Fine structure of neurosecretory cells and sinus gland in the eyestalk of the freshwater crab *Travancoriana schirnerae* Bott, 1969 (Decapoda: Gecarcinucidae)

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Abstract. This study elucidated the fine structure of neurosecretory cells and sinus gland in the optic ganglia of the freshwater crab *Travancoriana schirnerae* Bott, 1969 (Decapoda: Gecarcinucidae). The eyestalk ganglion showed the presence of four well defined ganglia arranged below the ommatidium: lamina ganglionaris, medulla externa, medulla interna and medulla terminalis of which the lamina ganglionaris, was devoid of neurosecretory cells. Groups of neurosecretory cells seen distributed along the medulla externa, interna and terminalis regions constitute the X-organs. Electron microscopic observations of the eyestalk ganglia revealed ten types of neurosecretory cells, mostly apolar with a few unipolar and bipolar cells classified according to the size, shape and density of the cell and nucleus, cell organelles/inclusions, together with the arrangement and properties of chromatin. These cells were characterized by the presence of large nuclei with unusually condensed chromatin, inclusions like vacuoles and vesicles of varying size, shape and density and organelles like Golgi, endoplasmic reticulum, ribosomes and mitochondria and neurosecretory material. The sinus gland of *T. schirnerae* was positioned laterally between the externa and interna regions, composed of axonal endings of the neurosecretory cells of the optic ganglia with interspersed glial cells. The axon terminals were enclosed with several small to large membrane bound homogenously dense neurosecretory granules which also occur in the preterminal areas of the axons. Based on size, shape and density of granules and axoplasmic matrix, seven terminal types could be distinguished in the sinus gland of *T. schirnerae*. Mostly, the granules contained in a terminal were of the same type; rarely, the same terminal enclosed granules of varying size, shape and density. The neurosecretory cell types and axon terminal types represent the types of neurohormones they contained. A precise knowledge of the morphology and cytology...
of neurosecretory cells in the XO-SG complex of the eyestalk that secrete neurohormones controlling major physiological processes such as growth and reproduction is imperative for successful captive breeding of a species of aquaculture potential.

**Keywords:** Eyestalk; Freshwater crab; Neurosecretory cells; Sinus gland; *Travancoriana schirnerae.*

**Introduction**

The optic ganglion in the eyestalk of decapod crustaceans is an important source of hormones that control major physiological processes such as growth and reproduction (Bliss and Welsh, 1952; Knowles, 1955; Knowles and Carlisle, 1956; Matsumoto, 1958). Upadhyaya (2000) divided the optic ganglia into four parts: lamina ganglionaris, medulla externa, interna and terminalis, of which the lamina ganglionaris region is devoid of neurosecretory cells (NSC). The NSCs observed in groups on medulla externa, interna and terminalis regions are termed as medulla externa X organ (ME-XO), medulla interna X organ (MI-XO) and medulla terminalis X organ (MT-XO). Neurosecretory granules (NSG) synthesized in cell bodies of these NSCs are transported via their axons to axon terminals. The axonal endings from NSCs of the optic ganglia, brain and thoracic ganglia together with interspersed glial cells constitute the sinus gland (SG), a neurohemal organ concerned with the storage and release of a number of neurohormones like the moult inhibiting hormone, gonad inhibiting hormone, crustacean hyperglycemic hormone, retinal pigment concentrating hormone and pigment dispersing hormone that regulate important physiological processes like moult and growth, gonad development, blood sugar level, colour change, tegumentary and retinal pigment position, secondary sexual characteristics, locomotion and neuronal activity (Knowles and Carlisle, 1956; Carlisle and Knowles, 1959; Naylor et al., 1973; Beltz, 1988; Mohamed and Diwan, 1991; Fingerman et al., 1993; Charmantier et al., 1997).

Several light and electron microscopic studies have been made to describe the X organ-sinus gland (XO-SG) system of brachyurans (Bliss and Welsh, 1952; Matsumoto, 1954; Parameswaran, 1956; Durand, 1956; Potter, 1954; Smith, 1974; Andrew et al., 1978; Dircksen, 1992). Enami (1951) classified the NSCs in the brain of the crab *Sesarma.* Lake (1970) has made substantial contributions to the studies on the NSCs types in the crab *Paragrapsus gaimardii.* Studies on the neurosecretory centres of the eyestalk and their involvement in moulting and reproduction have been carried out in *Carcinus maenas* by Smith (1975). Hisano (1974) detailed the light and electron microscopic studies of the NSCs types in the optic ganglia of the prawn *Palaemon paucidens.* Van Herp et al. (1977) made light microscopic study of the eyestalk NSCs of *Palaemon serratus.* Andrew et al. (1978) evaluated the structure of NSCs in the crayfish eyestalk. Bharathi et al. (2014) made a comparative study of NSCs in female *Penaeus indicus* after unilateral eyestalk ablation. Pandey et al. (2015) described the histomorphological changes in NSCs of eyestalk, brain and thoracic ganglia in the flower tail shrimp *Metapenaeus dobsoni* during different stages of ovarian maturation. Rajendiran and Vasudevan (2016) focused on the histological mapping of NSCs and the identification of possible sites of crustacean hyperglycemic hormone immunoreactivity in the optic ganglia of *Portunus pelagicus.* Though sufficient information is available on morphology, cytology and distribution of the NSC types in the optic...
ganglia, brain and abdominal ganglia (Enami, 1951; Durand, 1956; Matsumoto, 1954; Potter, 1958) and SG of marine decapods (Charmantier-Daures and Segonzac, 1998, Andrews et al., 1971), limited work has been attempted on freshwater brachyurans (Diwan and Nagabhushanam, 1975). Moreover, the crab species mentioned in this study, widely distributed in the wetlands of Wayanad, is edible and forms a cheap source of animal protein for the malnourished local tribes. A precise knowledge of the morphology and cytology of neurosecretory cells in the XO-SG complex of the eyestalk that secrete neurohormones controlling major physiological processes such as growth and reproduction is imperative for successful captive breeding of a species of aquaculture potential. In this perspective, the present work on fine structural analysis of the NSCs and SG found in the optic ganglia of *Travancoriana schirnerae* is undertaken.

**Methodology**

Adult intermoult crabs (carapace width 4.5-5 cm) collected from the paddy fields of Ondayangadi, Wayanad (11° 49’ 20.3” N and 76° 01’ 47.1” E) were allowed to acclimatize to the laboratory environment for a few days. They were fed with cooked beef liver, pulses and decaying aquatic vegetation. The wet weight, carapace width and moult stage of the animals were recorded before ablating the eyestalks. The eyestalks were carefully excised under a Trinocular stereo zoom dissection microscope and frozen for 24 hrs. The optic ganglia were dissected out by removing the exoskeleton using fine scissors and needles.

For ultrastructural studies, the tissues were fixed in 3% Glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h, washed twice in the same buffer, postfixed in 1% osmium tetroxide for 90 minutes and dehydrated in graded ethanol series. After clearing in propylene oxide, the tissue was left in a mixture of propylene oxide and araldite resin (1:1) overnight, then increased to 1:3 ratio followed by pure araldite resin for 2-3 h. Tissues were then embedded in flat embedding moulds and kept in an oven at 60°C for 48 h for polymerization (Frasca and Parks, 1965).

Once the polymerization completed, semithin sections of 0.5 µm thickness were cut under a UC 7 Ultramicrotome (Leica Microsystems, Germany), stained with 1% toluidine blue and observed under a Leica DM 500 Research Microscope with Dewinter camera attachment and Biowizard software for light microscopic observations. For ultrastructural studies, 60 nm thick sections were cut and stained with uranyl acetate followed by lead citrate. Stained sections were mounted on copper grids and scanned under a JEM 1400 Plus Transmission Electron Microscope (JEOL, Japan) operating at 60 KV. Selected regions were captured using a Gatan SC 1000B CCD camera and the electron micrographs were analyzed for fine structural details.

**Results**

**Light microscopy**

The longitudinal section of eyestalk ganglion of *T. schirnerae* showed the presence of four well defined ganglia arranged below the ommatidium: lamina ganglionaris (LG), medulla externa (ME), medulla interna (MI) and medulla terminalis (MT), of which the topmost or distal portion is the LG, devoid of neurosecretory cells (NSCs) (Figure 1A). Below the LG is the ME and the proximal region of the optic ganglion is the MT which tapers to form the optic nerve. Medulla interna is located between the ME and MT regions. Groups of NSCs were seen distributed along the ME, MI and MT regions constitute the X-organs (XO). The NSCs located dorsolaterally and at the surface between the LG and ME constitute the ME-XO while those
observed laterally in the MI as well as the MT regions constitute the MI-XO and MT-XO, respectively.

Light microscopic observations revealed seven types of NSCs in the X-organs of optic ganglia: type I, II, III, IV, V, VI and VII based on size and shape of the nucleus as well as the arrangement of chromatin. Frequency distribution of the cell types is given in Table 1. Maximum number of NSCs were found in ME-XO region and minimum in MT-XO. Type I were the most abundant cell types (36%) noticed in ME-XO followed by Type V (24%), VI (19%), IV (11%), VII (4%), II and III (3% each). MT-XO showed less number of cells with all the seven types. The abundance was in the order type I, V, VII, II, IV, VI and III. Type I was the most abundant cells in MI-XO followed by type V, IV, III, VI, II and VII (Figures 1B-D).

The SG of *T. schirnerae* appeared as a deeply stained oval structure, located laterally between the ME and MI (Figure 1E). Light microscopic observations revealed the presence of numerous highly basophilic round or oval bodies, called the axon terminals.

![Figure 1](image_url)

**Figure 1.** Light micrographs of eyestalk ganglia of intermoult *T. schirnerae* showing various ganglionic regions, neurosecretory cells types and the sinus gland. (A) Eyestalk ganglia demonstrating lamina ganglionaris, medulla externa, interna and terminalis regions (B-D) Neurosecretory cells of ME-XO, MI-XO and MT-XO regions (E) Sinus gland between ME and MI regions. LG: Lamina ganglionaris; ME: Medulla externa; MI: Medulla interna; MT: Medulla terminalis; SG: Sinus gland; T1: Type 1; T2: Type 2; T3: Type 3; T4: Type 4; T5: Type 5; T6: Type 6; T7: Type 7
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### Table 1. Frequency distribution of cell types in the ME-XO, MI-XO and MT-XO regions of the optic ganglion in *T. schirnerae*.

| Type of NSC | ME-XO | MI-XO | MT-XO |
|-------------|-------|-------|-------|
| I           | 265 (36%) | 56 (38%) | 32 (43%) |
| II          | 23 (03%)  | 8 (05%)  | 7 (10%)  |
| III         | 23 (03%)  | 11 (08%) | 4 (05%)  |
| IV          | 81 (11%)  | 27 (12%) | 6 (08%)  |
| V           | 176 (24%) | 38 (26%) | 11 (15%) |
| VI          | 141 (19%) | 9 (06%)  | 6 (08%)  |
| VII         | 26 (04%)  | 8 (05%)  | 8 (11%)  |

**Ultrastructure**

Electron micrographs of the eyestalk ganglia showed ten types of NSCs namely type I, II, III, IV, V, VI, VII, VIII, IX and X, classified according to the size, shape, electron density of the cell and nucleus, cell organelles/inclusions, together with the arrangement and properties of chromatin. The NSCs were characterized by the presence of nuclei with unusually dense chromatin, inclusions like vacuoles and vesicles of varying size, shape and density and organelles like Golgi, endoplasmic reticulum (ER), ribosomes, mitochondria and neurosecretory granules (NSG). The NSCs found in the eyestalk ganglia of *T. schirnerae* were mostly apolar (Type II, III, IV, V, IX and X) with a few unipolar (Type I, VI and VII) and bipolar cells (Type VIII).

**Type I**

Round or irregular, unipolar cells, measured 6.33-8.00 µm diameter with large electron lucent nuclei (4.67-5.33 µm). The nucleocytoplasmic ratio (NPR) was found in the range 0.58-0.84. Besides forming strong dense irregular blocks at the periphery of the nucleus, the chromatin was seen as small dense granules scattered throughout the nucleoplasm. The moderately dense cytoplasm was characterized by the presence of a large number of vesicles and vacuoles of varying size, shape and density: clear vesicles (membrane bound vesicles measuring 0.12-0.45 µm diameter), condensing vesicles (membrane bound vesicles filled with a dense homogenous ground substance, 0.40-0.48 µm in diameter) and autophagic vacuoles (0.80-0.83 µm) with cell debris. Fine electron dense NSGs (0.02-0.04 µm) were seen distributed in clusters throughout the cytoplasm. Besides these inclusions, the cytoplasm contained organelles like mitochondria (width 0.31-0.39 µm) with moderately dense matrix and parallel cristae, cisternae and vesicles (0.04-0.10 µm diameter) of Golgi and rough endoplasmic reticulum (RER) cisternae (0.02-0.03 µm width) (Figures 2A-B).

**Type II**

Rectangular cell bodies with a size range of 4.67-7.79 µm. Nuclei were elongate or oval with wavy margins and measured 2.67-5.17 µm across. In addition to the small electron dense granules which fill the nucleoplasm, dense blocks of chromatin were perceptible towards the periphery. These cells were distinguished with a NPR of 0.57 to 0.66. The moderately dense cytoplasm contained clear vesicles (0.29-0.69 µm), condensing vesicles (0.34-0.87 µm), autophagic vacuoles (0.41-0.93 µm), multivesicular bodies (MVB) (0.48-0.54 µm), clusters of moderately dense NSGs (0.05-0.08 µm) in proximity to Golgi vesicles, mitochondria (0.58-1.22 µm) with irregular or poor fragmented cristae and scattered ER cisternae (0.06-0.12 µm width) (Figure 2C).
Figure 2. Electron micrographs showing type I to V neurosecretory cells in the optic ganglion of *T. schirnerae*. (A-B) Type I neurosecretory cell (C-D) Type II and III neurosecretory cells accommodating various organelles and inclusion bodies (E) Perikarya of type IV cell depicting various types of vesicles, vacuoles and cell organelles (F) Type V cells with dense nuclei. A: Axon; AV: Autophagic vacuole; CH: Chromatin; CL: Clear vesicle; CV: Condensing vesicle; ERV: ER bound vesicle; G: Golgi vesicles; L: Lysosome; M: Mitochondria; MVB: Multivesicular body; N: Nucleus; NG: Neurosecretory granules; NU: Nucleolus; RER: Rough endoplasmic reticulum.

**Type III**
Round or irregularly shaped cells (3.61-6.67 µm), filled with moderately dense cytoplasm carrying oval to elongate, large, medium dense nuclei (2.68-4.67 µm). A remarkable feature noticed was their high NPR (0.70-0.74). The strongly dense chromatin forms a ring at the periphery and small blocks of chromatin were seen scattered in the nucleoplasm. The nucleoplasm has dense granulations and uninucleolated, with
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the nucleoli (0.3 µm) occupying peripheral positions. The perikaryon accommodated inclusions like clear vesicles (0.12-0.48 µm in diameter), condensing vesicles (0.23-0.45 µm), autophagic vacuoles (0.28-1.9 µm in diameter) packed with cell debris, scattered NSGs (0.05-0.06 µm) and organelles like a few mitochondria (0.46-0.53 µm across) having irregular or fragmented cristae and scattered cisternae of RER (0.04-0.08 µm thick) (Figure 2D).

**Type IV**

Larger cells (8.0-8.08 µm diameter) with small, round or spherical nuclei (2.87-3.30 µm) and large amount of cytoplasm. The NPR observed was low (0.36-0.41). The entire nucleoplasm was uniformly filled with an unusually dense chromatin. The cytoplasm enclosed many large, irregular-shaped membrane bound vesicles filled with electron lucent coarse granules (NSGs 0.05-0.06 µm), clear vesicles (0.20-0.55 µm), ER bound vesicles (0.10-0.30 µm), condensing vesicles (0.10-0.4 µm), autophagic vacuoles (0.15-0.45 µm) along with a few moderately dense mitochondria (0.5-0.65 µm) having irregular cristae, Golgi vesicles (0.05-0.07 µm) with moderate or dense contents and lysosomes (0.21-0.65 µm diameter) (Figure 2E).

**Type V**

Round or oval cells with wavy margins (2.84-4.67 µm width), characterized by large round or oval nuclei (2.52-3.53 µm) and negligible amounts of cytoplasm (NPR 0.75-0.89 µm). The nucleoplasm was partially or fully occupied by strongly dense chromatin. In partially dense nuclei, the core accommodates dense granules (Figure 2F).

**Type VI**

Unipolar cells (5.0-5.55 µm diameter) with large oval nuclei (4.5-5.16 µm) and small amounts of cytoplasm (NPR 0.90-0.93). The dense chromatin forms a ring attached to the inner nuclear membrane. Along with clear vesicles (0.26-0.60 µm), a few round or oval mitochondria (0.26-0.71 µm) with poor cristae were discernible in the perikarya. The axonal region contained numerous moderate to dense NSGs (0.03-0.04 µm), vesicles with granular contents, autophagic vacuoles (0.13-0.23 µm) and a few MVBs (0.52 µm across) (Figures 3A-B).

**Type VII**

Oval to elongate (3.57-5.16 µm across), unipolar cells possessing elongate nuclei (3.07-4.29 µm) with wavy margins (NPR 0.79-0.83). Besides the dense blocks seen attached to the inner nuclear membrane, dense granules of chromatin were found strewn in the nucleoplasm. The cytoplasm enclosed scattered NSGs, clear vesicles (0.125-1.0 µm), condensing vesicles (0.25-0.37 µm), autophagic vacuoles (0.41-0.83 µm) and stacks of cisternae of RER (0.04-0.03 µm). Neurosecretory granules (0.04-0.06 µm) and ER bound vesicles (0.41-0.50 µm) were also noticed in the axonal region (Figure 3C).
Figure 3. Electron micrographs of Type VI, VII, VIII, IX and X neurosecretory cells in the optic ganglion of *T. schirnerae*. (A) Unipolar Type VI neurosecretory cell with large nucleus and sparse cytoplasm (B) Axon of type VI cell enclosing vesicles and neurosecretory granules (C) Unipolar Type VII cells with large irregular blocks of chromatin, various types of vesicles and autophagic vacuoles in the perikarya (D) Large bipolar type VIII cell with blebbing of outer nuclear envelope, neurosecretory granules and lysosomes (E) Type IX neurosecretory cell with clumps of dense chromatin bordering the nucleus with transparent core area (F) Type X cells with numerous vesicles and unusually dense chromatin. A: Axon; AV: Autophagic vacuole; B: Nuclear blebbing; CH: Chromatin; CL: Clear vesicle; CV: Condensing vesicle; DV: Dense core vesicle; ERV: ER bound vesicle; G: Golgi vesicles; L: Lysosome; M: Mitochondria; MVB: Multivesicular bodies; N: Nucleus; NG: Neurosecretory granules; NU: Nucleolus; RER: Rough Endoplasmic reticulum; V: Vesicle.
Type VIII
Large, triangular shaped, bipolar cells (width 8.40-8.57 µm) possessing oval to elongate nuclei (width 3.0-3.28 µm) with distinct nucleoli. The nucleus was seen pushed to one side of the cell and showed blebbing of the outer nuclear membrane (0.12-0.4 µm). Patches of dense chromatin were arranged along the peripheral nucleoplasm. Autophagic vacuoles (0.12-0.36 µm diameter), ER bound vesicles (0.44 µm), MVBs (0.56-0.96 µm), mitochondria (0.36-0.68 µm) with irregular or fragmented cristae, a few scattered RER cisternae (0.04-0.08 µm wide), clusters of Golgi vesicles (0.04-0.15 µm in diameter) with lucent or moderately dense contents and small to large oval, membrane bound lysosomes with homogenous dense matrices (0.2-0.52 µm in diameter) and NSGs (0.04-0.06 µm) were apparent in the electron lucent cytoplasm. These cells were found to have low NPR (0.35-0.38) (Figure 3D).

Type IX
Oval to round cells (6.07-7.05 µm width) with irregular margins, possessing round, peripherally placed nuclei (3.64-4.27 µm). Small clumps of dense chromatin border the circumference of the nucleus with transparent core area. These cells were usually noticed with a NPR of 0.59-0.61. The cytoplasm was characterized by an abundance of dense core vesicles (0.10-0.16 µm diameter), clear vesicles (0.27-0.38 µm), autophagic vacuoles (0.16-0.01 µm in diameter), ER bound vesicles (0.20-0.22 µm), scattered NSGs (0.05-0.06 µm) and a few mitochondria (0.43-0.48 µm) with irregular or fragmented cristae (Figure 3E).

Type X
Oval cells with irregular margins (6.00-6.20 µm diameter). The nucleus (2.27-3.0 µm across) has an elongate or irregular shape with the strongly dense chromatin occupying almost the entire nucleoplasm with dense granular regions. Cytoplasm with moderate density enclosed numerous Golgi vesicles of varying sizes and densities, vacuoles (0.125-0.45 µm) and mitochondria (0.50-0.58 µm) with poor cristae. These cells were observed with a low NPR (0.38-0.48) (Figure 3F).

In the optic ganglia of T. schirnerae, myelinations of axons were observed. The axolemma of the myelinated axons were surrounded by a dense cover called the microtubular sheath (0.4-0.60 µm thick). Outside the microtubular sheath is the myelin sheath, composed of bundles of myelin fibres which lie parallel to the longitudinal axis of the axon. The space between the microtubular sheath and the myelin sheath is the submyelinic space, filled with cytoplasm which forms numerous pseudopodia like projections. The submyelinic space contained organelles like mitochondria (0.10-0.30 µm width) and cisternae of RER (0.03-0.06 µm thick), condensing vesicles (0.23-0.30 µm) and clear vesicles (0.16-0.31 µm) (Figure 4D). Large blood sinuses and hemocytes - both granular (6.87-7.50 µm) and agranular (2.9-5.0 µm) were detected in the hemal sinuses of the eyestalk ganglia (Figure 4C).

Non neurosecretory cells
Large, rectangular electron lucent cells (8.67-11.50 µm) arranged without intercellular spaces in the optic ganglia. The electron lucent nucleoplasm contained a few small patches of scattered chromatin. The cytoplasm contained organelles like oval to elongate, moderately dense mitochondria (0.5-1.0 µm) with irregular cristae and dilated RER cisternae (0.15-0.94 µm thick), but no inclusions like clear vesicles, condensing vesicles, autophagic vacuoles or NSGs which indicate their non-neurosecretory nature (Figure 4A).
Table 2. Neurosecretory cells in the optic lobe of *T. schirnerae*.

| Cell type | Cell size (μm)/shape | Nucleus size (μm)/shape | NPR  | Nature of chromatin | Nature of cytoplasm | Cytoplasmic inclusion/organell |
|-----------|----------------------|-------------------------|------|---------------------|---------------------|-------------------------------|
| I         | 6.33-8.00 unipolar, round/irregular | 4.67-5.3 round to oval | 0.58-0.84 | strongly dense, irregular blocks at the periphery of nucleus | moderately dense, granular | dear vesicles, condensing vesicle, autophagic vacuoles and neurosecretory granules (NSG) mitochondria, Golgi vesicles, RER cisternae |
| II        | 4.67-7.79 rectangular | 2.67-5.17 elongate/oval | 0.57-0.66 | dense blocks towards the peripheral nucleoplasm | moderately dense | dear vesicles, condensing vesicles, autophagic vacuoles, multivesicular bodies (MVB), NSG Golgi vesicles, mitochondria, ER cisternae |
| III       | 3.61-6.67 round/oblong/irregular | 2.68-4.67 oval | 0.70-0.74 | forms strongly dense ring at the peripheral nucleoplasm and blocks scattered in the nucleoplasm | moderately dense | dear vesicles, condensing vesicles, autophagic vacuoles, NSG mitochondria, RER |
| IV        | 8.0-8.08 irregular | 2.87-3.30 spherical | 0.36-0.41 | unusually dense, uniformly fills the entire nucleoplasm | electron lucent | clear vesicles, ER bound vesicles, condensing vesicles, autophagic vacuoles and NSG mitochondria, Golgi vesicles, lysosomes |
| V         | 2.84-4.67 oval | 2.52-3.53 round/oval | 0.75-0.89 | unusually dense, fills partially or fully the nucleoplasm | cytoplasm negligible | - |
| VI        | 5.00-5.55 unipolar, oval | 4.50-5.16 Oval | 0.90-0.93 | dense blocks attached to inner nuclear membrane | electron lucent | clear vesicles, autophagic vacuoles, MVB and NSG mitochondria |
| VII       | 3.57-5.16 unipolar oval to elongate | 3.07-4.29 elongate with wavy margins | 0.79-0.83 | forms dense blocks attached to the inner nuclear membrane and as granules strewn in the nucleoplasm | moderately dense | clear vesicles, condensing vesicles, autophagic vacuoles and NSG cisternae and vesicles of RER |
| VIII      | 8.4-8.57 bipolar, triangular | 3.0-3.28 Elongate | 0.35-0.38 | as patches along the peripheral nucleoplasm. | moderately dense | autophagic vacuoles, MVB, NSG mitochondria, cisternae and vesicles of RER, Golgi vesicles and lysosomes |

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**Table 2.** Neurosecretory cells in the optic lobe of *T. schirnerae*.

| Cell type | Cell size (μm)/shape | Nucleus size (μm)/shape | NPR | Nature of chromatin | Nature of cytoplasm | Cytoplasmic inclusion/organell |
|-----------|----------------------|-------------------------|-----|---------------------|---------------------|-------------------------------|
| IX        | 6.07-7.05 oval/irregular | 3.64-4.27 round | 0.59-0.61 | dense ring bordering the circumference of the nucleus | moderately dense | dense core vesicles, clear vesicles, autophagic vacuoles and NSG ER bound vesicles and mitochondria |
| X         | 6.00-6.2 oval | 2.27-3.0 irregular | 0.38-0.48 | strongly dense, occupies almost the entire nucleoplasm with dense granular regions | moderately dense | clear vesicles, autophagic vacuoles Golgi vesicles and mitochondria |

**Figure 4.** Electron micrographs illustrating non-neurosecretory cell, glial cell, axon of myelinated neuron, hemocytes and blood capillaries in the optic ganglion of *T. schirnerae*. (A-B) Glial cells (C) Granular and agranular hemocytes (D) Cross section of axon of myelinated neuron. A: Axon; AG: Agranulocytes; BC: Blood capillary; CH: Chromatin; CL: Clear vesicle; CP: Cytoplasmic process; DER: Dilated endoplasmic reticulum; G: Granulocytes; GC: Glial cell; M: Mitochondria; MS: Myelin sheath; MT: Microtubular sheath; N: Nucleus; NC: Non neurosecretory cell; RER: Rough endoplasmic reticulum; SS: Submyelinic space.
Fine structure of the sinus gland

The SG of *T. schirnerae* was positioned laterally between the ME and MI regions of the eyestalk ganglia. It is composed of axonal endings of the NSCs of the optic ganglia with interspersed glial cells. The axon terminals are enclosed with several large membrane bound homogenously dense NSGs which also occur in the preterminal areas of the axons. The axon terminals differ from each other in size, shape and density of granules and axoplasmic matrix. Mostly, the granules contained in a terminal were of the same type. Rarely, the same terminal enclosed granules of varying size, shape and density. Glial cells ramify between these terminals. Based on size, shape and density of granules contained, seven terminal types could be distinguished in the SG of *T. schirnerae*, designated as A1, A2, A3, A4, A5, A6 and A7 (Figures 5A-F) (Table 3).

Type A1 were irregular in shape (1.69-2.06 µm width), composed of numerous small sized, round, moderately dense granules (200-400 Å across). The A2 (3.21-4.66 µm) terminals were irregular in outline containing small dense granules (600-3300 Å in diameter). Their axoplasm also included electron lucent vesicles (0.06-0.4 µm in diameter) and concentric membranous lamellae. Type A3 were pear shaped, 2.77-3.07 µm in width, enclosing medium sized membrane bound homogeneously dense round to oval granules (1500-3100 Å in diameter). These were the second most abundant type which usually appeared in clusters. The oval to pear or irregular shaped A4 (2.77-4.31 µm across) were the most frequently encountered terminals, packed with large, oval to round, membrane bound, homogeneously dense granules with a diameter of 2300-6900 Å. Type A5 were mostly round or oval in outline (4.57-7.28 µm across), accommodating small to large membrane bound, homogeneously dense granules (2800-7700 Å diameter). Often these granules have a shrunken appearance, characterized by a clear area between the dense core and the limiting membrane. Their axoplasm contained a few clear vesicles (0.30-1.14 µm in diameter) and concentric membranous granules (Table 3).

**Table 3. Neurosecretory granules in the sinus gland of *T. schirnerae*.**

| Type | Size (µm) | Shape | Neurosecretory granules | Nature |
|------|-----------|-------|-------------------------|--------|
| 1    | 1.69-2.06 | irregular | 0.02-0.04 | round to oval | moderately dense |
| 2    | 3.21-4.66 | irregular | 0.06-0.33 | small granules | highly dense |
| 3    | 2.77-3.07 | pear shaped | 0.15-0.31 | round to oval | highly dense |
| 4    | 2.77-4.31 | oval/ pear/ irregular | 0.23-0.69 | oval to round | highly dense |
| 5    | 4.57-7.28 | round/oval | 0.28-0.77 | oval to elongate | strongly dense, shrunken appearance, clear area between the dense core and limiting membrane |
| 6    | 3.5-4.18 | Round | 0.18-0.45 | round or oval | strongly dense |
| 7    | 2.15-5.16 | Spherical | 0.07-0.71 | oval or pear shaped | strongly dense |
Figure 5. Ultra-thin sections of axon terminals in the sinus gland of *T. schirnerae*. (A-F) Axon terminals 1-7 enclosing various types of neurosecretory granules and inclusions in the sinus gland. Note the endocytotic invaginations of the axon terminal membrane in Figure E. A1: A1 axon terminal; A2: A2 axon terminal; A3: A3 axon terminal; A4: A4 axon terminal; A5: A5 axon terminal; A6: A6 axon terminal; A7: A7 axon terminal; CM: Concentric membrane; GC: Glial cell; M: Mitochondria; N: Nucleus; NG: Neurosecretory granules; NT: Neural tubules; Arrows indicate endocytotic invaginations of the axon terminal membrane.

lamellae. The A6 endings (3.5-4.18 µm) were round with dense membrane bound granules with a size range of 1800-4500 Å and filled with a dense homogeneous substance containing small to large, round or oval electron lucent vesicles (0.54-1.63 µm in diameter) and concentric membranes. The A7 endings were spherical (2.15-5.16 µm diameter) structures enclosing oval or pear shaped, membrane bound homogenously dense granules (0.07-0.71 µm in diameter). Their axoplasm enclosed numerous clear vesicles (0.15-
0.31 µm diameter) and concentric lamellae.

**Glial cells**

Were seen in the optic ganglia as well as in the SG intermingled with the axonal terminals. Elongate cells (2.63-6.84 µm width) mostly with the nuclei (2.11-5.79 µm across) pushed to one half of the cell. Distinct nucleoli can be seen occupying central or peripheral positions in the nucleoplasm. The NPR measured was in the range 0.76-0.81. The dense chromatin was arranged as a peripheral thin rim or forms several blocks attached to the inner nuclear membrane. The cytoplasm accommodates ovoid mitochondria (0.40-0.95 µm) with irregular or fragmented cristae and clear vesicles (0.05-0.5 µm) (Figure 4B). These cells send out several cytoplasmic processes called the glial cell processes which surround the axon terminals in the SG. These processes frequently enclosed mitochondria (0.25-0.63 µm width), Golgi complexes, glycogen granules and dilated RER cisternae (Figures 6A-B).

**Figure 6.** Glial cell and axon terminal in the sinus gland of *T. schirnerae*. (A) Glial cell nucleus interspersed among the axon terminals (B) Cytoplasmic processes of glial cell surrounding the axon terminal. AT: Axon terminal; CH: Chromatin; CP: Cytoplasmic process; DER: Dilated endoplasmic reticulum; M: Mitochondria; N: Nucleus; NG: Neurosecretory granule.

**Discussion**

Light and electron microscopic studies of the eyestalk of *T. schirnerae* revealed four ganglionic regions: the lamina ganglionaris, medulla externa, interna and terminalis along with the neurohaemal organ, the sinus gland. The microanatomy of the optic ganglia of *T. schirnerae* is identical to that of other crabs such as *Eriocheir japonicus*, *Chionoecetes opilio*, *Potamon dehanni*, *Neptunus trituberculatus*, *Sesarma intermedia* (Matsumoto, 1958), *Diogenes bicristimanus* (Nagabhushanam and sarojini, 1969); shrimps like *P. indicus* (Mohamed et al., 1993), *Metapenaeus ensis*, (Gunawardene et al., 2002) and the lobster *Nephrops norvegicus* (Giulianini et al., 1998; Fernandez and Radhakrishnan, 2010). Similar to the observations of the present study, many investigators reported the presence of three distinct X-organs: ME-XO, MI-XO and MT-XO in the eyestalks of *Pandalus borealis* (Carlisle, 1959), *Macrobrachium rosenbergii* (Dietz, 1982) and *P. indicus* (Mohamed et al., 1993). However, Hisano (1976) and Dietz (1982) have described only a single MI-XO complex in the optic ganglia of *P. paucidens* and
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*M. rosenbergii.* In *P. japonicus* (Nakamura, 1974) and *P. serratus* (Van Herp et al., 1977) the optic ganglia were observed without MI-XO.

The present study confirmed the presence of several neurosecretory cell types: seven light microscopically and ten ultrastructurally in the X-organs of the eyestalk ganglia. In agreement with these findings, many of the investigators have recognized diverse cell types in the eyestalks of crustaceans (Potter, 1958; Matsumoto, 1958): two in the lobster *Thenus orientalis* (Padmaja et al., 2010); three in crabs like *Sesarma* (Enami, 1951), *Eriocheir* sp. (Matsumoto, 1954) and *Paratelphusa hydrodromous* (Parameswaran, 1956) and prawn *P. japonicus* (Nakamura, 1974); four in the crayfish *Orconectes virilis* (Durand, 1956), shore crab *P. gaimardii* (Lake, 1970) and *P. indicus* (Bharathi et al., 2014) and five in *P. borealis* (Carlisle, 1953). On the other hand, Rajendiran and Vasudevan (2016) noticed tripolar and multipolar cells in addition to the unipolar and bipolar cells in the optic ganglia of *P. paucidens*. Similar to the current study, NSGs were evident in *Paratelphusa* (Parameswaran, 1956), *P. gaimardii* (Lake, 1970) and *P. indicus* (Mohamed et al., 1993). The presence of Golgi complexes, mitochondria and ER in the NSCs of *T. schirnerae* corroborates their role in the synthesis of NSGs. The involvement of Golgi and mitochondria in the synthesis of neurosecretory material was established by Nishiitsutsuji-Uwo (1960) and Beams and Kessel (1968). It is stated that the RER, Golgi and vesicles are involved in the production, processing and storage of peptide hormones. The proteinaceous material synthesized in the ER is transported to the Golgi cisternae by the small vesicles. The presence of MVBs and lysosomes and their close association with NSGs in the NSCs of *T. schirnerae* is considered as a characteristic of active NSCs and suggest their role in degrading the neurosecretory material of the NSGs (Shivers, 1967). Lytic bodies and MVBs were characteristic of active neurosecretory cells in *Gadus morrhua* (Lederis, 1962), crayfish (Shivers, 1967) and the teleost pituitary (Hopkins, 1969).
In agreement with the observations made by Hsu et al. (1980) and Ke Xu and Terakawa (1999) in *P. chinensis*, the optic ganglia in *T. schirnerae* demonstrated the presence of myelinated axons with dense microtubular sheath, submyelinic space and myelin sheath surrounding the axolemma. Conversely, in *Gecarcinus lateralis* the axons of SG were non-myelinated having thin limiting membranes (Hodge and Chapman, 1958). The myelin sheaths of invertebrate nerve fibres have a unique structure that differs from vertebrate myelinated fibres in that the nucleus of Schwann cell is located within the innermost lamina of the myelin sheath (Heuser and Doggenweiler, 1966; Ke Xu and Terakawa, 1999). Roots (1995) suggested that the myelin sheath has evolved independently in chordates, annelids and arthropods. Nevertheless, ultrathin sections of *C. irroratus* brain proved the position of Schwann cell nucleus outside the myelin sheath, quite similar to the myelinated nerve fibres of vertebrates (McAlear et al., 1958). In the current investigation, it is possible that the myelin sheath covering the axons acts as an electrical insulator and may provide mechanical support for the axon. It is also suggested that the microtubular sheath surrounding the axon may eliminate the wide gap between the axon and the myelin sheath.

In the present investigation, the SG was found occupying a position between the MI and ME. A more or less similar positioning was reported for the SG in *P. paucidens* (Hisano, 1976), *P. serratus* (Van Herp et al., 1977), *M. rosenbergii* (Dietz, 1982), *P. stylifera* (Nagabhushanam et al., 1986), *C. maenas* (Dircksen, 1992), *P. indicus* (Mohamed et al., 1993) and *M. ensis* (Gunawardene et al., 2002). On the other hand, the SG was found dorsal to the MI in *G. lateralis* (Hodge and Chapman, 1958), adjacent to the MI in *P. gaimardii* (Lake, 1970), in the MT ganglion in *Sesarma* (Enami, 1951), at the dorsolateral surface of the MT in *Cardisoma carnifex* (Weatherby, 1981), on the abaxial side of MT in the crab *Charybdis lucifera* (Chandy and Kolwalkar, 1985), laterally between the MI and MT regions in *P. pelagicus* (Rajendiran and Vasudevan, 2016), dorsally at the junction of the MI and MT in *D. bicrhistimanus* (Nagabhushanam and Sarojini, 1969).

In *T. schirnerae*, the SG is a deeply stained oval structure. Earlier studies reported different shapes for SG in crustaceans. Enami (1951) distinguished the SG as a triangular structure in *Sesarma* while flattened ovoid in *P. gaimardii* (Lake, 1970), crescent shaped in *P. serratus* (Van Herp et al., 1977), ellipsoid in *C. carnifex* (Weatherby, 1981) and in *P. indicus* (Mohamed et al., 1993), as a palmate leaf in *M. rosenbergii* (Dietz, 1982). Conversely, in *N. norvegicus* (Giulianini et al., 1998) and *P. homarus* (Fernandez and Radhakrishnan, 2010), no specific shape could be assigned to the SG.

As reported in the SG of *Callinectes sapidus* (Andrews et al., 1971) and *Homarus gammarus* (Castany et al., 1997), seven different types of axonal endings were identified in the SG of *T. schirnerae*. On the contrary, the SG of *G. lateralis* (Bliss and Welsh, 1952; Hodge and Chapman, 1958) and *P. paucidens* (Hisano, 1976) described four distinct types, five in *Procambarus clarkii* (Bunt and Ashby, 1967) and *Uca pugnax* (Silverthorn, 1975) and six in *P. borealis* (Carlisle, 1959) and *C. carnifex* (Weatherby, 1981). Histological observations identified six axon terminal types (Smith, 1974; Dircksen, 1992) in *C. maenas*.

In crustaceans, the number of NSG types varied from two to seven. According to the present study, seven NSG types were identified in the axon terminals of *T. schirnerae*. Similar observations were made in *C. sapidus* (Andrews et al., 1971) and *H. gammarus* (Castany et al., 1997). However, two...
types of NSGs were identified in *G. lateralis* (Hodge and Chapman, 1958); three in *C. maenas* (Meusy, 1968); four in *Pachygrapsus marmoratus* (Bressac, 1976); five in *P. clarkii* (Bunt and Ashby, 1967) and *U. pugnax* (Silverthorn, 1975) and six types in *C. carnifex* (Weatherby, 1981). In agreement with the findings in *T. schirnerae*, each terminal type contained a single type of NSG in *H. gammarus* (Castany et al., 1997). On the other hand, Andrews et al. (1971) and Dircksen (1992) observed the occurrence of several granule types in a single terminal in *C. sapidus* and *C. maenas*.

Our observations on the presence of glial cells or supporting cells intermingled with axonal terminals in the SG of *T. schirnerae* was supported by the reports made in SG of many crustaceans like *O. virilis* (Andrew et al., 1978), *G. lateralis* (Hodge and Chapman, 1958), *C. maenas* (Dircksen, 1992), *H. gammarus* (Castany et al., 1997) and *C. carnifex* (Weatherby, 1981). According to Mohamed et al. (1993), glial cells were present in the neurosecretory system of *P. indicus*. In compliance with the findings of Bunt and Ashby (1967) in *P. clarkii*, the glial cells in *T. schirnerae* were observed with cytoplasmic processes, organelles like mitochondria and ER and glycogen granules. Several authors reported the glial cells as store house of glycogen and the vesicular and granular inclusions in the cytoplasm of glial cells are involved in the nutritive, metabolic and catabolic functioning of neurons (Pentreath, 1989). The presence of glycogen and lipids in invertebrate glial cells was reported by Wigglesworth (1960) and Kuffier and Nichols (1966). The glial cells intermingled with the axonal terminals in the SG of *T. schirnerae* may possibly suggest their involvement in bringing, taking away and exchanging of substances to and fro with the neurons. The presence of glycogen in glial cells of *T. schirnerae* has led to the suggestion that these cells supply energy substrate to the NSCs that can survive without external energy source for several hours.

**Conclusions**

Fine structural analysis of the optic ganglia of *T. schirnerae* revealed four ganglionic regions and ten neurosecretory cell types. The neurosecretory cells were characterized by nuclei with unusually dense chromatin and other organelles like Golgi bodies, mitochondria, endoplasmic reticulum, ribosomes and lysosomes and inclusions like clear vesicles, condensing vesicles, dense core vesicles, multivesicular bodies and neurosecretory granules. The sinus gland with seven different terminal types enclosing neurosecretory granules of varying size, shape and density reveal the presence of a number of neurohormones synthesized and secreted by the neurosecretory cells of the optic ganglia in *T. schirnerae*. Further investigations have to be carried out on the immnocytochemistry of the neurosecretory cell types and contents of the axonal endings to confirm the kinds of neurohormones they carried.

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**Conflict of interests**

The authors declare that they have no conflict of interests.
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