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SCANNING ELECTRON MICROSCOPIC STUDY (SEM) OF ADULT MALE REPRODUCTIVE SYSTEM OF PUMPKIN BEETLES, *AULACOPHORAFOVEICOLLIS* (LUCAS) AND *AULACOPHORANIGRIPENNIS* (MOTSCHULSKY) (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

Reproduction in insects is an essential physiological process from the view point of propagation of the insect species. The testis, vas deference seminal vesicle and MARG of pumpkin beetles A. foveicollis (Lucas) and A.nigripennis (Motschulsky) were studied morphologically using scanning electron microscopy (SEM). In the present study morphological observations of testis, vas deference seminal vesicle and MARG of A. foveicollis and A. nigripennis the following surface observation have been examined. The testis were flattened leaf like structures, yellow coloured and surrounded by thick fibrous connective tissues and the entire structure with crystalline like layers of a peritoneal connective sheath. The testicular follicles with a number of pinocytotic pits, and itwas notable for its secretory function. Testicular tubes were covered with folded or ruffled connective tissues and covered with myoepithelial cells. The vas deferens of the A. foveicollis showed thick outer tubular structure with folded cuticle and myoepithelial cells. The epithelial layer consisted of numerous pinocytotic pits and secretory granules proved the epithelium to enhance its secretory activity. The seminal vesicle of the insect shows the tubular nature with outer ruffled muscles. The lumen contained more amount of secretory materials. The MARGs of the pumpkin beetle exhibit tubular structure with myoepithelialcells were covered with thin smooth cuticular muscles. The innervation of tracheae in the testes and accessory glands was observed in A.nigripennis. Testicular lobes of A.nigripennis were lined by a peritoneal layer an epithelial sheath, of the non-cellular layer (tunica propria). The vas deferens of A. nigripennis showed slender tube with externally covered by the smooth circular muscles with a number of secretory globules. The epithelial layer consisted of numerous pinocytotic pits and also secretory granules like that of A. foveicollis.The lumen of the seminal vesicle contained densely accumulated secretory materials with spermatozoa and also single layer of epithelial cells outside lumen. The seminal vesicle is externally covered by smooth circular and longitudinal muscle. The more morphological observations of testis, vas deference, seminal vesicle and MARG of A. foveicollis and A.nigripennisare helpful to trace the phylogenetic evolution of insect species.

Key words: Aulacophorafoveicollis, *Aulacophoranigripennis, Electron microscopic study.*

INTRODUCTION

Reproduction in insects is an essential physiological process from the view point of propagation of the insect species and as mentioned earlier, naturally results in the high biotic potential of insects, making them a successful group of animals. The various aspects of reproductive activities such as sexual excitement, mating, oviposition, transfer of seminal fluid during mating and the functions of male reproductive glands have been studied in different orders of insects (Ravisankar and Venkatesan, 1988; Selvisabhanayakam, 1995). Studies on the testes, vas deferens, seminal vesicles and male accessory reproductive glands are very essential to understand the problems related to the reproductive physiology of insects. Studies on the male reproductive organs generally reveal the existence of various in the shape, size, location and functions and these variations have not been well documented especially in the order Coleoptera (Chrysomeloidae). The male reproductive system of the *A. foveicollis* and *A. nigripennis* (Coleoptera: Chrysomelidae) consists of a testes, a lateral ducts, vasa deferentia, converging posteriorly from the testes, a median exit tube and the ejaculatory ducts, receiving the vasa deferentia anteriorly and communicating with the apex of the aedaegus posteriorly, and one more pair of accessory glands. The vas deferens is usually a simple tube with the small surrounded by circular muscles. A part of the vas deferens is sometimes swollen and forms the seminal vesicle of the storage of mature sperm as the latter leaves the testis. The position where this seminal vesicle is formed varies from the anterior extremely to the middle of the vas deferens. The length and width of the vas deferens also varies in different groups. From each testicular follicle a tiny duct, the vas efferens (vasa efferentia) leads to a common duct, the vas efferens (vasa deferentia). The vasa deferentia from both testes join to form the ejaculatory duct (ductus ejaculatorious) which terminates in the penis or aedeagus, at the genetial pore or the gonopore. Some cells of vasa deferentia are glandular and they secrete their products into the lumen (Riemann and Giebultowiex, 1991). The morphology of seminal vesicle in adult reproductive system appeared to be white oval shaped body. The seminal vesicle was richly supplied with tracheoles. It lies in the posterior region of the abdomen in between the vas deferens and the testes. The posterior region of seminal

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vesicle was connected to the vas deferens. The seminal vesicle has dual function that is secretory and storage of sperms.

MATERIALS AND METHODS

Field collection of beetles: The adult pumpkin beetles were collected in the vicinity of Annamalai university, Chidambaram, Tamil Nadu. Collections were made in the early morning by hand picking because the beetles are very sluggish so all the insects are collected before 11a.m. The beetles were brought to the laboratory and reared in plastics cages, having a dimension30X2 cm, at a laboratory temperature of 29 ± 1°C with 12hours light and 12 hours dark photoperiod. The floor of the cage was covered with fine sand, moderately moistened with water daily in order to maintain the humidity of the cage. The insects were fed daily with fresh leaves of cucumber (*Cucumissativus*), bottle gourd (*Lagenariasiceraria*), Water melon (*Citrulluslanatus*), Muskmelon (*Cucumismelo*) and Round gourd belongs to same family Cucurbitaceae. The dissection made under binocularmicroscope because the beetle is very tiny.

Scanning electron microscopic study (SEM): All (testis, vas deferens, seminal vesicle and MARG) the samples of insects were dried in vacuum for getting good moisture free specimen was needed. Then the samples were coated-gold with full deposition for 3 minutes using polaron SC 500 sputter coater. Few tungsten line coating was given this coating has given primary to prevent charging samples and clarity of pictures. Then the samples were mounted in stereo scan 440-model electron microscope (UK). The ascertaining voltage given was 20kw and the beam current used was in between 18-25 p.a (pica amperes) notching distance was between 39 mm to 1 mm. The secondary electron images were taken for all the samples with varied magnifications from 50 × to 10,000 × (Kotze and Soley, 1990).

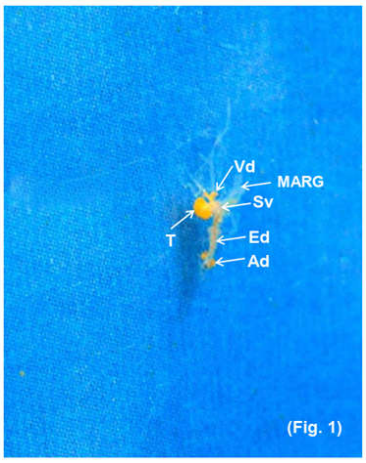
RESULTS

Male reproductive system of *A. foveicollis:* In *A. foveicollis* the male reproductive organs were found towards the tip of the abdomen. The opening from the reproductive organs was near the posterior end of the body and surrounded by external genitalia. There were claspers, used to hold the female during mating and the penis. The accumulated sperms were held in the body of the male in dilations of the vasa deferentia known as seminal vesicles. Bilobed testes was dorsally placed at the right side of the fifth abdominal segment. It was externally covered with the fat body and posteriorly it opened into the vasa deferentia. The follicles were connected with the vas deferens by a relatively slender tube of vas eferens and opened into vasa deferentia is the paired canals leading from the testes and was mesodermal in origin. Vasa deferentia leads to connected single seminal ducts and open into an ejaculatory duct. Finger like small tube of accessory gland secrete the fluid, which was connected to the seminal vesicle in the posterior end (Fig. 1). The testis were flattened leaf like structures, yellow coloured and surrounded by thick fibrous connective tissues and the entire structure with crystalline like layers of a peritoneal connective sheath (Fig. 3). Posteriorly the follicular tubules open into the vasa deferentia. The testicular follicles were observed with a number of pinocytotic pits, and it was notable for its secretory function that was taken from the testes itself (Fig. 5). Internally, each testicular lobe was separated by the interstitial connective tissues. Internally, each follicle seems to be consisted of an outer muscular layer and inner apical cells which were folded with numerous secretory vesicles. Thin and short prolonged canal in the centre part of testis known as efferent ducts, arose from each testicular tube and join to the vasa deferentia duct. Testicular tubes were covered with folded or ruffled connective tissues (Fig. 4) and it was covered with myoepithelial cells. Internally each follicle consisted of an outer muscular layer and inner apical folded cells with numerous secretory vesicles (Fig. 5). In each follicular cyst, below the apical cells, SEM reveals the occurrence of groups of germ cells.The morphology of vas deferens in adult male reproductive system of *A. foveicollis* appeared to be white and slendor tubular shaped structure. The vasa deferentia received the sperm or sperm bundles from the vasa deferentia and facilitate their transport to the ejaculatory duct.

The vas deferens of the *A. foveicollis* showed thick outer tubular structure with folded cuticle and myoepithelial cells (Fig. 6). The tubules consisted of coir rope like appearance. Circular and longitudinal muscle layers were arranged predominantly. The epithelial layer consisted of numerous pinocytotic pits and also it was noted that the secretory granules proved the epithelium to enhance its secretory activity (Fig. 6 and 7). The morphology of semial vesicle in the adult reproductive system appearedto be dilated tube of vasa deferentia which was yellowish in colour(Fig. 8). The seminal vesicle was richly supplied with tracheoles. It lies in the posterior region of the abdomen in between the vas deferens. The semial vesicle have dual function that is secretory and storage of sperms. The seminal vesicle of the insect shows the tubular nature with outer ruffled muscles (Fig. 9 and 10). The SEM architecture of seminal vesicle consisted of outer rope like structure of connective tissues with numerous myoepithelial cells. The epithelial cell consisted of numerous pinocytotic vesicles and the secretory granules were responsible for both secretion and absorption of some materials. The lumen contained more amount of secretory materials (Fig. 11). The MARGs of the pumpkin beetle exhibit tubular structure with myoepithelialcells which were covered with thin smooth cuticular muscles (Fig. 12). The secretoryepithelial cell consisted of many pores and secretory vesicles. The lumen contained secretory substances and many vesicles. The nucleus was found to be larger, and cytoplasm contained more amounts of secretory materials.

Morphology of male reproductive system of *A. nigripennis*

The male reproductive organs of the *A. nigripennis* comprised of single testis, vas deferens, single seminal vesicle and accessory glands (Fig. 2). The testes of the *A. nigripennis* was noted with globular (or) spherical like structure and yellow in color, usually located near the dorsum abdomen. The testes was connected to the seminal vesicles by the vas deferens. The dilated tube of seminal vesicle led to median ejaculatory duct. The short slendor tube of MARG was connected in between the seminal vesicle and ejaculatory duct. The innervation of tracheae in the testes and accessory glands was observed in *A.nigripennis* (Fig. 2). Testicular lobes of *A.nigripennis* were lined by a peritoneal layer an epithelial sheath, of the non-cellular layer (tunica propria) (Fig. 13).



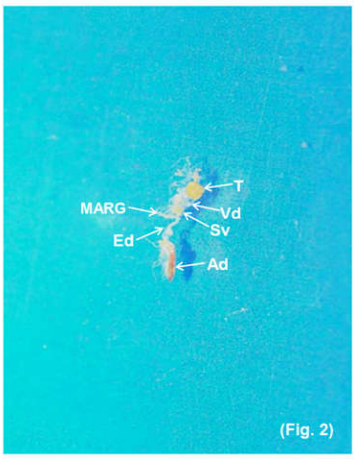
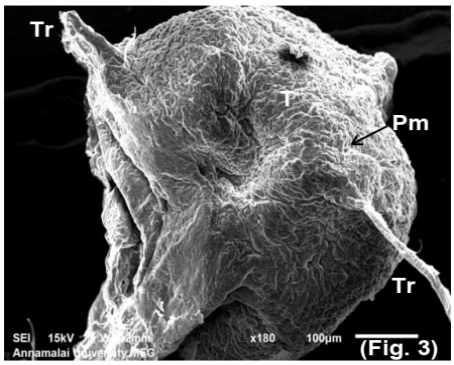
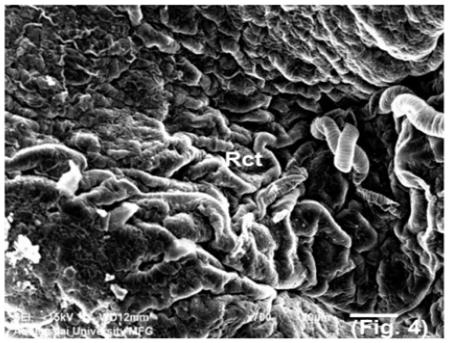


Fig. 1 and 2. Whole mount of male reproductive system of A.foveicollis and A.nifripennis

T-Testis,Sv-Seminal vesicle, Vd-Vasa differentia, MARG -Male accessory reproductive gland, Ed-Ejaculatory duct, Ad-Adeagus





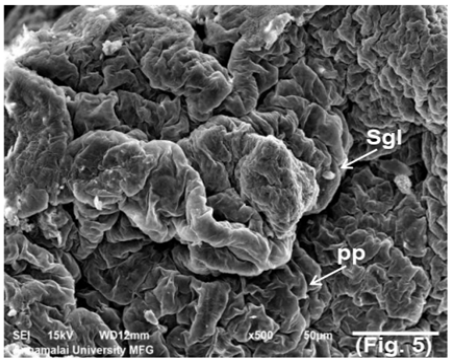
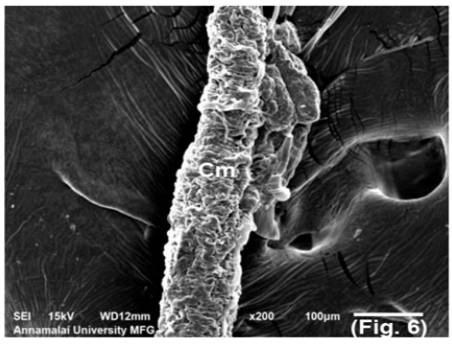
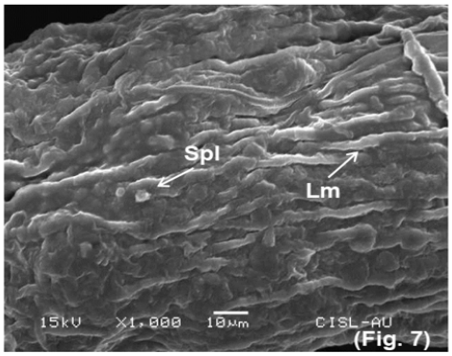


Fig.3-5. Scanning electron micrograph showing the testes of A.foveicollis





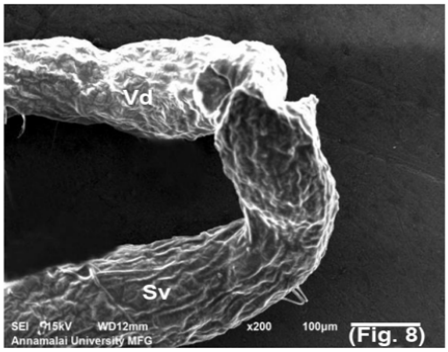
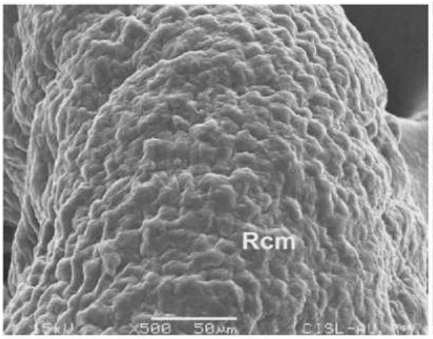
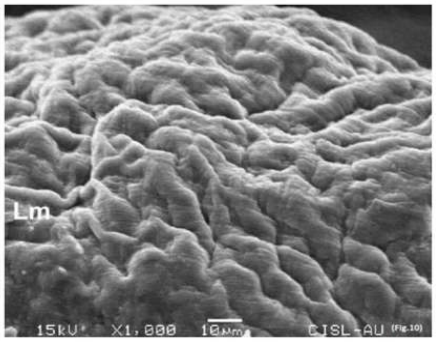


Fig.6-8. Scanning electron micrograph showing the vas deference of A.foveicollis

T-Testes, Tr-Trachea, PM-Peritnealmembrane, Rct-Ruffled connective tissue, Sgl-Secretory globule, pp-Pinocytoticpits, Lm-Longitutinalmuscle, Cm-Circular muscle, Sv-Seminal vesicle





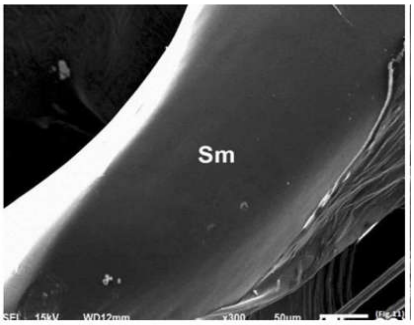


Fig. 9-11. Scanning electron micrograph showing the external view of seminal vesicle of A.foveicollis

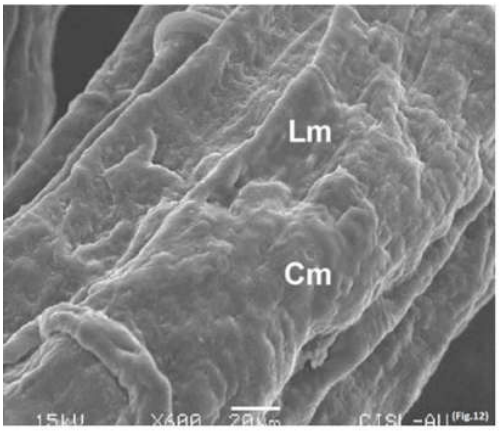
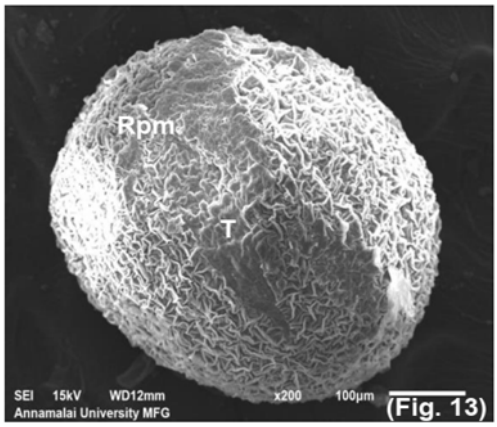
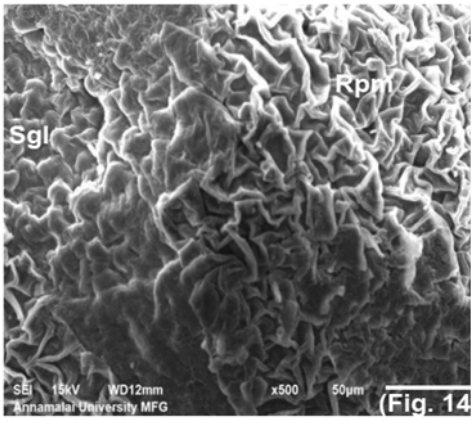
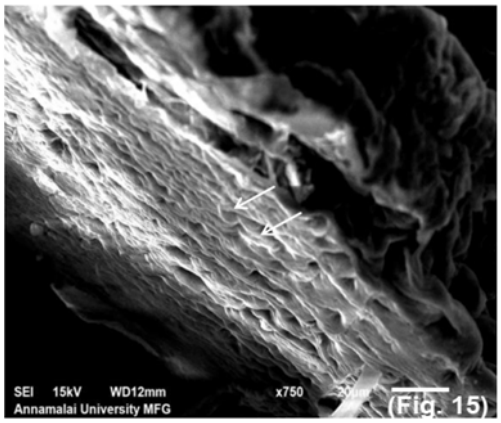
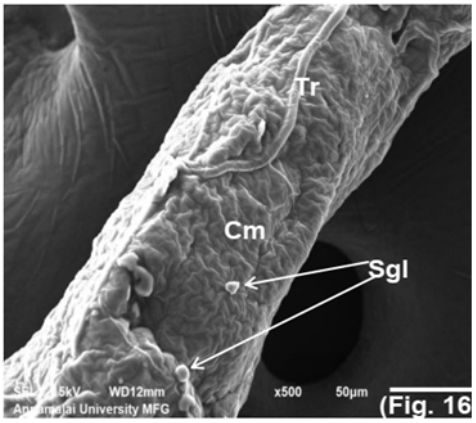


Fig. 12.Rcm-Ruffled circular muscle, Lm-Longitutinalmuscle, Sm-Secretory materials, Cm-Circular muscle Scanning electron micrograph showing the external view of MARG of A.foveicollis









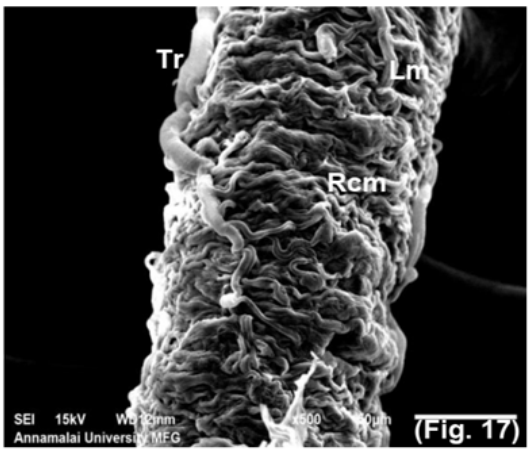
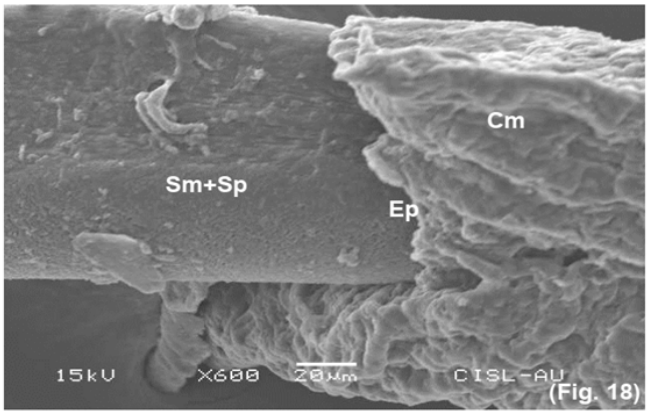


Fig. 13-17. Scanning electron micrograph showing the structure of testes of A.nigripennis T-Testes,Rpm-Ruffled peritrophicmembrane,Sgl-Scretory globule, Arrow indicate follicular cyst. Scanning electron micrograph showing the structure of vas deference and seminal vesicle of A.nigripennis Cm-Circular muscle Tr-Trachea, Sgl-Secretory globule, Lm-Longitutinalmuscle,Rcm-Ruffled circular muscle



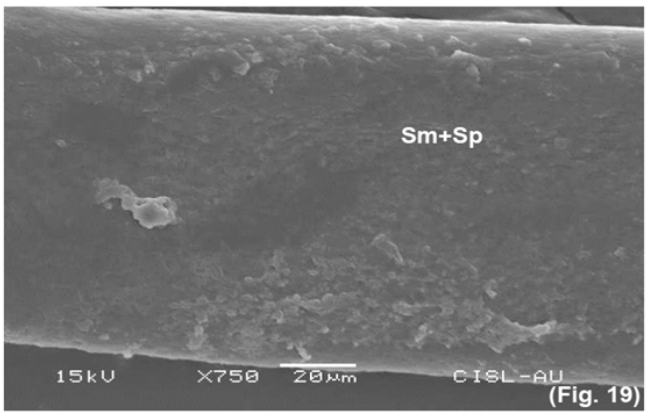


Fig. 18&19. Scanning electron micrograph showing the lumen of the seminal vesicle of A.nigripennis Sm+Sp-Secretorymaterial+ spermatozoa, Ep-Epithelium, Cm-Circular muscle

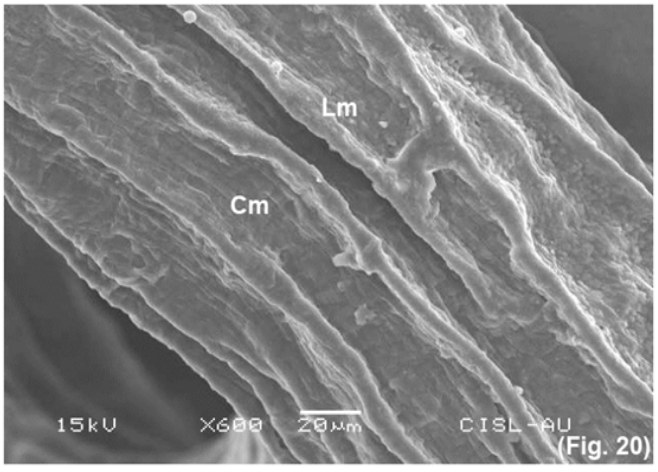


Fig. 20. Scanning electron micrograph showing the lumen of the MARG of A.nigripennisLm-Longitutinal muscle, Cm-Circular muscle

They were an arranged with intersecting horizontal and vertical lining that look like a network of threads (Fig. 14). Within the testicular follicles of *A.nigripennis* are three development zones (Fig. 15) the growth zone, where groups of spermatogonia become separated from the germarium and form into spherical clusters. These groups of cells become enclosed by several cells which form the wall of the sperm cyst. The vas deferens (through which the sperm travels) and the 'seminal vesicle' (where the sperms are stored prior to mating) were short and as cylindrical tube (Fig. 17). The seminal vesicle connects to the bulbusejaculatorius which is long and slender tube covered by circular muscles. The vas deferens of *A. nigripennis* showed slender tube with externally covered by the smooth circular muscles with a number of secretory globules. The epithelial layer consisted of numerous pinocytotic pits and also secretory granules were noticed like that of *A. foveicollis* (Fig. 16). The lumen of the seminal vesicle contained densely accumulated secretory materials with spermatozoa and also single layer of epithelial cells outside lumen were noted (Fig. 18 and 19). The male accessory glands (mesadenia) in *A. nigripennis* was tubular and milky white in color and open into the seminal vesicle at the proximal part of the ductus ejaculatorius (Fig. 20). A single accessory gland is situated in the postero-ventral region of the male abdomen. They are shown to have an abundant supply of tracheae.The seminal vesicle is externally covered by smooth circular and longitudinal muscle (Fig. 20).

DISCUSSION

The anatomical and morphological studies on male reproductive system has been made in several orders of insects, resulting in considerable improvements with its histological and ultrastructural studies of the structures that make up this organ for reproduction (Mikheyev, 2004; Ferreira *et al.,* 2004; Lemos *et al.,* 2005; Freitas *et al.,* 2010). The present study has shown that the male reproductive system of *A. foveicollis* and *A. nigripennis* is composed of a bilobed testes, vas deferens, seminal vesicle (single) and single ejaculatory duct. Such anatomical organization is consistent with that of other Chrysomelidae insects, but greatly varied from the other Coleopteran insects. The general rule of reproductive system in insect groups are composed of a paired testis with numerous testicular follicles, a pair of vas deferens, a pair of seminal vesicle and single ejaculatory duct. In the present study, on great contrast to the general rule, a testicular structure in male *A. foveicollis*and *A. nigripennis* were typically present with completely bilobed inseparable follicles in the form of fused spherical testis with single vasa deferentia, single seminal vesicle, one accessory gland and a ejaculatory duct. The present findings of unpaired testis had been attempted in some other insects such as *Eriosomalo nigerwon*, (Gautam, 1994), *Glyphinabetulae*, *Anoeciacorni* (Wieczorek and Swiatak, 2009) and Adephaga Coleopteran species, Locusta sp. (Chapman and Davis, 2004). Gemeo *et al.,* (1998) had reported that two testes have fused, and appears to be a single testis of bright green colour in *Agrotisipsilon* (Noctuidae). Lemos *et al.,* (2005) had observed intense red colour in *Podisusnigrispinus* (Pentatomidae).

The colour pigmentation observed in the present study is in agreement with Heming, (2003) and Winnick *et al.,* (2009) whose studies opined that this coating is likely to function to protect the developing gametes from harmful ultraviolet radiation. Bordas (1990) has observed the testicular tubules are enclosed in a peritoneal membrane. Davidson (1989) reported that the testicular follicles are covered with peritoneal membrane. The same phenomenon was also observed by Arunakumari (1983) in *Dysdercus similes* and Umamaheswari (2005) in *Spherodema rusticum.* The testicular structure of *A. foveicollis* and *A. nigripennis* are with short follicular lobe more or less flattened and somewhat leaf like. The follicles are held together by connective tissue and whole testes are covered by the peritoneal membrane, which has yellow

coloured pigmentation. In many insects, the seminal vesicles are not extensions of the vas deferens, they are separate paired tubular and sac like structures located posterior to the male accessory reproductive gland (Winnick, 2009). Whereas, in *A. foveicollis* and *A. nigripennis*, the seminal vesicles are dilations of the vas deferens and this type of structure were reported by Riemann and Giebultowicz, (1991) and Alves *et al.,* (2006) in lepidopteran and Vivekananthan (2011) in Coleopteran insects *M. indica*. In the present study, it has been reported that SEM changes in the MARGs exhibited tubular structure with myoepithelial cells which were covered with tracheoles. The tubules externally were covered with thick folded cuticle. The secretory epithelial cells consisted of numerous pores and secretory vesicles with short microvilli. The epithelial cells were also associated with minute secretory vesicles and secretory globules. Similar results had also been observed by Sumathi (2002) in *Gryllotalpaafricana*. According to Preethi Radhakrishnan *et al.,* (2009) on *Bactroceratryoni*, the epithelial layer consisted of numerous pinocytotic pits and microvilli also prove to trigger the epithelium to enhance it is secretory activity. In the present study, the testis of the pumpkin beetles, exhibited tubular structure with large number of primodial germ cells or spermatogonia. In *A.nigripennis* reproductive organs are very tiny and cellular structure are very small when compared to that of *A.foveicollis* which may be due to the evolutionary changes within the species.

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