ISSN- 0102-9010

LIGHT AND ELECTRON MICROSCOPIC ASPECTS OF GLANDS AND PSEUDOGLANDULAR STRUCTURES IN THE LEGS OF BEES (HYMENOPTERA, APINAE, EUGLOSSINI)

Carminda da Cruz-Landim and Aldirene Costa Franco

Department of Biology, Institute of Biosciences, Paulista State University (UNESP), 13506-900 Rio Claro, SP, Brazil.

ABSTRACT

The presence of glands and pseudoglandular structures in the legs of male and female of Euglossine bees was studied. Insect gland cells belonging to class I or class III are present in all pairs of legs and in all leg segments. Pseudoglandular structures are present in the male pretarsus, which lacked a typical tarsal gland. A special, probably absorptive epidermis, is present in addition to class III glandular cells in this segment. The basitarsus epidermis has special features which differed from other bees, and males have a tibial organ in the hind pair of legs. Light, scanning and transmission electron microscopy of the component parts of these structures showed that the tibial organ consists of three morphologically distinct parts, which could be distinguished in *Euglossa cordata, Eulaema mandibularis* and *Eufrisea violacens*. However, in *Exaerete smaradigina*, zone III was absent and there was a poorly defined zone IV.

Key words: bees, Euglossini, exocrine glands, tarsal gland, tibial organ.

INTRODUCTION

The Euglossinae is a subfamily of neotropical bees that are usually brightly metallic colored. This family consists of four genera (*Eulaema, Euplusia, Eufrisea* and *Euglossa*) of free living species and two genera (*Exaerete* and *Aglae*) of parasitic species. The free living species are solitary, communal, or quasi-social depending on species [11,14,15,19,25,26].

Euglossine bees, mainly the males, pollinate several families of neotropical plants, including the Orchidaceae, Gesneriaceae, Solanaceae and Araceae [13,21,23]. The relationship of the male bees to these flowers has been termed the "male euglossine syndrome" by Dressler [13] and "androeuglossophily" by Wiehler [24] because of the strange behavior the bees display when visiting the flowers.

Euglossine male bees have feathery brushes of hairs on the front leg tarsi and greatly inflated hind tibiae. On the outer superior border the tibiae have a slit with two thick rows of hard hairs [7,22]. The males scratch the flowers with their front tarsal brushes and transfer the fluid that exudates from the wounded tissues to the inflated hind tibiae [12]. Roberts *et al.* [20] observed that *Eufrisea purpurata,* an Amazonian species, often scratched the walls of houses sprayed with DDT and tried to collect the powder.

Orchids (and other flowers visited by euglossine males) are very fragrant but lack nectar and attract no other bees or insects [25]. Since these flowers have no food for the bees, the source of attraction is the fragrance, which the males collect with their hind leg tibiae. The enlarged hind tibiae contain an oval sac, usually referred as to the tibial organ, where the collected fluid is stored [8,22]. Euglossini females have normal hind tibiae and generally do not visit the same flowers as males. However, the females collect pollen and nectar from the flowers they visit.

Glandular structures have been reported in several segments of insect legs, particularly in Hymenoptera [3,4,10,14,17], and are therefore expected in euglossine bees. Based on these findings, we used light and electron microscopy to investigate the occurrence of structures present in the legs of male and female euglossine bees.

Correspondence to: Dr. Carminda da Cruz-Landim
Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Av. 24-A nº 1515, CEP 13506-900, Rio Claro, SP, Brasil, Tel. (55)(19) 526-4131, Fax (55)(19) 534-0009, E-mail: cclandim@rc.unesp.br

MATERIAL AND METHODS

Foraging male and female *Euglossa cordata, Eufrisea violacens*, *Eulaema mandibularis* and *Exaerete smaradigina* were collected in the gardens of the Instituto de Biociências (UNESP), Rio Claro, SP, Brasil.

The entire leg or its segments (coxa, trochanter, femur, tibia, basitarsus and pre-tarsus) were fixed for, light microscopy in Dietrich fixative [2], embedded in historesin (Leica) and processed for examination. Sections 6 µm thick were stained with hematoxylin and eosin. Legs fixed in Dietrich were also dehydrated in an ethanol series and critical point dried for examination by scanning electron microscopy (SEM). The samples were sputtered- coated with gold and viewed in a Jeol JMS-P15 (Japan) electron microscope operated at 60 kV. For transmission electron microscopy (TEM), the leg segments or dissected tibial organs, were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, post-fixed in buffered 1% osmium tetroxide, dehydrated in a graded acetone series and embedded in Epon-Araldite mixture. To improve the penetration of fixative, the tissues were placed in a microwave oven set to maximum power for 7-8 s. Thin sections were double stained with uranyl acetate and lead citrate in an ultrastainer and examined in a Zeiss EM9 S2 electron microscope. Semithin sections (1 µm) were stained with 1% toluidine blue for light microscopy. Part of the material was post-fixed in 2% osmium tetroxide in 0.1 M imidazol buffer, pH 7.4 to detect unsaturated lipids.

RESULTS AND DISCUSSION

In addition to the tibial organ present in the tibiae of the hind pair of legs, the euglossines have glands in all segments of the three pairs of legs, except for the tarsomeres (Table I).

Two types of glands were distinguished in euglossine legs: class I or epithelial glands, and class III or unicellular glands, based on the classification by Noirot and Quennedey [17]. As will be shown, some structures in the legs do not fit into the typical morphology of these or other glands of insects.

Distribution and morphology of the glands

Table I shows the distribution of the glands and pseudoglandular structures in all leg segments. When a class III gland was present in a given segment, the secretory pores could frequently be seen very well from the outside with SEM. Because the difficulty in sectioning the very hard cuticle, the presence of these pores was often the only sign of the presence of these glands (indicated as Ap in Table 1). In other cases, the presence of type III glands was not observed because of the bad preservation or tearing of the material during sectioning. In some of these cases (marked as "not observed" in Table I), the existence of the gland was assumed when previous studies had demonstrated that it is always present in other bees. Thus, tarsal glands have been observed in all Hymenoptera [1,3,4,5,6,8,10,17,18] and other insects [5] studied, but were not found in *E. violacens* (males and females) and in *E. mandibularis* (females). Nevertheless, it was assumed that such a gland exists in these individuals. The lack of their observation was attributed to the difficulty of obtaining good preparations.

The glandular structures in the distal segment of the hind legs of euglossine males are different from those already described for bees [1,3,5,7,13].

Table 1. Glands present in Euglossinae legs

Species	Sex and number of bees examined	Leg segments						
		Coxa	Trocanter	Femur	Tibia	Basitarsus	Tarsomeres	Pre-tarsus
Euglossa cordata	25 females	III	III	III	III	I ?	0	TG
	20 males	III	III	III	TO	I	0	TG ?
Eufrisea violacens	4 females	-	-	-	III	I ?	0	-
	4 males	-	-	-	TO	I	0	-
Eulaema mandibularis	4 females	-	-	III	III	-	0	-
	4 males	-	III	III	TO	-	0	TG ?
Exaerete smaradigina	2 females	Gp	Gp	Gp	-	-	0	GG
	2 males	-	-	III	TO	-	0	TG

III = Class III gland, **I** = Class I gland, **I ?** = possible class I gland, **TO** = tibial organ, **TG** = tarsal gland, **TG ?** = possible tarsal gland, **Gp** = gland pore, **-** = not observed, **0** = glands absent.

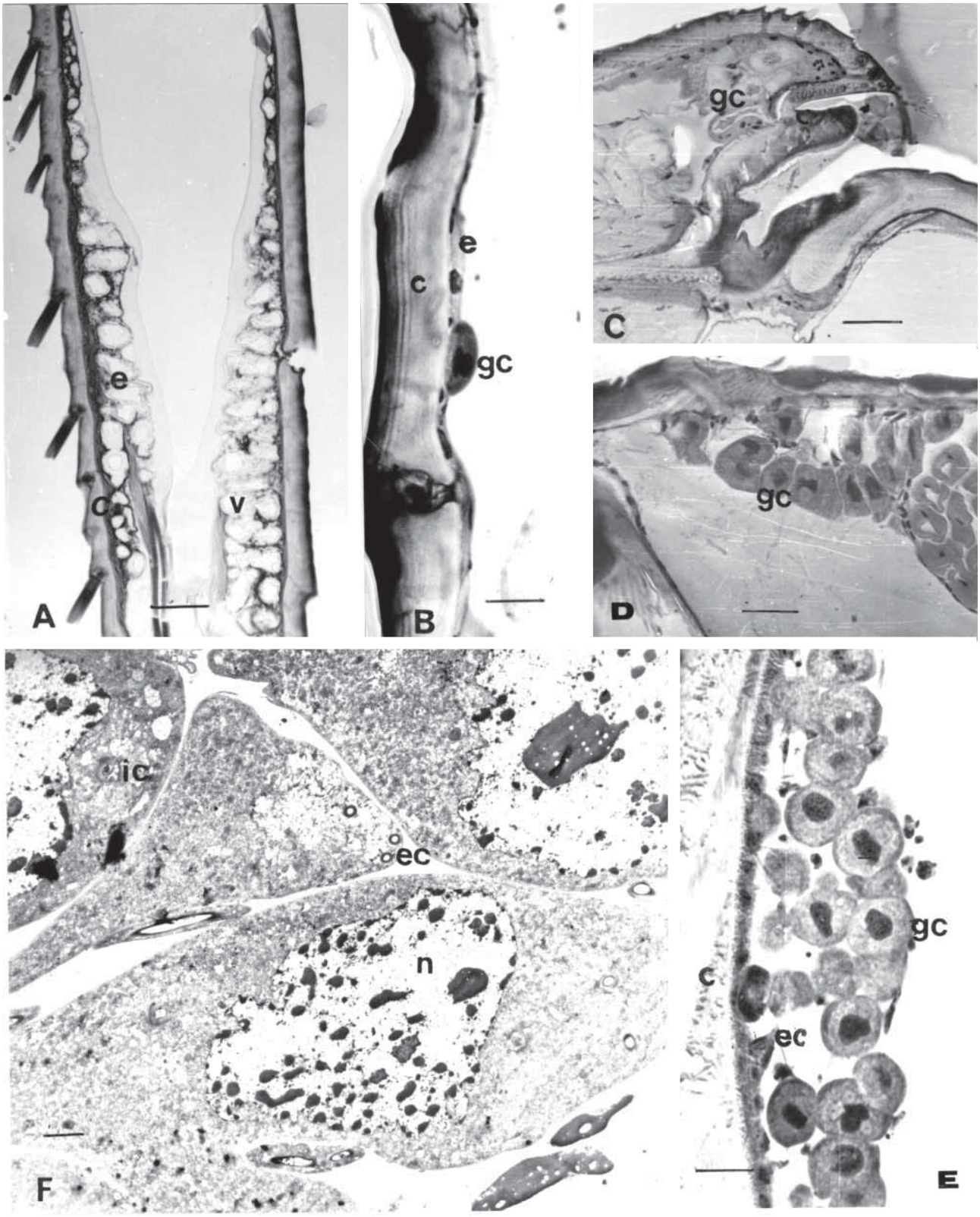

Figure 1A. Micrographs of Euglossinae leg inner structures. Basitarsus of *Euglosa cordata* showing the epidermis (e) consisting of vacuolated (v) cells. **B.** Tibia of *Exaerete smaradigna* showing a single class III gland cell (gc) apposed to the epidermis (e). **C.** Femur of *E. cordata* showing class III gland cell (gc) near the femur-tibial articulation. **D**. trochanter of *E. cordata* showing gland cells (gc) near the trochanter-coxa articulation. **E.** Femur of *E. smaradigna* showing gland cells (gc) along the dorsal side. **F**. TEM of class III gland cells from the coxa of *E. cordata*. c=cuticle, n=nucleus, ic=intracellular canal, ec=extracellular canal. Bars: A and E = 20 µm, B = 25 µm, C and D = 30 µm, F = 1 µm.

Only a sac-like tarsal gland constituted of epithelial cells is usually present in this segment. However, in males of *E. cordata, E. mandibularis* and *E. smaradigina*, the presence of a differentiated epidermis and class III gland cells was observed, in addition to a modified sac. Thus, three different structures are present in the pre-tarsus of these males: 1) an outer or dorsal epidermis with projections that faced the inside of the segment and supported by cuticular cores formed by spine-like structures that project inwards from cuticle, 2) a thin-walled sac, apparently not secretory, which occupied the center of the segment, and 3) a ventral or inner group of class III gland cells (Fig. 1A). Because of the difficulty in obtaining good preparations, it was impossible to confirm whether these structures were present in all pairs of legs, although they are present in the pre-tarsus of hindlegs

The cuticular projections consist of elongated, spine-like structures made up of coiled fibers located inside apical invaginations of the epithelial cell plasma membrane. These structures penetrate the cuticle and appear to be continuous with the contents of the cuticle canal pores. Since adequate preservation of this epithelium was not possible, its ultrastructural features cannot be described, althought they apparently contain a large amount of fibrous elements which form a cytoskeleton. This arrangement is also suggested by the striated aspect of the cytoplasm seen in light microscopy. In females of the studied species, the pre-tarsus contained only the tarsal gland formed by an invagination of the epidermis, as in other bees [1,8,18].

The basitarsus of Euglossini bees (males and females) also has a peculiar epidermis. In other bees [8,9,10,14], the epidermis of the dorsal side of the basitarsus is differentiated to produce a class I gland structure [9]. In the present species, the epidermis lining the inner surface of the segment is formed by very large and vacuolated cells (Fig. 1A). The vacuoles are larger towards the distal end of the basitarsus, where they left only a very thin, outer ring of cytoplasm. These vacuoles are apparently empty, but in some of them it was possible to perceive a layer suggestive of a cuticular lining. Since no electron micrographs were obtained of this part of the legs, the detailed structure of these vacuoles cannot be described.

The other leg segments also contain glands (Table 1). Female tibia has only a few class III gland cells that are closely apposed to the epidermis (Fig. 1B). However, in the femur (Fig. 1C,D), trochanter (Fig. 1E) and coxa (Fig. 1F), there are groups of class III gland cells in the outer, posterior corners of the segments. The number of cells present in *Exaerete* femurs (Fig. 1E) was greater than in the other species studied. In this case, the cells extend along the segment rather than, concentrating near the posterior articulation. In females the glands are more developed than in males, i.e., with a greater number of cells.

TEM examination of type III gland cells in the *E. cordata* coxa revealed very large irregular nuclei, with a prominent nucleolus and clusters of heterochromatin. The cytoplasm around the intercellular canaliculus appeared vacuolated, which suggests the accumulation of secretion (Fig. 1F).

The male tibial organ

Euglossine males have wide and thick hind leg tibiae. A series of structures located within the leg form the "male tibial organ".

Cruz-Landim *et al*. [7] recognized three zones in this organ. The interior of the tibia is almost completely occupied by an always inflated bag that histologically consisted of two regions (Fig. 2B). A superior or dorsal region connected to the outer slit in the tibia represents zone I (Fig. 2A,B). This part of the sac is very thin-walled, with an inner lining of cuticle from which many ramified spines projected radially into the interior of the sac. These spines form a framework that supposedly prevents the sac from collapsing (Fig. 3A,B). Below this is a part of the sac formed by a highly folded epithelium identified as zone II (Fig. 3B). This epithelium is formed by cells that are not as flat as those of zone I and is also lined with cuticle, but without spines. This part of the sac was prevented from collapsing by the epithelial infoldings (Fig. 3A). Zone III is represented by the modified epidermis subjacent to the pilous outer slit. This epidermis had features similar to those described for the pretarsus (Fig. 2C, 3A).

The tibial organ of *E. smaradigina* had a different arrangement in wich the structures are more delicate. Zone I has less developed spines and zone

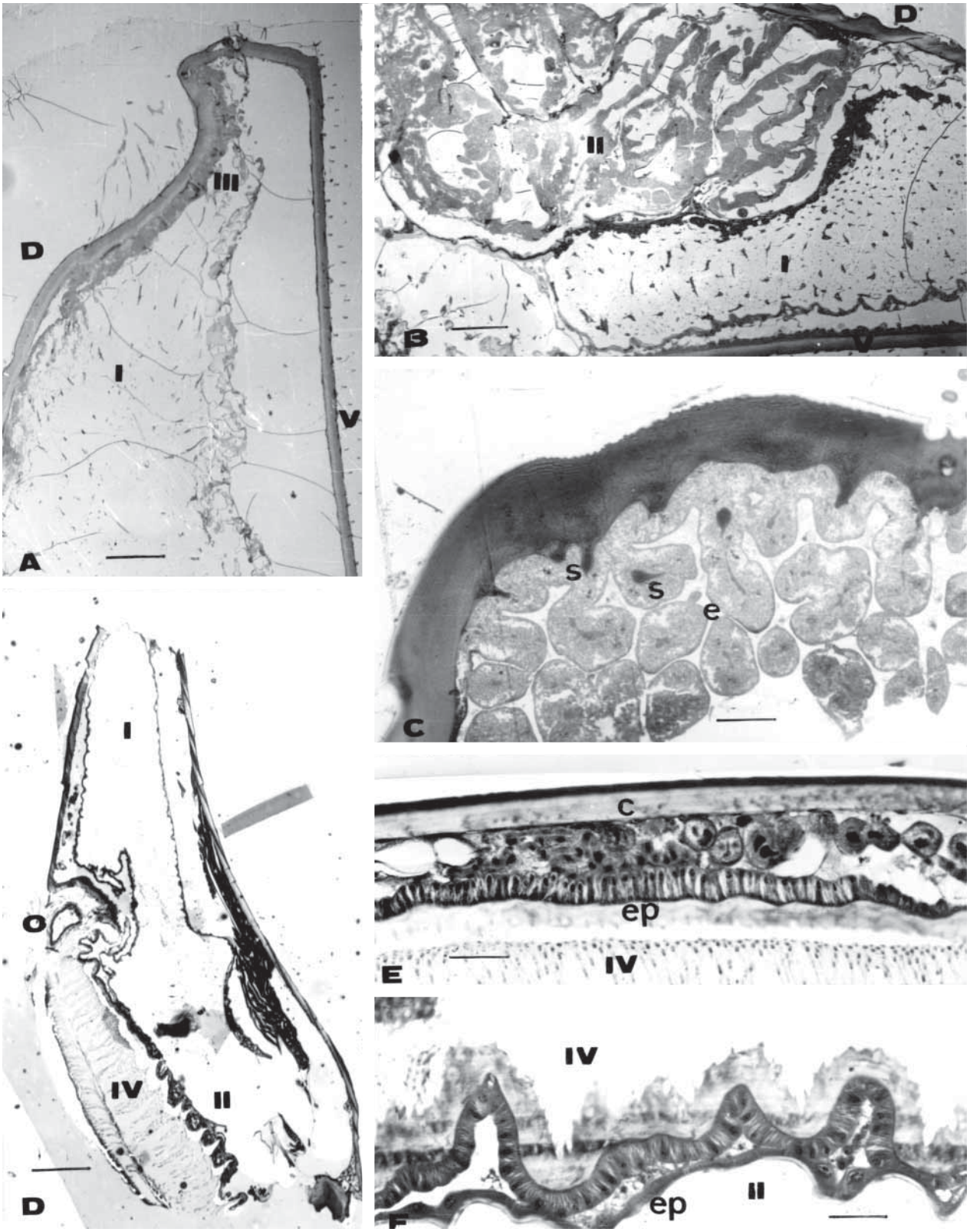

Figure 2. Male tibial organ. **A,B,C**. Tibial organ of *Euglossa cordata* showing in (**A)** a view of zones I and III and in (**B)** zones I and II . **C**. Detail of zone III. **D, E, F**. Tibial organ of *Exaerete smaradigna*. Note the absence of zone I, the presence of a zone IV and of class III gland cells (in E). c=cuticle, ep=epithelium, O=outer opening, s = cuticular spinus, Bars: A and D = 100 µm, B = 50 µm, C, E and F = 20 µm.

II has fewer invaginations (Fig. 2D). Zone III was absent, and instead a different zone, to referred here as IV, appeared behind the outer slit (Fig. 2D,E). This zone contains a folded epithelium (Fig. 2D,F) in a region occupied by what appears to be very thin cells closely applosed to each other. The epithelium surrounds the mass of thin cells, but its outer surface is smooth and without folds (Fig. 2E). Class III gland cells are present between the epithelium and the cuticle (Fig. 2E). The latter glandular epithelium consists of prismatic cells with very a well evident longitudinal striation (Fig. 2F).

Electron microscopy provided a better understanding of the structure of this organ. Scanning electron microscopy showed that the hairs present in the outer slit are hollow (Fig. 3B), and that the cuticular projection is covered by the epidermis (Fig. 3A), as shown in Figure 2C, formed zone III. The cuticle of this region is crossed by the hair roots and shows numerous canal pores (Fig. 3B,C,D,E). Transmission electron microscopy showed that a layer of oil was present on the outer surface of this cuticle (Fig. 3D). The epithelium is formed by cells of median height and, as seen in the pretarsus, the epithelial cells had apical invaginations of the plasma membrane which are filled with a substance that continues within the cuticular canal pores (Figs. 4A,B,C). In cross-section, a space was seen between the cell membrane and the material that occupied the lumen invaginations (Fig. 4B).

Scanning electron microscopy showed that the interior of the sac in zone I is totally filled with ramified spines. The outer surface of this sac in zone I has perforations corresponding to the points of spine insertion into the wall. The ramifications of the spines were perpendicular to the axis and forms a closed frame parallel to the sac wall. Towards the center of the sac, the number of branches decreases and the framework became looser.

Sections prepared for SEM showed that the main lumen of the spines was hollow and filled with an oil-like substance (Fig. 5A).

Zone II was located above and external to zone I, and separated from it by a depression beginning at the point from where a canal enters and connects the sac to the outside (Fig. 2D). Transmission electron microscopy showed that this part is probably formed by an invagination of the tibial epidermis, and is therefore lined internally by a cuticle (Fig. 4C, 5C).

The hemolymph within the tibia would bathe the infolds of this epithelium. The epithelial cells contain numerous lipid droplets and lipid-like stores is seen adhering to the cuticle (Fig. 5B,C) which in turn stains deeply by the osmium imidazol treatment.

The description above refers to *Euglossa, Eulaema* and *Eufrisea. Exaerete* was not studied by SEM and TEM.

Although not seen with LM, class III cells were seen with TEM (Fig. 5E).

The morphology of the structures just described shows that some of them clearly have a glandular nature, while others appear to be organs for the absorption and storage of different products. The glandular structures are class III gland cells and the tarsal gland present in the female pre-tarsus. The differentiated epithelium present in the basitarsus requires further study in order to determine the precise cell structure.

The modified epidermis present in the male pretarsus and zone III of the tibia appeared to have an absorptive function. The substances absorbed would be those collected by the hairs present in the outer slit of the tibia. Once inside the tibial hemolymph, these substances may be absorbed by zone II spines and stored in the corresponding sac. The relationship between zones II and III is not clear. These two zones, although morphologically different and spacially separated, appeared to have a continuous lumen, the content of which appears to be lipidic in both cases. The cells of the zone II epithelium also had lipid droplets in their cytoplasm. The quality of the TEM preparations was not sufficient to determine whether this lipid was imported or synthetized within these cells. Fixation with imidazol buffered osmium confirmed the unsaturated nature of the lipid stores.

The mechanisms by which the material absorbed and stored in this organ is further delivered to be used by the male is unclear. The presence of an opening, independent of the slit, may indicate that there is a local outlet for substances, different from that for their intake. There is evidence that the canal connected to this opening is linked to zone III. However, there is no explanation for the ab-

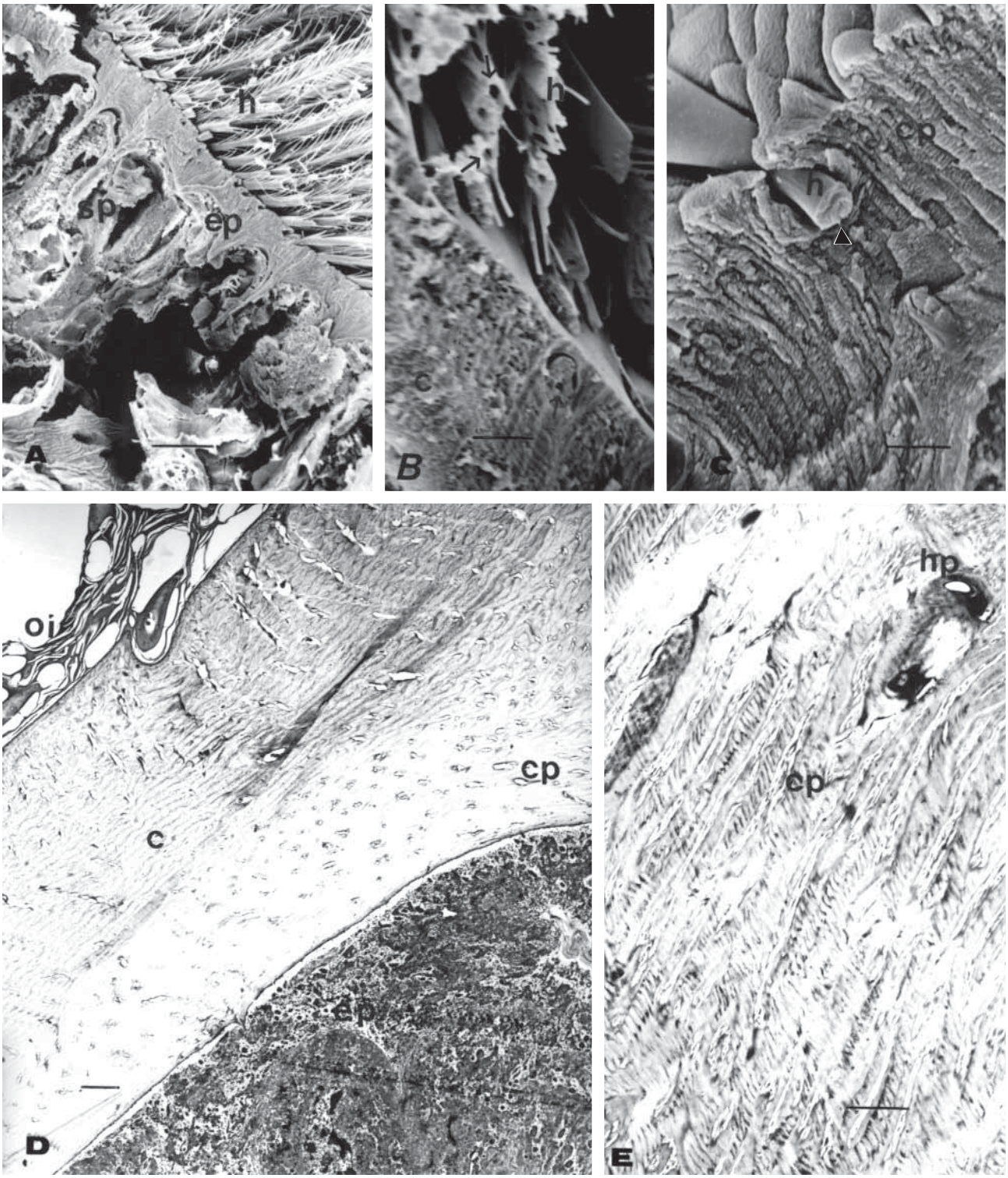

Figure 3. Cuticle of the tibia. **A**. SEM of zone III showing the cuticular spines (sp) and the epidermis (ep). Note the outer hairs (h). **B**. SEM detail showing the canal (arrows) in the hairs (h). **C.** SEM of the canal pore (cp) in the cuticle and their root (arrowhead) crossing the cuticle (c). **D.** TEM of the cuticle (c) and epidermis (ep) of region III. Note the oil-like material (oi) deposited outside and the canal pores (cp). **E**. Hair pore (hp) and canal pore (cp) in the cuticle (c). Bars: A = 200 µm, B = 150 µm, C = 50µm, D and E = 1 µm.

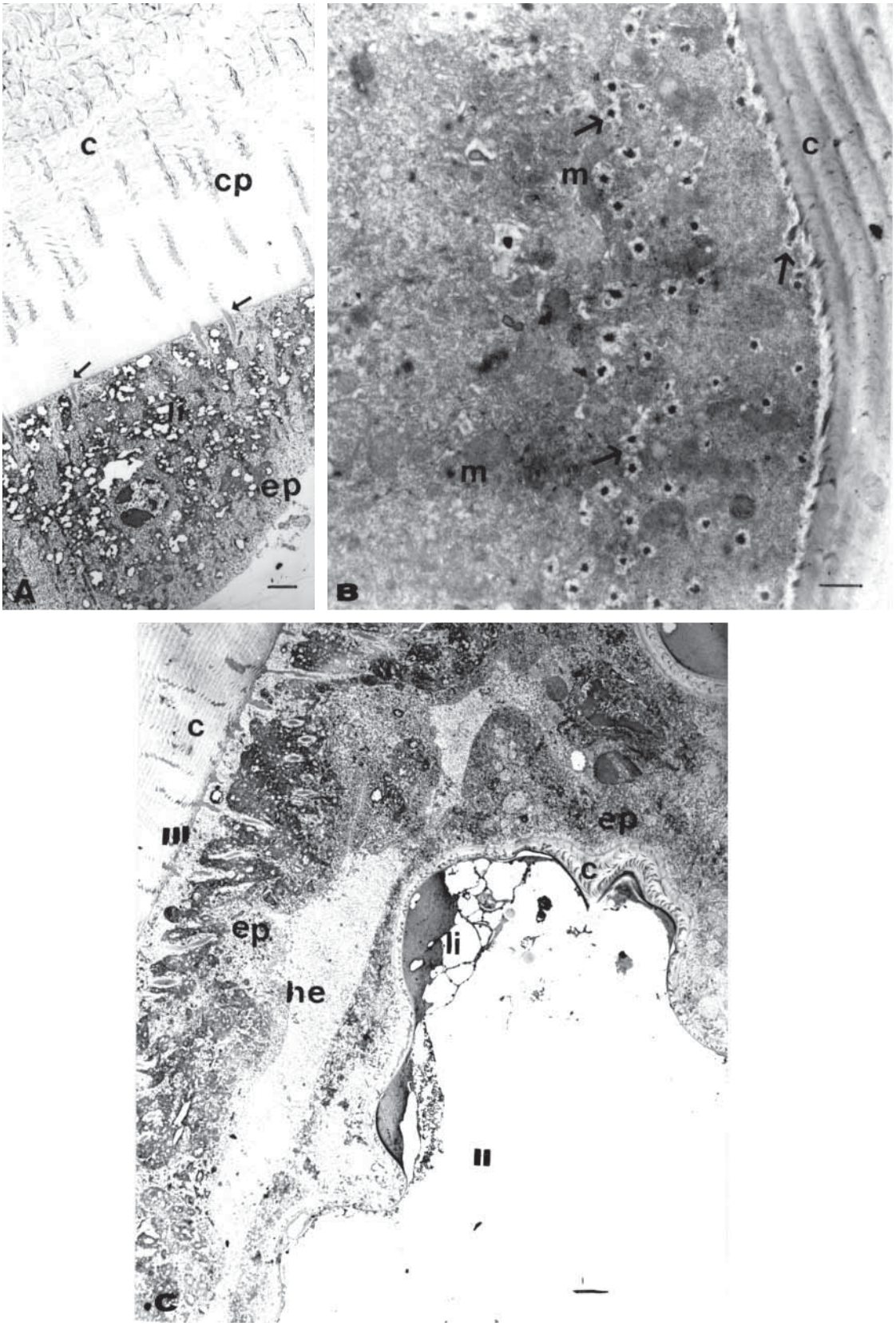

Figure 4. A, B. TEM of zones II and III showing sections of the "spines" (arrows) **C**. Zone II showing the epithelium (ep) and lipid (li) content. m = mitochondria; he = hemolymph. c = cuticle. Bars = 1 µm.

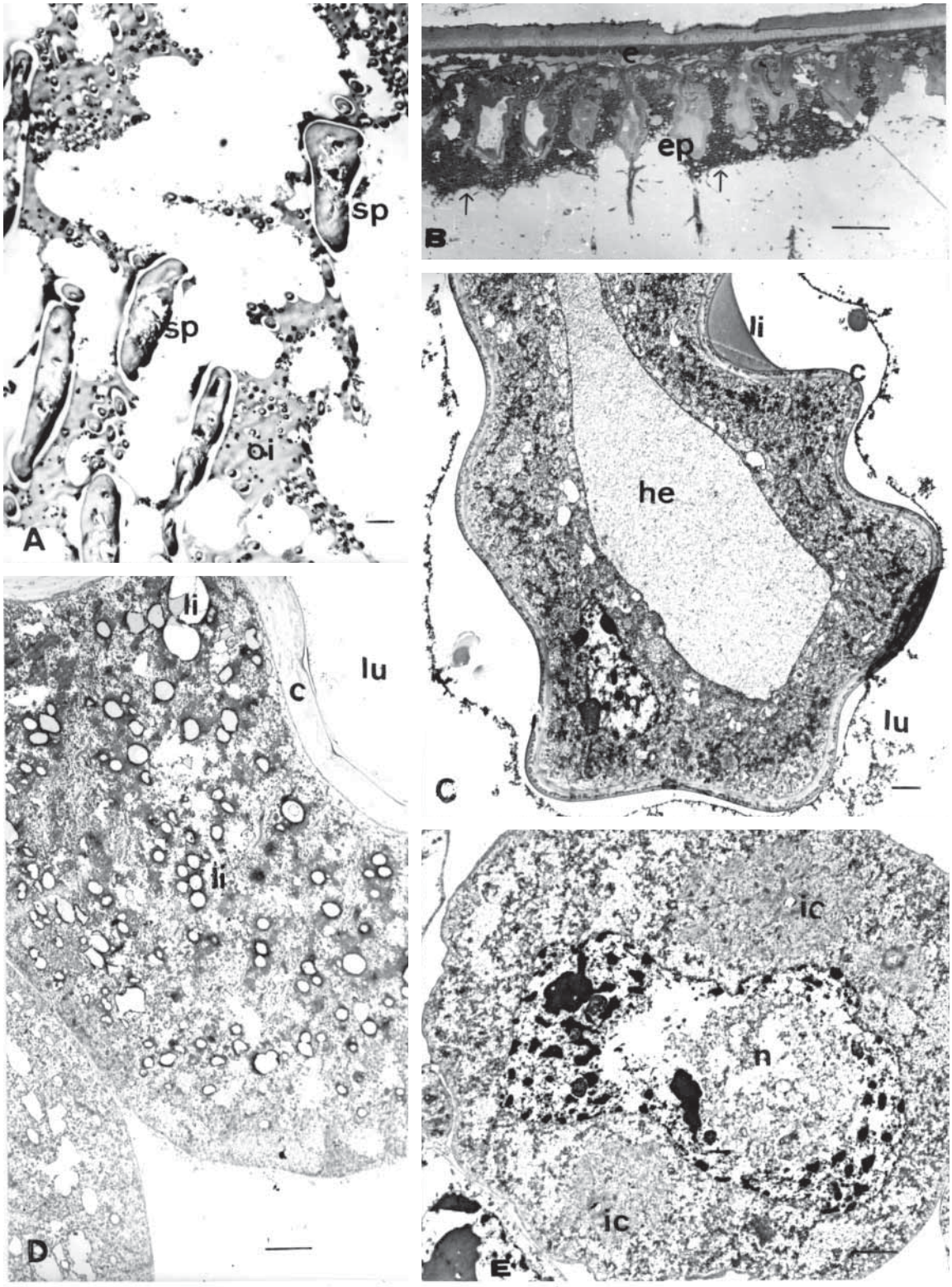

Figure 5. A. Cross-sections of zone I spines (sp) surrounded by oil-like (oi) deposits. **B.** Light microscopy of zone II showing the folded epithelium (ep) and the lipid content (arrows) retained among them. **C.** TEM of one fold of zone II showing the cuticular (c) lining facing the lumen (lu). Note the lipid (li) adhered to the cuticle (c). **D.** Detail of the epithelium showing lipid (li) droplets stained by osmium-imidazol in the cytoplasm. **E.** Class III gland cell in the tibia. n = nucleus, ic = intercellular canal. Bars = 1 μm

sorption of substances by zone I and for delivery by zone II since there is no evidence for metabolization of the sac contents by epithelial cells. On the other hand, the lipids absorbed may be condensed in zone II before being eliminated through the canal connected to the cuticle.

ACKNOWLEDGMENTS

This work was supported by CNPq (520.566/97-5) and FAPESP (92/4700-9).

REFERENCES

1. Arnhart L (1923) Das Krallenglied der Honigbiene. *Arch. Benenk*. **5,** 37-86.
2. Beçak W, Paulete-Vanrell J (1970) *Técnicas de Citologia e Histologia*. Livraria Nobel S.A., São Paulo.
3. Billen JPJ (1984) Morphology of tibial gland in the ant *Crematogaster scutellaris*. *Naturwiss* **77,** 234-235.
4. Billen JPJ (1997) Morphology and ultrastructure of the metatibial gland in the army ant *Dorylus molestus* (Hymenoptera, Formicidae). *Belg. J. Zool.* **127,** 159-166.
5. Brousse-Gaury P (1983) Sur l'existance d'une gland tibiale chez *Lapidura riparia* Pallas et *Forficula aurivularia* L. (Insectes, Dermaptères). *C. R. Acad. Sci.* Ser. III **297,** 203-208.
6. Cederberg B (1977) Evidence for trail marking in *Bombus terrestris* workers (Hymenoptera, Apidae). *Zoon* **5,** 143-146.
7. Cruz-Landim C, Stort AC, Cruz MAC, Kitajima EW (1965) Orgão tibial dos machos dos Euglossinae: estudos ao microscópio óptico e eletrônico. *Rev. Brasil. Biol.* **20,** 323-342.
8. Cruz-Landim C, Cunha MAS (1965) Glande tarsale des abeille sans aiguillon. In: *Actes du Vème Congrès pour l'Etude des Insectes Sociaux*, Toulouse, France, 5-10 July, Laboratoire d'Entomologie de la Faculté des Sciences, pp. 219-225.
9. Cruz-Landim C, Silva de Moraes RLM (1994) Ultrastructural localization of new exocrine glands in legs of social Apidae (Hymenoptera) workers. *J. Adv. Zool.* **15,** 60-67.
10. Cruz-Landim C, Silva de Moraes RLM, Salles HC, Reginato RD (1998) Note on glands present in Meliponinae (Hymenoptera: Apidae) bee legs. *Rev. Brasil. Zool.* **15,** 159-165.
11. Dodson, CH (1966) Ethology of some bees of the tribe Euglossini (Hymenoptera: Apidae). *J. Kansas Entomol. Soc.* **39,** 607-629.
12. Dodson, CH, Dressler, RL, Hills HG, Adams RM, Williams NH (1969) Biologically active compounds in orchid fragrances. *Science* **164,** 1243-1249.
13. Dressler RL (1968) Pollination by euglossine bees. *Evolution* **22,** 202-210.
14. Franco AC, Cruz-Landim C (1999) Ocorrência morfologia de glândulas nas pernas de *Centris* e *Epicharis* (Hymenoptera: Anthophoridae). *Rev. Brasil. Zool.* **16** (Suppl. 2), 1-10.
15. Michener, CD (1974) *The Social Behaviour of the Bees*. The Belknap Press, Harvard University Press: Cambridge, M.A.
16. Noirot C, Quennedey A (1991) Glands, gland cell, glandular units: some comments on terminology and classification. *Ann. Soc. Ent. Fr.* **27,** 123-128.
17. Patricio EFRA, Silva de Moraes RLM (1997) Do the workers of the stingless bees *Frieseomelitta silvestri* and *F.varia* (Apidae, Trigonini) use their legs only for walking ? **4** (Suppl.), 162.
18. Pouvreau A (1991) Morphology and histology of tarsal glands in bumble bees of the genera *Bombus, Pyrobombus* and *Megabombus*. *Can. J. Zool.* **69,** 866-872.
19. Roberts DR, Alecrim WD, Heller JM, Ehrnadt SR, Lima JB (1982) Male *Eufrisea purpurata*, a DDTA-collecting euglossine bee in Brazil. *Nature* **297,** 62-63.
20. Roberts RB, Dodson CH (1967) Meeting biology of two communal bees, *Euglossa imperialis* and *Euglossa ignita* (Hymenoptera: Apidae), including description of larvae. *Ann. Entomol. Soc. Am.* **60,** 1007-1014.
21. Soares AA, Campos LAO, Vieira MF, Melo GAR (1989) Relações entre *Euglossa (Euglossella) mandibularis* e *Cyphomandra calycina* (Solanacea). *Ciênc. Cult.* **41,** 903-905.
22. Vogel S (1963) Das sexuelle Anlockungsprinzip der Catasetinen und Stanhopeen Bluten und die wahre Funktion chressogenannten Futtergewlves. *Osterneichischen. Bot. Zeits.* **110,** 308-337.
23. Vogel S (1968) Die Ölblumensymbiosen-Parallelismus und andere Aspecte ihrer Entwicklung in Raum und Zeit. *Z. Zoll. Syste. Evolut-Forsch* **26,** 341-362.
24. Wiehler H (1976) A report on classification of *Ahimenes, Eucodia, Gloxinia, Goyazia* and *Anetanthus* (gesneriaceae). *Selbyana* **1,** 374-404.
25. Williams NH (1968) A preliminary bibliography on euglossine bees and their relationships with orchids and other plants. *Selbyana* **2,**345-355.
26. Wilson EO (1974) *The Insect Societies*. The Belknap Press, Harward University Press: Cambridge, M.A.

Received: October 26, 2001
Accepted: January 29, 2002