

SCENT APPARATUS WITH REFERENCE TO ENZYMATIC
ACTIVITY OF RED PUMPKIN BUG *CORIDIUS JANUS* (FABR.)
(HETEROPTERA : DINIDORIDAE) WITH COMMENTS ON
THE SYSTEMATIC POSITION OF THE FAMILY

BY

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SYNOPSIS

Except GUPTA's (1960) erroneous and misleading presentation of stink glands in the two species of the genus *Aspongopus* (*Coridius*) the Dinidorid scent apparatus is entirely unknown, although this important structure in other Pentatomoid species has been studied by BRINDLEY (1930), MALOUF (1933) and more recently by ROTH (1961), RAI and TREHAN (1964), GILBY and WATERHOUSE (1967), AHMAD and KHANUM (1968) and AHMAD and KHAN (in manuscript). In the light of MOORE and WALLBANK's (1968) findings in Bombardier beetles (*Carabidae* : *Brachininae*) which discharge their secretion into a second chamber where an enzyme-catalysed chemical reaction between pre-formed components takes place with explosive force, it is apparent that the glands and the reservoir are highly active areas, but in stink bugs the enzymatic activity within these organs are little studied.

In the present paper the authors have studied the scent apparatus of *Coridius janus* (FABR.) an important cucurbit pest of West Pakistan with regard to its mode of action and enzymatic activity. The structure as compared to in other Pentatomoids suggests a distinct family rank of *Dinidoridae* within the superfamily *Pentatomoidea*.

INTRODUCTION

The family status of *Dinidoridae* is disputed, is clear from MIYAMOTO's (1961) remark « Some authors (LESTON, 1955, PENDERGRAST, 1957,

SCUDDER, 1959 and etc.) have recognized this group differing from the other subfamilies of the *Pentatomidae* but others (CHINA and MILLER, 1955 and 1959) denied ». During the recent years various aspects of morphology by (MIYAMOTO, 1961; KUMAR, 1962; AHMAD and ABBASI, 1971 and AHMAD *et al.*, (in press), are used in order to solve the above problem.

The scent apparatus in the tribe *Carpocorini* (of *Palomena prasina*, BRINDLEY, 1930), *Eurydemini* (of *Bagrada cruciferarum*, RAI and TREHAN, 1964), *Pentatomini* (of *Nezara viridula*, MALOUF, 1933 and GILBY and WATERHOUSE, 1967) and in *Halyini* (of *Halys dentatus*, AHMAD and KHANUM, 1968) within the family *Pentatomidae* and in other Pentatomoid families *Acanthosomatidae* (of *Elasmostethus griseus*, BRINDLEY, 1930 and in *Cydnidae* (of *Scaptocoris divergens*, ROTH, 1961) has been studied. GUPTA (1960) has described and illustrated the stink gland in the two species of *Aspongopus* (*Coridius*) of the family *Dinidoridae* but his results are misleading and confusing. BRINDLEY (1930) working on the comparative morphology of scent apparatus in the fourteen families of *Heteroptera* utilized this character in ascertaining the relationships between various families. GUPTA (1961, 64) and AHMAD and KHANUM (1968) (in a review and working on a coreid) and (a pentatomid) respectively suggested that this character might well be used in showing relationships between various families or even between the lower categories.

QURESHI and AHMAD (1970) have shown the taxonomic value of this structure in the genus *Poecilocerus* (*Orthoptera* : *Pyrgomorphidae*).

Although an extensive literature is available on the chemical secretions of the scent glands of the true bugs (BLUM and TRAYNHAM, 1960; WATERHOUSE *et al.*, 1961; ROTH and EISNER, 1962; REMOLD, 1963; GORDEN *et al.*, 1963; WATERHOUSE and GILBY, 1964; GILCHRIST *et al.*, 1966; EISNER and MEINWALD, 1966; GILBY and WATERHOUSE, 1965 and 1967) very little work on the enzymatic activities within the reservoir or in the glands has been undertaken. EISNER and MEINWALD (1966) in their extensive review « On the defensive secretions of Arthropods » included the stink bugs in the third category with cockroaches, earwigs, stick insects, grass hoppers, caterpillars of the family *Notodontidae*, beetles of the families *Carabidae* and *Tenebrionidae*, whip Scorpions and Millipedes, in which the scent glands discharge their contents as a spray to a distance of several feet with maximum effectiveness and minimum wastage. MOORE and WALLBANK (1968) while working on the « Chemical composition of the defensive secretions in carabid beetles and its importance as taxonomic tool », reported an enzyme-catalysed chemical reaction within the second chamber between the preformed components resulting into an explosive force.

In the present paper the functional morphology of the scent apparatus is studied and is compared with the account available in the existing literature as reported above and the enzymatic activity of proteinase,

dipeptidase, amylase, cellulase, alpha-glucosidase, beta-galactosidase, beta-fructosidase, acid phosphatase and alkaline phosphatase is determined.

MATERIAL AND METHODS

The adult red pumpkin bugs were collected from May 1969 through September 1969 on pumpkin and other cucurbits in Malir gardens near Karachi. These bugs eject their yellow repugnant liquid secretion almost instantaneously as soon as these are disturbed. Adult fresh specimens were dissected under a dissecting binocular and the structure of scent apparatus was studied after removing the overlying viscera. The drawings were made using ocular grid to the scale mentioned.

Preparation of the homogenate

Adults of known age (approximately one month) were chilled at -10°C for about 15 minutes and then were dissected in Ringers solution for taking out the glands and the reservoir. Reservoir and the glands were separated and homogenized in a Teflon pyrex tissue grinder containing 2 ml buffer (pH = 8.3) for three minutes. Homogenate of the glands and the reservoir was prepared according to the requirement representing scent apparatus of two bugs per ml.

Determination of Enzymatic Activity

(1) Determination of proteinase activity was accomplished quantitatively by using chromophoric protein derivative, azocasein as substrate according to the procedure described by TOMARELLI *et al.* (1949). Substrate solution was prepared as mentioned in their procedure and was incubated at 38°C . Proper number of tubes were also placed in a rack which in turn was placed in a water bath. One ml of the substrate and one ml of the homogenate were pipetted in the test tubes for each determination.

Substrate blank was run by using 1 ml bicarbonate buffer instead of the substrate solution. After 30 minutes' incubation, digestion was stopped by adding 8 ml of 5% trichloroacetic acid. For the rest of the procedure and calculation TOMARELLI *et al.* (1949) methods were followed.

(2) For the determination of dipeptidase the homogenate was prepared in phosphate buffer. Half ml of the homogenate was mixed with half ml of the substrate (glycylglycine) and the mixture was incubated at 38°C for 3 hours. Samples of 3 microlitres were taken from the mixture using microsyringe and were applied to whatman chromatographic paper No. 1. Spots of the standard substrate glycine and boiled homogenate were

similarly applied. Descending paper chromatographic experiments were carried out for nearly 48 hours using *n*-butanol-acetic acid-water (120 : 30 : 50 v/v) as solvent system and by dipping the paper in the solution of 0.25 ninhydrine, the colour of the spots was developed.

(3) For the determination of amylase, celulase, alpha-glucosidase, beta-galactosidase and beta-fructosidase substrates used were, starch, cellulose, maltose, lactose and raffinose respectively. The hydrolysis of these substrates was conducted according to KHAN and FORD's (1967) method and the hydrolysis products were detected chromatographically as described in the above reference.

(4) Determination of acid and alkaline phosphatases was made quantitatively according to the procedure described by NAQVI *et al.* (1967).

Disodium *p*-nitrophenyl phosphate was used as substrate and *p*-nitrophenol as colorimetric standard. Optimum pH was determined for both the enzymes as it intensely influences the enzymatic activity.

RESULTS

Scent Apparatus (Fig. 1)

After removing the crop and the over lying viscera, a pair of white recemose, elongated, bean-form glands are exposed attached to the anterior sides (lateral ducts) of a very large dark orange pear-shaped reservoir. On its ventral surface lies the convoluted accessory gland which also opens in the lateral ducts of the reservoir. Lateral ducts of each side in between the meso and metathoracic coxal cavities open in their respective broad, sub-quadrangular semi-transparent membranous vestibules. The opening on each side is guarded by a valvular apparatus. In turn each vestibule opens to the exterior through a large ovoid opening located slightly lateral in between the meso and meta-thoracic coxal cavities on the metasternum.

The Glands (Fig. 1)

The glands are recemose spongy in appearance having more or less elongated bean-form. Each gland lies symmetrically adjacent to the pterothoracic abdominal nerve, facing each other, away from the lateral margins. The glandular tissue is shining white and is easily distinguished from the surrounding fat bodies which resemble loose mass of aggregate cells. Each gland opens in the lateral ducts of the reservoir through a visible relatively thin membranous duct.

The Reservoir (Fig. 1 and 2)

The reservoir is dark orange in colour and is a very large pear-shaped structure wider than long and widest in the middle with a sinuate posterior margin lying medially in the depression of the metasternum extending on to the second abdominal sternum. Anteriorly it is continued into relatively narrow, membranous ducts which lie on each side immediately posterad to the mesosternal ridges and adjacent to the anterior ridges of the metathoracic coxal cavities. The wall of the reservoir appears thicker and more cuticular as compared to the glands. It is very slightly depressed dorso-medially and dorso-laterally and ventrally has the ridges and the furrows. In the freshly killed specimens the reservoir seems to be full of secretion and its walls show remarkable smoothness.

The Accessory Gland (Fig. 2)

The accessory gland appears like extremely convoluted and coiled tube on the ventral surface of the reservoir occupying extreme lateral position nearly adjacent to the ventral edges of the reservoir, having almost the same colour as that of the reservoir. It is only distinguishable from the ventral wall of the reservoir as the raised tubular structure. On the postero-ventral side of the reservoir it is very well fitted in the ventral groove and is very hard to recognize. It opens on each side in the lateral ducts of the reservoir adjacent to the ventro-medial depression.

The vestibule (Fig. 1 and 3)

The vestibules are semimembraneous, whitish, uniformly broad, sub-quadrangular structures lying on each side between the ridges of the meso and metathoracic coxal cavities and slightly laterad to them. On their proximal end these are connected with the distal portion of the ducts of the reservoir through valvular apparatus. When pressed at the base, the secretion appears moving in their cavities. At their distal end these broadly open through an ovoid aperture or ostiole. The vertical lunate ridge of the ostiole is absent.

The Valvular Apparatus (Fig. 1 and 3)

The valvular apparatus is a semi-lunate, flap-like structure fitted nicely underneath the ridges of each metathoracic coxal cavity. Each valvular apparatus lies on the antero-lateral side of the duct of the reservoir and governs the flow of the secretion from the lateral ducts of the reservoir into the vestibules. The apex of the outer arm is nicely fitted underneath the raised ridges of the metathoracic coxal cavities whereas the

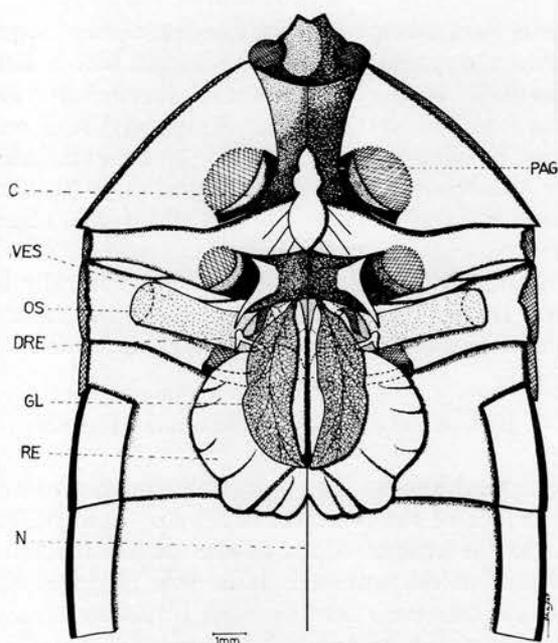


Fig. 1. — Scent apparatus of *Coridius janus* (FABR.), entire, dorsal view.

KEY TO THE LETTERING

ACG : Accessory gland.

C : Coxal Cavity.

DEP : Ventral depression
of the reservoir.

DRE : Duct of the reservoir.

GL : Scent gland.

IAM : Inner arm of the valvular
apparatus.

LDR : Lateral duct of the reservoir.

N : Ganglionic nerve.

OAM : Outer arm of the valvular
apparatus.

OS : Ostiole.

PAG : Ptero-thoracic-abdominal
ganglion.

PT : Proximal part of the vestibule.

RE : Reservoir.

VER : Reservoir ventrally.

VES : Vestibule :

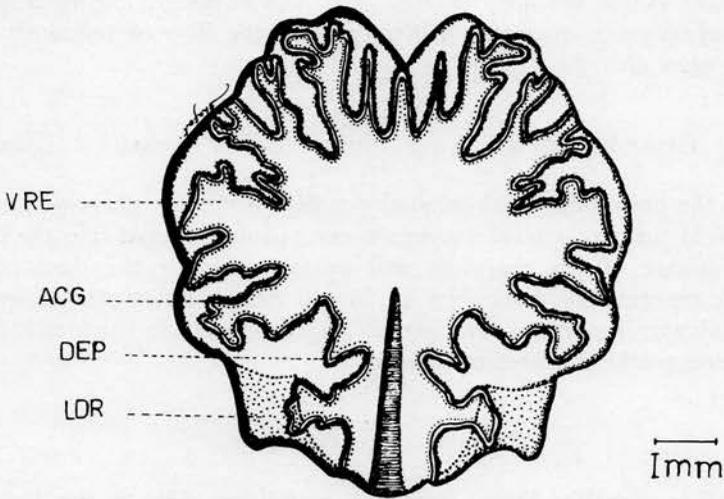


Fig. 2. — Reservoir, ventral view, showing convoluted accessory gland.

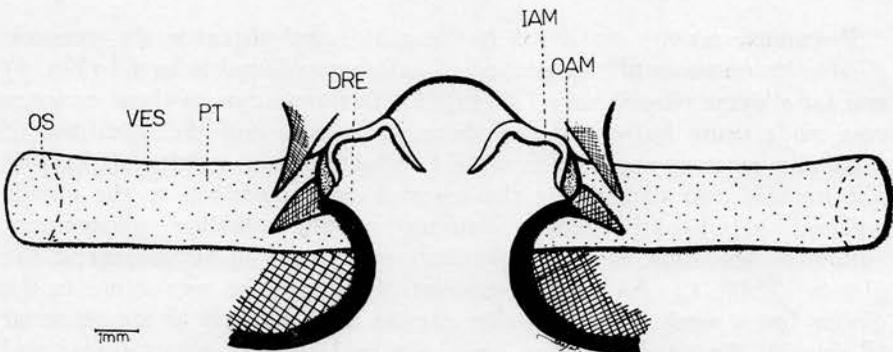


Fig. 3. — Anterior portion of Scent apparatus magnified, dorsal view, showing lateral ducts of the reservoir, valvular apparatus and the vestibules.

inner arm is free. The two arms are separated through an interlying membrane. When the apex of the inner arm is pulled, the membranous portion of valvular apparatus is lifted up and the flow of yellowish liquid may be seen into the vestibules.

The Tracheation and the Nerve Supply (Fig. 1)

From the pterothoracic abdominal ganglion a median nerve and several (at least 3) pairs of lateral nerves appear to run posterad into the dorso-medial groove of the reservoir and at the sides of the ducts of the reservoir respectively. Probably the lateral nerves innervate the muscles of the valvular apparatus. The glands and the reservoir show rich supply of the fine tracheal branches.

Mode of Action

Secretion probably flows from the glandular cells to the lumen of the glands which ejects it into the reservoir through the lateral ducts probably utilizing chemical energy, which is released there. The accessory gland also probably discharges its content into the reservoir through the lateral ducts. The ejection might also have been effected through a cumulative pressure in the glands and the reservoir. The muscular contraction probably pulls the outer arm of the valvular apparatus which releases the valve which in turn allows the secretion to enter into the vestibules from where it is ejected out side through the ostioles.

Enzymatic Activities in the Glands and in the Reservoir

Proteinase activity was weak in the glands and absent in the reservoir (Table 1), optimum pH for acid phosphatase was found to be 4.3 (Fig. 4) and for alkaline phosphatase 7.2 (Fig. 5). Determination of these enzymes was made using buffers of the above pH values and the quantities of these enzymes are reported in table 1. Slightly more activity of the acid phosphatase was detected in the reservoir in comparison to the glands whereas significantly higher activity of the alkaline phosphatase (although less than acid phosphatase) was found to be present in the glands (Table 1). An average amount of dipeptidase was found in the glands but a weak activity of this enzyme was detected in the reservoir (Table 2). Amylase, cellulase, alpha-glucosidase, beta-galactosidase and beta-fructosidase were not detected in the glands as well as in the reservoir (Table 2).

A spot of doubtful significance was obtained of alpha-glucosidase in the case of the glands and of beta-galactosidase in the case of the reservoir, on the chromatographic paper (Table 2).

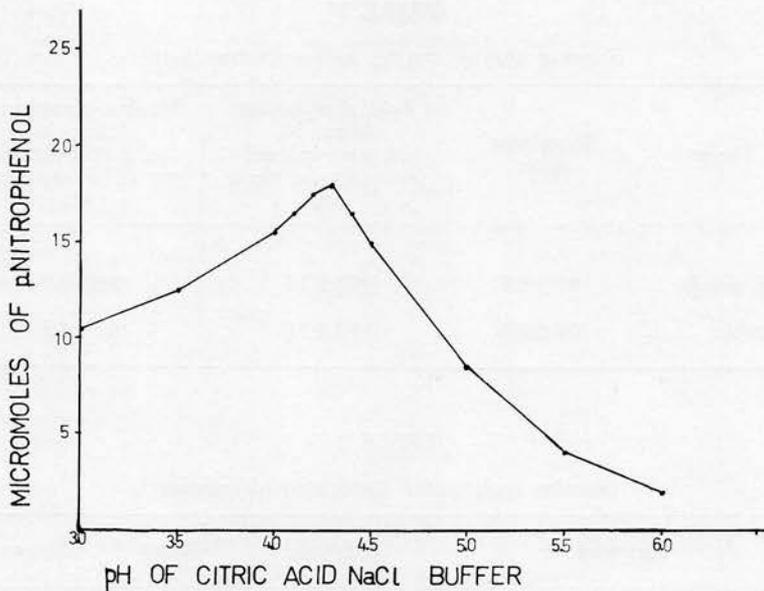


Fig. 4 — Graph showing peak indicating optimum pH value for the acid phosphatase activity.

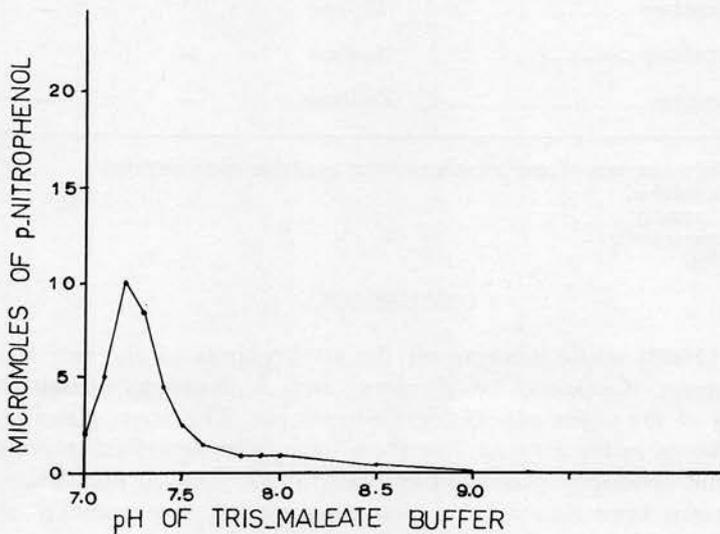


Fig. 5. — Graph showing peak indicating optimum pH value for the alkaline phosphatase activity.

TABLE 1

Showing enzyme activity in quantitative units

Tissue	Proteinase units	Acid phosphatase Micro M of p-nitrophenol per milligram tissue wt.	Alkaline phosphatase Micro M of p-nitrophenol per milligram tissue wt.
Stink glands ...	4.0±0.5	13.0±2.5	9.0±1.0
Reservoir	0.0±0.0	14.5±3.0	4.5±1.5

TABLE 2

Showing quantitative distribution of enzymes

Enzymes	Substrate	Glands	Reservoir
Dipeptidase	Glycylglycine	++	+
Amylase	Starch	—	—
Cellulase	Cellulase	—	—
Alpha-glucosidase	Maltose	?	—
Beta-galactosidase	Lactose	—	?
Beta-fructosidase	Raffinose	—	—

N. B. — Only enzymes whose substrates were available were detected.

— Not detectable.

+ Weak activity.

++ Average activity.

? Doubtful.

DISCUSSION

GUPTA (1960) while working on the stink glands of the two species of *Aspongopus* (*Coridius*) i.e. *A. janus* and *A. brunneus*, erred in the recognition of the scent glands for the reservoir. The scent glands have not been shown in his diagram nor these have been described in his text. Similarly the accessory glands (BRINDLEY, 1930; AHMAD and KHANUM, 1968) has not been illustrated in his diagram nor any mention of its presence or absence has been made in the text. He, however, has concluded from the histological study of the reservoir (not really of the glands as he has mentioned) that the lateral sides of the sac are more secretory in nature, probably thereby referring to the accessory gland which in

these species extend lateral to the extreme ventral edges of the reservoir. ROTH (1961) working on the odoriferous glands of a cydnid *Scaptocoris divergens* has also shown the two rows of the secretory cells in his figure 2 A through arrows which according to him lie closely appressed to the surface of the reservoir which he has referred to as the accessory gland.

GUPTA (1960) could not recognize the valvular structure in the scent apparatus he studied. Similarly RAI and TREHAN (1964) working on the interal anatomy of *Bagrada cruciferatum* erred in the recognition of the glands for the reservoir for they have reported a single sac-like gland adhering to the metathoracic basisternum and the sternum of the second abdominal segment. The description speaks for itself that it refers to the reservoir and not to the scent glands. They could not recognize the accessory gland or the valvular structure in the scent apparatus. ROTH (1961) has not made any mention of the valvular apparatus, or the lateral ducts of the reservoir however his description « At the base of the vestibule the membranous reservoir has a large muscle which upon contraction allows the secretion to pass up the vestibule, out of the ostiole », clearly mentions the membranous reservoir at the base of the vestibule, thereby referring probably to the lateral semi-membranous ducts of the reservoir and his large muscle allowing the secretion to pass up the vestibules through contraction either refers to the valvular apparatus itself or to the muscle which is attached to this structure. GILBY and WATERHOUSE (1967) in *Nezara viridula* (*Pentatomini*) and AHMAD and KHANUM (1968) in *Halys dentatus* (*Halyini*) have called this structure occluding mechanism and valvular apparatus respectively. The scent glands in *Coridius janus* seems to be very different in shape from all those to date described in the superfamily *Pentatomoidea*. These are elongated almost of bean-shape as compared to almost oval, leaf like or subrounded glands of *Carpocorini*, *Pentatomini* and *Halyini* described by BRINDLEY (1930), GILBY and WATERHOUSE (1967) and AHMAD and KHANUM (1968) respectively. BRINDLEY (1930) has described the reservoir as subrectangular in *Carpocorini* and almost similar shape has been presented (except smaller variations) by GILBY and WATERHOUSE (1967) and AHMAD and KHANUM (1968) in *Pentatomini* and in *Halyini* respectively but in *Coridius janus* the reservoir is distinctly pear-shaped, widest in the middle and extremely narrowed proximally.

The vestibules of pentatomid species also as illustrated by GILBY and WATERHOUSE (1967) and AHMAD and KHANUM (1968) differ from that in *Coridius janus* for in the former species these are swollen in the middle and are extremely narrowed distally whereas in the present species these are sub-rectangular, sac-like of the uniform width through out their length. The accessory gland of *Carpocorini* (BRINDLEY, 1930), *Pentatomini* (GILBY and WATERHOUSE, 1967) and of *Halyini* (AHMAD and KHANUM, 1968) in all the Pentatomid tribes to date described differs from *Coridius janus* the present species in that in the latter the tubular

form of the accessory gland shows numerous convolutions which certainly can hardly be counted as compared to only a few convolutions in the above species. Also the accessory gland of the present species occupies the extreme lateral position almost reaching to the ventral edge of the reservoir whereas in the above species it is ventro-median in position. Similar position has been shown in *Scaptocoris divergens* (Cydnidae) by ROTH (1961) but with more convolutions. On the basis of the above peculiarities of the scent apparatus (large pear-shaped reservoir, extremely curved and convoluted accessory gland and broad and relatively short vestibules) the present authors regard *Coridius janus* isolated in the superfamily *Pentatomoidea* and agree with LESTON, 1955 and 1958, PENDERGRAST, 1957, SCUDDER, 1959; MIYAMOTO, 1961, and KUMAR, 1962, that the group *Dinidoridae* deserves family rank.

The scent apparatus in general is considered by BRINDLEY (1930) « A character not only ancient but which has been relatively little affected in its evolution by environmental conditions » and in particular with reference to *Pentatomidae* his comments « with the remarkable linear accessory gland which curves round the ventral surface of the organ might have arisen by enlargement and elongation from a primitive type such as that of *Nabis* » clearly suggest that more convoluted accessory glands must be considered a specialized feature. Although in the same work he himself has mentioned, « The view taken here is that the modification of the stink glands has always taken the form of reduction or loss of parts ». In the light of this point of view the highly convoluted accessory gland of *Dinidoridae* seems to be a primitive feature which by loss of part gave rise to simple unconvoluted almost straight accessory gland which finally completely disappeared in the families *Miridae*, *Tingidae* and *Coreidae*. BRINDLEY (1930), in *Anorbus rubeiginosus* (*Coreidae*) and GUPTA (1964) in *Leptocorisa costalis* (*Coreidae*) have reported large reservoir and the latter has illustrated broad and comparatively short vestibules which on the other hand either relates *Dinidoridae* in at least some features with advanced groups like *Coreidae* or probably reflect convergent evolution. KUMAR (1962) with reference to *Dinidoridae* has commented. « It is interesting to note that internal male reproductive organs of both *Dinidoridae* and *Acanthosomatidae* show resemblances to *Pyrrhocoridae* but their salivary glands are very different, in former two cases they are of typical pentatomid type while in the latter case they are of typical coreid type which suggests that the *Pyrrhocorid* resemblances may be a matter of parallel evolution only ».

The highly convoluted accessory glands is shared by *Scapticoris divergens* (Cydnidae) (as illustrated by ROTH, 1961) with the present species of *Dinidoridae*. KUMAR (1962) has also noted that Cydnids in the characters of the salivary glands represent the transitory state between *Pentatomidae* at one hand and *Acanthosomatidae*, *Dinidoridae* and *Scutelleridae* on the other. MIYAMOTO (1961) has concluded, « thus the *Dinidoridae* seem to show rather primitive characters among the *Pentatomo-*

dea », although he has also noted « two rows of gastric caeca have affinity to those of *Lygaeoidea*, *Coreoidea* and some lower families of the *Pentatomioidea*. Structure of the pylorus is allied to both, the *Pentatomioidea* and *Coreoidea*, not to the *Lygaeoidea* ». Probably some more work (covering larger number of species and an observation of larger number of characters) would show *Dinidoridae* an early off shoot of some pentatomoids which evolved and specialized in many characters.

The proteinase activity which has been found weak in the glands and absent in the reservoir is probably due to the hydrolysis of proteins present in the scent gland cells for the preparation of the secretory fluid. Similarly the presence of average amount of dipeptidase in the glands and weak in the reservoir again represents more peptide-bond breakage in the glands and less so in the reservoir. The presence of these enzymes more in the glands probably also refers to the enzyme-catalysed chemical reactions in these areas, between the preformed components resulting in the liberation of chemical energy which probably is used in ejecting the secretion in the lateral ducts of the reservoir and weaker indication of these enzymes in the reservoir probably refers to the activities in the accessory gland of the reservoir, the secretory cells of which also transfer their secretions in the lateral ducts of the reservoir. This conclusion is in agreement with the findings of MOORE and WALLBANK (1968). The main lumen of the reservoir is non secretory and is muscular in structure and probably through cumulative pressure and through muscular contraction of its walls ejects the secretion into the vestibules, through its lateral ducts when the muscular contraction releases the valves (ROTH, 1961). Slightly more activity of the acid phosphatase in the reservoir probably confirms the above conclusion that the transfer and absorption of the scent fluid are mainly carried out in this region. Similar function of this enzyme has been reported by NAQVI *et al.* (1967) and NAQVI *et al.* (1968). A relatively higher activity (although less than acid phosphatase) of the alkaline phosphatase in the glands probably indicates the main secretory role of the cells of the scent glands in which this enzyme seems to play an active role as has been reported by ASHRAFI *et al.* (1969).

The absence of amylase, cellulase, alpha-glucosidase, beta-galactosidase, and beta-fructosidase in both, the scent glands and in the reservoir and the presence of proteinase and dipeptidase (more in the glands and weak in the reservoir) probably indicates that only the proteins are involved in the synthesis of scent fluid. This conclusion is also in agreement with GUPTA (1960) « when the gland (the reservoir) is treated with concentrated H_2SO_4 , its insolubility shows as if it is composed of epicuticle and exocuticle as the endocuticle is soluble in concentrated H_2SO_4 » « the non appearance of the violet colouration in the gland (when treated with freshly prepared solution of the iodine in KI and a drop of concentrated $ZnCl_2$) shows the absence of chitin ». Doubtful appearance of the spots in the case of the glands of alpha-glucosidase and

beta-galactosidase in the case of the reservoir may be due to some experimental error as these were only detected in one replicate alone.

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