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ANATOMICAL AND HISTOLOGICAL STUDIES ON THE MALE REPRODUCTIVE SYSTEM OF THE ADULT WATER BEETLE, *HYDROUS ACULEATUS* SOLIER AND THE GROUND BEETLE, *PIMELIA THEVENETI* SENAC. WITH SPECIAL REFERENCE TO THEIR PHYLOGENY

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Key words : *Hydrous aculeatus*, Hydrophilidae, *Pimelia theveneti*, Tenebrionidae.

ABSTRACT

The male reproductive system of the aquatic beetle, Hydrous aculeatus (Hydrophilidae) consists of two testes, two vasa deferentia, two vesiculae seminalis, a long ejaculatory duct and ejaculatory sac. The ejaculatory duct receives two pairs of accessory glands (ectadenia and mesadenia). Each testis consists of numerous testicular follicles. The male reproductive system of the ground beetle, Pimelia theveneti (Tenebrionidae) is made up of two testes, two vasa deferentia, two vesiculae seminalis, ejaculatory duct and two pairs of accessory glands which are referred to as ectadenia and mesadenia. There are many similarities and some differences in the histological structure of the male reproductive system of the two beetles and the observations demonstrated that the ground beetle more advanced than the aquatic beetle.

INTRODUCTION

In an earlier-work, Dekinesh et al. (1990) gave a detailed description of the structural adaptations of the central nervous system of three Egyptian beetles living in different habitats, namely *Hydrous aculeatus* Solier (aquatic beetle), *Pimelia theveneti* Senac. (ground beetle) and *Onitis alexis* Klug (flying beetle). They mentioned that the central nervous system of the aquatic and the ground beetles

represent, a more or less, primitive state, while that of the flying beetle is more specialized and adapted to withstand severe changes in the environmental surroundings. Moreover, the anterior displacement and the concentration of the abdominal ganglia, which occurs in the ground and flying beetles, respectively, could provide a probable evidence about the aquatic nature of the ancestors of coleopteran insects. Also, El-Kady *et al.* (1991) reported many structural similarities in the alimentary canal of these three beetles, which may be attributed to their monophyletic origin, yet there are clear ecomorphological differentiations in their alimentary canals. These differentiations may be attributed to the environmental variations and the available foods in their different habitats.

The gross anatomy of the male reproductive system in some tenebrionid beetles had been investigated: *Tribolium castaneum* and *T. confusum* (El-Kifl, 1953), *T. anaphe* (Hafeez and Gardiner, 1964), *Tenebrio molitor* (Jones, 1967; Happ et al., 1977; Alrubeui and Gorell, 1985), *Blaps sulcata* (Osman et al., 1982), *Troglogeneion zapoteca* (Aalbu, 1985), T. *brevicornis* (O'Dell *et al.*, 1990) and *Tribolium* spp. (Rummel and Grimnes, 1991).

The histology of the male reproductive system has been studied in limited tenebrionid species: *T. molitor* (Cameron, 1965; Heut, 1966; Gadzama, 1972; Ashraf and Brower, 1974; Gerber, 1976; Happ *et al.*, 1977; Dailey et al., 1980; Alrubeai and Gorell, 1985), *B. sulcata* (Osman et al., 1982), *T. brevicornis* (Sevener *et al.*, 1992), *T. freemani* (Melissa and Grimnes, 1994) and *T. castaneum* (Younes *et al.*, 1994).

No details has been done on the anatomy of the male reproductive system of Hydrophilidae except that on *H. triangularis* (Trimble, 1935) and *H. aculeatus* (Gonaidy, 1957). No study had been done on the histology of the male reproductive system of most of

Hydrophilid genera except that on *Speonomus hydrophilus* (Cazals and Lysiane, 1982). According to the available literature, no study had been performed on the histology of the male reproductive system of *H. aculeatus*.

Recently, investigators have demonstrated the value of the internal anatomy as a taxonomic category and as an indicator of phylogentic relationships. The phylogeny of insects is based on the morphological relationships due to the absence of complete fossil evidences. The present comparative study has been carried out with the purpose of exploring the structure of the male reproductive system of the ground and water beetles and further to clarify their position in insect phylogeny.

MATERIALS AND METHODS

The two coleopteran species were collected from the vicinity of El-Fayoum Province: *Pimelia theventi* Senac. (Tenebrionidae) was collected from the cultivated palm trees, and *Hydrous aculeatus* Solier (Hydrophilidae) was collected from the canals and swamps. The obtained insects were maintained under laboratory conditions.

For the gross anatomical studies, the adult beetles were anaesthesized with ether and dissected in Ringer's saline solution under a binocular microscope. For histological preparations, the different parts of the reproductive system were cutt off, fixed for 12 hours in alcoholic Bouin. After fixation, the organs were then washed in 70% ethyl alcohol, dehydrated, and cleared in xylol before being embedded in paraffin wax. The sections were cut with a rotary microtome at a thickness of 5 microns, mounted on slides, stained with Ehrlich's haematoxylin and counter-stained in alcoholic Eosin. The diagrams (drawings) were made with the help of camera lucida. The photomicrographs, observations and measurements were made

with a research microscope equipped with a camera and an ocular micrometer. At least five sections were used to obtain the average measurement of each structure.

OBSERVATIONS AND DISCUSSION

A - Anatomy

The male reproductive system of *Hydrous aculeatus* (Fig. 1) consists of two testes, two vasa deferentia, two vesicula seminalis, a long ejaculatory duct and ejaculatory sac. The ejaculatory duct receives two paired accessory glands, ectadenia and mesadenia.

The testes lie anteriorly in the abdominal cavity and are maintained in position by the tracheae and fat-body. Each testis consists of a great number of testicular follicles enclosed in a testicular sheath. Each testis has a cylindroconical shape and leads into the distal end of the vas deferens. The vas deferens extends posteriorly as a fine tube and turns forwards, where it enlarges noticeably to form the vesicula seminalis. The latter opens ventrally at the anterior end of the ejaculatory duct. The ejaculatory duct is a long cylindrical tube, widens near its posterior end and narrows again. This wide portion of the ejaculatory duct has been asigned as ejaculatory sac. The accessory glands consist of two pairs joining the ejaculatory duct (1) the mesadenia, are two paler and narrower tube like structures in comparison with the ectadenia, (2) the ectadenia, are two white large C-shaped thick tubes. Trimble, 1935 (in *Hydrous triangularis*) wrongly, regarded the ectadenia of one side as the testis and its vas deferens, while the dilated anterior part of the ejaculatory duct and its narrow curved parts which illustrated by him as seminal vesicle and the ejaculatory duct, respectively. Our observations agree with those described by Gonaidy (1957) on the same insect, but with respect to the ejaculatory duct we termed the wider part as an ejacultory sac.

In polyphagan beetles, two or more of mesadenia are present, but in adephagan beetles, the ectadenia are the only accessory glands (Imms, 1977). In beetles, one to four pairs of accessory genital glands of both ectadenal and measadenal may occur together (Tembhare, 1984). One pair of male accessory genital glands were found in *Speonomus hydrophilus* (Cazals and Lysiane, 1982).

The male reproductive system of the ground beetle, *Pimelia theveneti* (Fig. 2). comprises two testes, two vasa deferentia, two vesiculae seminalis, ejaculatory duct and two pairs of accessory glands which referred to as ectadenia and mesadenia.

The testes (Fig. 2) are yellowish in colour and lie on either sides of the alimentary canal, partly below the ileum. They are maintained in a fixed position by the surrounding lobes of the fat body and tracheal branches. Each testis consists of numerous, globular testicular follicles. Each follicle is connected with the vas deferens by a relatively well developed slender vas efferens. The vas deferens extends posteriorly for half its length and changes its cours to run anteriorly. At its basal portion, it dilates to form the vesicula seminalis before joining the ejaculatory duct, which is a clyindrical long tube, narrows, gradually towards its posterior end and passes into the aedeagus. Two pairs of accessory glands open into the anterior end of reproductive tract, near the union of the vesiculae seminalis and the ejaculatory duct. The mesadenia make a V-shaped insertion with the ejaculatory duct, while the ectadenia are curved and coiled.

Two pairs of male accessory genital glands were described in *Tribolium anaphe* (Hafeez and Gardiner, 1964), in *Tenebrio molitor* (Happ *et al.*, 1977), in *Blaps sulcata* (Osman *et al.*, 1982), and in *T. castaneum* (Younes *et al.*, 1994).

B - Histology

The histological structure of the testis of *Hydrous aculeatus* is illustrated in figures (3-6). Each testis is completely divided into a number of testicular follicles. The testis is ensheathed with a coat of fine fibrous connective tissue measuring about 20 μm thick. Each testicular follicle is enveloped by a squamous mesothelial layer. Romoser (1973) stated that the mesothelial layer seems to perform a nutrient transport function to the germ cells within the testicular follicles. The testicular follicles are connected with the vas deferens. The different stages of spermatogensis could be observed in the testicular follicles. The germarium occupies the upper part of the testicular follicle and contains spherical spermatogonial cells (Fig. 4 and 5). The spermatocytes possess relatively large nuclei. The spermatids are small cells. At the base of the testicular follicle, the spermatozoa are characterized by possessing filamentous tails. Such arrangement had been early reported in *Passalus cornutus* by Krause (1946). He described the testis as nudule shaped and comprising two of short, radially arranged blind tubes. Each tube gives rise fine duct, which ultimately unite to form the vas deferens. The periphery of each tube contained the spermatogonial cells, the central part was filled with mature spermatids and the basal part was filled with spermatozoa.

Wigglesworth (1972) mentioned that the testis is made up of a series of tubular follicles varying greatly in number and arrangement in insects. Crawson (1981) stated that the testicular follicles in Coleoptera differ greatly in number, form, and arrangement. The number may be reduced, two or even one, or increased to scores or even hundreds as in some Meloidae. The male reproductive system of *Speonomus hydrophilus* consists of two testes, each made of one follicle in which spermatogenesis is cystic (Cazals and Lysiane, 1982).

The vas deferens (Fig. 7) consists of a single layer of secretory cubodial epithelial cells measuring about 10 μm in thickness. The epithelial cells possessing large spherical nuclei measuring about 5 μm in diameter. The muscularis of the vas deferens is mainly formed of circular muscle layer measuring about 10 μm in thickness. The epithleium has no cuticular lining. Similarly, there is a coat of muscles surrounding the epithelial cells of the vas deferens in *Phyllophaga anxia* (Berberet and Helms, 1972), in *Zygogramma exclamationis* (Gerber *et al.*, 1978), and in *Blaps sulcata* (Osman et al., 1982). However, there is no muscles around the vas deferens of *Coccinella septumpunctata* (Singh et al., 1979) and in *Tribolium castaneum* (Younes *et al.*, 1994).

The vesicula seminalis is similar to the vas deferens however, the lumen of the vesicula seminalis is comparatively wider. The wall of the vesicula seminalis (Fig. 8) is composed of an inner epithelium and an outer muscularis. The epithelium is not lined with intima and consists of columnar secretory cells measuring about 80 μm in thickness, each with spherical nucleus of about 20 μm in diameter. The muscularis is mainly formed of circular muscle layer measuring about 15 μm in thickness. The lumen of vesicula seminalis is filled with semen.

Histologically, the ectadenes (Figs. 9 and 10) have a thick layer of mucosa of about 120 μm thick and composed of numerous tubular glands. Each tubular gland contains secretory material similar to that present in the lumen of the ectadene. The mucosa is surrounded with a thin layer of circular muscle layer measuring about 12 μm in thickness and has no intima lining. The muscular layer is surrounded by a thin peritoneal membrane. The lumen has been observed to contain acidiophilic secretion. Similarly, there is no intima and longitudinal muscle fibres have been observed in the ectadenes of *Blaps sulcata* (Osman et al., 1982).

The histological structure of the mesadenes shows a columnar epithelium (Fig. 11) measuring about 30 μm in thickness and have oval nuclei of about 8 μm in length and 5 μm in width. The epithelium is surrounded by a muscularis of about 25 μm thick, consisting of inner circular muscle fibres and outer longitudinal muscle fibres. A similar structure had been described in the mesadenes of *Tribolium castaneum* (Younes et al., 1994).

The ejaculatory duct (Fig. 12) consists of simple columnar epithelium measuring about 12 μm in thickness, provided with spherical nuclei of about 4 μm in diameter. The epithelium is lined internally with thin intima. The muscularis is well developed measuring about 90 μm thick and comprises alternated longitudinal and circular muscle fibres. Hinton (1974) mentioned that the sperms transferred within the male reproductive ducts by muscle contractions of the vasa deferentia and ejaculatory duct.

The ejaculatory sac (Fig. 13 and 14) is composed of the epithelium internally and the muscularis externally. The epithelium consists of secretory cells measuring about 40 μm in thickness and possessing densely stained spherical nuclei. The muscularis is differentiated into an inner longitudinal muscle layer measuring about 10 μm in thickness and an outer circular muscle layer of about 20 μm in thickness. Outside the muscularis there is a numerous secretory vesicles. The ejaculatory sac needs histochemical studies in order to define the nature and functions of its secretion. In *Antheraea pernyi* a polypeptide secreted by the ductus ejaculatorius activates the sperms which, prior to ejaculation, are motionless in the seminal vesicles (Shepherd, 1974 and 1975).

The histological structure of the testis of *Pimelia theveneti* is illustrated in figures (15-18). Each testis has numerous acinous testicular follicles. The testicular follicles connect the vas deferens by a relatively well developed slender vas efferens. The testicular

follicles are enclosed by a thin layer of syncitial epithelium. The different stages of spermatogenesis are observable in the testicular follicle. At its apical portion, it contains a spermatogonial masses, below which there are clusters of spermatocytes and spermatids. The sperm bundles occupied a major portion of the testicular follicles.

The vas efferens (Fig. 15) consists of a thin layer of epithelium measuring about 3 μm in thickness. The nuclei are spherical of about 2 μm in diameter. The vas efferens has neither intima nor muscle coat.

Histologically, the vas deferens (Fig. 19) consists of inner epithelial cells and an outer coat of muscle fibres. The epithelium has no intima, and made up of columnar cells measuring about 25 μm in thickness and possessing spherical nuclei of about 5 μm in diameter. The muscularis is differentiated into an inner circular muscle layer and an outer longitudinal muscle. The circular muscle layer is about 8 μm thick, while the longitudinal muscle layer is about 4 μm in thickness.

The vesicula seminalis (Fig. 20) is composed of an inner epithelium and an outer muscularis. The epithelium consists of a layer of cuboidal cells measuring about 23 μm in thickness, each with spherical nucleus of about 5 μm in diameter. The muscularis is differentiated into an inner circular muscle layer of about 15 μm thick and an outer longitudinal muscle layer measuring about 5 μm in thickness.

Histologically, the ectadenes (Fig. 21 and 22) have a highly columnar epithelium measuring about 80 μm in thickness, in which the nuclei are spherical shaped. Outside the epithelium there is a layer of muscle. The muscle layer consists of an inner circular muscle

fibres measuring about 20 μm in thickness and an outer longitudinal muscle fibres measuring about 5 μm in thickness.

The wall of the mesadenian gland (Fig. 23) is composed of a layer of glandular highly columnar epithelial cells. These cells measuring about 20 μm in thickness and possess spherical nuclei measuring about 5 μm in diameter. The epithelium has no cuticular lining. The epithelium is surrounded externally by the muscularis which is differentiated into an inner circular muscle layer, about 10 μm thick and an outer longitudinal muscle fibres of about 5 μm thick. A similar structure had been described in the mesadenes of *Tenebrio molitor* by Happ *et al.* (1977) and in *Blaps sulcata* by Osman et al. (1982).

The ejaculatory duct (Fig. 24) is provided with a powerful muscular coat mainly consisting of circular muscle fibres, measuring about 250 μm in thickness. The epithelium is made up of cuboidal cells measuring about 25 μm in thickness and possessing spherical nuclei of about 4 μm in diameter. The epithelium is lined with thin intima.

Melissa and Grimnes (1994) stated that the general pattern for the male reproductive accessory complex in family Tenebrionidae appears to consist of two pairs of accessory glands which are attached to the seminal vesicles and the ejaculatory duct. The first set of glands, the tubular accessory glands (TAGs), are elongated glands with a single cell type and a uniformly staining soluble material within the lumen. The second set of glands are highly musculated, have long thin cells and contain opaque secretions. The second pair of glands has been variously identified as BAGs, PAGs or RAGs. The morphology of the bean-shaped accessory gland (BAGs) of *Tenebrio molitor* and its individual cell types have been described (Dailey et al., 1980). The lesser mealworm, *Alphitobius diaperinus*, also has

BAGs (Hopkins et al., 1993). The accessory glands of *Tribolium brevicornis* has been shown to be pear-shaped (PAGs) (O'Dell et al., 1990) and five cell types have also identified in *Tribolium brevicornis* (Sevener et al., 1992). Rod-shaped (PAGs) glands have been identified in *Tribolium anaphe* (Hafeez and Gardiner, 1964) and *Tribolium freemani* (Rummel and Grimnes, 1991).

Dekinesh et al. (1990) found structural similarities in the central nervous system of the two beetles discussed herein. Also, El-kady et al. (1991) found structural similarities in the alimentary canals of the same insects and they mentioned that the alimentary canal of the aquatic beetle *H. aculeatus* is more or less primitive state, while that of the ground beetle resembles a more specialized derived state. Odum (1971) mentioned that the range of climatic variations is more pronounced in the water than in the terrestrial habitat. Finally, there are many similarities and some differences in the male reproductive system of the two beetles discussed and the observations cited in the present work demonstrated that the ground beetle more advanced than the aquatic beetle and may support the consept that the polyphagous coleopterans originated from a common ancestor and the ancestors of land arthropods are aquatic (Boudreaux, 1979 and Hennig, 1981).

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FIGURES

Fig. 1. : A diagram showing the male reproductive system of the aquatic beetle, *Hydrous aculeatus* Solier.

Fig. 2. : A diagram showing the male reproductive system of the ground beetle, *Pimelia theveneti* Senac.

Fig. 3. : S.S of the testis of *H. aculeatus* showing the testicular follicles (Tf).

Fig. 4. : L.S of the testicular follicles of *H. aculeatus* showing the spermatogonia (Sg) and the spermatocytes (Sc.).

Fig. 5. : L.S of the testicular follicles of *H. aculeatus* showing the spermatocytes (Sc) and the spermatids (St).

Fig. 6 : L.S of the testicular follicles of *H. aculeatus* showing the spermatids (St) and spermatoza (Sz).

Fig. 7 : T.S of the vas deferens of *H. aculeatus.*

Fig. 8 : T.S of the vesicula seminalis of *H. aculeatus.*

Fig. 9 : T.S of the ectadenia accessory gland of *H. aculeatus.*

Fig. 10 : A magnified part of T.S of the ectadenia accessory gland of H. aculeatus showing the glandular epithelium (Ep), the circular muscle (Cm) and the secretion (S).

Fig. 11 : T.S of the mesadenia accessory gland of *H. aculeatus.*

Fig. 12 : T.S of the ejaculatory duct of *H. aculeatus.*

Fig. 13 : T.S of the ejaculatory sac of *H. aculeatus*.

Fig. 14 : A magnified part of the ejaculatory sac showing the secretory epithelium (Ep), the circular muscle (Cm) and the secretory vesicles (V).

Fig. 15 : L.S of the testis of *Pimelia theveneti* showing two testicular follicles (Tf) and the vasa efferentia (Ve).

Fig. 16 : T.S of the testis of *P. theveneti* showing the testicular follicles (Tf).

Fig. 17, 18 : L.S of the testicular follicle of *P. theveneti* showing a group of spermatogonia (Sg), spermatocytes (Sc), spermatids (St) and spermatozoa (Sz).

Fig. 19 : T.S of the vas deferens of *P. theveneti*.

Fig. 20 : T.S of the vesicula seminalis of *P. theveneti*.

Fig. 21. : T.S of the ectadenia accessory gland of *P. theveneti*.

Fig. 22. : A magnified part of T.S of the ectadenia accessory gland of *P. theveneti*.

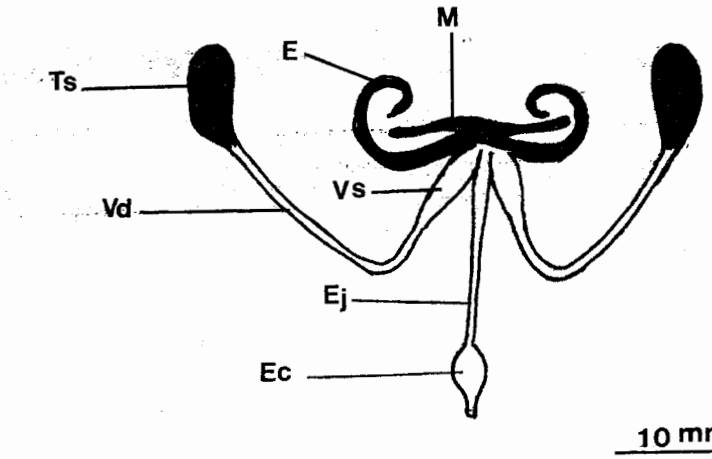
Fig. 23. : T.S of the mesadenia accessory gland of *P. theveneti*.

Fig. 24. : T.S of the ejaculatory duct of *P. theveneti*.

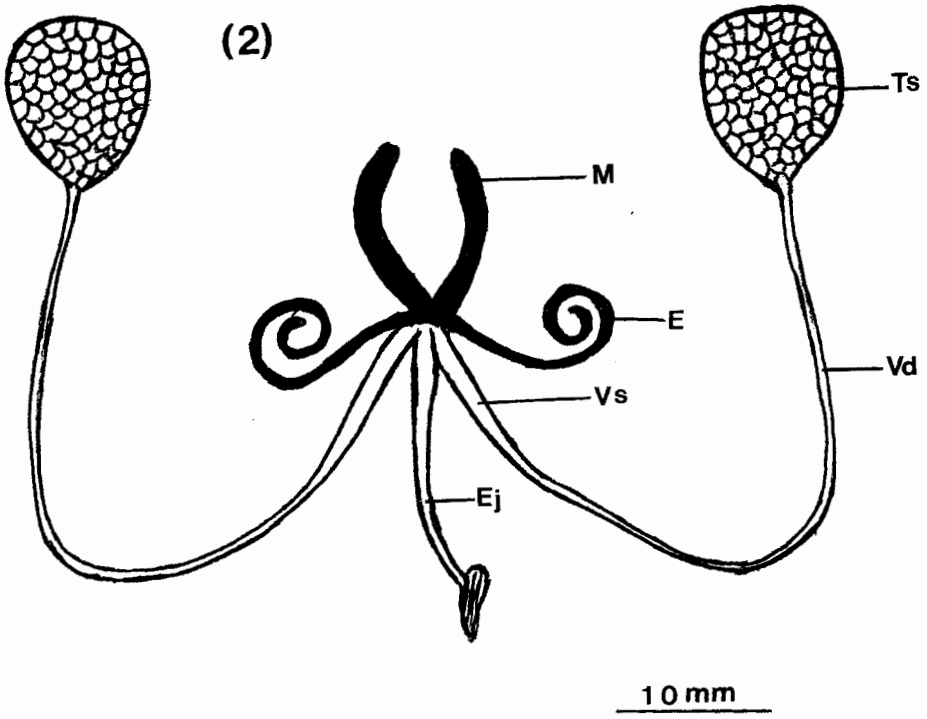
LIST OF ABBREVIATIONS

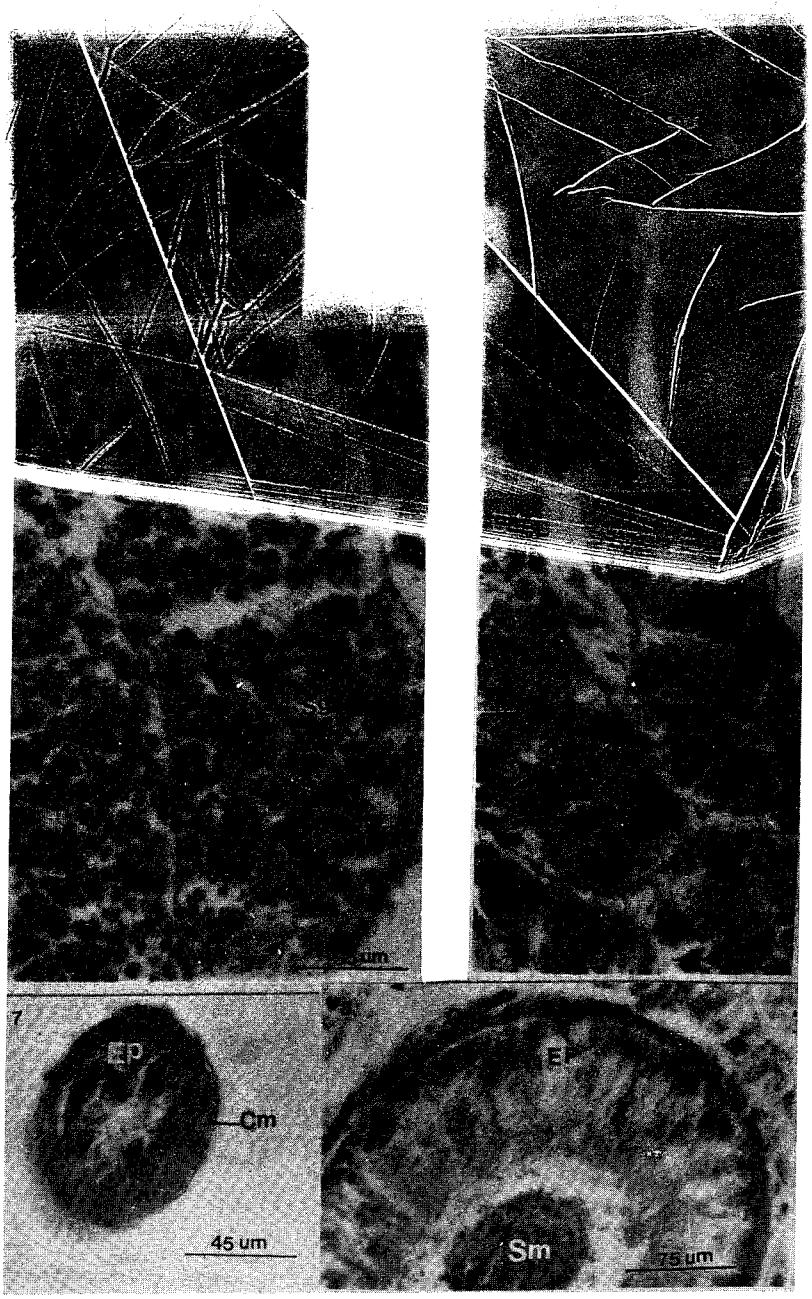
Cm : Circular muscles
Ct : Connective tissue
E : Ectadenia
Ec : Ejaculatory sac
Ej : Ejaculatory duct
Ep : Epithelium
In : Intima
L : Lumen
Lm : Longitudinal muscles
M : Mesadenia
Me : Mesothelial layer
Mu : Mucosa
N : Nucleus
Pm : Peritoneal membrane
S : Secretion
Sc : Spermatocytes
Sg : Spermatogonia
Sm : Semen
St : Spermatids
Sy : Syncitial epithelium
Sz : Spermatozoa
Tf : Testicular follicle
Tg : Tubular gland
Ts : Testis
V : Vesicles
Vd : Vas deferens
Ve : Vas efferens
Vs : Vesicula seminalis.

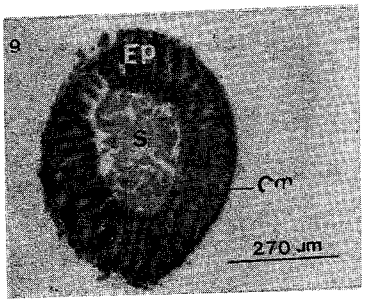
(1)

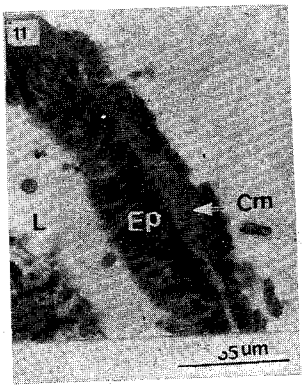


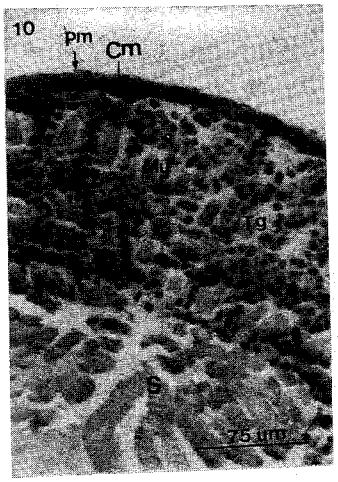
(2)

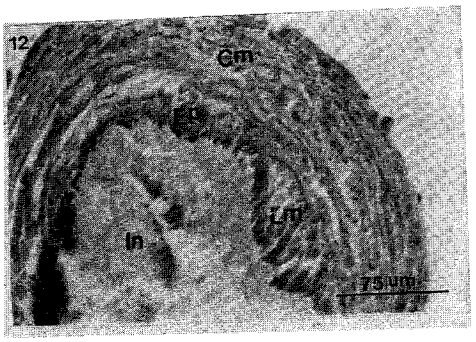


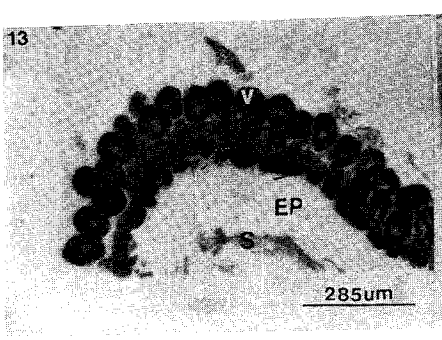

7
EP
Cm
45 um
EP
Sm
75 um

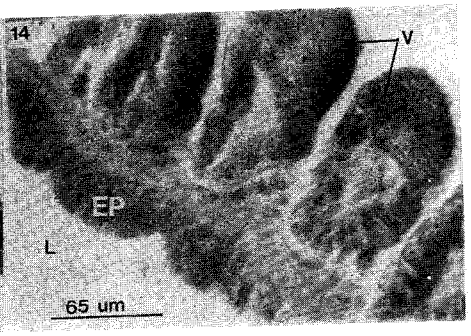

9
EP
S
Cm
270 um


11
L
EP
Cm
25um

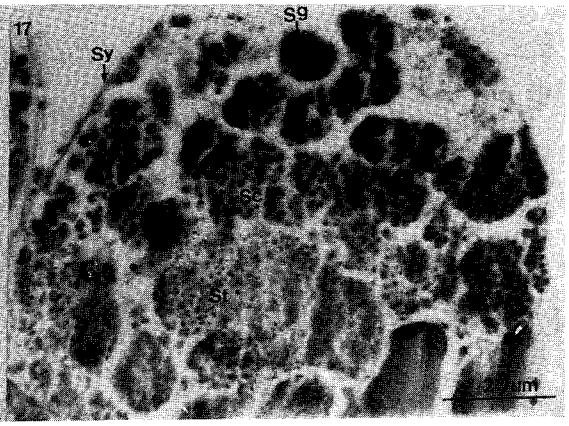

10
Pm
Cm
EP
S
75 um

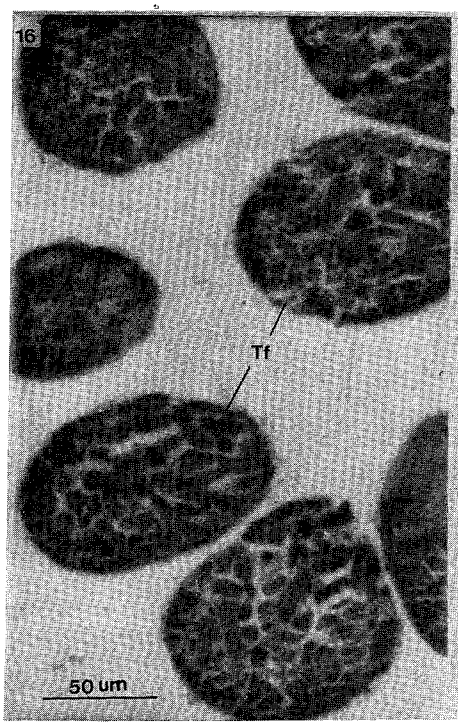

12
Cm
EP
In
L.m
75 um

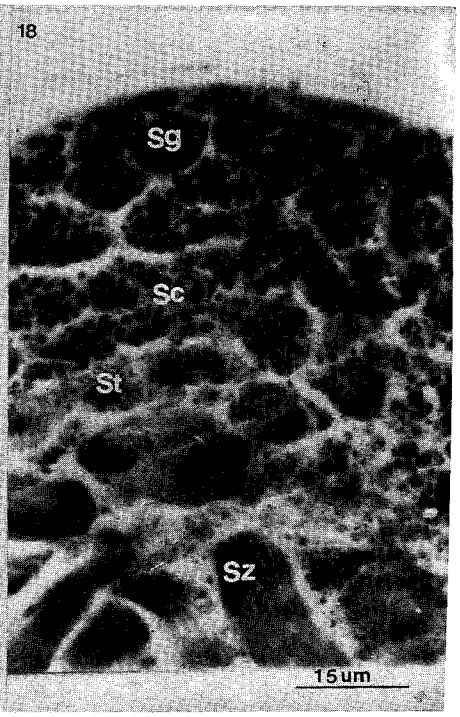

13
V
EP
S
285um

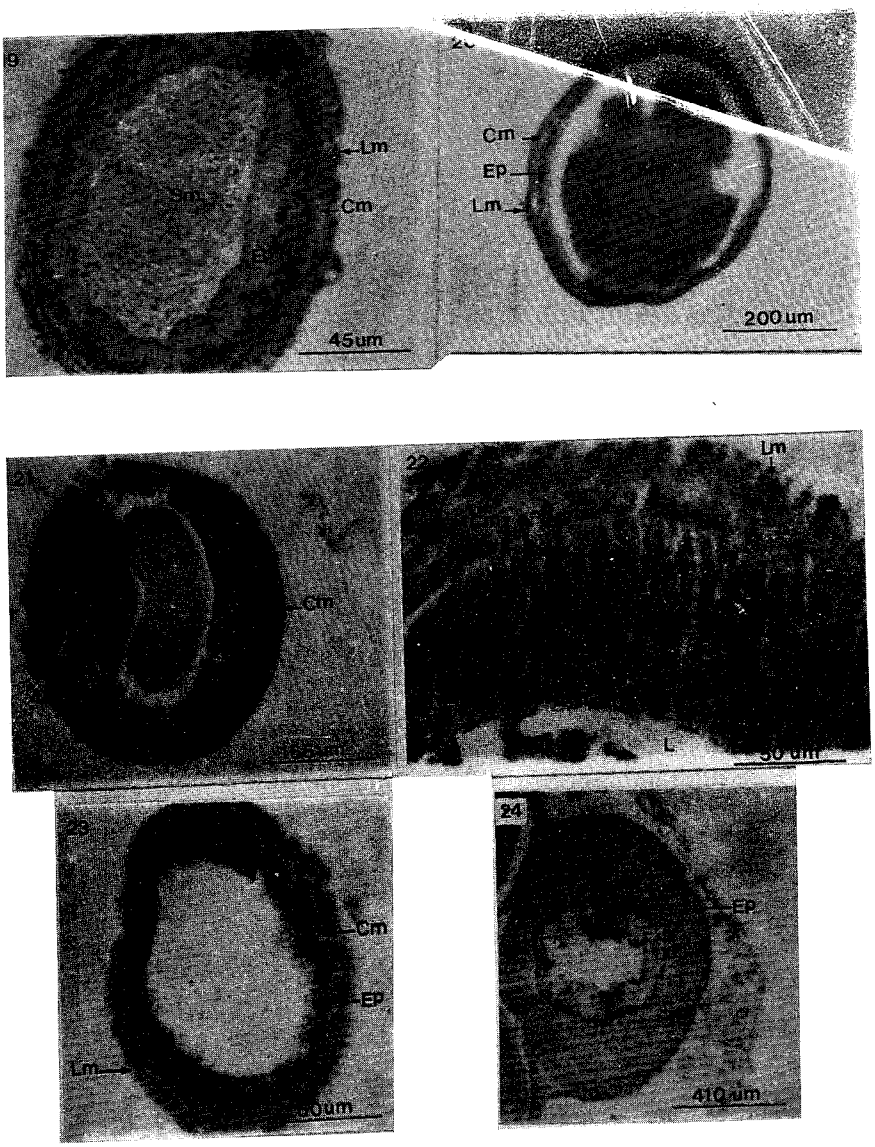

14
V
EP
L
65 um


15
Tf
Ve
70um


17
Sg
Sy
Sc
St


16
Tf
50 um


18
Sg
Sc
St
Sz
15 um


Lm
Cm
45um
Cm
Ep
Lm
200 um
Cm
Lm
L
Ep
Cm
Ep
Lm
410 um

دراسات تشريحية وهستولوجية على الجهاز التناسلى الذكرى للخنفساء المائية البالغة هيدرس أكيولاتس والخنفساء الأرضية بيميليا ثيفينتى مع الإشارة بوجه خاص إلى نشأتهم التطورية

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قسم علم الحيوان – كلية العلوم – جامعة المنوفية
شبين الكوم – مصر

يتركب الجهاز التناسلى الذكرى فى الخنفساء المائية هيدرس أكيولاتس (هيدروفيليدى) من خصيتين ووعائين ناقلين وحويصلتين منويتين وقناة قاذفة تنتهى بكيس ، وتتسلم القناة القاذفة زوجين من الغدد المساعدة التناسيلية (ectadenia and mesadenia) . ويتكون الجهاز التناسلى الذكرى للخنفساء الأرضية بيميليا ثيفينتى (تنيبر يونيدى) من خصيتين ووعائين ناقلين وحويصلتين منويتين وقناة قاذفة وزوجين من الغدد المساعدة التناسلية (ectadenia and mesadenia) . وقد أوضحت الدراسة العديد من أوجه الشبه وبعض الإختلافات فى التركيب الهستولوجى والتشريحى للجهاز التناسلى الذكرى فى كلا النوعين من الخنافس إضافة إلى ذلك أوضحت الدراسة أن الخنفساء الأرضية أكثر تطوراً من الخنفساء المائية .