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Haemocyte types and count in *Bradinopyga geminata* (Rambur) (Anisoptera: Odanata)

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Abstract
Seven haemocyte types were identified in the haemolymph of adult *Bradinopyga geminata* (Rambur) (Anisoptera: Odanata) *viz*., Prohaemocytes, plasmatocytes, granulocytes, oenocytoids, adipohaemocytes, spherulocytes and vermicytes. Prohaemocytes, plasmatocytes and granulocytes were the most numerous circulating haemocytes in the haemolymph of *Bradinopyga geminata*. Prohaemocytes were the smallest cell type, spherical in shape with a centrally located large nucleus. The plasmatocytes were polymorphic with or without cytoplasmic extensions. The granulocytes were spherical or oval in shape with characteristic basophilic granules in the cytoplasm. Oenocytoids were the largest of all the cell types with a large nucleus and a homogenous cytoplasm. Adipohaemocytes were polymorphic, varied from small to large cells mostly irregular in shape with prominent lipid-like inclusions. The spherulocytes were characterized by the presence of highly basophilic or acidophilic spherules. Vermicytes were fusiform with a centrally located nucleus. The total and differential haemocyte counts were also done.

Keywords: *bradinopyga geminata*, haemocytes

Introduction
Insect haemocytes are a mixture of cell types with varying functions. They are morphologically distinct cell types [1, 2], comparable to vertebrate leucocytes [3] constituting important and crucial components of the haemolymph in the open circulatory system of insects as well as in other arthropods and invertebrates [4]. The role of haemocytes is to defend the insects against pathogens and parasites that enter their haemocoel [5]. Functionally, haemocytes are the generally accepted cellular defense units in insects and are partially responsible for their immune responses [6, 8].
A primary defense response is encapsulation, a process in which haemocytes attach to the foreign organisms, immobilize by surrounding and killing them. They recognize a variety of foreign targets as well as alterations to self. These haemocytes possess the ability to discriminate stranger agents, mediate phagocytosis, cytotoxicity, encapsulation, wound repair and coagulation [4, 9, 10]. Classification of haemocytes is based on morphology or a combination of morphological and functional characters [11, 14], however, they were first discovered by Swammerdam [15]. Overall five to seven types of haemocytes have been frequently described in insects *viz*., prohaemocytes, plasmatocytes, granulocytes, spherulocytes, adipohaemocytes, oenocytoids and coagulocytes [4, 5, 16, 17].
The odonate *Bradinopyga geminata* (Rambur), commonly called Rock dweller, is black in colour with white patches, frequently seen on the dark surfaces, such as rocks, uncemented compound walls etc., to avoid the predators. Usually the female adults lay their eggs in the open water tanks on the top of houses. Moreover, the nymphs are highly voracious feeders of mosquito larva and can be used to control the mosquitoes. This study aims at characterizing the haemocyte types, as well as the total and differential haemocyte counts of these normal adult dragonflies since the success of the immune response depends on the number and types of haemocytes involved in these mechanisms [18].

Materials and methods
The adult *Bradinopyga geminata* perched on walls and water tanks were gently captured using an insect net from the Madras Christian College campus, Tambaram, Chennai, Tamil Nadu, India.

Haemolymph collection and preparation of slides
The dragonflies were delicately punctured with a needle in the abdominal region and the haemolymph was bled directly on a glass slide and allowed to air dry for 20-30 minutes. During this time, the haemocytes adhered to the glass slide. Cells were then fixed in methanol for five minutes. After air drying of the fixative, haemocytes were stained with Giemsa-Rosenfeld for 3-4 minutes and the slides were rapidly washed with double distilled water. After air drying, the glass slides were dehydrated and mounted in DPX [19].

Haematology
Haemolymph was obtained by delicately puncturing the abdomen with a micro needle. Haemocyte counts were performed on individual adults. Total Haemocyte Counts (THCs) were done by loading diluted haemolymph to a Neubauer haemocytometer [20]. The haemolymph (5µL) was mixed thoroughly with 5µL Versene saline (NaCl 0.9g, KCl 0.942g, $CaCl_2$ 0.82g, $NaHCO_3$ 0.002g and EDTA 2g in

100mL of distilled water). The total haemocyte count was calculated using the formula:

$$\frac{\text{Haemocytes in 51mm squares x dilution x depth of the chamber}}{\text{No. of 1mm squares counted (16)}}$$

THCs were expressed as number of cells for each µL of haemolymph. Differential Haemocyte Count (DHC) was performed on slides stained with Giemsa-Rosenfeld. One hundred and fifty cells identified from four randomly selected fields were counted per adult under the light microscope. Photomicrographs of the stained cells were taken using a Nikon camera. DHC were expressed as the mean of each haemocytes type in the total cells counted.

Results

The haemolymph of *Bradinopyga geminata* constituted 1709 cells per µL based on the haemocyte count using Neubauer haemocytometer. Seven haemocyte types were distinctly identified in the slides stained with Giemsa-Rosenfeld in the haemolymph of adult *Bradinopyga geminata viz.*, prohaemocytes, plasmatocytes, granulocytes, oenocytoids, adipohaemocytes, spherulocytes and vermicytes (Figure 1). Figure 2 shows the per cent granulocytes maximum in the circulating haemolymph of adult *Bradinopyga geminata* followed by plasmatocytes, prohaemocytes, spherulocytes, adipohaemocytes, oenocytoid and vermicytes. Prohaemocytes (PR) are the smallest haemocytes, spherical or oval in shape with a centrally located large nucleus surrounded by a narrow band of cytoplasm (Figure 1A). These haemocytes represented up to 17.35% of the circulating haemocytes. The plasmatocytes (PL) showed a high degree of polymorphism; they were oval, spherical or spindle shaped with or without cytoplasmic extensions. The nuclei was either spherical or oval according to the spreading ability of the cell (Figure 1 D). These cells were present frequently and represented 23.54% of the circulating haemocytes. Granulocytes (GR) were the most frequently observed cell type (35.85%), spherical or oval in shape and were variable in size. A number of small basophilic granules were identified in the cytoplasm after staining with Giemsa. The basophilic nucleus were round and generally occupied the central position in the granulocytes (Figure 1C). Oenocytoids (OE) were the largest of all the cell types. Mostly spherical and sometimes oval in shape, with a large nucleus and a homogenous cytoplasm containing fine acidophilic granulation (Figure 1B). These cells were rare and represented only 2.89% of the total circulating haemocytes. Adipohaemocytes (AD) while exhibiting polymorphism varied from large to small cells and had an oval or irregular shape. The cytoplasm was basophilic after Giemsa staining, and showed on the cell surface a variable number of prominent, large lipid like inclusions, sometimes obscuring the nucleus. These cells constituted 5.71% of the total haemocyte count. Spherulocytes (SP) while being present in moderate numbers in the haemolymph (12.8%) were spherical or oval in shape and generally larger than PL and GR. The SP were characterized by the presence of highly basophilic or acidophilic spherules and small spherical vacuoles distributed in the cytoplasm (Figure 1E). Vermicytes (VE) were the least frequently present haemocyte type in the circulating haemolymph (1.86%). They were fusiform in shape with tapering extremities and a centrally located elongated nucleus (Figure 1F).

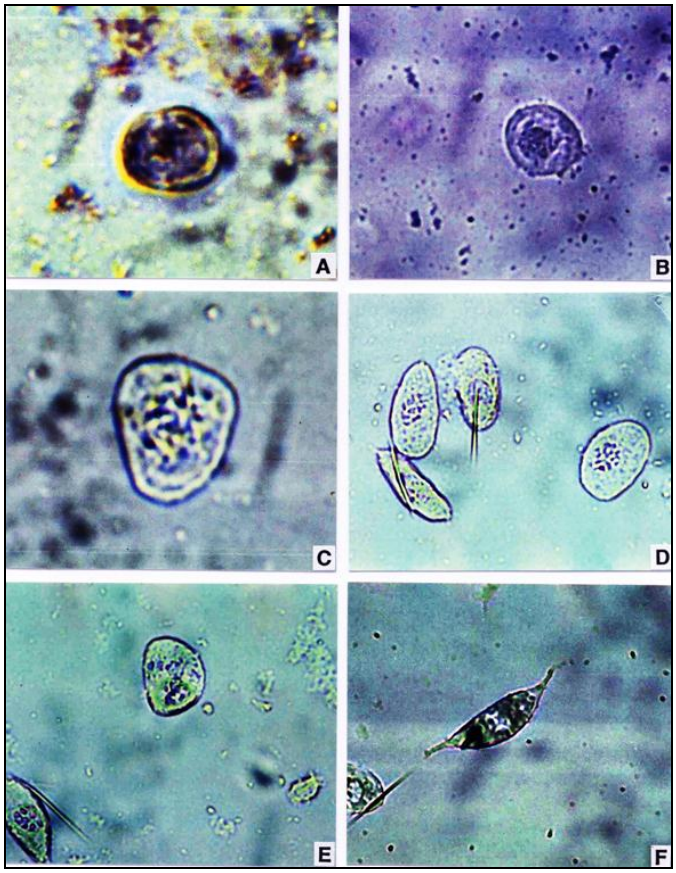

Fig 1: A. Prohaemocyte (X640) B. Oenocytoid (X270) C. Granulocyte (X410) D. Plasmatocytes (X270) E. Spherulocyte (X270) F. Vermicyte (X270)

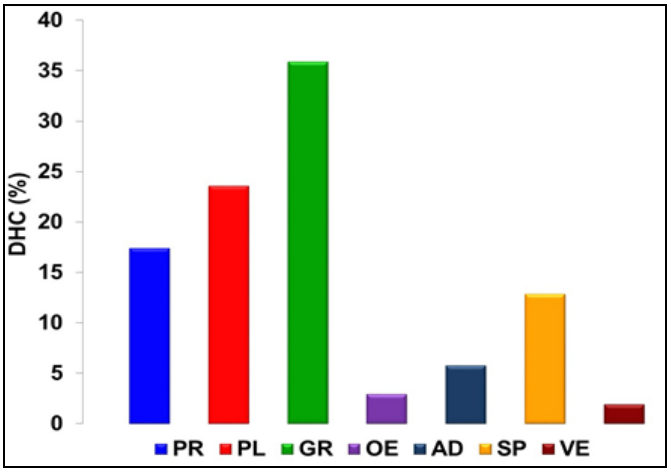

Fig 2: DHC (%) in adult *Bradynopyga geminata*

Discussion

The prohaemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids of *Bradinopyga geminata* presented morphological features similar to those of other insect species [5, 11, 19, 21, 23]. Gupta [24] has described seven basic types of haemocytes including coagulocytes which are

fragile cells that can be observed "*in vitro*" only [25]. However, in this study, this cell type was not evident. The prohaemocytes were distinguished by their small size and large nucleo-cytoplasmic ratio. Prohaemocytes were comparable to those of other insect species described by several authors [5, 26, 28]. The plasmatocytes were observed to be polymorphic cells. They were spherical to oval and putforth protoplasmic extensions. Whitten [29], Pal and Kumar [17] have reported similar cell type in dipterans. The granular haemocyte of *Bradinopyga geminata* is similar to the granulocyte of other dipterans *Sarcophaga ruficornis*, *Musca domestica* and *Chrysomya meghacephala* [17, 19]. Oenocytoids are equal in size to the granulocytes or even larger. They are similar to the other insect species but may be absent in some insects [30]. Falleiros *et al*. [9] has not considered adipohaemocyte as cellular type since, ultrastructurally they showed a similar morphology to that of granulocyte except in the presence of large amounts of lipid droplets in the cytoplasm. Lea and Gilbert [31] considered adipohaemocytes to be functional variants of other cellular types with lipid droplets as observed in *Musca domestica* [17]. Spherulocytes are also not present in all insect species [30]. They may vary in size among other dipterans like *Anastrepha obliqua* [19]. Several authors have questioned the classification of the vermicyte as a distinct cell type as they consider it to be a functional variant of plasmatocyte [32, 33] which is distinctly reported in *Sarcophaga ruficornis* [17]. The present study contributes to the characterization of the haemocytes of *Bradinopyga geminata*. Further studies are required to understand the cell types in the larval stages of this species as well as their functional efficacy since they live in an aquatic environment, encountering pathogens and pollution.

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