MORPHOLOGY OF SALIVARY SYSTEM

I. 1. INTRODUCTION

Reduviids are the abundant predatory fauna of agroecosystems, semi-arid zones, scrub jungles and tropical rainforest ecosystems (Ambrose, 1999). Their success in every ecosystem/trophic niche is due to their morphological and physiological adaptations in predation. Their potent salivary system is one such physiological adaptation. The paralytic action of salivary toxins of reduviids was well documented (Edwards, 1961; Haridass and Ananthakrishnan, 1981a; McMahan, 1983; Morrison, 1989; Ambrose, 1999; Ambrose and Maran, 1999 a; Maran and Ambrose, 1999). The salivary system of reduviids conforms to the general heteropteran plan of principal and accessory glands, which are divisible into secretory and conducting parts (Baptist, 1941; Barth, 1954; Southwood, 1955; Louis and Kumar, 1973). The morphology of salivary glands is diverse in different subfamilies, which could be utilized as a reliable taxonomical tool (Louis and Kumar, 1973). The principal gland is unilobed or bilobed or multilobed, whereas accessory gland is unilobed and vesicular, exhibiting distinct functional and histological differences (Haridass and Ananthakrishnan, 1981a). The principal gland is divided into anterior lobes and posterior lobes, suggesting the differential functions of the lobes involving division of labour (Haridass and Ananthakrishnan, 1981a), with histological variations. The anterior lobes of
principal glands secrete zootoxic enzymes which are used to paralyse the prey, whereas the posterior lobe secretes digestive enzymes. The accessory gland is typically vesicular (Baptist, 1941; Southwood, 1955; Edwards, 1961), and differ histologically from the lobes of principal glands and secretes watery saliva (Miles and Slowiak, 1976; Haridass and Ananthakrishnan, 1981a; Morrison, 1989), which is used in the lacerate flush mode of feeding in reduviids (Miles, 1972). Miles (1967, 1968, 1972) and Hori (1969) demonstrated that the different lobes of salivary glands have differential secretory activities, but Baptist (1941) disagreed with this view.

Baptist (1941) and Barth (1954) reviewed the structure of salivary glands of blood sucking Triatominae. But, information on the functional morphology of salivary glands of entomosuccivorous reduviids is scanty, except the work of Haridass and Ananthakrishnan (1981a) in Haematorrhophus nigrovioleaceus (Reuter) (Ectrichodiinae), Lestonemus affinis (Serville) (Peiratinae) and Triatoma rubrofasciata De Geer (Triatominae), Morrison (1989) in Acanthaspis pedestris (Stål) (Reduviinae), Santha (1986) in Catamius brevipes (Serville) (Peiratinae), Sivaraj (1986) in A. pedestris, Udayakumar (1986) in Ectomorcos tibialis (Distant) (Peiratinae) and Vellingirinathan (1986) in Lophocepha guerini (Laprote) (Harpactorinae). But detailed and systematic work on the salivary glands of reduviid predators is scanty. Hence the author studied the functional morphology as well as histomorphology of salivary glands of three harpactorine predators viz., Rhynocoris fuscipes (Fabricius), Rhynocoris kumarii Ambrose and Livingstone and Rhynocoris marginatus (Fabricius). The author selected these three harpactorines, because they are excellent biocontrol agents with good searching ability, a high degree of host
Plate 1.  
1. *R. fuscipes* predating upon *Pelopidas mathias* (F)  
2. *R. kumarii* predating upon *S. litura*  
3. *R. kumarii* predating upon *M. pustulata*  
4. *R. marginatus* predating upon *S. litura*  
5. *R. marginatus* predating upon *D. cingulatus*  
6. Polymorphic forms of *R. marginatus* predating upon *M. pustulata*
specificity and higher reproductive capacity and are amenable to mass culture in laboratory and they kill more prey than they need to satiate themselves (Balduf, 1950; Evans, 1962; Schaefer, 1988; Ambrose, 1999). Such information of these potential biological control agents will be very useful to understand their interaction with their pest prey spectra in nature as well as in the introduced or released situations.

I. 2. MATERIALS AND METHODS

I. 2.1. COLLECTION AND MAINTENANCE

The adults and nymphal stages of *R. fuscipes* were collected from blackgram (*Phaseolus mungo*) fields of Melapalayam (77° 44' 55" E and 8° 43' 14" N) and Thachanallur (77° 43' 68" E and 8° 47' 76" N) agroecosystem; *R. kumarii* were collected from Muppandal (77° 35' E and 8° 14' N) and Marunthuvazhmalai scrubjungles (77° 50' E and 8° 7' N) and of *R. marginatus* were collected from Sivanthipatti (77° 47' E and 8° 30' N) and Melapattam scrubjungles (77° 44' E and 8° 30' N). They were reared in plastic containers (220 ml) under optimal laboratory conditions (Temperature : 30 – 35°C; Relative humidity : 75% - 85%; Photoperiod 11 – 13 hrs). They were fed ad libitum on head crushed larvae of *Corcyra cephalonica* Stainton.

I. 2.2. DISSECTION

To isolate the salivary glands, the tergal plates of anaesthetised predators were carefully removed by making a circular lateral incision around the abdomen with a single edge razor blade in saline solution (NaCl – 6.5 gms; Kcl – 0.25 gms;
CaCl$_2$ 0.25 gms; Na$_2$CO$_3$ - 0.25 gms; distilled water 1000 ml). The gut, reproductive, nervous and trachel systems along with any adhering tissues were carefully removed to fully expose the glands and ducts. The main salivary duct was next detached from the sclerotised mouth parts closer to the salivarium. The gland removed from the predator was rinsed and placed in saline, weighed in a precision balance (Pricisa 125 A; Switzerland) and fixed in Bouin's fluid. The morphometry of salivary glands was carried out under microscope with ocular and stage micrometers.

I. 2. 3. HISTOLOGICAL PROCEDURE

The Bouin's fixed salivary glands were washed in distilled water, dehydrated in graded alcohol, cleared in xylene, impregnated and embedded in paraffin wax (melting point 58 to 60°C). Sections were cut at 6 to 8 µ, stained with haemotoxylin - eosin (Davanport, 1960). The stained slides were cleared in graded alcohol followed in xylene and mounted with DPX. The cleared slides were observed under the high power of the microscope (60 X and 100 X) and the cells were measured with ocular and stage micrometers.

I. 3. RESULTS

I. 3. 1. GROSS MORPHOLOGY

The salivary gland comprises of a pair of principal glands and accessory glands in R. fuscipes, R. kumarii and R. marginatus (Fig 1 a, b and c).

The principal salivary glands (PSG) are located on either side of the anterior midgut and extended into the abdominal cavity. The PSG is elongately bilobed with an anterior lobe (AL) and a posterior lobe (PL).
Figure 1. Salivary gland complex of a) R. fuscipes b) R. kumarii and c) R. marginatus
The AL is elliptical with acutely tapering cephalic end and continues as the fine suspensory ligament (SL) into the head capsule running dorsal to the oesophagus and beyond which it could not be traced. The transparent caudal end of the anterior lobe is swollen. The junction of anterior and posterior lobes is distinctly constricted, called the hilus (H).

The PLs of R. fuscipes, R. kumarii and R. marginatus are elongately slender, but their shape is not consistent. They are swollen anteriorly at the hilus. They are slightly sinuous throughout its length. Often they are found highly enlarged at certain regions indicating secretory activity. Such enlargements are transparent, while the other areas remain opaque. But they slightly tapered and curved at an angle and fastened with the middle of the crop and the posterior mid gut by means of tracheal branches. A nerve that runs along the length of the oesophagus forms a mesh work at the hilus.

The main duct of the principal salivary gland runs forward, usually following the contour of the alimentary canal and from both sides enter the neck and a little distance beyond the 'U' turn of the accessory salivary gland duct, unite to form a common duct that ultimately enters the lumen of the salivary pump.

The AL of the principal salivary gland secretes zootoxic enzymes, while the PL secretes digestive enzymes.

The accessory salivary system (ASS) of R. fuscipes, R. kumarii and R. marginatus are similar. The ASS has the vesicular part that remains intimately
attached to the anterior half of the crop. Midway it sends the accessory gland duct, that runs anteriorly, parallel to the posterior lobe of the principal salivary gland. It then continues to run parallel to the afferent salivary duct, enters the head capsule and loops around the hypopharyngeotentorial complex near the gena. It then turns back, becomes narrower, runs parallel to the oesophagus as descending limb and opens apparently independent of the afferent duct into the hilus. Thus the duct up to the loop is ascending and slightly broader than the descending duct. The ascending and descending ducts are demarcated by a narrow constriction at the hypopharyngeotentorial complex.

The vesicular part of the accessory salivary gland is distinctly glandular and possesses a moniliform tubular secretory appendix and lies closely to the posterior region of the crop and the posterior midgut. In freshly dissected salivary glands, when the inseparable vesicle is released from the crop, it seems to be formed of an anterior narrow limb that is almost intimately connected to the junction of oesophagus and the crop and a comparatively broader and more saccular posterior limb which is lying closely opposed to the crop.

Accessory salivary glands are filled with watery fluid, which recirculates water from the gut to ensure a copious flow of watery saliva and helps the predator to flush out the predigested food from the body of the prey.

Observations showed interesting variations in the state of salivary gland activity at different stages of predation. In starved predators, the lobes of the
principal and accessory glands were completely filled with secretions. When these predators attacked a prey, the anterior lobes were flaccid. The posterior and accessory gland lobes were filled with their respective secretions. But when these predators completed their feeding events, the anterior lobe, the posterior lobe and the accessory glands seemed empty, suggesting the secretions were spent during feeding.

The ducts of both principal and accessory glands are having cuticular linings resembles the spiral thickening in tracheal tubes.

I. 3. 2. Morphometry

The morphometry of salivary glands of R. fuscipes, R. kumarii and R. marginatus is presented in Table 1.

I. 3. 2. 1. R. fuscipes

The principal salivary gland of R. fuscipes weighed 1.335 ± 0.115 mg. The length and width of its anterior lobe were 1.162 ± 0.041 and 0.993 ± 0.045 mm, respectively. The length of the posterior lobe was 2.669 ± 0.113 mm, whereas its width was 0.968 ± 0.023 mm. The accessory gland measured 1.151 ± 0.044 mm long, 1.101 ± 0.038 mm, broad.

I. 3. 2. 2. R. kumarii

The principal salivary gland of R. kumarii weighed 3.535 ± 0.164 mg. The length and width of its anterior lobe were 3.049 ± 0.105 and 1.275 ± 0.062 mm, respectively. The length of the posterior lobe was 5.231 ± 0.156 mm, whereas its width was 1.114 ± 0.041 mm. The accessory gland measured 1.358 ± 0.074 mm long and 1.771 ± 0.034 mm broad.
Table 1: Morphometry of salivary glands of *R. fuscipes*, *R. kumarii* and *R. marginatus* (n = 20; Mean ± SE)

<table>
<thead>
<tr>
<th>SALIVARY GLAND</th>
<th>MORPHOMETRY</th>
<th><em>R. fuscipes</em></th>
<th><em>R. kumarii</em></th>
<th><em>R. marginatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal</td>
<td>Weight (mg)</td>
<td>1.335 ± 0.115</td>
<td>3.535 ± 0.164</td>
<td>2.535 ± 0.239</td>
</tr>
<tr>
<td></td>
<td>Anterior lobe length (mm)</td>
<td>1.162 ± 0.041</td>
<td>3.049 ± 0.105</td>
<td>2.829 ± 0.072</td>
</tr>
<tr>
<td></td>
<td>Anterior lobe width (mm)</td>
<td>0.993 ± 0.045</td>
<td>1.275 ± 0.062</td>
<td>0.957 ± 0.0006</td>
</tr>
<tr>
<td></td>
<td>Posterior lobe length (mm)</td>
<td>2.569 ± 0.133</td>
<td>5.231 ± 0.156</td>
<td>4.987 ± 0.022</td>
</tr>
<tr>
<td></td>
<td>Posterior lobe width (mm)</td>
<td>0.968 ± 0.023</td>
<td>1.114 ± 0.041</td>
<td>1.020 ± 0.010</td>
</tr>
<tr>
<td>Accessory</td>
<td>Length (mm)</td>
<td>1.151 ± 0.044</td>
<td>1.358 ± 0.074</td>
<td>1.980 ± 0.027</td>
</tr>
<tr>
<td></td>
<td>Width (mm)</td>
<td>1.101 ± 0.038</td>
<td>1.771 ± 0.034</td>
<td>1.816 ± 0.022</td>
</tr>
</tbody>
</table>
I. 3. 2. 2. *R. marginatus*

The principal salivary gland of *R. marginatus* weighed $2.535 \pm 0.239$ mg. The length and width of its anterior lobe were $2.829 \pm 0.072$ and $0.957 \pm 0.0006$ mm, respectively. The length of the posterior lobe was $4.987 \pm 0.022$ mm, whereas its width was $1.020 \pm 0.010$ mm. The accessory gland measured $1.980 \pm 0.027$ mm long and $1.816 \pm 0.022$ mm broad.

I. 3. 3. **Histomorphology**

The principal salivary glands of *R. fuscipes*, *R. kumarii* and *R. marginatus* have single layer of binucleate cells enclosing a spacious cavity for storing their secretions. The cells of anterior lobes of these three predators are smaller and flattened with less viscous cytoplasm having numerous secretory granules. Their nuclei are flattened and elongated. The cells in the anterior lobes of *R. fuscipes*, *R. kumarii* and *R. marginatus* are $17.928 \pm 1.050$, $21.40 \pm 0.669$ and $14.742 \pm 0.619$ μm wide with nuclei of $8.120 \pm 0.326$, $9.784 \pm 0.196$ and $8.501 \pm 0.296$ μm wide, respectively. In contrast, the cells of the posterior lobes are larger with highly viscous cytoplasm with numerous granules and vacuoles. Each cell has two spherical nuclei with many chromatin granules. These cells are $28.578 \pm 0.678$, $37.342 \pm 1.750$ and $31.464 \pm 0.940$ μm wide with nuclei of $15.721 \pm 0.461$, $13.679 \pm 0.689$ and $17.242 \pm 0.741$ μm wide in *R. fuscipes*, *R. kumarii* and *R. marginatus*, respectively (Table 2). The wall of accessory salivary gland is made up of extremely flattened syncytial epithelial cells enclosing a wide lumen. The cytoplasm is devoid of secretory granules and vacuoles. The cells in the hilar valve are columnar, uninucleate and without any granules. The ducts of main and accessory glands are made up of a single layer of cuboidal cells (Plate 2).
Table 2: Morphometry of salivary gland cells in *R. fusipes*, *R. kumarii* and *R. marginatus* (n = 25; Mean ± SE).

<table>
<thead>
<tr>
<th>REDUVIIDS</th>
<th>ANTERIOR LOBE CELLS</th>
<th></th>
<th></th>
<th>POSTERIOR LOBE CELLS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ENTIRE (μ)</td>
<td>NUCLEUS (μ)</td>
<td>ENTIRE (μ)</td>
<td>NUCLEUS (μ)</td>
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<td></td>
<td>RANGE MEAN</td>
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<td>RANGE MEAN</td>
<td>RANGE MEAN</td>
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<tr>
<td><em>R. fusipes</em></td>
<td>9.00 – 26.85</td>
<td>17.928 ± 1.050</td>
<td>4.75 – 9.50</td>
<td>8.120 ± 0.326</td>
</tr>
<tr>
<td><em>R. kumarii</em></td>
<td>15.75 – 29.25</td>
<td>21.40 ± 0.669</td>
<td>9.50 – 11.87</td>
<td>9.784 ± 0.196</td>
</tr>
<tr>
<td><em>R. marginatus</em></td>
<td>9.00 – 20.25</td>
<td>14.742 ± 0.619</td>
<td>4.75 – 9.50</td>
<td>8.501 ± 0.296</td>
</tr>
</tbody>
</table>
A, B - Anterior lobe cells of *R. fuscipes*.
C, D - Anterior lobe cells of *R. kumarrii*.
E, F - Anterior lobe cells of *R. marginatus*.
A, B - Posterior lobe cells of *R. fuscipes*.  C, D - Posterior lobe cells of *R. kumarii*.

E, F - Posterior lobe cells of *R. marginatus*.

G, H and I - Accessory gland cells of *R. fuscipes*, *R. kumarii* and *R. marginatus*. 

NU - Nucleus
The salivary system was innervated by a complex nervous system from the suboesophageal ganglion and stomatogastric system.

I. 4. DISCUSSION

The morphology of salivary glands of *R. fuscipes*, *R. kumarii* and *R. marginatus* conforms to the general heteropteran plan. (Baptist, 1941; Barth, 1954; Southwood, 1955; Louis and Kumar, 1973; Haridass and Ananthakrishnan, 1981a and Morrison, 1989). The structure and number of lobes of the principal gland are found to be specific to different subfamilies. The principal glands of these three harpactorine predators *R. fuscipes*, *R. kumarii* and *R. marginatus* have an anterior and a posterior lobes as observed by Haridass and Ananthakrishnan (1981a) in *Sycanus collaris* Fabricius and *Sphedenolestes bowringi* Distant. Similar numbers of anterior and posterior lobes were also observed for the members of subfamilies Peiratinae, Reduviinae, Salyavatinae and Rhaphidosomatinae (Harpactorinae) (Haridass and Ananthakrishnan, 1981a). But in the members of Ectrichodiinae three lobes i.e., one anterior lobe, one posterior dorsal and one posterior ventral lobe were observed (Haridass and Ananthakrishnan, 1981a). The unilobed salivary system is found in Triatominae. Louis and Kumar (1973) suggested the trilobed condition of the salivary system as primitive and its reduction to bilobed condition as observed in the three harpactorines; an advanced character and unilobed as most advanced. Accordingly, Ectrichodiines is considered as the most primitive and Triatominae as the most advanced. Haridass and Ananthakrishnan (1981a) also reported a similar view and correlated the salivary system architecture to pyloric and rectal glands in reduviids.

In *R. fuscipes*, *R. kumarii* and *R. marginatus*, the anterior lobe secretes zootoxic enzymes which the predators use to immobilize their prey, and the posterior lobe secretes digestive enzymes (discussed in preceding chapters), as observed for
L. affinis and H. nigrovioleaceous (Haridass and Ananthakrishnan, 1981a) and A. pedestris (Morrison, 1989; Ambrose and Maran, 1999). This is contrary to the results obtained by Edwards (1961) in *Platymeris rhadhamanthus* Gearstacker. He found the presence of zootoxic enzymes both in the anterior and posterior lobes besides digestive enzymes secreted by the posterior lobe. The secretion in the anterior lobe is lesser in quantity, viscous and transparent, whereas the posterior lobe secretes larger quantity of highly viscous and milky white secretions as reported by Haridass and Ananthakrishnan (1981a).

The accessory salivary glands of *R. fuscipes*, *R. kumarii* and *R. marginatus* are typically of vesicular type as observed for other heteropterans by Baptist (1941), Southwood (1955) and Edwards (1960). They are also vesicular in other harpactorines, reduviines and salyavatines (Haridass and Ananthakrishnan, 1981a; Vellingirinathan, 1986; Agnes, 1990); elongated vesicle with triradiate tubular branches in ectrichodiines, elongated vesicle in peiratines and saccular vesicle in triatomines (Haridass and Ananthakrishnan, 1981a; Santha, 1986). Accessory glands are filled with watery fluid which helps the predator to flush out the predigested food from the body of the prey, very similar to the lacerate – flush mode of feeding of Pentatomomorpha in which the watery saliva is useful in flushing out the food from its source (Miles, 1972; Miles and Slowiak, 1976).

A common valvular system exists for both principal and accessory glands. For instance, in *T. rubrofasciata*, the accessory gland's secretion flows into the main salivary duct separately and independently of those coming from the main gland.
The hilus provides a regulatory system for sending out secretions from different lobes of the salivary system. In *L. affinis*, and *H. nigrovioleaceous*, the valves in the hilus make it possible not only to send the secretions independently from the accessory glands, but also to send separately the secretions issued from the anterior and posterior lobes of the principal gland (Haridass and Ananthakrishnan, 1981 a). Such an independent flow for the anterior and posterior lobes of the principal gland is also observed for *R. fuscipes*, *R. kumarii* and *R. marginatus*.

The present observation on the functional morphology and histology of salivary gland of *R. fuscipes*, *R. kumarii* and *R. marginatus* corroborates the observations of Haridass and Ananthakrishnan (1981a) in that both the anterior and posterior lobes are possessing binucleate cells. But Morrison (1989) observed uninucleate cells in anterior lobe and binucleate cells with highly viscous cytoplasm in posterior lobes of *A. pedestris*. Such variations are found among members of different subfamilies of Reduviidae.

A complex nervous supply from the suboesophageal ganglion and stomatogastric system to the salivary system reported in the present observation corroborates the observations of Baptist (1941) and Miles and Slowiak (1976). A double nerve supply separately to the anterior and posterior lobes facilitates independent discharge of saliva (Miles, 1972). The histochemical analysis by Agnes (1990) suggests excretory function to the salivary system in addition to the salivary secretory function (Schuh and Slater, 1995).