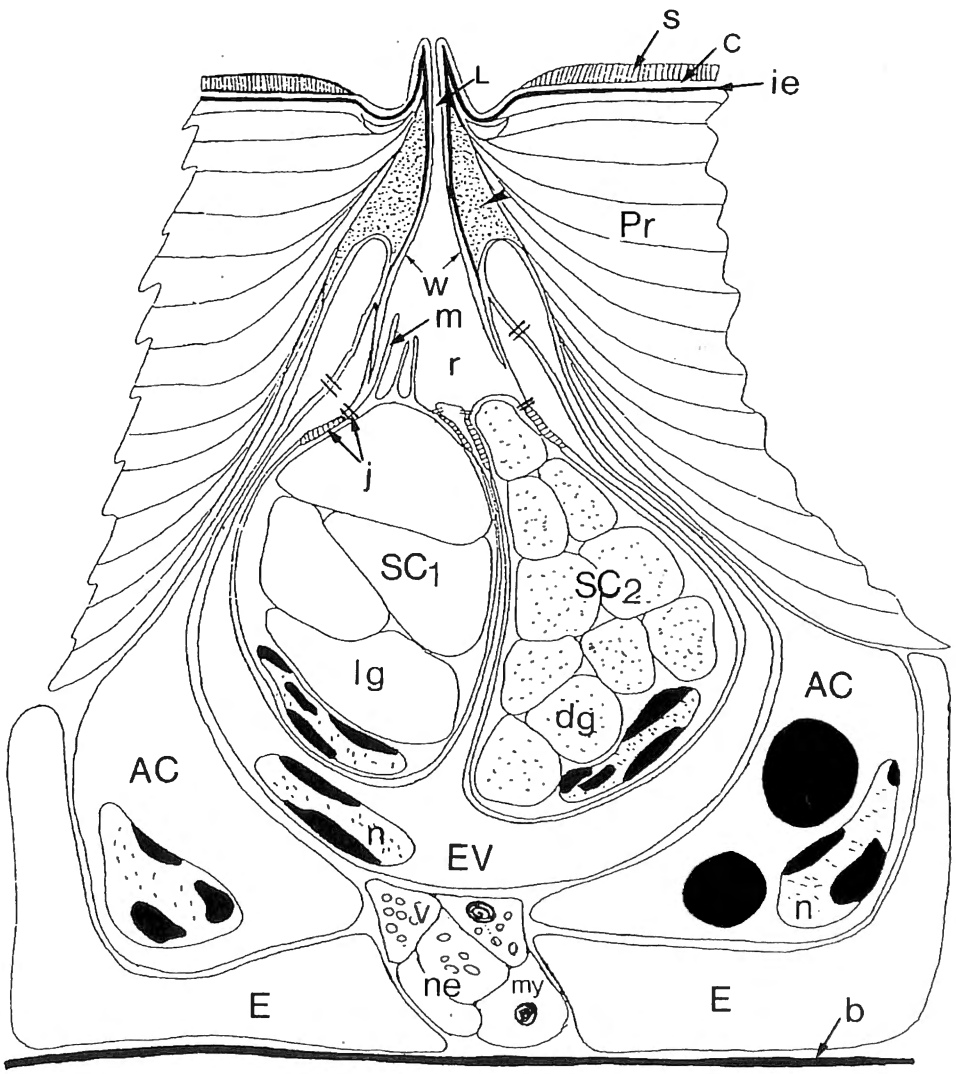


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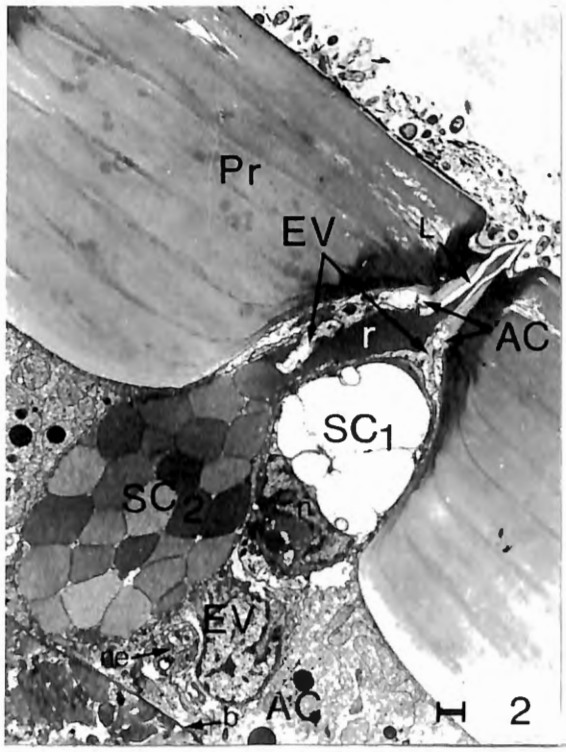
Fig. 1. — Diagram showing the cellular organisation of a pycnogonid epidermal gland. AC, accessory cells ; b, basal lamina ; c, cuticulin layer ; dg, electron-dense granules ; E, epidermal cells ; EV, enveloping cell ; ie, inner epicuticle ; j, intercellular junctions including a zonula adherens and a septate desmosome ; L, excretory duct lumen ; lg, electron-lucent granules ; m, microvilli ; my, myelin sheath ; n, nucleus ; ne, nerve extensions ; Pr, procuticle ; r, collection reservoir ; s, surface coat ; SC1, type-1 secretory cell ; SC2, type-2 secretory cell ; v, synaptic-like vesicles ; w, cuticular wall of the excretory duct ; arrow head, modified procuticle.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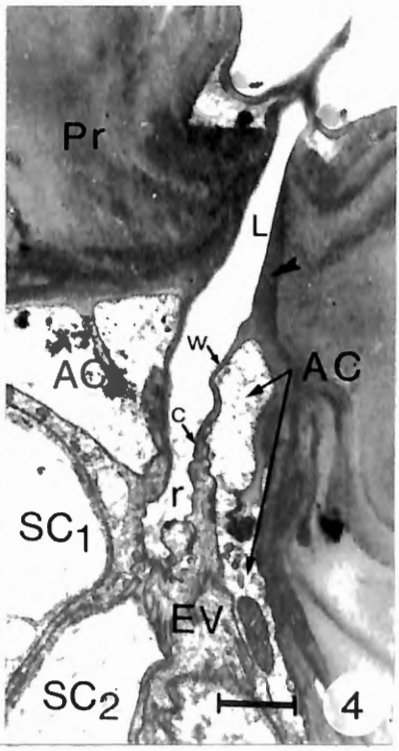
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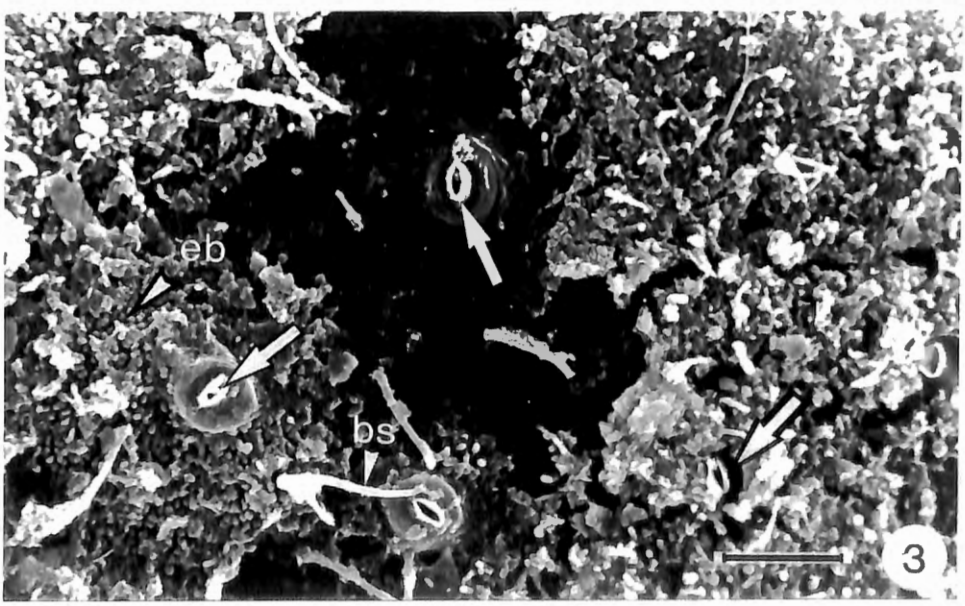
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reservoir that collects the secretion products. Basically, the enveloping cell is in contact with nerve extensions showing synaptic-like vesicles and myelin sheaths.

The accessory cells are slightly modified epidermal cells lining the cuticular wall of the hole and surrounding the cuticular sheath of the excretory duct.

The cuticle of the excretory duct is composed of three different layers : the cuticulin layer, the inner epicuticle, and the procuticle (Figs 1 and 4), layers previously identified in the sclerite cuticle of *P. littorale* (COMPÈRE *et al.*, 1993). Toward the lower end of the duct, the procuticle and epicuticle gradually become thinner, whilst the cuticulin layer subsists and still appears to line the duct through the enveloping cell where it appears abruptly to end.

## DISCUSSION

On the basis of their organisation, the epidermal gland cells of pycnogonids can be classified as class 3 secretory cells, described by NOIROT and QUENNEDEY (1974) in insects and arachnids.

In agreement with these authors' definition of class 3 gland cells, the epidermal gland cells of pycnogonids have no direct contact with the cuticle and are connected to the external medium by an excretory duct that runs through a specialised cell, sometimes forming a collection reservoir. The enveloping cell may correspond with the specialised canal-forming cells of insect glands, the canal cell and the intermediate cell.

While the principal originality of pycnogonid epidermal gland cells is their location inside cuticle holes, they also differ in the following respects from those of insects : (1) the excretory duct does not issue from a microvillous terminal apparatus invaginated into the secretory cells ; (2) the enveloping cell differs from canal or intermediate cells in that it completely surrounds the two secretory cells.

Our results support the hypothesis, proposed by FAHRENBACH (1994), that the epidermal glands of pycnogonids play a defensive role. Such a role would fit with

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Fig. 2. — General view of an epidermal gland in the leg femur of a female *Nymphon gracile* Leach. AC, accessory cells ; b, basal lamina ; EV, enveloping cell ; ie, inner epicuticle ; L, excretory duct lumen ; n, nucleus ; ne, nerve extensions ; Pr, procuticle ; r, collection reservoir ; SC1, type-1 secretory cell ; SC2, type-2 secretory cell. Scale bar = 1 µm.

Fig. 3. — Cuticle surface of the femur of a female *Nymphon gracile* Leach. eb, epibiotic organisms ; bs, bipartite sensillum ; arrows, openings of the epidermal gland ducts. Scale bar = 10 µm.

Fig. 4. — Detail of the apex and excretory duct of an epidermal gland in the tibia of a female *Nymphon gracile* Leach. AC, accessory cells ; c, cuticulin layer ; EV, enveloping cell ; L, excreting duct lumen ; Pr, procuticle ; r, collecting reservoir ; SC1, secretory cell of the type 1 ; SC2, secretory cell of the type 2 ; w, cuticular wall of the excreting duct ; arrow head, modified procuticle. Scale bar = 1 µm.

the following facts : (1) the presence of nerve extensions in contact with the enveloping cell strongly suggests that these glandular formations are under nervous control, presumably enabling the animals to discharge their secretion product when disturbed ; (2) the epidermal glands are distributed over the whole body surface ; since they do not seem to be involved in the deposition of cuticular material, a defensive role might explain this distribution ; (3) when disturbed, *P. littorale* was found by TOMASCHKO (1994) to defensively secrete a mixture of eight ecdysteroids. This discharge of ecdysteroids significantly deterred from feeding the common shore crab *Carcinus maenas* (L.), recognised as a general predator in the habitat of this pycnogonid species.

We may thus reasonably hypothesise that the peculiar epidermal glands of pycnogonids are involved in secreting ecdysteroids or other substances rendering these vulnerable animals inedible by predators. This hypothesis, however, must be taken with caution and remains to be confirmed. Mimetism is another possible means by which pycnogonids might defend themselves (FAGE, 1949).

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## REFERENCES

COMPÈRE, P. (1990) — Fine structure and elaboration of the epicuticle and pore canal system in tergite cuticle of the land isopod *Oniscus asellus* during a molting cycle. In P. JUCHAULT and J.P. MOCQUARD : *Biology of terrestrials isopods III.* Poitiers : 169-175.

COMPÈRE, PH., PH. THIRY, J.-C. BUSSERS and G. GOFFINET (1993) — Ultrastructure of the integument of the sea spider *Pycnogonum littorale* (Ström) (Pycnogonida). *Belgian J. Zool.*, **123** (suppl. 1) : 11-12.

DAVENPORT, J., N. BLACKSTOCK, D.A. DAVIES and M. YARRINGTON (1987) — Observations on the physiology and integumentary structure of the Antartic pycnogonid *Decolopoda australis*. *J. Zool.* (Lond.), **211** : 451-465.

DOUGHTIE, D.G. and R.K. RAO (1982) — Rosette glands in the gills of the Grass Shrimp, *Palaemonetes pugio*. Comparative morphology, cyclical activity and innervation. *J. Morphol.*, **171** : 41-67.

FAGE, L. (1949) — Classe des Pycnogonides. In P.P. GRASSÉ : *Traité de Zoologie, Tome VI, Chélicérates*. Masson, Paris : 906-941.

FAHRENBACH, W.H. (1994) — Microscopic anatomy of Pycnogonida : 1. cuticle, epidermis and muscle. *J. Morphol.*, **222** : 33-48.

KING, P.E. (1974) — British sea spiders. Arthropoda : Pycnogonida. Key and notes for the identification of the species. In Linnean Society of London : Synopsis of the British Fauna n° 5. Academic Press, London, New York : 67 pp.

MILLONIG, G. (1976) — Laboratory manual of biological electron microscopy. SAVIOLO, M., Vercelli : 67 pp.

NOIROT, C. and A. QUENNEDEY (1974) — Fine structure of insect epidermal glands. *Annu. Rev. Entomol.*, **19** : 61-80.

TOMASCHKO, K.H. (1994) — Defensive secretion of ecdysteroids in *Pycnogonum littorale* (Arthropoda, Pantopoda) — *Z. Naturforsch.*, **49c** : 367-371.