

Ultrastructure of dermal and defence glands in *Cyphophthalmus duricorius* JOSEPH, 1868 (Opiliones: Sironidae)

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Abstract: The structure of dermal glands and defence glands is described in a species of Cyphophthalmi. The dermal glands consist of different cell types that discharge their secretion into a microvilli bordered cavity before reaching the cuticle-lined duct system. The paired sac-like defence glands are composed of an excretory channel, a non-secretory area and a secretory area. These three parts are characterized by a different arrangement of cells. The secretory area includes a number of glandular units, probably derived from dermal glands, producing a heterogeneous secretion that is discharged via numerous small ducts into the wide cuticle-lined lumen.

Key words: Cyphophthalmi, defence gland, dermal gland, ozophore

Introduction

The Cyphophthalmi represent a subgroup of small, „mite-like“ Opiliones of about 115 species living in caves and leaf litter (MORITZ 1993, GIRIBET 2000, GIRIBET, BOYER 2002). They are regarded by some authors as closely related to Palpatores (Cyphopalpatores; e.g., MARTENS *et al.* 1981, MARTENS 1986), whereas others consider them as the most early derivative Opiliones constituting the sister group to all other Opiliones (GIRIBET *et al.* 1999, 2002, KARAMAN 2005). The first view was mainly based on morphological characteristics of the ovipositor and penis. In contrast the second interpretation used aside of numerous morphological characters also molecular data sets. Furthermore, Opiliones are placed differently in cladograms depicting arachnid phylogenies. For example, some authors consider them closely related to Acarinomorpha (Ricinulei and Acari; e.g., WEYGOLDT, PAULUS 1979a, b, PAULUS 2004), whereas others suggested a more or less close relationship to Scorpiones (e.g., HAMMEN 1989, SHULTZ 1990, WHEELER, HAYASHI 1998, GIRIBET *et al.* 2002). Evidently, much more has to be learnt about these arachnids until it is possible to reach generally accepted conclusions.

In the frame of a general study on cyphophthalmic ultrastructure we present here preliminary results on two glandular systems using *Cyphophthalmus duricorius* (Sironidae): dermal glands and defence glands. Further glands such as coxal glands (nephridia) and the male tarsal glands are currently under investigation and further opilionid taxa will also be included.

Material and Methods

The individuals of *Cyphophthalmus duricorius* JOSEPH, 1868 were collected from leaf litter in Styria (Austria) by R. Schuster in May 2005. The study is based on 10 adult specimens of both sexes. For

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transmission electron microscopy (TEM) examinations the cyphophthalmids were cut between prosoma and opisthosoma with a razor blade in buffered glutaraldehyd (2.5% glutaraldehyd in 0.1M phosphate buffer, pH 7.2). The tissues remained in the fixative for one night. After washing the material in 0.1M phosphate buffer (two times), it was postfixed in osmium tetroxide (2%), and then washed in phosphate buffer (three times) again. Before embedding in Spurr's resin (SPURR 1969) the material was dehydrated in graded ethanols (from 60 up to 100%). Ultrathin sections were made with a Leica Ultracut UCT, and then stained with uranyl acetate and lead citrate. Observations were done with a transmission electron microscope Zeiss EM 10 A.

For scanning electron microscopy (SEM) one animal was dissected in a phosphate buffer (same as above) for studying the defence glands. The sample was then transferred into the fixative (see above), and then treated like the material for TEM until the dehydration in pure ethanol. Afterwards the sample was transferred in amylacetate and critical point dried with a BAL-TEC CPD. Subsequently, the material was covered with palladium-gold by using the Polaron Mini Sputter Coater SC 7620. The sample was studied with a scanning electron microscope LEO DSM 940 A.

Results

No structural differences between the sexes were observed with regard to the glands studied.

1. Dermal glands

The integument of *C. duricorius* is richly invested with small dermal glands on the dorsal side of the body as well as on the ventral side. The gland openings are distributed irregularly with a maximum density of 8 openings per 100 μm^2 . These glands consist of a set of three types of cells: secretory, collar and canal cells which are surrounded by intercalary cells (Fig. 1A).

The secretory cells (Fig. 1A-C), are rather large, containing a prominent nucleus, which is surrounded by numerous cisternae of rough endoplasmic reticulum, free ribosomes and mitochondria. The mitochondria (0.1–0.4 μm in diameter) are round, sometimes elongated, located mainly in the distal part of cells. Small lipid droplets were also observed. Golgi bodies produce distinct, densely staining granules (0.5–2.1 μm in diameter). A different distribution of granules was found depending on the secretory activity of the cell. In early stages there are round or oval granules differing in their electron density. In late stages a number of the electron lucent granules merge and fill almost the whole cell. The apex of the secretory cell bears microvilli (1.0–1.8 μm long), which extend into a funnel-shaped cavity, called secretory reservoir, where the secretion accumulates (Fig. 1A, B).

The collar cell surrounds the secretory cells of each gland and a small part of the proximal part of the canal cell (Fig. 1A). The collar cell contains rough endoplasmic reticulum, mitochondria and granules. Its nucleus is situated at the base. Golgi bodies and lipid droplets are present as well. The granules (0.5–0.7 μm in diameter) of the collar cell are surrounded by a distinct membrane. Similar to the secretory cells, also the collar cell bears microvilli (Fig. 1A, B). However, in contrast to the secretory cells, the microvilli are shorter (0.2–0.4 μm long). A distinct marginal fold is connecting the secretory cells and the collar cell (Fig. 1A, B). This fold stabilises this area as it anchors the glandular cells (secretory cells + collar cell). The fold includes densely packed microtubules encircling this area of the reservoir (Fig. 1B).

The microvilli border encloses the secretory funnel-shaped reservoir. The contents of the reservoir pass through a canal formed by a canal cell. Although the canal can be divided in two strikingly different parts, it seems that the duct is composed of one canal cell only. The proximal part of the canal cell (0.1 μm long) has a thick but less dense cuticle of a peculiar fine structure.

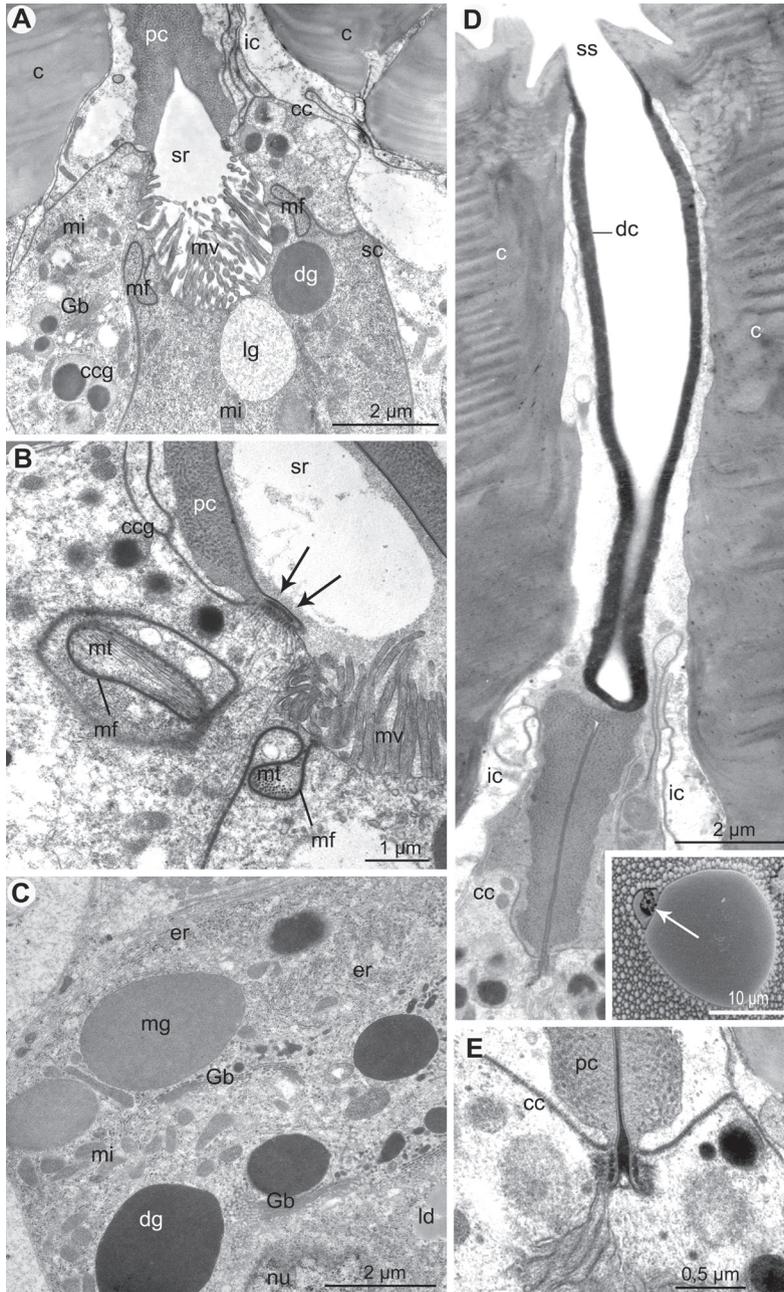


Fig. 1. The dermal glands. **A:** Glandular unit of a gland. Both, the secretory and the collar cell form a microvilli border, which extends into the secretory reservoir. **B:** The connection between the secretory cell and the collar cell is characterized by marginal folds provided with many microtubules. The linking of the collar cell to the proximal canal cuticle is provided by a peculiar attachment zone (arrows). The proximal beginning of the duct is wide open. **C:** Detail of the secretory cell. Note the Golgi bodies and secretions. **D:** Longitudinal section through the canal of a dermal gland. The canal cell is surrounded by intercalary cells. The proximal beginning of the duct is closed. Inset: SEM figure of the orifice of a dermal gland (arrow) in the surface cuticle next to a smooth tubercle. **E:** The proximal beginning of the canal with the collar cell linked to the canal cell by septate junctions.

Its electron-lucent inner (i.e., adjacent to the lumen) layer projects into the reservoir lumen. The canal cell is proximally linked by septate junctions with the collar cell (Fig. 1E). The collar cell attaches to the cuticle by a peculiar junction (comparable to hemidesmosomes) (Fig. 1B). The distal part of the canal cell (length about 0.3 μm) is provided with a thin but dense cuticle. It seems that the thin cuticle of the distal part is interlocked in the thick cuticle of the proximal part composing a valve like structure. The canal surrounded by the canal cell is distinctly narrowed in this region before extending to the surface. Near the orifice, the cuticle of the distal canal merges with the integument cuticle. The duct terminates at the surface with a small opening surrounded by tiny cuticular lips arranged in a rosette like manner (Fig. 1D).

2. Defence glands

In *C. duricorius*, as in other Cyphophthalmi, the openings of the sac-like defence glands are located on dorsolateral elevations, the ozophores (Fig. 2A). They are approximately 110 μm high. The slit-like opening is located under a small lid-like protuberance (Fig. 2B, C) and measures about 20 μm . The glands are composed of a short excretory canal, a distal non-secretory part and a proximal secretory part.

Examined by TEM, the secretory slit is bordered by a cuticle forming a thick and externally smooth dorsal lid that overhangs the ventral border (Fig. 2B). Starting from the secretory slit, the cuticle becomes thinner towards the excretory canal (Fig. 2D). At this part muscles are attached to the canal (Fig. 2E, F). Likely, the muscles play an important role for the opening of the glands and consequently for the expulsion of the secretory products. The excretory canal continues to the non-secretory part and finally to the secretory part. Both regions are composed of an epithelium, covered by a thin cuticle. The flat epithelium of the non-secretory area is composed of cuticle-supporting cells only. Characteristic for the non-secretory part are the foldings of the wall. Because of this feature we propose a division of the non-secretory area in to three parts. In the first part the intima shows simple ridges (Fig. 2F); in the second part regular folds are present (Fig. 2G) and, finally, in the third part close to the secretory area the intima is strongly and irregularly folded (Fig. 2H). These differences may reflect the different rigidity of the cuticle of the three regions. The cuticle-supporting cells of the non-secretory part are provided with ovoid nuclei and glycogen granules. Other cell organelles like small mitochondria (0.2 μm in diameter) were more obvious in the second and third part of the non-secretory area. In the last part of the non-secretory area muscles are also present (Fig. 2H). The secretory part extends into the interior of the body as a rather wide large sac (Fig. 2A). This part of the gland is more complex. Like the non-secretory part, the secretory part of the defence glands is also characterized by many folds of the wall (Fig. 2I). These folds are stronger here than in the non-secretory part. Contrary to the excretory canal and the third part of the non-secretory area, no muscles have been observed in the secretory area. The wall of the secretory part consists of secretory cells and duct cells, forming glandular units, and cuticle-supporting cells. The secretory cells consist of an ovoid nucleus (0.4 μm in diameter), numerous cisternae of rough endoplasmic reticulum, many mitochondria, lipid droplets and granules. The mitochondria are elongated and are mostly concentrated at base and at apex of the cell. The granules are either electron-lucent or electron-dense. The lucent granules are smaller (0.07 μm in diameter) while the dark granules (0.07-0.1 μm in diameter) are larger. The dark granules were more often observed and sometimes appeared in groups of two or three granules. Furthermore, lysosomes were observed in the secretory cells. The cells of the secretory part are connected to each other by interdigitations and bear many microvilli projecting into an elongated cavity (Fig. 2J). The duct cells begin at this cavity (Fig. 2J, K). The duct cells are similar to those of the dermal glands. But the thick, less dense cuticle is not seen. The cuticle-supporting cells of the secretory area contain an elongated nucleus, some small mitochondria and glycogen granules. The lumen of the defence gland may contain sometimes a heterogeneous secretion (Fig. 2I).

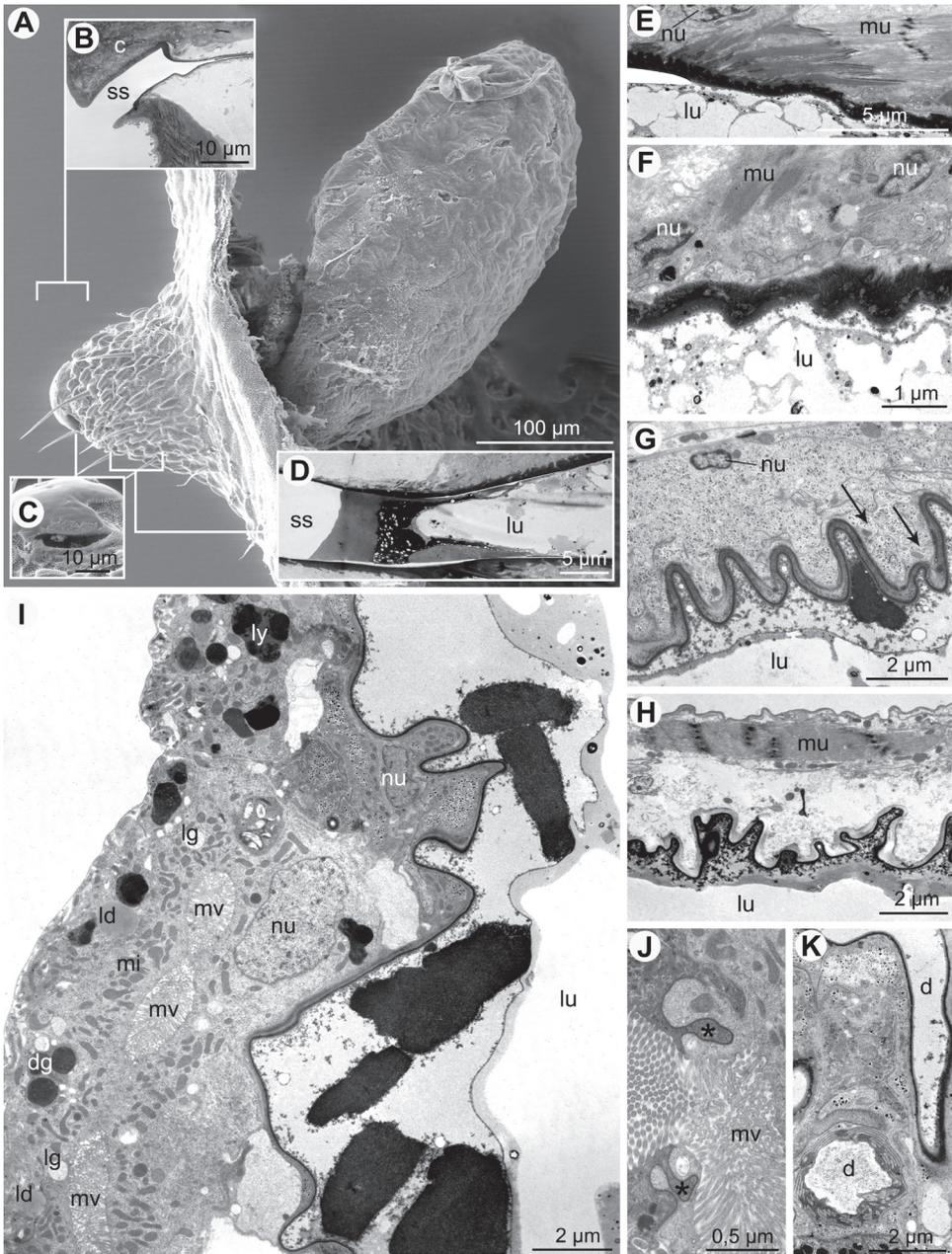


Fig. 2. The defence gland. **A:** SEM figure of the ozophore with the defence gland. **B:** Lid-like structure of the opening of the ozophore. **C:** SEM view of the secretory slit of the defence gland. **D:** Secretion in the excretory canal next to its exit. **E:** Longitudinal section through the muscle attachment. **F:** Wall of the first part of the non-secretory area. **G:** The second part of the non-secretory area with regular folds. Mitochondria (arrows) are located under the folds. **H:** Third part of the non-secretory area with irregular folds. Note the muscles under the epithelium. **I:** The secretory part of the glandular sac with some microvilli bordered cavities of the glandular units. **J:** The secretory cells are linked to each other by interdigitations (asterisks). **K:** Ducts are also observed in the secretory epithelium.

Abbreviations: c = cuticle, d = duct, dg = dense granules, lg = lucent granules, ld = lipid droplets, lu = lumen, ly = lysosomes, mi = mitochondria, mu = muscles, mv = microvilli, nu = nucleus, ss = secretory slit.

Discussion

1. Dermal glands

Numerous dermal glands are known from a number of Opiliones. They may help to modify integumental properties. Sometimes these secretions serve for camouflaging (e.g., Trogulidae). In Cyphophthalmi these glands are very frequent but rather inconspicuous because their openings are very tiny and usually covered by a thin film of secretion. Hence they have not been often recognised (e.g., MARTENS 1979, HAMMEN 1989). In Sironidae it seems likely that the secretions help to keep the surface hydrophobic.

The dermal glands correspond to the class 3 glands according to the classification of NOIROT, QUENNEDY (1974). This type of glands is composed by different cells. The secretory cell is extruding its secretion products into a microvilli bordered cavity from where the secretion passes within the cuticle-lined duct towards the exterior. The similarity of the ultrastructure of these dermal glands with the glands of the male adenostyle (tarsal gland) described by MARTENS (1979) from the same species is remarkable. The occurrence of secretion in the secretory and collar cells demonstrate that both cell types have a secretory activity. Their products are released into a funnel-like cavity lined by microvilli, which are formed by the secretory and collar cells. In the secretory cells, the formation of the granules runs through different stages. Before extrusion into the secretory reservoir, the granules merge into larger granules, which are electron-lucent. The proximal beginning of the duct is provided with a distinct probably sealing structure which may be involved in the control of the expulsion of the secretions.

2. Defence glands

The defence, repugatory or scent glands are present in all Opiliones. In the Cyphophthalmi these glands are located between the second and third pair of legs on the dorsolateral side of the body on specific elevations, called the ozophores (JUBERTHIE 1970, GIRIBET *et al.* 2002). The defence glands are considered first to provide chemical defence against putative predators. It is possible that their secretion is released as fine sprays or as a droplet (JUBERTHIE 1976, MARTENS 1978, HOLMBERG 1986). In some studies the secretions have been suggested to be used for territorial marking (JUBERTHIE 1976) or as alarm pheromones (MACHADO *et al.* 2002). The chemical composition of the defence secretion was known for the Laniatores and Palpatores (EKPA *et al.* 1985, JONES *et al.* 1976). In a recent study the composition of the gland secretion of *Cyphophthalmus duricorius* was reported by RASPOTNIG *et al.* (2005) for the first time of a species of Cyphophthalmi.

It seems evident that the defence glands are derived from dermal glands: The lid-like structure covering the opening is simply an enlarged tubercle of the surface cuticle, the body of the gland is provided with a (cuticular) intima, and the glandular units found in the secretory part correspond largely in structure with the dermal glands. Discharge of the secretion may be achieved by an increase of haemolymphic pressure. Alternatively, it could be that the gland is kept constantly under pressure and the muscles attaching to the non-secretory part may serve as dilators of this part releasing the secretions when stimulated. No sphincter muscles have been seen. Thus cuticle properties may be responsible for keeping the opening closed when undisturbed. The appearance of a huge number of mitochondria is probably evident for the high activity of the defence glands. Together with the presence of the microvilli bordered cavities in the secretory part, an effective transport of secretions may be assumed.

The defence glands of Cyphophthalmi, described by JANCZYK (1956) and JUBERTHIE (1961) by light microscopy, are ultrastructurally rather similar to those of Phalangidae (CLAWSON 1988), a family belonging to the Palpatores, a taxon regarded by GIRIBET *et al.* (1999, 2002) as paraphyletic.

The similarity of these glands in Cyphophthalmi and “Palpatores” may seem to support a taxon “Cyphopalpatores” as suggested by MARTENS *et al.* (1981) and MARTENS (1986). However, since the peculiarities of these glands of other Opiliones than Cyphophthalmi and Phalangiidae are not known, such a conclusion would be overhasty and further studies have to be awaited.

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Ултраструктура на дермалните и защитните жлези при *Cyphophthalmus duricorius* JOSEPH, 1868 (Opiliones: Sironidae)

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(Резюме)

В статията се описва устройството на дермалните и защитните жлези при сенокосеца *Cyphophthalmus duricorius*. Дермалните жлези се състоят от различни по тип клетки, които изпразват секретите си в периферни кухини с микровили, преди да достигнат кутикулната канална система. Двойката защитни жлези с торбовидна форма са съставени от един екскреторен канал, една несекреторна и една секреторна области. Тези три части се характеризират с различна подредба на клетките. Секреторната област включва няколко жлезисти участъка, които произвеждат хетерогенни секрети и по всяка вероятност произлизат от дермалните жлези. Тези жлези отделят секретите си чрез малки канали, водещи в широкия кутикулен лумен.