www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 17: 231-243 (2017) DOI: 10.4194/1303-2712-v17_2_01

RESEARCH PAPER

Ultrastructure of Haemocytes of the Freshwater Crab *Travancoriana schirnerae*

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Received 03 April 2016
Accepted 05 September 2016

Abstract

The light and ultrastructural observations on haemocyte profile of the freshwater crab *Travancoriana schirnerae* demonstrated three cell types: agranulocyte, granulocyte I and granulocyte II. Agranulocytes are the smallest of the haemocyte types, comprised 38% of the haemocytes, devoid of granules and showed high nucleoprotoplasmic ratio. Their cytoplasm contained numerous free ribosomes, vesicles of smooth endoplasmic reticulum, few mitochondria with parallel cristae and vacuoles. Granulocytes I (37%) are the largest of the haemocyte types, characterized by the presence of numerous granules (26 to 61) of varying size, shape and densities and organelles like mitochondria, free ribosomes and peripheral vacuoles and displayed low nucleoprotoplasmic ratio. Granulocytes II (25%) contained large electron dense granules (10 to 23) and organelles like free ribosomes and vacuoles and showed intermediate nucleoprotoplasmic ratio. Unlike granulocytes, agranulocytes exhibited prominent pseudopodial projections suggesting a phagocytory function. This study revealed the unusual occurrence of a binucleate agranulocyte and a rare phenomenon called clasmatosis of granulocyte II, wherein part of the cell fragments off along with cytoplasmic contents devoid of nucleus.

Keywords: Agranulocyte, clasmatosis, granulocyte, nucleoprotoplasmic ratio.

Introduction

In crustaceans, the immune activities are executed effectively by an array of innate, complex cellular and humoral reactions. Haemocytes are the primary components of the immune system. The circulating blood cells have been broadly classified into hyaline cells and granular cells depending on the presence or absence of cytoplasmic granulations (Bauchau, 1981; Hose *et al*., 1990). These cells perform both immune and non-immune roles. The immune functions include phagocytosis, encapsulation, cell-mediated toxicity and coagulation (Johansson and Söderhäll, 1989; Jiravanichpaisal *et al.*, 2006; Sivakamavalli *et al.*, 2012). The non-immune roles comprise moulting, haemocyanin production and transport and storage of nutrients (Bauchau and Plaquet, 1973; Vacca and Fingerman, 1975). Hyaline cells initiate haemolymph coagulation and hardening of the exoskeleton after moulting (Vacca and Fingerman, 1983; Omori *et al*., 1989). Granulocytes play diverse roles, being involved in phagocytosis, agglutination, coagulation, encapsulation and storage of haemocyanin and glycoproteins (Wood and Visentin, 1967; Busselen, 1970; Stang-Voss, 1971; Wood *et al*., 1971; Ravindranath, 1980).

Light microscopic and ultrastructural investigations with regard to the morphological and functional aspects of haemocytes have been extensively studied in economically important marine decapods. Ultrastructural details of haemocytes of the Chinese mitten crab *Eriocheir sinensis* were reported by Bauchau and De Brouwer (1972). In the shore crab *Carcinus maenas*, Johnston *et al*. (1973) described the haematological details with regard to carbohydrate metabolism. Vranckx and Durliat (1977) analyzed the circulating cells in a number of decapods. Bodammer (1978) and Clare and Lumb (1994) described the morphology, fine structure of haemopoietic tissue and haemocytes and phenoloxidase (PO) activity in the blue crab *Callinectes sapidus*. Tsing *et al*. (1989) demonstrated the morphology, cytochemistry and haemograms of penaeid and palaemonid shrimps. Hose *et al*. (1990) reported a haemocyte classification scheme integrating morphology, cytochemistry and function in three decapods: *Loxorhynchus grandis*, *Homarus americanus* and *Panulirus interruptus.* The fine structure of circulating haemocytes in the Indian white shrimp *Fenneropenaeus indicus* was described

by Laxmilatha and Laxminarayana (2004).

Compared to their marine counterparts, reports on the morphological and fine structural aspects of haemocytes in freshwater crustaceans are relatively sparse. Vázquez *et al*. (1997) explored the morphology of three haemocyte types in *Macrobrachium rosenbergii*. Haemocyte types of two freshwater palaemonids and a peneaid were analyzed by Gargioni and Barracco (1998). Comparatively very few studies are devoted to freshwater crabs; those reported are restricted to haemocyte counts and morphological aspects. Yavuzcan-Yildiz and Atar (2002) reported the haemocyte classification and differential counts in the freshwater crab *Potamon fluviatilis.* Similar studies were conducted by Nayan *et al.* (2010) in *Sartoriana spinigera*. Gupta *et al*. (2013) and Rulprakash *et al*. (2013) identified different types of haemocytes in the freshwater crabs *Paratelphusa masoniana* and *P. hydrodromous*, respectively. Against this background, the present investigation on the fine structure of haemocytes of the freshwater crab *Travancoriana schirnerae* is undertaken. The results of this study will provide information on the cytology of haemocytes to support further investigations on their physiology and function.

Materials and Methods

Adult intermoult *T. schirnerae* of carapace width 4.5–5.0 cm (n=5) were collected from the paddy fields of Ondayangadi, about 5 km northeast of Mananthavady (11.82° N and 76.02° E, altitude 767 m) in Wayanad district of Kerala, India during March 2014. The crabs were transported immediately and acclimatized to the laboratory conditions for four days. Haemolymph (1 ml) was carefully drawn by a needle (2 ml syringe attached to a 26 gauge needle) inserted into the body cavity through the arthrodial membrane of the 4th walking leg and transferred immediately to a test tube containing 1 ml cold Karnovsky fixative. It was thoroughly mixed and allowed to stand for two hours for proper fixation. The sample was centrifuged at 1000 rpm for five minutes. After discarding the supernatant, the pellet was carefully washed thrice with 0.1 M phosphate buffer (pH 7.4), post-fixed in 1% osmium tetroxide for 90 minutes and washed with buffer. The pellet was dehydrated in graded series of ethanol, cleared in propylene oxide and left in a mixture of propylene oxide and araldite (1:1) overnight in a rotator at room temperature. The pellet was then embedded in araldite and allowed to polymerize at 60°C for 48 hours.

Semithin (0.5 µm) and ultrathin sections (80 nm thick) were cut with glass knives in a Leica UC6 ultramicrotome. Semithin sections were stained with 1% toluidine blue and observed under a Leica DM 500 Research Microscope. Light micrographs were taken with a DG 330/210 camera using Biowizard software. Ultrathin sections were stained with uranyl acetate and lead citrate and observed under a Tecnai G2 Spirit Biotwin transmission electron microscope (TEM) operating at 80 KV. Electron micrographs were taken with a Mega View-III CCD camera using Analysis software and analyzed for fine structural details.

The relative percentage of each haemocyte population was calculated from the semithin sections, which is more precise for judgement because of inbuilt better resolution. The number of cells belonging to each cell type was counted from five visual fields to determine the differential counts. From each field 60 cells were counted, totaling 300 cells per specimen. Totally, two semithin sections were used for counting. The morphological dimensions of the haemocytes and granules were measured from the electron micrographs using Analysis software. The nucleoprotoplasmic ratio (NPR) was calculated by dividing the width of the nucleus by the width of the cell. Measurements from only those haemocytes with clearly visible nuclei were considered for calculating the NPR.

Results

Light Microscopy

Light microscopic observations of the haemocyte profile of *T. schirnerae* distinguished two haemocyte types – granulocytes and agranulocytes – based on the presence or absence of cytoplasmic granulations (Figure 1A). Semithin sections revealed that granulocytes occurred in greater numbers than the agranulocytes (Ag). Agranulocytes were the smallest cells, comprising 38% of the total haemocytes with prominently large nuclei occupying much of the cytoplasmic space. Their homogenous cytoplasm totally lacked granules. In some, rarely one or two mildly stained granules were spotted (Figure 1B).

Based on the distribution of granules, granulocytes were distinguished into granulocyte I (GI) and granulocyte II (GII). Granulocytes I (37%) were characterized by the presence of numerous deeply stained granules concentrated either towards the periphery or uniformly dispersed in the cytoplasm (Figure 1C). Granulocytes II represented 25% of the total haemocytes, with fewer numbers of granules encircling the nucleus (Figure 1D). In some GII, granules were dispersed throughout the cell, obscuring the nuclei (Figure 1E). In both the granulocyte types, the nuclei were centric or eccentric in position.

Ultramicroscopy

Ultrastructural observations corroborated the light microscopic findings on the cell types – Ag, GI and GII (Figure 1F).

Agranulocytes:Small, elongate cells (5.15×2.57–7.69×3.50 µm) with large nuclei (3.45×1.15–

4.28×1.70 µm) occupying much of the cytoplasmic space and exhibited high NPR (Table 1). The smooth and distinct nuclear envelope enclosed dense heterochromatin attached to the inner nuclear membrane interrupted by one or two peripheral nucleoli. These cells were generally devoid of granules. Rarely one or two granules were spotted (diameter 0.19 to 0.94 µm) in the cytoplasm. Numerous free ribosomes, vesicles of smooth endoplasmic reticulum (SER), few vacuoles and mitochondria with parallel cristae were dispersed in the cytoplasm. Golgi bodies were rare or absent. The presence of prominent pseudopodial projections was another distinctive feature of Ag (Figure 2A, B). Rarely, binucleate Ag were noticed (Figure 2C).

Depending on the size, shape and density, six granule types were distinguished in the granulocytes (Figure 3, Table 2).

Type 1: Small to large, round (diameter 0.17–1.58 µm), rod (0.51×0.26–0.99×0.25 µm) or tear-shaped (0.29×0.22–1.15×0.35 µm) granules with a homogenous dense matrix (Figure 3A). Smaller sized type 1 granules were typically encountered in GI while larger sized type 1 granules occurred abundantly in GII.

Type 2: Elongate granules of intermediate density (0.34×0.17–1.18×0.38 µm), with or without limiting membranes; often spotted in GI but

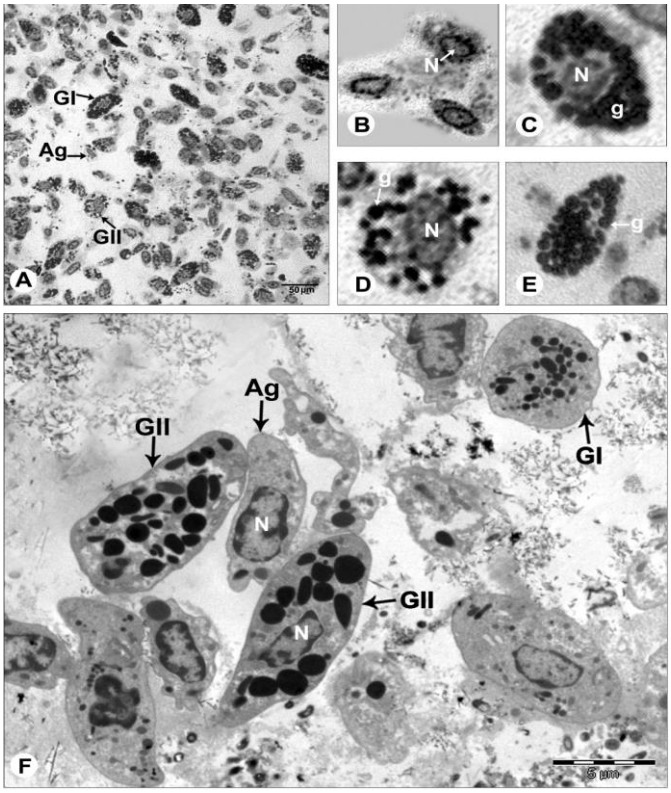

Figure 1. Haemocytes of *T. schirnerae.* (A) Light micrograph depicting haemocytes stained with toluidine blue (scale bar 50 µm). (B) Agranulocytes. (C) Granulocyte I. (D) Granulocyte II. (E) Granulocyte II with granules totally obscuring the nucleus. (F) Transmission electron micrograph of haemocyte types (scale bar 5 µm). Ag: Agranulocyte; GI: Granulocyte I; GII: Granulocyte II; g: Granule; N: Nucleus.

Table 1. Haemocyte morphology of the freshwater crab *T. schirnerae*

Parameter	Agranulocyte	Granulocyte I	Granulocyte II
Cell size	5.15×2.57–7.69×3.50 (6.80±1.01×3.55±6.34)	7.65×3.08–15.40×7.01 (10.89±3.06×4.72±1.80)	7.03×6.22–9.26×5.16 (7.84±0.97×4.25±1.32)
Nucleus size	3.45×1.15–4.28×1.70 (3.79±3.04×1.68±3.59)	1.86×1.46–6.13×3.06 (3.25±1.63×1.97±0.82)	2.34×1.92–4.46×2.23 (3.41±0.74×2.54±0.69)
Average NPR	0.55±0.05	0.26±0.11	0.43±0.11
Avg number of granules/cell	+ (18)	40.36±10.5 (28)	11.5±6.19 (30)
Granule size	0.19×0.10–0.94×0.79 (0.49±0.3×0.31±0.2)	0.16×0.11–1.15×0.35 (0.56±0.31×0.41±0.23)	0.49×0.61–1.58×1.11 (1.09±0.30×0.86±0.21)

The unit 'µm' is used for all the measurements. '+' denotes granules nil or rarely one or two. Number in parentheses indicates the number of cells counted for granules. NPR = nucleus size/cell size.

uncommon in GII (Figure 3B).

Type 3: Round, electron lucent granules (diameter 0.49–0.53 µm) frequently occurred in GI (Figure 3C).

Type 4: Spherical granules (width 0.36–0.55 µm) with a central core of intermediate density and a lucent periphery; moderate numbers (5–8) occurred in GI, very few (2 or 3) in GII (Figure 3D).

Table 2. Comparative features of the different granule types of *T. schirnerae*

Parameters	Type 1	Type 2	Type 3	Type 4	Type 5	Type 6
Shape and size (µm)	round (diameter) 0.17–1.58 (1.15±0.43) rod (length×width) 0.51×0.26–0.99×0.25 (0.74±0.15×0.32 ±0.08) tear-shaped (length×width) 0.29×0.22–1.15×0.35 (0.79±0.26×0.27 ±0.07)	elongate with or without limiting membranes (length×width) 0.34×0.17–1.18×0.38 (0.74±0.27×0.33 ±0.11)	round (diameter) 0.49–0.53 (0.50±0.01)	spherical (diameter) 0.36–0.55 (0.44±0.05)	elongate (length×width) 0.25×0.10–0.56×0.19 (0.39±0.11×0.18 ±0.05)	spherical (diameter) 0.39–1.21 (0.79±0.33)
Occurrence	in GI and GII	often spotted in GI; uncommon in GII	frequent in GI	moderate in GI; very few in GII	in GI only	frequent in GI; rare in GII
Nature of contents	homogenously dense	intermediate density	electron lucent	central core of intermediate density and lucent periphery	intermediate density enclosing dense circular areas	Reticulate central core surrounded by lucent periphery

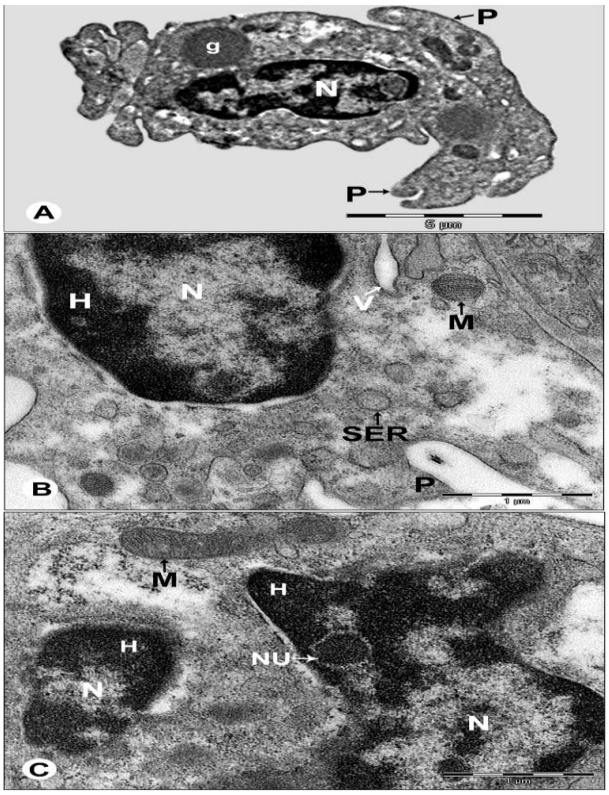

Figure 2. Ultrastructural images of agranulocytes. (A) Agranulocyte illustrating large nucleus and prominent pseudopodia (scale bar 5 µm). (B) Cytoplasm displaying cell organelles. (C) Binucleate agranulocyte. Scale bar 1 µm, applies to B and C.

g: Granule; H: Heterochromatin; M: Mitochondrion; N: Nucleus; NU: Nucleolus; P: Pseudopodium; SER: Smooth endoplasmic reticulum; V: Vacuole.

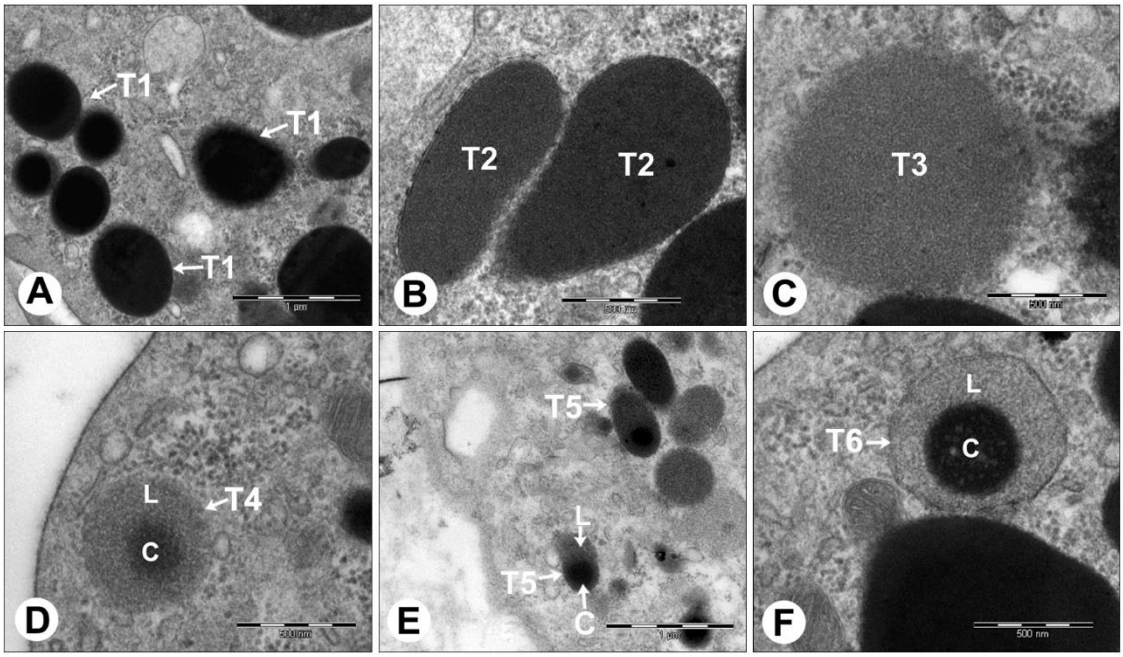

Figure 3. Transmission electron micrographs of different granule types. (A) Type 1. (B) Type 2. (C) Type 3. (D) Type 4. (E) Type 5. (F) Type 6; scale bar 1 µm, applies to A and E; 500 nm for B–D and F.
C: Central core; L: Lucent peripheral area; T1: Type 1 granule; T2: Type 2 granule; T3: Type 3 granule; T4: Type 4 granule; T5: Type 5 granule; T6: Type 6 granule.

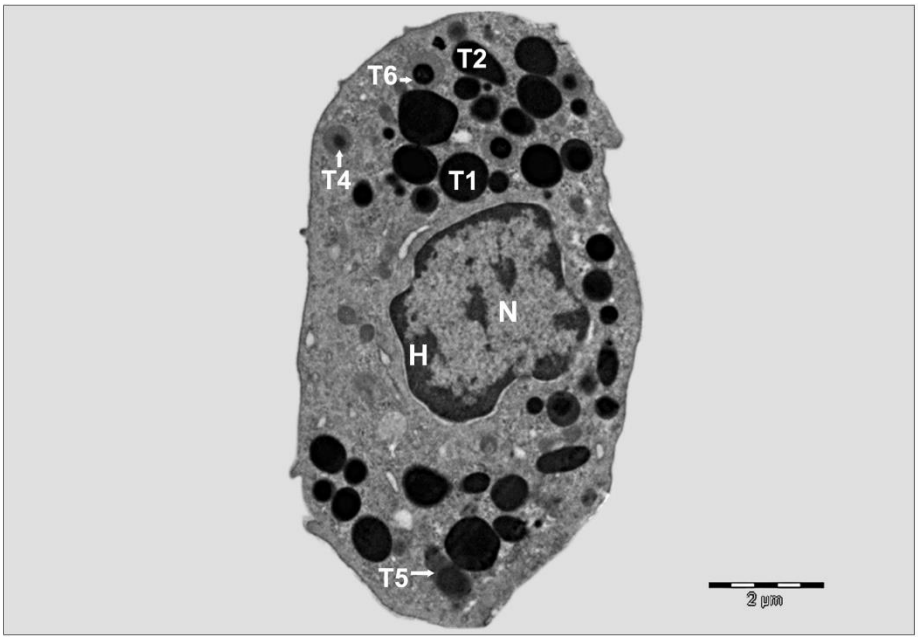

Figure 4. Fine structural image of granulocyte I describing granule types (scale bar 2 µm).
H: Heterochromatin; N: Nucleus; T1: Type 1 granule; T2: Type 2 granule; T4: Type 4 granule; T5: Type 5 granule; T6: Type 6 granule.

Type 5: Elongate granules (0.25×0.10–0.56×0.19 µm) of intermediate density enclosing dense circular areas. These granule types were observed in GI (Figure 3E).

Type 6: Spherical granules (0.39–1.21 µm) with reticulate central core surrounded by lucent periphery, with or without limiting membranes; frequent in GI but rare in GII (Figure 3F).

Granulocytes I: Typically oval to elongate cells (7.65×3.08–15.40×7.01 µm) with small, centric or eccentric nuclei and without pseudopodia (Figure 1F). The outer nuclear membrane was smooth and distinct. When compared to Ag and GII, the heterochromatin band of GI was less dense and a few patches were

seen disbursed in the extensive euchromatic region (Figure 4). These cells exhibited low NPR (Table 1). The cytoplasm contained vesicles of SER, free ribosomes, mitochondria with parallel cristae and a few peripheral polymorphic vacuoles (Figure 5A). Pseudopodial projections were not prominent. The presence of numerous small and occasional large type 1 granules differentiated GI from GII. Total number of granules in GI ranged from 26 to 61 and was concentrated either peripherally or scattered throughout the cytoplasm. Though all the six granule types were observed, type 1 dominated the cytoplasm (Figures 4, 5B).

Granulocytes II: Oval or elongate cells of intermediate size (7.03×5.16–9.26×6.22 µm) with centric or eccentric nuclei (2.34×1.92–4.46×2.23 µm) (Figure 1F). A discontinuous layer of dense heterochromatin was attached to the inner nuclear membrane. The NPR was found intermediate to Ag and GI (Table 1). Granulocytes II were the most prominent of the cell types as they carried distinctly large, dense, type 1 granules (diameter 0.61–1.11 µm) (Figure 6A). The number of granules per cell ranged from 10 to 23. Instances of granules totally obscuring the nuclei were also evident (Figure 6B). Except for free ribosomes, vesicles of SER, mitochondria and a few vacuoles, no other organelles were not perceptible in the cytoplasm (Figure 6C). Granule

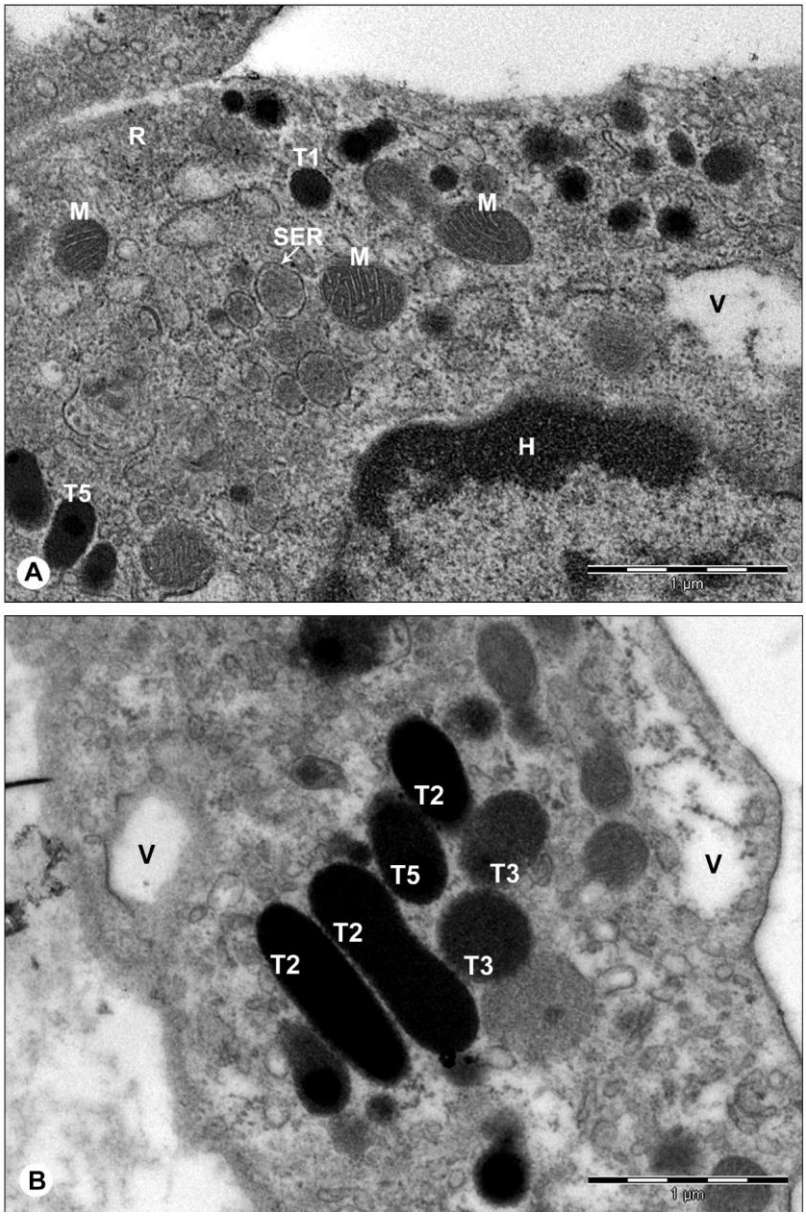

Figure 5. Granulocyte I at higher magnification depicting cell organelles and granule types. (A) Cell organelles. (B) Granule types; scale bar 1 µm, applies to A and B.
H: Heterochromatin; M: Mitochondrion; R: Free ribosomes; SER: Smooth endoplasmic reticulum; T1: Type 1 granule; T2: Type 2 granule; T3: Type 3 granule; T5: Type 5 granule; V: Vacuole.

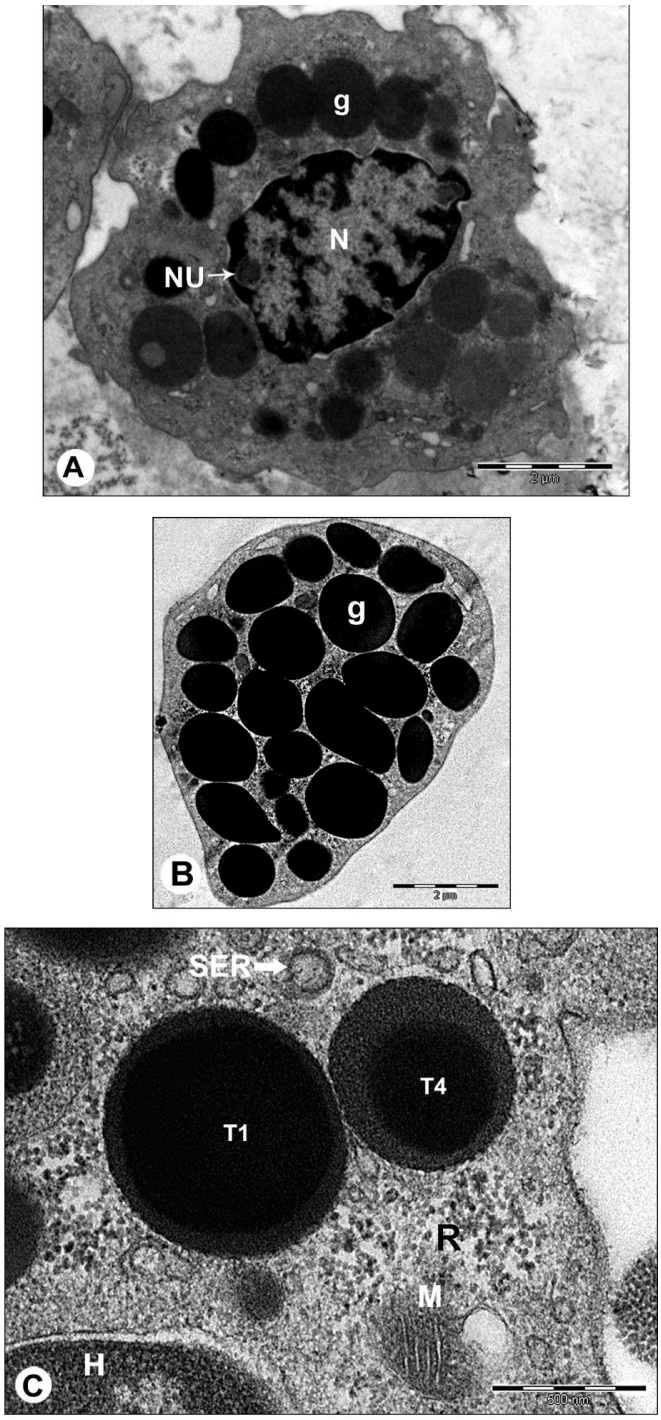

Figure 6. Electron micrographs of granulocyte II. (A) Granulocyte II portraying nucleus and granule types. (B) Large type 1 granules obscuring the nucleus. (C) Cell organelles in granulocyte II. Scale bar 2 µm, applies to A and B; 500 nm for C. g: Granule; H: Heterochromatin; M: Mitochondrion; N: Nucleus; NU: Nucleolus; R: Free ribosomes; SER: Smooth endoplasmic reticulum; T1: Type 1 granule; T4: Type 4 granule.

types 2 to 6 were traced occasionally in GII. An unusual instance of a granule releasing its content into the haemolymph was noticed in GII (Figure 7A).

A rare phenomenon called clasmatosis was noticed in GII where the cytoplasm is seen fragmenting without the nucleus. The cell fragmentation seems incomplete as the cytoplasm along with some granules was seen moving into the fragmenting portion without the nucleus (Figure 7B).

Discussion

The ultrastructural observations on circulating haemocyte profile of *T. schirnerae* revealed three cell types: agranulocytes, granulocytes I and II. This is in agreement with the general observations reported in

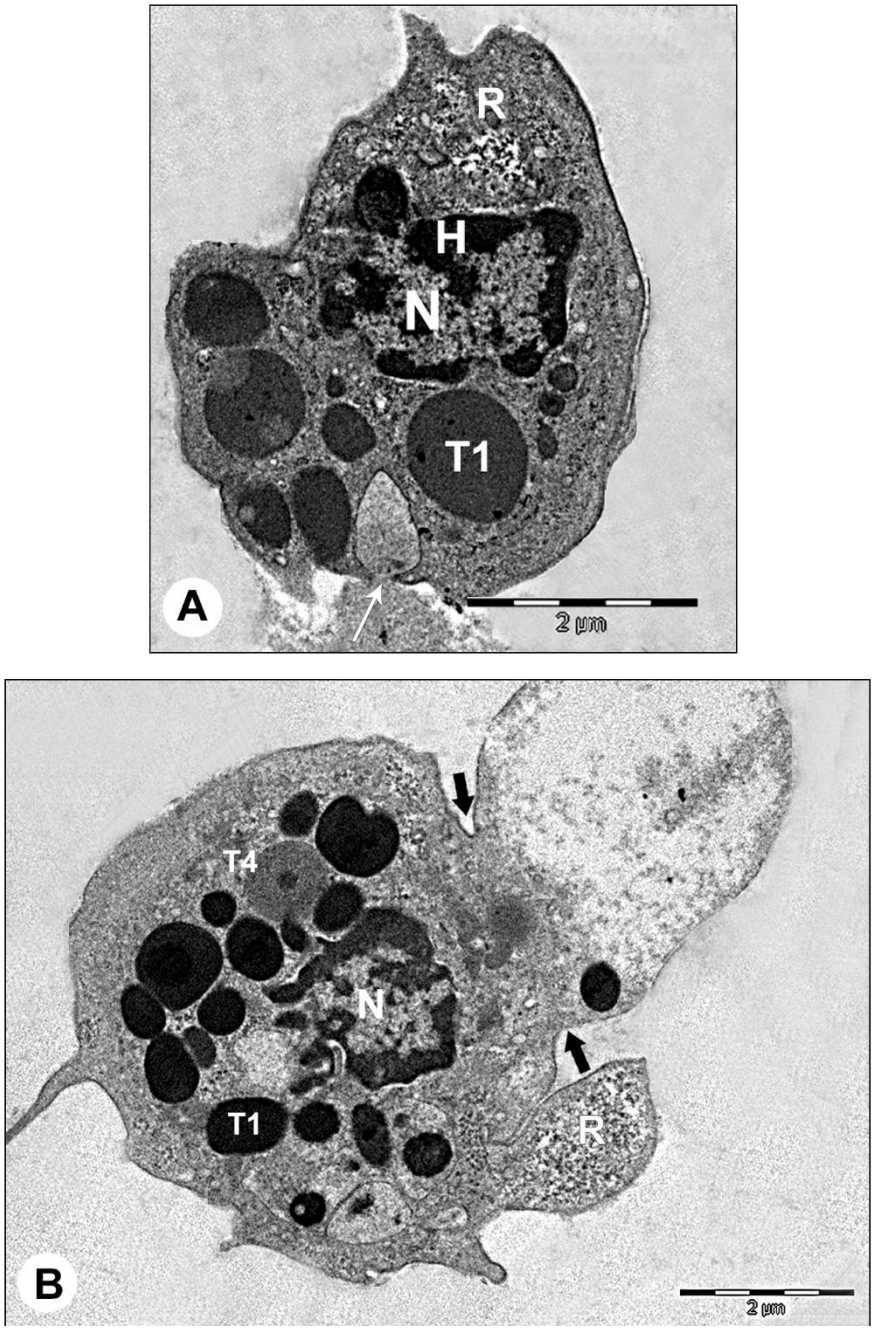

Figure 7. Transmission electron micrographs of granulocyte II. (A) Release of granular content into the haemolymph. (B) Granulocyte II undergoing clasmatosis. Scale bar 2 µm, applies to A and B.
H: Heterochromatin; N: Nucleus; R: Free ribosomes; T1: Type 1 granule; T4: Type 4 granule; Arrow indicates release of granular content into the haemolymph; Bold arrow indicates granulocyte II undergoing clasmatosis.

other crustaceans, based broadly on granule distribution, morphology and cell dimensions. Although the terminologies differ, features of the haemocyte types reported here are comparable to those reported for marine crabs (Mix and Sparks, 1980; Hose *et al*., 1990; Clare and Lumb, 1994), lobsters (Hearing and Vernick, 1967; Cornick and Stewart, 1978) and penaeids (Tsing *et al*., 1989; Jayasree, 2009). Yavuzcan-Yildiz and Atar (2002) and Rulprakash *et al*. (2013) reported three haemocyte types in freshwater crabs *P. fluviatilis* and *P. hydrodromous*, respectively. On the other hand, Williams and Lutz (1975) distinguished two major cell types in *C. maenas*, four in *H. americanus* (Cornick and Stewart, 1978), *Sicyonia ingentis* (Martin and Graves, 1985) and *E. sinensis* (Hong da, 2002) and five in the white leg shrimp *Litopenaeus vannamei* (Muhammad *et al*., 2013). Generally, crustaceans display three morphologically distinct haemocyte types – hyaline, semigranular and granular cells (Bauchau, 1981; Persson, 1986; Johansson and Söderhäll, 1989) comparable to the agranulocytes,

granulocytes I and II, respectively of the present study.

In the present investigation, the haemocyte profile comprised of 38% Ag, 37% GI and 25% GII. Similar observations were made by Clare and Lumb (1994) in *C. sapidus* with 48, 31 and 21%, respectively of hyaline haemocytes, small granule haemocytes (SGH) and large granule haemocytes (LGH). In penaeid shrimps, Vargas-Albores *et al.* (2005) reported SGH in abundance (51%) followed by hyaline cells (29%) and LGH (19%). Tsing *et al.* (1989) and Gargioni and Barracco (1998) have reported the occurrence of varying proportions of haemocytes in palaemonids and penaeids. In *P. fluviatilis*, the most abundant cell type was semigranulocytes constituting 54.25% of total haemocytes; the percentages of hyalinocytes and granulocytes were 15 and 30.75% respectively (Yavuzcan-Yildiz and Atar, 2002). The total and differential counts of hemocytes may provide a useful means for evaluating the physiological condition of an animal (Le Moullac and Haffner, 2000). The wide difference in cell percentages may be attributed to various parameters such as sex, diet, length at captivity, moult stage (Stewart *et al.*, 1967; Tsing *et al*., 1989; Wang and Chen, 2005a), pathological conditions (Smith and Ratcliffe, 1980; Eddy *et al*., 2007) and environmental contaminants (Smith *et al*., 1995). A decrease in total haemocyte count (THC) and differential haemocyte count (DHC) due to salinity (Wang and Chen, 2005b; Nisha, 2006) and environmental stress conditions such as hypoxia (Le Moullac *et al*., 1998) was reported in the shrimps *F. indicus*, *F. paulensis*, *L. vannamei* and *P. stylirostris*. Smith *et al*. (1995) noticed reduction in haemocyte number due to increase in the haemolymph volume. In *C. pagurus* diagnosed with shell disease syndrome, there was no correlation between THC and the degree of infection but the percentage of basophilic and eosinophilic granulocytes increased (Vogan and Rowley, 2002). The values of total and differential haemocyte counts may provide a useful tool for further immunological investigations (Yavuzcan-Yildiz and Atar, 2002).

The Ag of *T. schirnerae*, generally devoid of granules, was comparable to the hyaline cells reported for other crustaceans (Hose *et al*., 1990; Vázquez *et al.*, 1997; Vargas-Albores *et al.*, 2005). Granules were rare in the hyaline cells of *E. sinensis* (Bauchau and De Brouwer, 1972) and *Scylla olivacea* (Sa-nguanrut *et al*., 2010) and in hyalinocytes of *F. indicus* (Laxmilatha and Laxminarayana, 2004). However, in different species, these cells display variation in number and size of granules. For instance, numerous small granules were reported in the hyaline cells of *H. americanus* while only a few large granules were spotted in the hyaline cells of *Penaeus paulensis* (Gargioni and Barracco, 1998) and *F. chinensis* (Zhang *et al*., 2006). In *L. grandis*, the hyaline cells are rich in granules that they are easily confused with granulocytes while in *P. interruptus*, the granules in hyaline and large granule haemocytes are approximately of the same size. Söderhäll *et al.* (1986) described the hyaline cells as the main phagocytic haemocytes in *C. maenas* whereas in *Pascifastacus leniusculus* phagocytosis is performed by both hyaline and semigranular cells. Hose *et al.* (1990) reported that the hyaline cells in *L. grandis*, *P. interruptus* and *H. americanus* lyse and initiate coagulation. The involvement of hyaline cells in the clotting processes in *C. sapidus* was demonstrated by Clare and Lumb (1994).

The presence of pseudopodial projections was a prominent feature noticed in the Ag of *T. schirnerae*. In *C. maenas*, Johnston *et al.* (1973) reported pseudopodial projections pertinent to in vivo haemocyte mobility. According to Williams and Lutz (1975), pseudo-podial projections in haemocytes were not a permanent feature, but developed according to the in vitro status of the organism. Fine structural studies on haemocyte coagulation in *E. sinensis* (Bauchau and De Brouwer, 1974) revealed instantaneous pseudopodia formation during the primary stages of clotting, accompanied by noticeable cytoplasmic changes. However, TEM observations of hyaline cells lacked pseudopodia in the freshwater crayfish *Astacus astacus* (Stang-Voss, 1971), *C. sapidus* (Bodammer, 1978) and penaeid shrimps (Vargas-Albores *et al*., 2005). The pseudopodial projections of Ag in the present study may possibly suggest that these cells may have a role in coagulation and phagocytosis. However, Laxmilatha and Laxminarayana (2004) reported the presence of pseudopodia-like extensions as a feature of the dense granulocytes of *F. indicus*.

The unusual occurrence of binucleate Ag in *T. schirnerae* can be compared to the binucleate hyaline haemocytes of *C. sapidus* (Bodammer, 1978) and the dividing nucleus observed in the Ag of *P. indicus* (Laxmilatha, 1991). The binucleate condition observed here may be quite fortuitous and thorough investigations are required to explore their occurrence, frequency and physiological significance.

In *T. schirnerae*, the size, shape and density played a decisive role in classifying granules into six distinct types which is in accordance with the classification of Bodammer (1978) and Clare and Lumb (1994) in *C. sapidus*. Laxmilatha and Laxminarayana (2004) reported the presence of two granule types – very dense and less dense – in the granulocytes of *F. indicus.* In the same species, Nisha (2006) identified small basophilic and large eosinophilic granules. From the features of granule types in *T. schirnerae*, we assume that the granule types are transitional stages progressing towards greater density, eventually becoming homogenously dense type 1 granules. The participation of electron dense granules in the production and storage of copper containing pigment (haemocyanin) was demonstrated in many decapods (Stang-Voss, 1971;

Bauchau and De Brouwer, 1972; Bauchau *et al*., 1975). Granules are the sites of prophenoloxidase (proPO) system in shrimps (Vargas-Albores *et al*., 1993; Hernández-López *et al*., 1996) and freshwater crayfishes (Smith and Söderhäll, 1991; Lanz *et al*., 1993a). In the present study, electron dense small and large type 1 granules dominated the cytoplasm of GI and GII, respectively. Comparable granular features were reported in SGH and LGH of *H. americanus* (Hearing and Vernick, 1967), *A. astacus* (Stang-Voss, 1971), *E. sinensis* (Bauchau and De Brouwer, 1972), *C. maenas* (Johnston *et al*., 1973) and *C. sapidus* (Bodammer, 1978).

The abundance of SER, free ribosomes and mitochondria found in GI of the present study suggests their involvement in metabolic and synthetic activity, comparable to the SGH of *F. indicus* (Laxmilatha and Laxminarayana, 2004). Since SER and ribosomes are found in close proximity to granules in the present study, we assume a lipoproteinaceous nature for the granules. As reported for phagocytic and granular ameobocytes of the crayfish *A. astacus* (Stang-Voss, 1971), Golgi bodies were not distinct in any of the haemocyte types in *T. schirnerae*. The absence or poor development of Golgi elements is a unique feature evident only in freshwater decapods (Johnston *et al.*, 1973; Clare and Lumb, 1994). Golgi bodies were readily discerned in LGH but not as discrete organelles in the hyaline haemocytes of *C. sapidus* (Bodammer, 1978).

The phenomenon of cytoplasmic fragmentation, devoid of nucleus (clasmatosis) was observed in GII of *T. schirnerae*. Similar observations were made by Ravindranath (1977) in granular haemocytes of the mole crab *Emerita asiatica*. Clasmatosis was extensively reported in the granular haemocytes of insects (Arnold, 1966; Gupta and Sutherland, 1966). Since clasmatosis is not reported in haemocytes of other crustaceans, further investigations are required to ascertain the physiological reasons and significance of this phenomenon.

Functionally, haemocytes are destined to play specific roles in the immune mechanism, for which their configuration, composition and distribution is uniquely built. In blue crabs and other crustaceans, studies on the functional aspects reveal that hyaline cells exclusively participated in phagocytosis (Johnson, 1976; Smith and Ratcliffe, 1978; Söderhäll *et al*., 1986; Thornqvist *et al*., 1994; Johansson *et al*., 2000). As the cellular features of Ag observed in the present study bear a striking resemblance to the hyaline cells mentioned above, we assume they too perform a similar task in the immune process. In contrast, granule containing hyaline cells of *L. grandis*, *H. americanus*, *P. interruptus* and penaeid shrimps were involved in the process of coagulation (Hose *et al.*, 1990; Vargas-Albores *et al.*, 2005).

Granular haemocytes are predominantly agents of encapsulation, storage and release of proPO system and cytotoxicity with limited role in phagocytosis (Söderhäll and Smith, 1983; Johansson and Söderhäll, 1985; Söderhäll *et al.*, 1985; Perazzolo and Barracco, 1997; Sung *et al.*, 1998; Johansson *et al*., 2000; Vogan and Rowley, 2002). Studies of Söderhäll and Smith (1983) and Johansson and Söderhäll (1985) have indicated overlapping functions for SGH and LGH in the immune process. In *P. leniusculus*, Söderhäll *et al.* (1986) revealed that both hyaline and semigranular cells function as agents of phagocytosis. Since the granular features of GI and GII of the present study resemble those reported for other crustaceans (Hose *et al.*, 1990; Vázquez *et al.*, 1997; Hong da, 2002; Laxmilatha and Laxminarayana, 2004; Vargas-Albores *et al.*, 2005; Nisha, 2006; Sanguanrut *et al.*, 2010) it is possible that these cells also execute similar roles in *T. schirnerae*. Further cytochemical investigations are required to ascertain their definite roles in the immune processes of *T. schirnerae*.

Conclusion

This study provided information on fine structural aspects of haemocytes of the freshwater crab *T. schirnerae* which will support further investigations on physiological and functional aspects of haemocytes of freshwater crabs. Our observations also revealed the unusual occurrence of a binucleate agranulocyte and a rare phenomenon called clasmatosis of granulocyte II which needs further clarification.

Acknowledgements

The financial assistance provided by Kannur University to the first author is gratefully acknowledged.

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