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Original Article

Light and electron microscopic studies on the Y organ of the freshwater crab *Travancoriana schirnerae*

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ABSTRACT

The fine structure of the premoult Y organ in the freshwater crab *Travancoriana schirnerae* revealed elliptical epithelial gland cells with large, eccentric, multinucleolated nuclei and ample cytoplasm. The cytoplasm showed numerous polymorphic mitochondria with tubular cristae, highly anastomosed tubules and vesicles of smooth endoplasmic reticulum (SER), rich free ribosomes, small amounts of cisternae of rough endoplasmic reticulum (RER), microtubules and was devoid of Golgi complexes. Mitochondria were of two types the more abundant micromitochondria with electron dense matrix and the less abundant macromitochondria with moderately dense matrix. The tubular SER was particularly concentrated towards the basal region of the cell, intermingled with mitochondria and dense patches of free ribosomes while the vesicular SER lie close to the lateral plasma membrane. Large vesicles with flocculent substances, a few electron dense granules and multivesicular bodies could also be noticed in the gland cell cytoplasm. Aggregations of microvesicles which appeared close to the lateral plasma membrane, in association with dilated SER cisternae and microtubules, possibly suggest the intercellular exchange of substances. The plasma membrane beneath the basal lamina was composed of invaginations and the apical surface possessed numerous microvilli which serve to increase the surface area for metabolic exchange. Towards the apical region, the lateral plasma membrane of adjacent cells was linked by tight junctions. The presence of extraordinarily abundant tubular SER, high proportion of mitochondria with tubular cristae and rich free ribosomes could well be elucidated in favour of steroid production by the gland cells.

1. Introduction

Moulting being the indirect version of growth, study of endocrine organs that regulate moulting is of increasing interest, especially among species of aquaculture potential. The Y organs or the ecdysial glands are paired cephalic endocrine organs of higher crustaceans (Malacostraca) and are absent in lower groups (Entomostraca). The gland controls moulting, reproduction and other physiological activities in crustaceans [1,2]. The Y organ has its origin from the epidermis; either remain attached to the epidermis as in crayfishes or become fully independent organ as in crabs [3]. Gabe [4] first described a glandular organ, which he named the Y organ and suggested that it is the ecdysial gland of crustaceans, homologous to the prothoracic gland of insects. Later on, extirpation and reimplantation experiments of Echalier [5,6] in *Carcinus maenas* substantiated Gabe's suggestion that the Y organ is

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involved in moult control. Echalier's results were validated by similar studies in other brachyurans [7], isopods [8] and penaeids [9].

The anatomical features of the Y organ showed large variations among species [2,10]. A number of investigations have been carried out in various decapods on the morphological and histological profile of the Y organ during different moult stages [11,12] and different phases of gonad maturation [13]. Preliminary cytochemical investigations of the organ in various crustaceans have been reported by Hoffman [14] and Simione and Hoffman [15]. The earliest account on penaeid Y organ was that of Dall [16] who described it as a ventral gland in *Metapenaeus* sp. Later on, Bourguet et al. [9] reported the same results in *Penaeus japonicus*. Unfortunately, very few authors have reported the cytological and anatomical details of this organ that there has been great confusion regarding its exact location and identification among researchers.

Mattson and Spaziani [17] and Sonobe et al. [18] have shown that in decapod crustaceans, the ecdysial gland synthesizes and secretes the moulting hormone (ecdysone), the only steroid hormone known thus far in arthropods. The moulting glands of arthropods secrete 3-dehyroecdysone or 25-deoxyecdysone in addition to ecdysone [19]. In arthropods, 20-hydroxyecdysone is the predominant circulating form of ecdysteroids. It was believed that ecdysone was the only ecdysteroid secreted by the Y organs of *Pachygrapsus crassipes* and *Uca pugilator* [20,21]. However, recent studies revealed that the Y organs in *Procambarus clarkii*, *Macrobrachium rosenbergii* and *Cancer antennarius* secrete ecdysone and 3-dehyroecdysone [18,22,23]. In *Menippe mercenaria*, the gland secretes 3-dehyroecdysone and 3-dehydro-25-deoxyecdysone [24,25].

Moulting in higher crustaceans is hormonally regulated by the moult inhibiting hormone (MIH) from the neuroendocrine complex of the eyestalks and ecdysteroids from the Y organ [3,26]. In vitro studies indicated that the MIH acts directly on Y organs to suppress the synthesis of ecdysteroids [27,28] and uptake of lipoprotein-bound cholesterol, the biosynthetic precursor of ecdysteroids [29,30]. Based on these and related findings, an established model for moult control in crustaceans suggests that MIH from the X-organ/sinus gland complex inhibits the gland during intermoult and moulting cycle is initiated when MIH secretion diminishes [26].

Numerous studies have investigated the ultrastructural aspects of Y organs in different species of marine brachyurans: *C. maenas*, *Hemigrapsus nudus* [31], *Portunus pelagicus* [32], *C. antennarius* [33] and *P. trituberculatus* [34]; caridean *P. japonicus* [12] and astacideans like *P. clarkii* and *Astacus astacus* [11,35]. The Y organ of the isopod *Ligia oceanica* has been described by Maissiat and Maissiat [36]. There have been very few studies that examined light and electron microscopic features of the Y organ in freshwater decapods [37]. The present study on histology and fine structure of the Y organ in the freshwater crab *Travancoriana schirnerae* is reported to fill this gap. This crab species, abundant in the wetlands of Wayanad (Kerala, India), is edible and forms a cheap source of animal protein to the poor, malnourished local tribes.

2. Materials and methods

Adult early premoult crabs were collected from the paddy fields near Mary Matha Arts & Science college campus, Mananthavady, Wayanad (Kerala, India). For ultrastructural studies, the Y organs were dissected out and fixed in Karnovsky's solution for 24 h. The tissue was washed twice in 0.1 M phosphate buffer (pH 7.2), postfixed in 1% osmium tetroxide and dehydrated in graded alcohol series. The tissue was then cleared in propylene oxide, infiltered in propylene oxide–araldite mixture (1:1) followed by pure araldite, embedded in the same and kept at 60 °C (48 h) for polymerization.

After polymerization, semithin sections (0.5 μm) were cut under Leica UC6 Ultramicrotome, stained with 1% toluidine blue and observed under a light microscope. For electron microscopic observations, ultrathin sections (60 nm thick) collected on copper grids were stained using uranyl acetate followed by lead citrate and observed under a Technai G2 SpiritBiotwin Transmission Electron Microscope. Interested areas were captured using *Megaview-III* CCD camera.

3. Results

Under light microscope, the organ appeared as a pair of pale yellowish, conical, glandular epidermal structures located in the cephalothorax, anterior to the branchial chamber and postero-lateral to the eyestalks. The gland was seen embedded in a brown fatty tissue covered by a thin connective tissue sheath. The size of the organ varied from 4.0 to 4.5 mm long and 2.5 to 3.0 mm wide.

3.1. Light microscopy

The gland was composed of anastomosing irregular lobules of epithelial cells covered by a basal lamina. The lobules were either in close apposition or separated by interconnected blood sinuses and capillaries. Hemocytes were frequently observed in the hemal spaces. Each lobule is composed of densely packed cells (30–50), 13.0–20.0 μm wide and polygonal in shape. A clear cell boundary was apparently retained by the gland cells. Their oval nuclei (5.0–8.0 μm in diameter), eccentric in position, contained 2–3 nucleoli and several distinctly aggregated chromatin granules. The cytoplasm was mild to moderately basophilic, granular in nature and the nucleocytoplasmic ratio (NPR) found in the range 0.30–0.48 (Fig. 1).

3.2. Electron microscopy

Ultrastructural observations revealed that the organ cells have oval nuclei and the ample cytoplasm was occupied by numerous organelles like mitochondria with tubular cristae, tubular and vesicular smooth endoplasmic reticulum (SER), free ribosomes, small amounts of rough endoplasmic reticulum (RER), microtubules, lysosomes and inclusions like microvesicles, multivesicular bodies and electron dense granules (Figs. 2A and B, 4A and B, 5A and 8A).

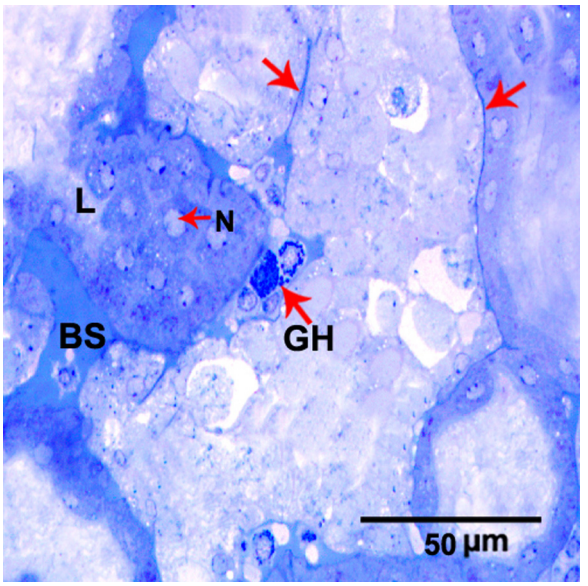

Fig. 1. Light micrograph of the Y organ of *T. schirnerae*. BS: blood sinus; GH: granular hemocyte; L: lobule; N: nucleus. Arrow indicates capillaries.

3.2.1. Nucleus

The nuclei of organ cells were oval, eccentric in position and often possessed 2–3 nucleoli. The heterochromatin condensed in small masses, concentrate adjacent to the inner nuclear membrane and surrounds a finely granulated nucleoplasm. The nucleoli appeared dark and peripherally arranged, close to the inner nuclear membrane (Fig. 2B).

3.2.2. Mitochondria

The Y organ cells contained abundant polymorphic mitochondria and the most significant feature was the tubular configuration of their cristae. Mitochondria were frequently associated with the tubular SER, plasma membrane infoldings of the basal region and the apical cytoplasm near the microvilli (Figs. 2B and 3A and B). They were particularly abundant in the perinuclear cytoplasm, encircling the nucleus. Based on the size, shape and density of matrix, two types of mitochondria can be distinguished: micro and macromitochondria. Micromitochondria were smaller in size, more abundant, round or oval in shape (0.12–0.39 μm in diameter) and had an electron dense matrix while macromitochondria were larger in size, scarce, elongate in shape (0.53–1.0 μm in diameter) with a moderately dense matrix. Both the micro and macromitochondrial matrices showed considerably higher electron density than the cell cytoplasm (Fig. 4A).

3.2.3. Smooth endoplasmic reticulum (SER)

The endoplasmic reticulum was well developed and consisted largely of the agranular profile. In accordance with the steroid secreting cells (SSC) of mammals, abundant SER was shown in the Y organ where it was unequally distributed in the form of highly anastomosed tubules and vesicles in the basal and peripheral cytoplasm, respectively. The tubular SER was better developed and tend to concentrate in the basal cytoplasm whereas vesicular SER was seen

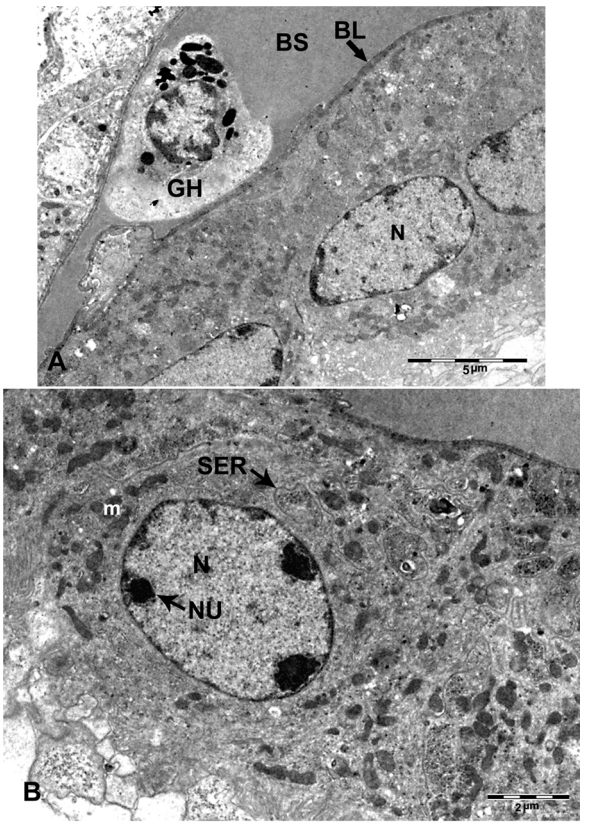

Fig. 2. (A) Ultrastructure of early premoult organ illustrating gland cells and hemocytes in the hemal space. (B) Electron micrograph of a gland cell at higher magnification. BS: blood sinus; BL: basal lamina; GH: granular hemocyte; m: micromitochondria; N: nucleus; NU: nucleolus; SER: smooth endoplasmic reticulum.

distributed towards more peripheral regions of the cells, near the lateral plasma membrane (Figs. 4B and 5A). Vesicles of varying sizes, filled with flocculent substances, were generally perceived in close proximity with a few dilated SER cisternae. These vesicles in association with the SER cisternae, from which they apparently originate, appear to fuse with each other to form larger vesicles. Occasionally, large SER vesicles may be seen among the anastomosing SER tubules in the basal cytoplasm (Fig. 5B).

Besides SER, small amounts of RER cisternae were observed in the perinuclear cytoplasm (Fig. 6). The gland cells exhibited many dense patches of free ribosomes throughout the cytoplasm, particularly abundant in the basal cytoplasmic region where a large number of tubular SER and mitochondria were present (Fig. 5B).

Aggregates of microtubular filaments lie close to the lateral plasma membrane, evident in areas where SER vesicles concentrated (Fig. 5A). Occasionally, lytic inclusions like lysosomes and multivesicular bodies were present (Figs. 4A and 7). Golgi elements were inconspicuous or absent in the organ cells.

3.2.4. Other inclusions

Aggregations of microvesicles with an average diameter of 400–900 Å appeared mostly close to the cell's periphery,

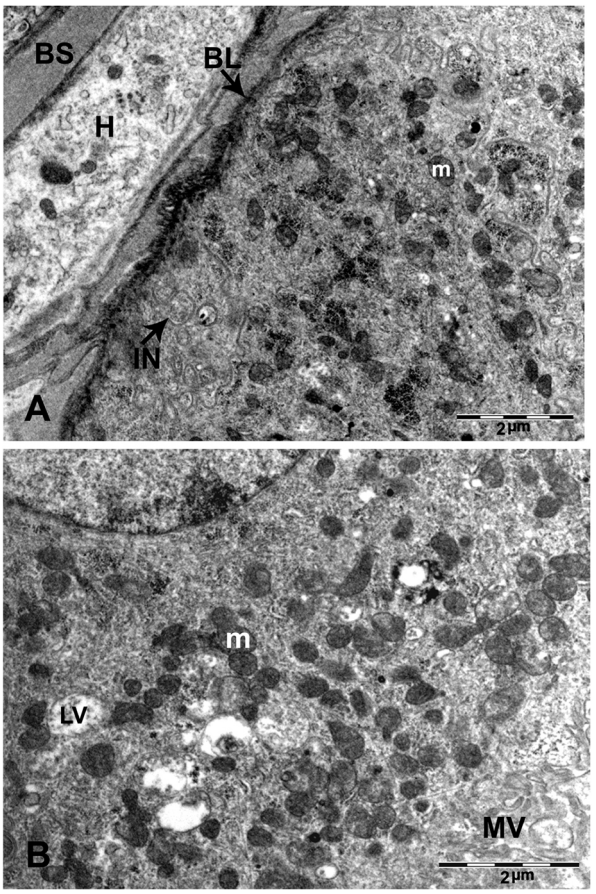

Fig. 3. (A) Plasma membrane infoldings near basal lamina. Note the mitochondria associated with the infoldings. (B) Apical region of gland cell demonstrating abundance of mitochondria and large vesicles filled with flocculent material. BS: blood sinus; BL: basal lamina; H: hemocyte; IN: infoldings of basal plasma membrane; LV: large vesicles filled with flocculent material; m: micromitochondria; MV: microvilli.

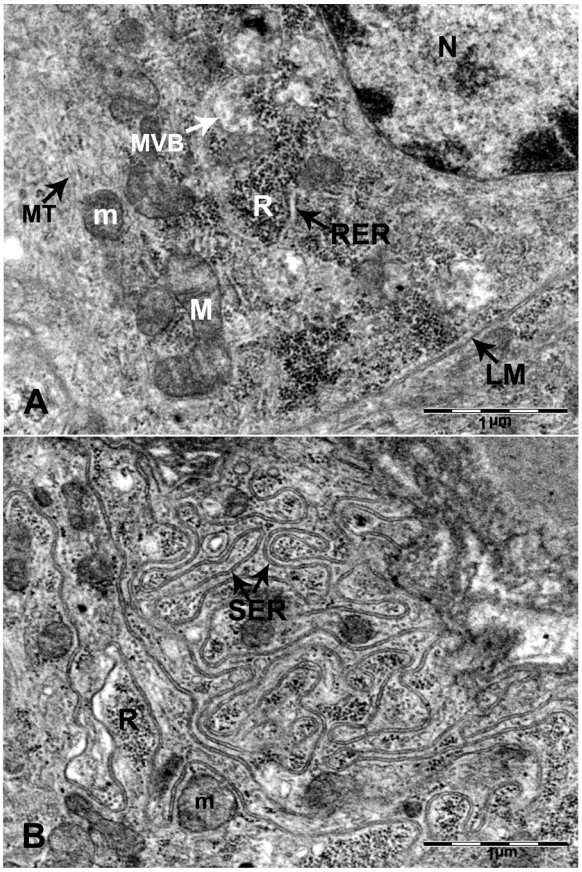

Fig. 4. (A) Electron micrograph of the gland cell showing macromitochondria with tubular cristae, dense patches of free ribosomes and multivesicular bodies. (B) Gland cell exemplifying association of tubular SER with mitochondria and patches of free ribosomes in the basal cytoplasmic region. LM: lateral plasma membrane; m: micromitochondria; M: macromitochondria; MT: microtubules; MVB: multivesicular bodies; N: nucleus; R: ribosomes; RER: rough endoplasmic reticulum; SER: smooth endoplasmic reticulum.

near the lateral plasma membrane and seem to be transported in groups from cell to cell (Fig. 5A). Larger vesicles filled with flocculent substances as well as electron dense particles may be noticed near the apical cell cytoplasm (Fig. 3B). A few oval or round electron dense granules measuring 70–160 nm in diameter were perceptible in the cytoplasm of the organ cells (Fig. 8A).

3.2.5. *Plasma membrane*

The profile of the plasma membrane of cells varied with location. The plasma membrane bordering the basal lamina consisted of invaginations which may serve to increase the surface area for metabolic exchange (Fig. 3A). In apical areas, the plasma membrane produces numerous microvilli (Fig. 8B). The lateral plasma membranes of adjacent cells may be in close apposition or separated by intercellular spaces. Specialized structures such as tight junctions were common in the apical region of the lateral plasma membrane of adjacent gland cells (Fig. 9A).

3.2.6. *Blood sinuses and capillaries*

Blood sinuses and capillaries were observed between the lobules. Granular hemocytes (4.0–9.0 μm wide) were often encountered adhering the basal lamina in the interlobular hemal spaces (Fig. 2A). These hemocytes had irregular outlines and possessed relatively large oval to elongate nuclei with one or two nucleoli and patchy heterochromatin condensed along the inner nuclear membrane. Their cytoplasm showed numerous round to oval dense granules of varying sizes, often surrounding the nucleus. The perinuclear cytoplasm also contained organelles like mitochondria, RER cisternae, vesicles of SER and large oval to irregular shaped vesicles with electron lucent or dense material (Fig. 9B).

4. Discussion

Ultrastructural observations on the premoult Y organ in *T. schirnerae* revealed that the organ cells were characterized by abundant polymorphic mitochondria with tubular cristae, highly developed tubular SER, rich free

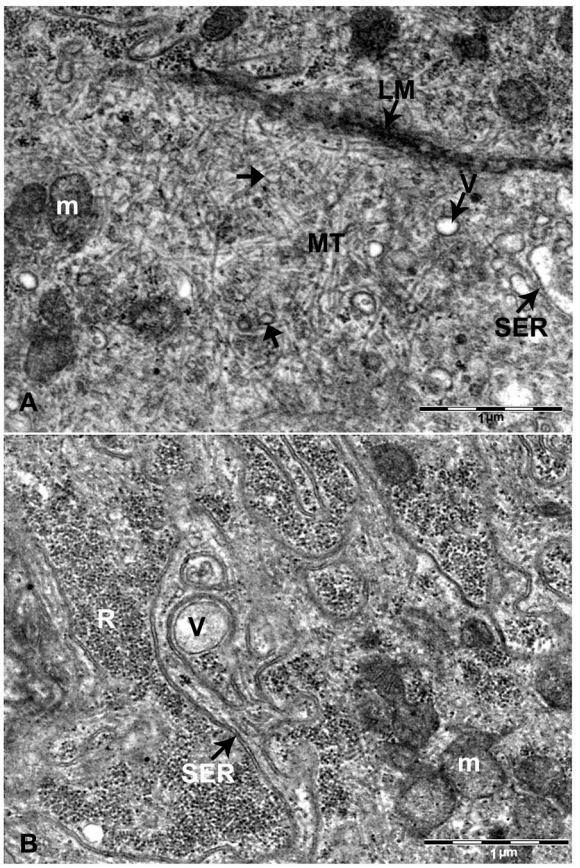

Fig. 5. (A) Electron micrograph depicting microvesicles and macrovesicles close to the lateral plasma membrane in association with aggregates of microtubules. (B) Basal cytoplasmic area rich in free ribosomes and tubular and vesicular SER. LM: lateral plasma membrane; m: micromitochondria; MT: microtubules; R: ribosomes; SER: tubular smooth endoplasmic reticulum; V: SER vesicle. Arrows indicate microvesicles.

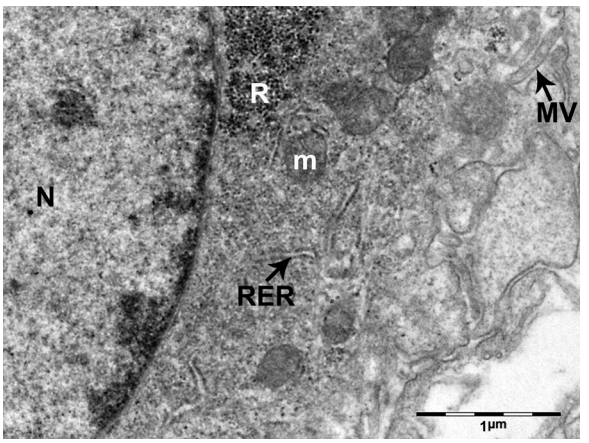

Fig. 6. Apical cytoplasm of gland cell demonstrating RER cisternae. m: micromitochondria; MV: microvilli; N: nucleus; R: ribosomes; RER: rough endoplasmic reticulum.

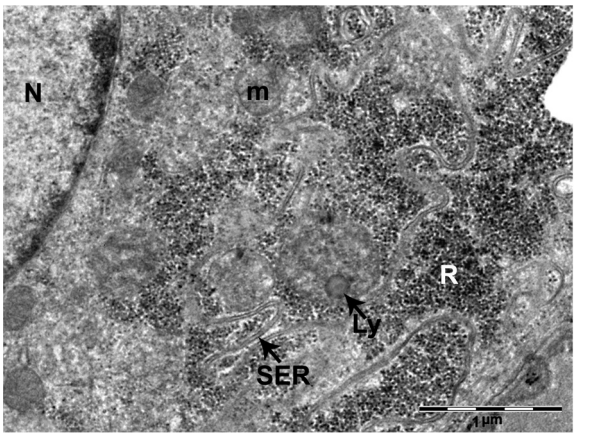

Fig. 7. Gland cell cytoplasm showing lysosomes and other organelles. Ly: lysosome; m: micromitochondria; N: nucleus; R: ribosomes; SER: tubular smooth endoplasmic reticulum.

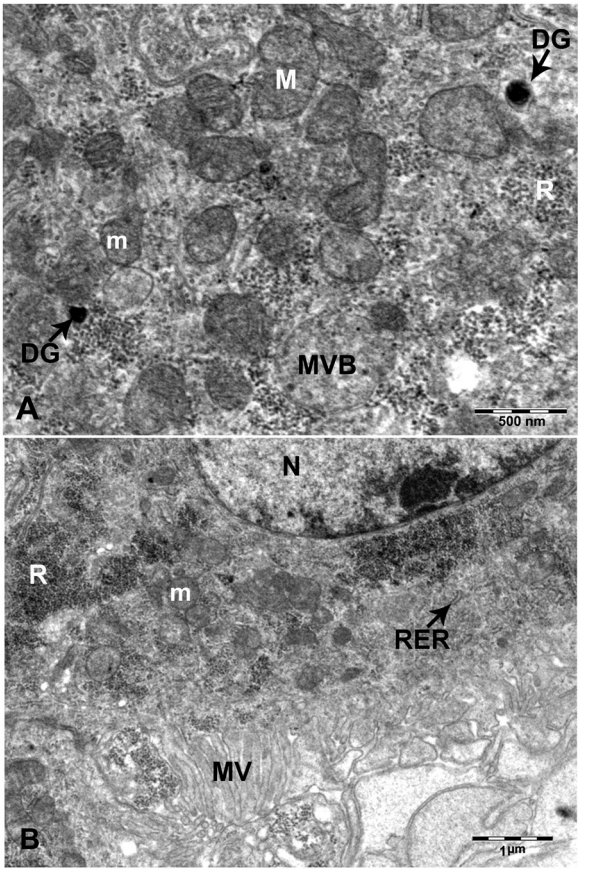

Fig. 8. (A) Gland cell portraying mitochondria and dense granules in the organ cell cytoplasm. (B) Gland cell illustrating numerous microvilli at the apical border. DG: dense granule; M: macromitochondria; m: micromitochondria; MVB: multivesicular body; MV: microvilli; N: nucleus; R: ribosomes; RER: rough endoplasmic reticulum.

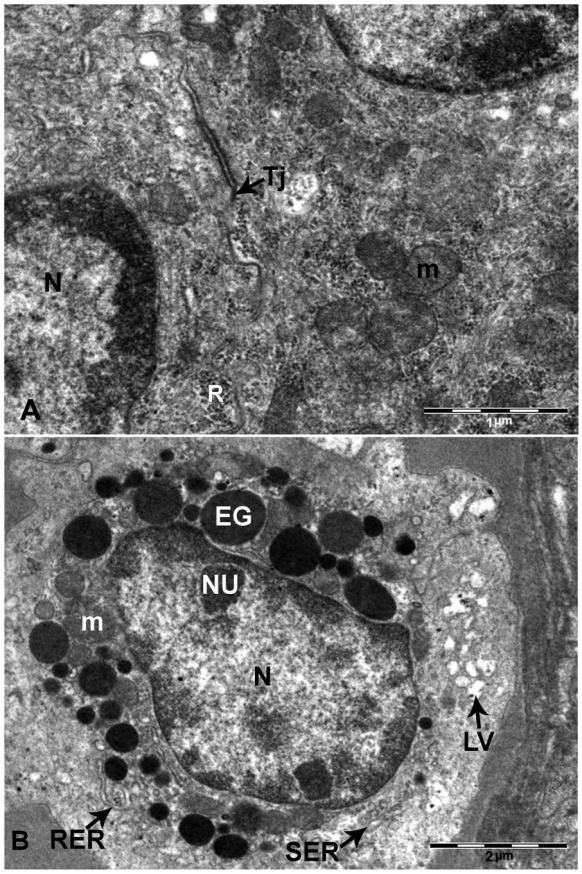

Fig. 9. (A) Plasma membrane of adjacent gland cells demonstrating tight junctions. (B) Granular hemocyte showing electron dense granules and organelles. EG: electron dense granule; LV: large vesicle; m: mitochondria; N: nucleus; Nu: nucleolus; R: ribosomes; RER: rough endoplasmic reticulum; SER: smooth endoplasmic reticulum vesicles; Tj: tight junction.

ribosomes, small quantities of RER and inconspicuous Golgi complexes, similar to those described for other decapods [11,31,38]. Besides organelles like agranular endoplasmic reticulum, tubular mitochondria and numerous free ribosomes, the Y organ cells of *A. astacus* displayed conspicuous Golgi bodies [35]. However, the gland cells in *C. antennarius* showed polymorphic mitochondria with tubular cristae, numerous free ribosomes and vesicles, but little in way of smooth or rough endoplasmic reticulum or Golgi complexes [33]. In *Metopograpsus messor*, an abundance of organelles such as mitochondria, Golgi and secretory vesicles was noted according to seasons [39].

The present study disclosed the fact that the cytology of Y organ of *T. schirnerae* has a close resemblance to the prothoracic glands of insects and vertebrate SSC [40,41]. In the prothoracic glands of larval *Philosamia cynthia ricini*, the cytoplasm contained rod shaped mitochondria with dense matrix intermingled with SER [40]. The mammalian SSC such as leydig cells of testis [41], adrenocortical cells [42] and lutein cells of ovary [43] exhibited abundant SER and tubular mitochondria. Rothwell [44] noticed that the three evident cytoplasmic features of steroidogenesis in leydig cells of domestic fowl include large circular or oval mitochondria, smooth surfaced random tubular configuration of the SER and scattered free ribosomes. The adrenal gland cells of the marsupial *Isoodon macrourus* contained large amounts of SER and mitochondria with tubulovesicular cristae [45]. The most important organelles that characterize mammalian SSC, namely abundant SER, mitochondria with tubular inner structure and rich free ribosomes are without doubt present in the Y organ cells of *T. schirnerae*, suggesting their role in steroidogenesis.

The Y organ cells of *T. schirnerae* contained copious mitochondria, categorized into micro or macromitochondria based on their size, shape and density of matrix. Similar results were reported from the Y organ cells of *Palaemon paucidens*, *A. astacus* and *M. messor* [11,35,39]. In *P. paucidens*, Aoto et al. [11] reported that the size and internal structure of mitochondria showed remarkable changes during the moult cycle. In *A. astacus*, besides normal mitochondria, there are giant forms in the Y organs of both the intermoult and premoult stages [35]. The Y organ displayed several micro and macromitochindria during the moult-reproductive season in *M. messor* [39]. In *P. trituberculatus*, the mitochondria were elongate and swollen with a dense matrix during premoult stages but were smaller in size and spherical or ovoid during intermoult [34]. In *Libinia emarginata*, the gland cell cytoplasm contained large numbers of mitochondria with tubular cristae and dense matrix which vary in size and shape [38]. Polymorphic mitochondria with tubular cristae and flocculent matrix were noticed during premoult in the gland cells of *C. antennarius* [33]. Considerable structural variability of mitochondria was described in the adrenal cells of cat [46]. Giant mitochondria containing crystalloid structures have been reported from the prothoracic glands of the lepidopteran insect *Spodoptera littoralis* [47]. The observations on copious occurrence and structural modifications of mitochondria in the present study are associated with the steroid synthetic activity of the organ cells.

The Y organ cells of the current study enclosed both tubular and vesicular forms of SER with tubular SER tend to concentrate in the basal cytoplasm and vesicular SER more near the lateral plasma membrane, in the peripheral cytoplasm. These observations were supported by the findings of Hinsch and Al Hajj [38] in *L. emarginata*, where the SER is a system of highly anastomosed tubules and vesicles distributed in the peripheral cytoplasm. Most of the ER in the organ cells of *P. paucidens* were smooth consisted of both tubular and vesicular forms which showed marked structural changes during the moult cycle [11]. In *H. nudus*, the SER appeared as random tubules and tubule sheets in the organ cell cytoplasm [31]. In *A. astacus*, the ER is better developed in premoult and consisted of agranular profiles [35]. It is noteworthy that the SER in the Y organ cells of crustaceans take either vesicular or undulated tubular form, somewhat different in appearance from the branched tubular form of mammalian SSC [48]. The difference in appearance of SER may reveal the fact that crustacean steroids are synthesized by conversion of cholesterol from outside [49] while mammalian steroid hormone is synthesized de novo with the aid of several enzymes contained in the smooth ER [41].

The premoult gland cells in *T. schirnerae* exhibited numerous dense patches of free ribosomes, intermingled with tubular SER and mitochondria in the basal and perinuclear cytoplasm, which point towards the involvement of these organelles in steroidogenesis. Similar observations were made in the Y organ cells of many decapods. In *P. paucidens*, ribosomes were seen scattered freely in the cytoplasm and in rare occasions, a few of them detected on ER [11]. The cytoplasm contained numerous free ribosomes during intermoult in *C. antennarius* [33]. In *M. messor*, the premoult Y organ cells displayed plenteous amounts of free ribosomes [39]. However, very few ribosomes were detected in *L. emarginata* [38].

The electron dense granules apparent in the cytoplasm of the present study were comparable to those evident in the premoult Y organ cells in *C. antennarius* [33] and *P. trituberculatus* [34], which is characteristic of an advanced state of gland activation.

This study described the presence of micro and macrovesicles in the peripheral cell cytoplasm, apparently derived from the SER. Likewise in *H. nudus*, aggregations of microvesicles appear mostly close to the cell's periphery and they seem to be transported in groups from cell to cell as well as in to the hemolymph [31]. In *C. maenas* Y organ cells, Chassard-Bouchaud and Hubert [50] found microvesicles which they regarded as vesicles derived from the SER filled with the moult hormone. The presence of microvesicles in steroid producing tissues may be interpreted in terms of reverse micropinocytosis [51].

As suggested for *L. emarginata* [38], the plasma membrane invaginations seen in this study are anatomical adaptations to increase the surface area thus facilitating metabolic exchange between the cells and the hemolymph. In *P. trituberculatus*, the amount of infoldings of the plasma membrane in the cells of Y organ changes during the moult cycle and in premoult organ, the infoldings appeared only at the surface that faces a blood sinus [34]. According to Herman [52], the cell surface irregularities may represent reverse pinocytosis to release the moulting hormone. In the present study, specialized structures such as tight junctions were noted in the apical region of the lateral plasma membrane between adjacent gland cells. These results were in accordance with the observations of Aoto et al. [11] in *P. paucidens*, where the cell border is complicated by tight junctions. Septate junctions are frequent in the apical area of the lateral plasma membrane in premoult gland cells of *A. astacus* [35]. Rarely specialized structures such as desmosomes or septate desmosomes were seen between adjacent gland cells in *L. emarginata* [38].

In the present study, aggregates of microtubules lie close to the peripheral cytoplasm where SER vesicles were usually noted. Microtubules were seen in association with the membranous invaginations in *L. emarginata* gland cells [38]. In *A. astacus*, microtubules were oriented mostly parallel to the long axis of the cellular processes [35]. The microtubules may act as cytoskeleton in membranous invaginations and their close relationship with the SER vesicles might serve to orient these vesicles.

In *T. schirnerae*, it is evident that in the apical area, the plasma membrane has several microvillli and this particular character was not reported in other decapod Y organs. The microvilli in vertebrate SSC were of special importance. In rats, the plasma membrane of ovarian luteal and adrenal cortical cells forms several microvilli which trap large number of high-density plasma lipoproteins, probably functioning in the release of cholesterol to these cells [53].

The granular hemocytes often encountered adhering the basal lamina in the interlobular hemal spaces of Y organ cells in *T. schirnerae* suggest their role in metabolic exchange between the gland cells and the hemolymph. Chassard-Bouchard and Hubert [50] have reported the same in the Y organ cells of *C. maenas*.

5. Conclusion

The ultrastructure of the Y organ cells exhibited features such as abundant mitochondria with tubular cristae, tubular SER and rich free ribosomes which closely resembles that of vertebrate steroid hormone secreting glands. Further research is needed for elucidating the release and storage mechanism of ecdysteroids by these glands.

Conflict of interest

The authors declare that there is no conflict of interest.

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