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Gross Morphology of Feeding Canal, Salivary Apparatus and Digestive Enzymes of Salivary Gland of *Catamirus brevipennis* (Servile) (Hemiptera: Reduviidae)

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ABSTRACT

Gross morphology of feeding canal, and salivary apparatus, protein content, enzyme profiles of *Catamirus brevipennis* Servile (Hemiptera: Reduviidae) salivary gland and alimentary canal were investigated. *C. brevipennis* has two each maxillary stylets inside and mandibular stylets outside. Maxillary stylets bear barbs where as mandibular stylets composed of filtering structures. Salivary gland is organized into larger multilobed principle gland (anterior and posterior lobes) and a shorter vesicular accessory gland. Posterior lobe of principle gland further constructed in to multilobes. At junction of anterior and posterior principle lobes of the principle gland shows a well-developed hilus. Short foregut (2.26±0.05 mm) was followed by very long midgut (6.02±0.06 mm) and hindgut (7.38±0.05 mm). Digestive enzymes like amylase, lipase and protease were found in both salivary gland and alimentary canal. Significantly low level of amylase and acid phosphatase and high level of protease was observed in the principle gland. Lipase activity was almost similar in both principle and accessory gland. Fore, mid and hindgut showed lipase and amylase activities. However, midgut and hindgut showed higher level of protease and acid phosphatase activity. This predatory bug is equipped purely for zoophagy by its functional morphological features and physiological nature.

Keywords: Salivary gland, alimentary canal, morphology, protein, enzymes, *Catamirus brevipennis*.

INTRODUCTION

Reduviids are the largest terrestrial predatory insects, mostly feeding on insects. They are considered as effective biological control agents against several agricultural pests (Ambrose, 1999; Sahayaraj *et al,* 2007). Proper assessment of the role of Reduviid predators in regulation of insect pests in diverse crop system was also proposed earlier (Whitcomb, 1981). *Catamirus brevipennis* (Servile) is one of the largest predators of the family Reduviidae present in scrub jungles, semiarid zones, tropical rain forest and agroecosystems of south India (Sahayaraj, 2007). It feeds on wide range of insect pests including *Helicoverpa armigera* (Hubner) (Bhatnagar *et al,* 1983), *Mylabris pustulata* (Faust) (Ambrose, 1999), *Spodoptera litura* (Fab.) (Sahayaraj, 2000). A consumer's ability to use plant or animal materials for food is indicated by the presence of specific digestive enzymes and by food and feeding canal

functional morphology (Hori, 2000; Zeng and Cohen, 2000b; Boyd, 2003). Morphology of Miridae stylet (Boyd, 2003), Nabidae, Anthocoridae, Tincidae (Cobben, 1978), Pentatomidae (Cohen, 1996) and Hygaeidae (Cohen, 1990) stylet were available in the literature.

For the better understanding of the prey-predator relation and use a predator in the pest management programme, it is necessary to understand the enzyme profiles of both salivary gland and alimentary tract. The enzymes present in the salivary gland helpful in extra oral digestion of the prey which mostly facilitated by amylase and protease (Chapman, 2000; Corzo *et al.,* 2001). The digestive enzymes of heteroptera include proteinase, lipase, phospholipase A1, amylase, pectinase, invertase, hyaluronidase and nucleases (Miles, 1972; Cohen, 1998a; Sahayaraj, 2007), α – amylase, α and β glucosidase (Mehrabadi *et al*., 2009). Role of various enzymes like peptidases and phospholipases (Cohen, 1990; Cohen and Hendrix, 1992), amylases (Boyd *et al*., 2002), pectinase (Hori, 2000) of watery saliva of Hemipteran predators were discussed earlier.

The zootoxic substances are found both in the anterior lobe and posterior lobe of the salivary gland (Edwards, 1961). During feeding, Reduviids not only consuming the haemolymph but also the interior contents including the organs and their networking macro and micro molecular complex including proteoglycans, collagens and elastins (Agusti and Cohen, 2000a). *C. brevipennis* venom also showed antimicrobial activity on selected human pathogens (Sahayaraj *et al,* 2006). The salivary gland complex contributes in two ways to viscosity reduction of prey fluids by producing enzymes that reduce the size of macromolecules (Cohen, 1995), and by secreting copious diluted saliva (Miles, 1972). Thus, the saliva's role in reducing the prey viscosity is by offering watery saliva to hydrolyze macromolecules (Cohen, 1998b; Torres and Boyd, 2009).

Predatory hemipterans do not confine their ingestion to the body fluids of their prey. They use a solid-to-liquid feeding method and attack the nutrient-rich organs and tissues of their prey (Cohen, 1995; 1998a; Swart and Felgenhauer, 2003). Though enormous information's available about the heteropteran maxillary stylet, alimentary canal and salivary gland morphology and enzyme profiles, very little information was available for Reduviid predators. The objective of this study was to observe gross morphology of stylet and salivary apparatus, total protein, qualitative and quantitative enzyme profiles of *C. brevipennis* salivary apparatus and alimentary canal.

MATERIALS AND METHODS

Predator collection and rearing

Fifty animals of different nymphal stages of *C. brevipennis* were collected from in and around Sivanthipatti semi-arid zone and agroecosystems, Tirunelveli, Tamil Nadu, India and were reared in plastic containers (6 x 15 cm) with *Corcyra cephalonica* (Stainton) fifth instar larvae under the laboratory conditions (Relative humidity 75 ± 10%, temperature 27 ± 2 °C and photoperiod of 13L : 11D). Laboratory emerged adult *Catamiarus brevipennis* male and female were randomly selected from the culture

and fed them with *C. cephalonica* continually for two to three weeks and then allowed to starve for 24 hrs prior to dissection, this helps for the accumulation of enzymes in the salivary gland as suggested by Boyd (2003).

Gross morphology

Twenty heads, along with mouthparts of *C. brevipennis* were placed in 95% ethanol for 24 hrs and then air dried for analyzing head size except stylet size and structure, where ten insect heads were placed, labium up on wax tray. Stylets were removed from the labium within insect pin. Stylet bundles were separated into their mandible and maxillary stylets. Place a drop of xylene and make temporary mounting. Measure their size and drew functional morphology of the stylet using camera lucida. The entire salivary apparatus was dissected out and placed in a petridish base was pasted with a graph sheet and the length was measured using the light microscope (5X x 10X) (Model number, AE – 11, Amba Optik, India). Similarly functional morphology of salivary apparatus was also carried out and expressed in mm. Using ocular (5X) and stage micrometer (10X), total length of rostrum, various parts of stylets, salivary apparatus and alimentary canal were measured. Photographs of stylets were taken using Olympus CX 41 attached with Olympus E-420.

Preparation of enzyme source

Only adult male and female were used in these tests. The Reduviids were starved for 24 hrs before dissection to standardize the insects and to allow an accumulation of enzymes (Boyd, 2003). 10 – 15 predators from each sex was placed at -2° C for 5 minutes and dissected in ice - cold Ringer's solution (137 mM NaCl, 8.51 mM KCl, 3.87 mM $CaCl_2$ and 1 mM $MgCl_2$, pH 7.0) (Li *et al*., 2009) under a dissection microscope (10x) (MV tex DM – 1, India). The entire salivary gland complex was dissected out, separated into principle (PG) and accessory gland (AG) weighed them separately. Then placed in one ml of phosphate buffer (0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, pH 7.2) (Yu *et al*., 2007) for all assays. Similarly, alimentary canal was dissected out and separated in to foregut (FG), midgut (MG) and hindgut (HG). The tissues were homogenized with 500µl of 0.2 M phosphate buffer (pH 7.2) with a tissue homogenizer (Remi 8000 RPM, Mumbai) and centrifuged at 5000 rpm for 15 mins at 4° C. The supernatant was used as the enzyme source as proposed by Boyd (2003) and Sahayaraj *et al.* (2007).

Total Protein Estimation

Total protein content of various parts of both salivary gland and alimentary canal were estimated (Lowry *et al.,* 1951) using the bovine serum albumin (BSA) as a standard.

Enzyme profile

In quantitative enzyme profile studies, amylase, protease, acid phosphatase, lipase and lactate dehydrogenase were estimated in different parts of alimentary canal and salivary gland.

Amylase

The amylase activity was observed following Ishaaya and Swiriski (1970) methods. Extract consists of 250 µl was added to the substrate starch soluble (0.2%) in phosphate buffer (pH-7.2) was taken in a test tube and incubated at 37 °C for 10 mins. After the incubation, 400 µl of dinitrosalicyclic (DNS) acid was added again incubate at 100 °C for 10 mins. The colour development was read in spectrophotometer at 575 nm. The amylase quantity was expressed in terms of the weight of the reducing sugar produced by the enzyme action per unit weight, per unit time, using maltose as standard.

General Protease assay

Protease activity was estimated by using 1 ml of 1% casein and 0.5 ml of the enzyme solution, were incubated at 37 °C in water bath for 30 mins. The reaction was stopped by adding 2 ml of 10% Trichloro acetic acid (TCA) and centrifuged at 5000 rpm for 10 mins. To the supernatant, add 5 ml Lowry's reagent C (2% Na_2Co_3 in 0.1N NaOH and 0.5% $CuSo_4.5H_2O$ in 1% potassium sodium tartarate) and incubated at 37 °C for 10 mins. Then add 500 µl of Folin's phenol reagent and their colour intensity was measured at 670 nm. The activity of enzyme was expressed in µ moles of tyrosine liberated/min/mg of protein (Morihara and Tsuzuki, 1977). The assay was performed three times for each sample.

Acid phosphatase

Acid phosphatase activity was estimated by adding 500 µl of substrate mixture containing EDTA (1.49%), citric acid (0.84%) and ρ – nitrophenyl phosphate (0.03%). To the above said mixture, add 1ml of enzyme source and 1 ml of acetate buffer (0.2M) and incubate at 37 °C for one hour. After incubation, add 10 ml NaOH (0.1N) was added and read at 420 nm. The amount of ρ – nitrophenol released as a result of the enzyme activity determined from a standard curve drawn using standard ρ – nitrophenol (10 µmoles/ml) (Sridhara and Bhat, 1963).

Lipase

The lipase activity was determined by Cherry and Crandall (1932) method using olive oil emulsion (25%) as the substrate. Add the enzyme source (500 µl) to the 1 ml of substrate and incubated at 37° C for 6 hrs. Then 1 ml of 95% of ethanol was added along with few drops of phenolphthalein. The mixture was titrated against 0.05N NaOH, the end point was the formation of pink colour. Increase in the titre value of the test mixture compared with the control was taken as an index of lipase activity.

Lactate dehydrogenase

The level of lactate dehydrogenase was found out using the method of King (1965) using NAD as the substrate. Mix 200 µl NAD with 100 µl of enzyme source in a test tube and incubate the mixture at 37 °C for 15 mins. After the incubation, the colour reagent 2, 4 – dinitro phenyl hydrazine (1 ml) was added and the mixture was again incubated for 15 mins at 37° C. The reaction mixture was cooled at room temperature and 10 ml of 0.4N NaOH was added. After 60s, the colour intensity was measured in spectrophotometer at 440 nm. The enzyme activity was expressed as mIU/mg/protein/

min using pyruvic acid as the standard. All the enzyme activities were performed three times for the each sample. A mIU is defined as the amount of enzyme that is required to catalyze the conversion of 1 mL of lactate to pyruvate or pyruvate to lactate per minute per mL of the sample under the prescribed assay conditions.

Statistical analysis

Data were analysed using the SPSS statistical software (Version 11.5). The significance of the differences between the salivary apparatus and alimentary canal were determined by the one way ANOVA and values of $P \leq 0.05$ were taken to imply statistical significance (Parker, 1979).

RESULTS

Gross Morphology

Rostrum and stylet

The head of *C. brevipennis* was 7.45±0.30 mm length. *C. brevipennis* had chase and pounced type of feeding behavior. It has short and curved rostrum about 3.90±0.03 mm in long can be moved about 90° forward (Fig. 1a). Among the three rostral segments, base (1.03±0.04 mm) and terminal (1.05±0.05 mm) segments were sub-equal in length and the middle (1.89±0.08 mm) is longer. As observed in other heteropterans, the stylet of *C. brevipennis* has two maxillary stylet (6 mm) inside and two mandibular stylets (4.525 mm) outside (Fig. 1b and 1c). These stylets are present in bundles. At anterior end, maxillary stylet has a sharp edged tip used for piercing the host body and barbs present inner side. Mandibular stylets ends with tubular opening to sucking the pre-digested food materials while in feeding. Throughout the mandibular stylet has filtering structure in four rows (Fig. 1c). The posterior ends of stylets are attached to the head by means of adductor muscle (Fig. 1d).

Salivary apparatus

The salivary gland of *C. brevipennis* consists of a paired multilobed principle gland (27.15±0.24 mm long), located on either side of the fore part of the midgut (6.08±0.37 mm) and extending upto the abdominal cavity; and paired vesicular accessory glands (2.06±0.05 mm long) (AG) usually located on either side of saccular midgut in the abdominal cavity. From the dorso – ventral mid region of accessory gland arise the accessory salivary duct (9.2 mm) (AD) traversing forwards, then turn running posteriorly to join the principle salivary gland at the hilus (HI) (Fig. 2a). The hilus was located at the junction of the anterior (3.50±0.03 mm) (APG) and well developed posterior principle glands (PPG) in *C. brevipennis.* Just above the main duct of the principle gland emerges, taking a forward course follow the contour of the total alimentary canal (52.00±0.70 mm) (Fig. 2b). The principle ducts from both sides enter into the neck, united to form a common salivary duct that ultimately finds its way into the lumen of the salivary pump as the efferent salivary duct. Posterior lobe of the principle gland was six times longer (21.4±0.52 and 23.5±0.22 mm long for male and female respectively) than the anterior lobe (3.50±0.03 to 4.24±0.05 mm for male and female respectively).

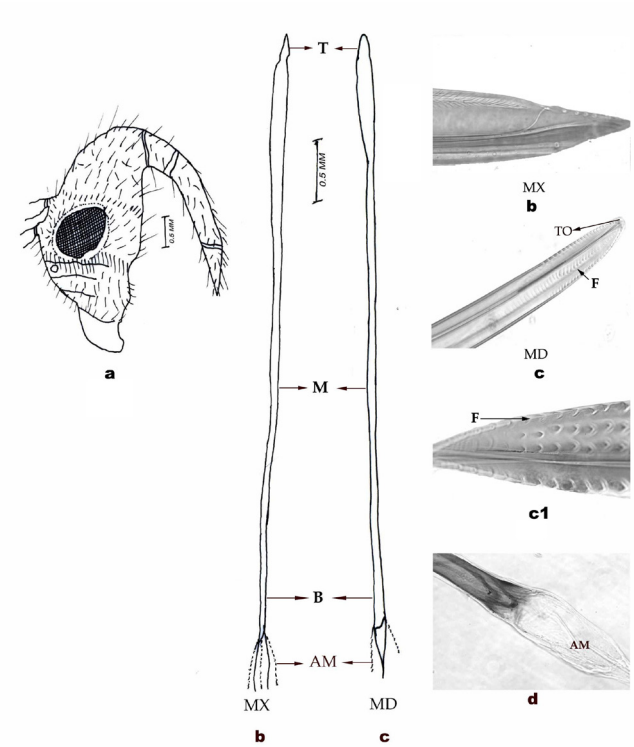

Fig. 1. *Catamirus brevipennis*, a) Entire head - lateral view; b) Maxillary (MX); c and c1) Mandibular (MD); d) stylets and muscles (B - base, F - filtering sutructure, M - Mid, T - terminal, TO - terminal opening).

Total protein content

Total protein content shows that posterior lobe of the salivary apparatus was act as a storage part (46.0 and 54.8 mg/100mg of wet tissue for male and female, respectively) and accessory gland was the reservoir of water (2.0 and 3.2 mg /100mg for male and female respectively). More total protein content was observed in the foregut followed by hindgut and midgut in *C. brevipennis* (Fig. 2b). Over all protein content was significantly maximum in females ($df = 5$; $F = 691.9$, $P < 0.001$) than the males.

Enzyme profile

In the quantitative enzyme profile, protease and acid phosphatase level was significantly ($p < 0.05$) higher in midgut and hindgut, respectively. Lipase and amylase level was insignificantly ($p < 0.05$) lesser in foregut and midgut, respectively. One way ANOVA results shows that comparison between the enzyme level of foregut with midgut ($df = 3$, $F = 71.25639$, $P < 0.00005$) and hind gut ($df = 3$, $F = 131.5355$, $P > .00005$) were significant. In salivary gland, amylase and acid phosphatase level was significantly higher and in accessory gland and protease level was higher in principle gland. Quantitative enzyme level of both accessory gland and principal glands shows that they were significant ($df = 3$, $F = 94.586$, $P < 0.00005$). Comparison between

foregut and salivary gland (df = 3, F = 94.5861 and 45.7681, P < 0.00005 for accessory and principal gland, respectively) and principal gland with alimentary canal (df = 3, F = 23.6016, 71.2563 and 131.5355, P < 0.00005 for fore, mid and hindgut, respectively) were significant (Table 2). The lactate dehydrogenase activity was not found in the both salivary gland and alimentary canal of *C. brevipennis.*

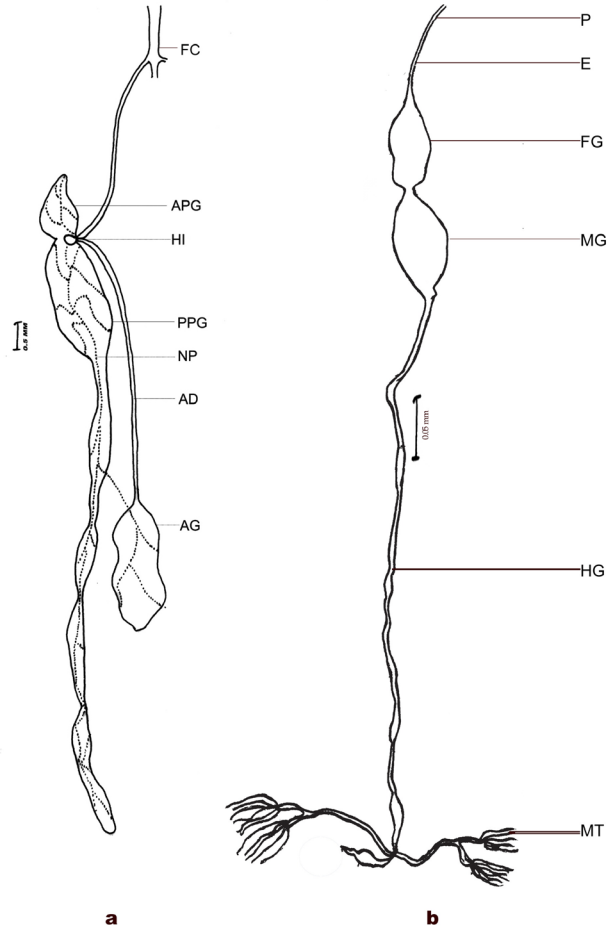

Fig. 2. *Catamirus brevipennis*, a) Salivary gland; b) Alimentary canal (APG - anterior principle gland, AD - accessory duct, AG - accessory gland, E - esophagous, FC - food canal, FG - foregut, HG - hindgut, HI - hilus, MG - midgut, MT - malphigian tubules, NP - nevre plexus, P - pharynx and PPG - posterior principle gland).

DISCUSSION

The mandibular stylets of *C. brevipennis* are typical of the hemipterans, whether phytophagous or zoophagous (Cohen, 1996; Boyd, 2001 and 2003; Wheeler, 2001). Cobben (1978) suggested that the inner surface of the right maxillary

stylets of heteropterans ranged from moderately serrated in predatory families (eg. Anthocoridae, Nabidae) to smooth in strictly phytophagous families (eg: Tinigidae), with intermediate in Miridae. Boyd (2001 and 2003) observed maxillary stylets of predatory mirids are more serrated than the phytophagous insects as observed in this study. It is probably used to disrupt prey by ripping and tearing tissues as suggested by Cohen (2000). In *C. brevipennis* barbs pointing towards the head as observed in predacious pentatomids (Cohen, 1996). Reduviids do not produce a salivary slange the apical serrations on the mandibular stylets are considered adaptive in holding on to tissue below the outer layer of the prey.

Haridass and Ananthakrishnan (1981) reported that Harpactorinae Reduviids are having one anterior and one posterior with an elongated vesicular and tubular extension of accessory gland. They also stressed that the separation of the principle salivary gland in Harpactorinae suggests the differential function of these lobe involving division of labour. In Reduviidae, anterior lobes secrete zootoxic substances which the predators used to immobilize their prey, whereas the posterior lobe concerned with the secretion of digestive enzymes (Haridass and Ananthakrishnan, 1981). This is contrary to the results obtained by Edwards (1961), who analyzed the salivary secretions of *Platymeris rhadhamanthus* Garest. (Hemiptera: Reduviidae) had found the presence of zootoxic substances both in anterior and posterior lobes besides digestive enzymes secreted by the posterior lobe. This study also reveals that digestive enzymes like invertase, lipase, amylase, and protease were distributed both in the principle and accessory glands of *C. brevipennis* salivary apparatus as observed by Sahayaraj *et al.* (2007). These enzymes can help the predator for pre-oral digestion and digestive enzymes observed in the alimentary canal helps for further digestion of partially digested food in the alimentary canal. However in *Creontiades dilutus* (Hemiptera: Miridae), chymotrypsin mainly involved in the pre-oral digestion (Colebatch *et al.,* 2001).

But the lactate dehydrogenase activity was not found in *C. brevipennis* this is because the lactate dehydrogenase is an enzyme used in toxicological diagnose of cell, tissue and organ damage. Furthermore, the potential of this enzyme as an indicative criterion in invertebrate toxicity tests (Oliveria *et al.,* 2006; Riberio *et al.,* 1999; Senthil Nathan *et al.*, 2006a and 2006b). Digestive enzymes are advantageous for zoophagous nature of reduviid particularly the proteases (Cohen, 2000). Proteases in general and trypsin in particular were recorded in the alimentary canal of *C. brevipennis* (Sahayaraj *et al*., 2007). Amylase is one of the key enzymes involved in digestion and metabolism of carbohydrate in insects. The higher amylase activity in alimentary canal shows that digestion of carbohydrate was mainly takes place while food is raveling through various parts of the gut region.

The enzymatic profile of predatory hemipterans plays an important role in the predation process. The process begins by the predator selecting a suitable prey for their suite of enzymes, digesting prey contents prior to ingestion (Cohen, 1996), further digesting it in the gut and then converting the food to energy for other needs (Applebaum, 1985; Terra and Ferreira, 1994). The types of digestive enzymes, especially those of salivary origin, are highly correlated with the feeding

habitats of hemipterans (Miles, 1972; Hori, 1975; Agusti and Cohen, 2000b; Hori, 2000; Zeng and Cohen, 2000a and 2000b; Boyd, *et al*., 2002; Boyd, 2003).

The digestive enzymes are hydrolases including amylase, lipase and protease which are specifically associated with zoophagous predatory Reduviids (Cohen, 1996). However, the presence of amylase is not expected in strict zoophagous insects, since it involved in digestion of starch granules and related carbohydrates from plants (Agusti and Cohen, 2000b; Boyd *et al,* 2003) and it may be useful in digestion of glycogen obtained from prey too (Cohen, 1996). In *C. brevipennis*, amylase and lipase activity was less when compared to protease and acid phosphatase this is due to the amylase enzyme are indicative of phytophagy (Cohen, 1996) and the plant feeding mirids have high level of amylase in their salivary gland (Agusti and Cohen, 2000a). α -amylase activity was recorded from heteropterous insects like *Lygus hesperus* (Rauscher *et al*., 1986; Agblor *et al,* 1994; Agusti and Cohen, 2000a) and *L. lineolaris* (Silva and Terra, 1997; Agusti and Cohen, 2000a). Previously Hori (1969 and 1970) recorded amylase from salivary glands of *Lygus disponsi*. Protease synthesis may be constitutive in these insects rather than being induced by food or a secretogogue. In contrast, Cohen (1993) reported that during feeding depletion of proteases occurs in salivary gland, at the same time trypsin- like activity increase in midgut of *Zelus renardii* (Heteroptera: Reduviidae) as observed in *C. brevipennis*. Carneiro *et al*. (2001) reported that two subtypes of acid phosphatase activities were detected in the venom gland secretory cells of *Bothrops jararaca.* In *Pimpla hypochondriaca*, Dani *et al.* (2005) reported that high acid phosphatase activity was present in the venom. In the case of *Pteromalus puparum* (Hymenoptera: Pteromalidae), the enzymatic activities of this venom protein were measured with the substrate of ρ - nitro phenol phosphate (Zhu *et al.,* 2008).

Edwards (1961) recorded no lipase activity in the saliva of *P. rhadhamanthus,* however it was recorded in the gut. This study reveals that lipase involved both in pre- oral digestion and digestion at various parts of alimentary canal. The protease is the main group of digestive enzymes involved in extra oral digestion in predacious animals (Cohen, 1993 and 1995). Protease catalyzes the production of peptides and amino acids from a protein substrate and is divided in to two groups as endopeptidase and exopeptidase. The endopeptidase is found in the saliva of heteropteran predators (Cohen, 1993 and 1998b). Protease activity is very high in principle gland of *C. brevipennis* shows that during pre-oral digestion, most of the ingested proteins was digested, if any does not digested, then it was digested when partially digested food is passing through both in midgut and hindgut. The acid phosphatase activity of *C. brevipennis* alimentary canal gradually increased from foregut to midgut and then to hindgut. Phosphatase is an important enzymes related to transport of materials across membranes. High rate of food consumption and subsequent absorption probably led to an enhanced activity of acid phosphatase.

CONCLUSION

Gross morphology of rostrum, stylet and salivary gland of *Catamirus brevipennis* reveals general morphology of predatory hemipteran bugs. *C. brevipennis* salivary

apparatus and alimentary canal contain amylase, proteinase, lipase and acid phosphatase. These enzymes are mainly facilitated extra oral digestion and digesting the prey. The extra oral digestion in predacious Reduviids allows them to prey on larger insects, but required a longer time with their prey until they are completely satiated. This is because of the high involvement of enzymes production and injection into the prey, requiring time to act for enzymes and subsequent time to recover these enzymes. Presence of these enzymes makes the animals are strict zoophagous and thus partially digest using salivary enzymes and the liquefied food was complete digested inside the gut using the digestive enzymes.

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Table 1. Total length (mm) and weight (mg) of *Catamirus brevipennis* (Servile) salivary gland and alimentary canal. (ALPG – anterior lobe of principle gland and PLPG – posterior lobe of the principle gland) and alimentary canal

Parts	Sex	Length (in mm)	Weight (in mg)
Accessory gland	Male	2.06±0.05	2.74±0.04
	Female	2.06±0.05	2.74±0.07
ALPG	Male	3.50±0.03	2.02±0.03
	Female	4.24±0.05	3.18±0.03
PLPG	Male	21.4±0.52	3.54±0.05
	Female	23.5±0.22	3.74±0.04
Foregut	Male	12.0±0.31	2.04±0.02
	Female	12.8±0.20	2.26±0.05
Midgut	Male	6.02±0.06	1.46±0.05
	Female	6.08±0.37	1.70±0.03
Hindgut	Male	32.4±0.50	6.78±0.03
	Female	34.8±0.37	7.38±0.05

Table 2. Quantitative enzyme profile of *C. brevipennis* salivary gland and alimentary canal.

Body parts	Amylase (in µg/g)	Protease (in µg/g)	Acid Phosphatase (in µmoles/g)	Lipase (in µequ/g)
Accessory gland	13.55^{a}	16.00^{a}	15.71^{a}	6.25^{a}
Principle Gland	8.75^{b}	31.70^{b}	13.31^{b}	6.37^{ab}
Foregut	16.00^{c}	15.53^{ac}	11.56^{c}	5.00^{c}
Midgut	15.51^{cd}	25.43^{d}	20.00^{d}	6.05^{abd}
Hindgut	16.00^{ce}	22.51^{e}	46.50^{e}	6.75^{abde}

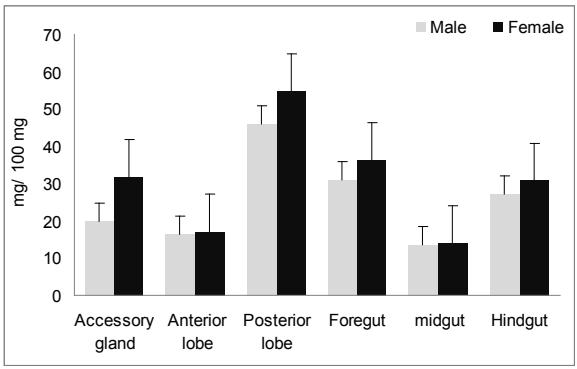

Fig. 3. Total protein content (mg/100mg) of salivary gland and alimentary canal of *C. brevipennis.*

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