

THELYCUM AND SPERMATOPHORE STRUCTURE
IN THE ANTARCTIC EUPHAUSIIDS
EUPHAUSIA SUPERBA AND *E. CRYSTALLOROPHIAS*

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Abstract: The structure of *Euphausia superba* and *E. crystallorophias*'s developing thelyca and male spermatophore, obtained from exuviae casted from animals maintained in the laboratory, have been examined using both light and scanning electron microscopy. This is the first report of adult *E. crystallorophias* being held in the laboratory for greater than 12 months.

The thelycum has a characteristic structure in both species examined, confirming its importance in the systematics of euphausiids.

Differences were also observed in the shape, but not in the surface structure of the spermatophore between species. The spermatophores in both species were formed from numerous overlapping circular chitinous plates.

Following spermatophore attachment, the thelycum in each species was observed to change. The most noticeable difference being the complete covering of *E. crystallorophias*'s thelycum with a chitinous substance once the spermatophore has been attached.

1. Introduction

Euphausia superba plays a key role in the marine oceanic ecosystem of the seasonal pack-ice zone (HEMPEL, 1985). *E. crystallorophias* on the other hand, is a neritic species (LOMAKINA, 1964) occupying the permanent pack-ice zone and often replacing *E. superba* in terms of abundance and becoming the dominant element in the food web of this region (KUHLE and SCHNEPPENHEIM, 1986). These two zones are however not mutually exclusive, and the distribution of these two species are known to overlap (RAKUSA-SUSZCZEWSKI and STEPNIK, 1980; WILLIAMS *et al.*, 1983, 1986).

Difficulties in differentiating between sub-adult *E. superba* and *E. crystallorophias*, especially if specimens are in a semi-digested state, as in Antarctic predator gut analysis studies, has led us to study both the thelycum and spermatophore of these two species.

The thelycum, or female copulatory organ in Euphausiacea was first recorded by CLAUS (1863). The potential usefulness of this organ as a diagnostic tool in species identification of adult female euphausiids was originally demonstrated by EINARSSON (1942).

MAUCLINE and FISHER (1969) in noting its potential as a taxonomic tool, commented on the lack of sufficient data to accurately assess its value. Later studies have confirmed its taxonomic value (LOMAKINA, 1972; COSTANZO and GUGLIELMO, 1976a, b; GUGLIELMO and COSTANZO, 1977). Its routine use for specific identification of females is still impeded however by the difficulties associated in producing instantly

recognisable drawings of this complex structure (MAUCLINE, 1980).

To overcome this problem, the present investigation uses the scanning electron microscope to study differences in the thelyca fine structure between *E. superba* and *E. crystallorophias*.

2. Materials and Methods

Live adult female *E. superba* were captured in Prydz Bay with a rectangular mid-water trawl net (BAKER *et al.*, 1973) during Australia's involvement in the second phase of the Second International BIOMASS Experiment (SIBEX II) in January 1985 (HARRINGTON and IKEDA, 1986). Live *E. crystallorophias* were collected in November 1985 from the edge of the continental shelf off Enderby Land, East Antarctica (HARRINGTON and THOMAS, 1987).

Both species were returned to Australia for further laboratory growth studies. Animals were maintained individually in 4 l glass bottles filled with seawater which was filtered through a 0.45 μm Millipore filter and kept at 0°C in constant darkness for 18 months. Seawater (34‰) was changed weekly.

E. superba was supplied with 2.0 mg C l⁻¹ of the pennate diatom, *Phaeodactylum tricornutum* each week, while *E. crystallorophias* was fed a mixture of *P. tricornutum* (150 ml) and *Phaeocystis pouchetii* (150 ml) per week. *P. tricornutum* has been shown to be a useful food source in the culture of *E. superba* (IKEDA, 1984, 1985). All algal species were cultured in f/2 medium (GUILLARD and RYTHER, 1962) at either 10°C (*P. tricornutum*) or 4°C (*P. pouchetii*).

Bottles were examined every 24 h for exuviae which were collected and preserved with 0.4 ml of buffered formalin (40%) in 20 ml of seawater (IKEDA and DIXON, 1982; THOMAS and IKEDA, 1987). Six mature and developing thelyca from each species were detached from the exuviae and post-fixed with 4% osmium tetroxide for 1 h. Examples of fertilised thelyca were obtained after maintenance of live previously mated individuals collected from the field. Specimens were then dehydrated in a graded ethanol series, critical point dried with CO₂ and coated with 30 nm of gold in a sputter coater before examination using a JEOL JSM-840 scanning electron microscope (SEM).

3. Results and Discussion

Throughout the experimental all individuals of both species displayed positive growth and advanced to maturity (THOMAS and IKEDA, 1987).

3.1. *Euphausia crystallorophias*

The thelycum of this species, as with *Euphausia superba*, is formed at the base of the sixth pair of thoracic legs from the sternite and coxal plates. The shape of the thelycum as it developed from sub-adult to mature adult is shown in Figs. 1a-1c. The time taken for this to occur was over a period of three moults (an average of 22 days for each intermoult period). The thelycum became progressively more chitinised and pigmented with each moult, so that once mature, it was firm to touch and coloured red (Fig. 1c). The inside edge of the sternite plate is covered with "finger-like" pro-

trusions and small pores (Figs. 1d and 1e). While distinct patches (approx. 10 μm in diameter) of short "bristle-like" setae cover the leading edges of the coxal plates (Fig. 1f).

Pores similar to those observed on the sternite plate (Figs. 1d and 1e) were also seen covering *E. superba*'s thelycum. The significance of these pores is unknown, although similar pores have been reported from the mouthparts of *E. superba* larvae (MARSCHALL, 1985). It is postulated that these pores may be either; (a) associated with some form of mucous production to inhibit the growth of micro-organisms on the exoskeleton, or (b) are integumental glands similar to those described by CLARKE *et al.* (1962) in copepods, which tend to be simple sacs underlying the cuticle and communicate with the environment via a pore penetrating the cuticle. Thus these pores covering the thelycum may aid the successful attachment of the spermatophore.

Once fertilised the thelycum is completely covered with a paste-like substance (Fig. 1g). From this, the spermatophores, 1.2 mm in length extrude, tapering at their base, to become half the diameter of the opposite end (Figs. 2a and 2b). Often only two spermatophores are attached to the thelycum, however, occasionally one or even three were recorded protruding from the substance covering the thelycum of some individuals examined. This fibrous substance may have two roles. First, it may help to lock the spermatophore into place. Second, it would deter other males from actively seeking and copulating with previously mated females by:

- 1) Obscuring any thelycum pigmentation, as pigmentation may be an important factor in attracting males to reproductively mature females. In both species once the structure of the thelycum became mature the intensity of the pigmentation was seen to increase.

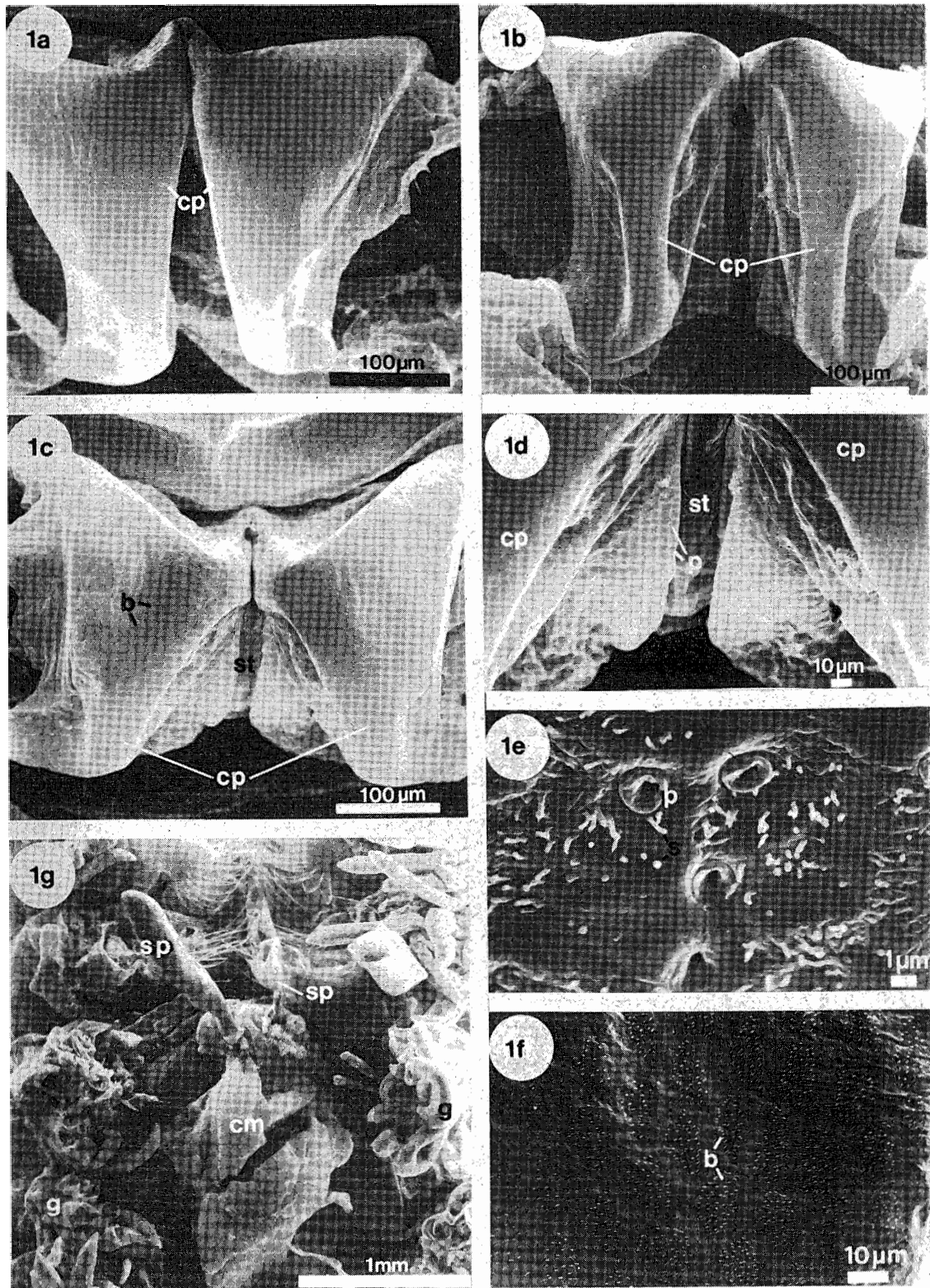
- 2) Blocking access to the thelycum, thus allowing only one successful mating for each brood.

The surface structure of *E. crystallorophias*'s spermatophore is formed from overlapping circular chitin plates (Fig. 2c). Edges of these plates covering the spermatophores were observed in some specimens to be folded. The appearance of folding in some specimens examined and not in others, has led the authors to believe that this is an artifact of the fixation technique used. Spermatozoa were found to be non-motile and oval in shape (Fig. 2d).

3.2. *Euphausia superba*

The differently shaped but still heavily chitinised and pigmented mature thelycum of *E. superba* is shown in Fig. 3a. Holdfasts which are thought to help spermatophore attachment (DENYS and MCWHINNIE, 1982) have a "cobble-stone" appearance and are studded with short sharp setae (Fig. 3b). Small pores can also be seen protruding from its surface. The outer edges of the coxal plates also have this "cobble-stone" appearance (Fig. 3c) while the inner region of the coxal plates, near the holdfasts, are much more deeply grooved and irregular (Fig. 3d). This irregularity may also assist in spermatophore attachment.

The procedure by which the spermatophore is transferred to the thelycum is described by (BARGMANN, 1937) and a typically fertilised *E. superba* thelycum is shown in Fig. 4a. The thelycum, once fertilised, alters its appearance from the mature form



Figs. 1a–1c. Ventral view of the developmental stages of E. crystallophias's thelycum from sub-adult to mature adult. cp (coxal plate); st (sternite plate); b (groups of bristles covering outer edge of coxal plate). Note:- anterior surface is directed to the bottom edge of the page.

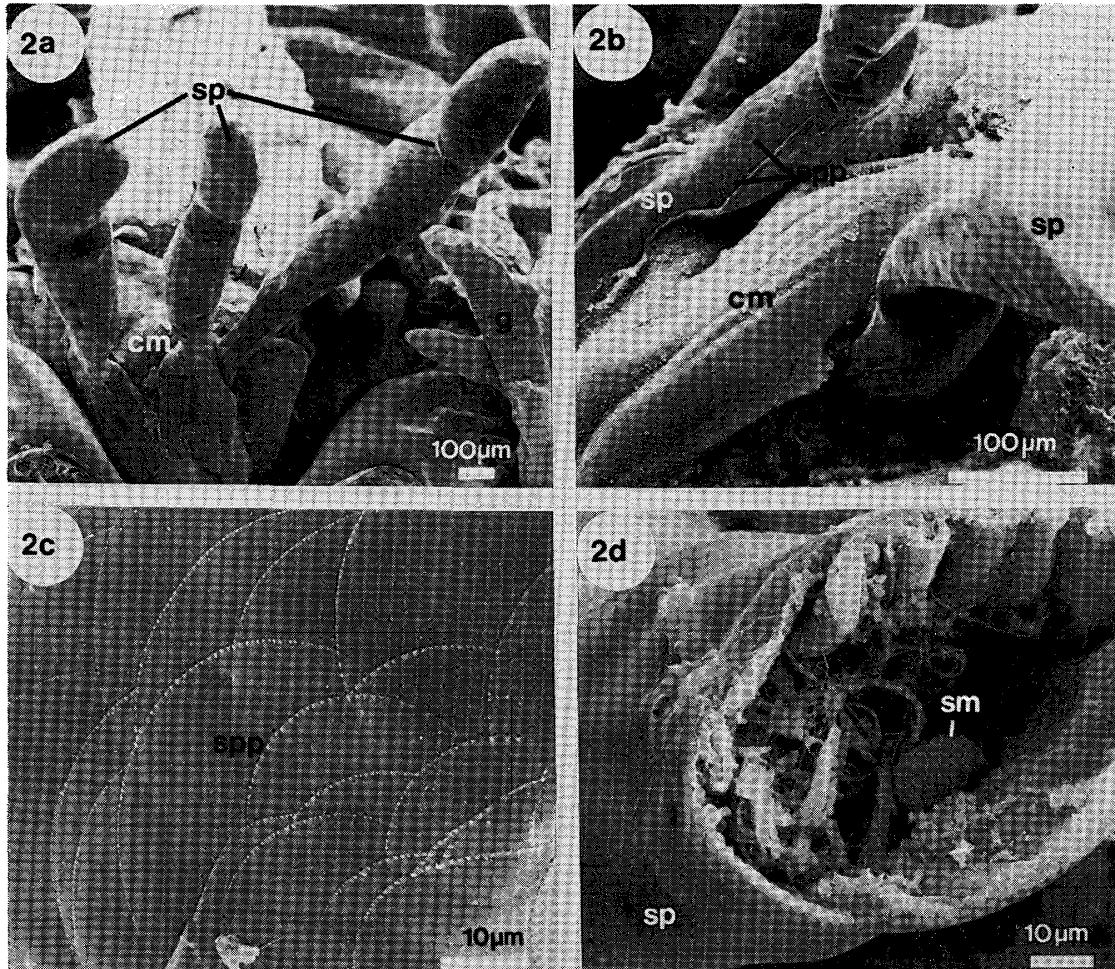


Fig. 2a. *E. crystallorophias* spermatophores (sp) protruding from the substance (cm) covering the thelycum.

Fig. 2b. Spermatophore surface structure (spp) and attachment sites are shown.

Fig. 2c. Circular chitin plates (spp) covering the surface of *E. crystallorophias*'s spermatophores.

Fig. 2d. Dissection of *E. crystallorophias*'s spermatophore showing internal and external structure as well as the spermatozoa (sm).

with the addition of usually two spermatophores and a sperm-plug. However, the thelycum is not covered, as in the case of *E. crystallorophias*, with a paste-like substance, although access to the thelycum, once fertilised, is blocked by the presence of a fibrous sperm-plug (Figs. 4a and 4b).

As with *E. crystallorophias*, *E. superba*'s spermatozoa are non-motile and approximately 10 µm in diameter (Fig. 4c). The spermatophore surfaces are also similar-

Fig. 1d. Higher magnification of the sternite plate (st) which forms part of the thelycum. Pores (p) and small setae (s) cover this plate.

Fig. 1e. Higher magnification of the pores (p) and setae (s) which cover the sternite plate of *E. crystallorophias*.

Fig. 1f. Groups of bristle setae (b) covering the ventral surface of the coxal plates (cp) which form *E. crystallorophias*'s thelycum.

Fig. 1g. Fertilised thelycum of *E. crystallorophias* showing the paste-like substance (cm) over the thelycum. Spermatophores (sp) are seen protruding from this substance which covers the thelycum. Gills (g) are seen lying either side of the thelycum.

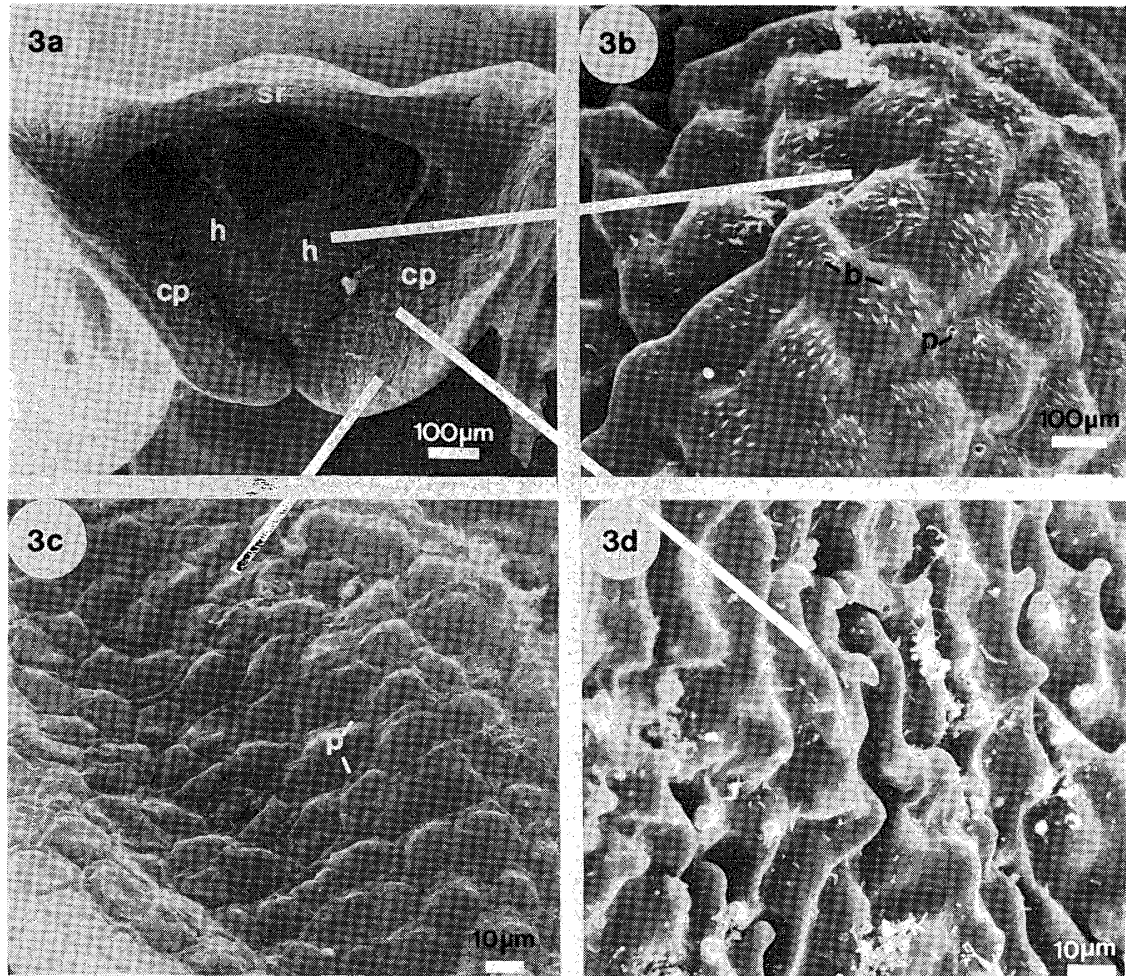


Fig. 3a. Ventral view of *E. superba* mature thelycum. Position of the coxal plates (cp), sternite ridge (sr) and holdfasts (h) are shown. Note:- anterior surface faces bottom of the page.

Fig. 3b. Groups of bristles (b) covering the surface of the thelycum holdfasts (h) from the position indicated in Fig. 3a.

Fig. 3c. Surface structure from the outer edge of the coxal plates forming *E. superba*'s thelycum, pores (p) from the position indicated in Fig. 3a.

Fig. 3d. Structure of the inner surfaces of the coxal plates forming *E. superba*'s thelycum from the position indicated in Fig. 3a.

ly covered with circular chitin plates which overlap (Fig. 4d). This type of surface structure would allow the swelling and contraction processes, hypothesised by both RAAB (1915) and BARGMANN (1937), which are needed to rapidly force the spermatozoa from the spermatophore into the thelycum. Spermatophore shape, however, differs between the two species, with *E. superba*'s being shorter and broader, *i.e.* more bulb-like in shape than *E. crystallorophias*'s spermatophore (compare Figs. 4e and 2a).

Both the thelycum and male petasma can be easily recognised using the light microscope in these two euphausiid species. Thus the structure of the thelycum and petasma (although not discussed here) has been usefully employed as an additional diagnostic characteristic to aid the separation of semi-digested dietary components from penguins and other seabird vomit samples (PUDDICOMBE and JOHNSTONE, 1987; THOMAS and GREEN, unpublished).

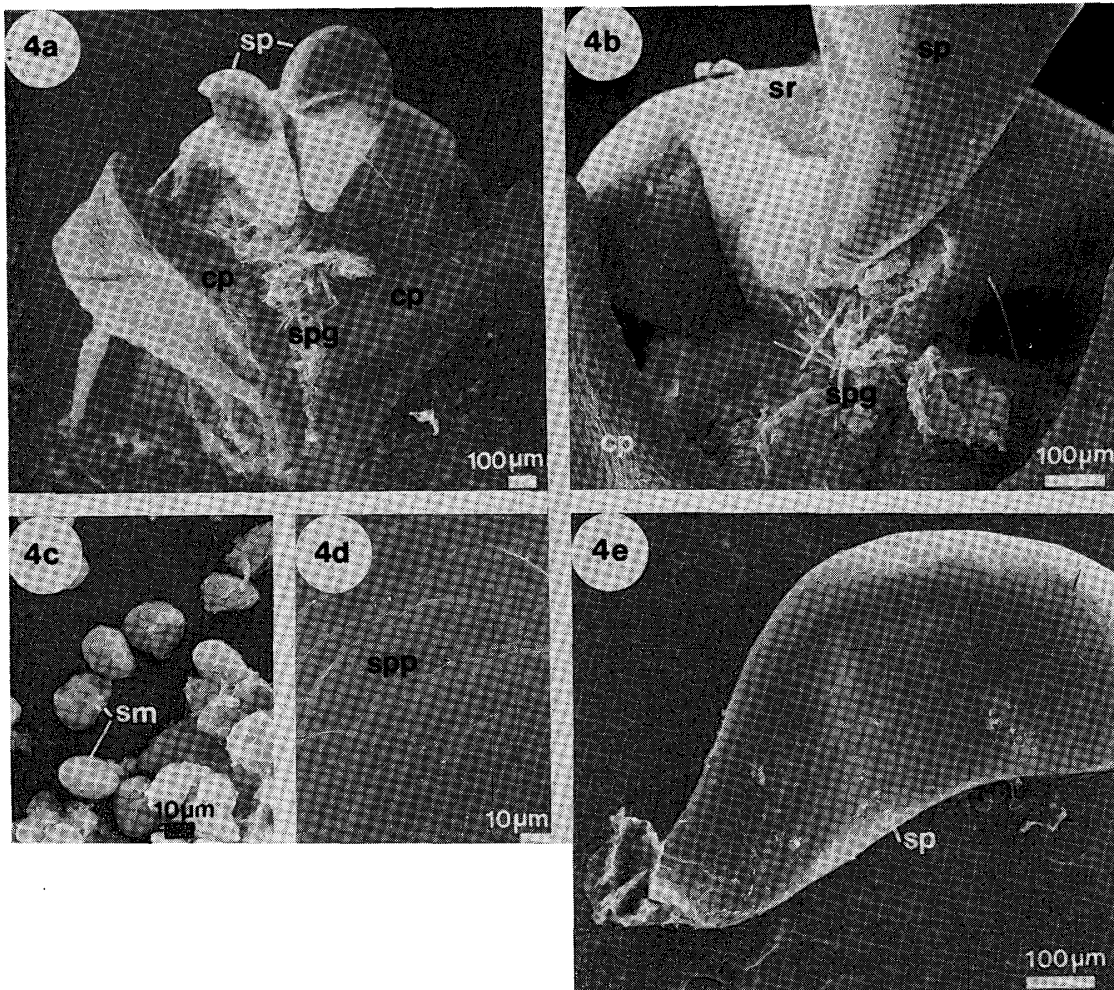


Fig. 4a. Anterior view of *E. superba*'s thelycum following spermatophore (sp) attachment. Fibrous sperm plug (spg) is indicated.

Fig. 4b. Spermatophore attachment site.

Fig. 4c. *E. superba*'s spermatozoa (sm).

Fig. 4d. Circular chitin plates (spp) covering the surface of *E. superba*'s spermatophore (sp).

Fig. 4e. Typical shape of *E. superba*'s spermatophore (sp).

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