B). Anatomy and histology of male reproductive system of *Heterometrus xanthopus* (Pocock) (Scorpionidae):

The members of species were regularly collected from the Alandi, Kharadi, Wagholi, Jejuri and other eastern portion of Pune and surrounding area (Maharashtra, India). The male members were separated from female. Generally males are shorter and slender than females. The tergum of male fused with sternum with indistinct pleural membranes. In male pedipalps and chelae are longer and narrower than females. Females are characterised by having smaller pectinal teeth.

Some of the collected specimens were brought to laboratory for further study and others were released in their natural habitat after observing their morphological characters, habits, habitats, population etc. In laboratory the specimens were maintained for few days to months. Generally fresh and mature specimens were used for anatomical and histological studies.

The male specimens chosen for further study were separated from other members and kept in empty glass jar containing cotton ball soaked with chloroform, covered with petridish, for anaesthesia. Anaesthetized specimens were immediately kept in wax tray containing scorpion Ringer's solution (Ahern and Hadley 1976). The specimens were dissected in very short time to avoid post-mortem changes. Dissection was carried under stereoscopic binocular microscope. The male reproductive system and its associated glands were observed and exposed under binocular microscope for camera lucida diagrams. Camera lucida was attached to binocular microscope to sketch diagrams. The magnification of drawing was also noted. Some dissected specimens were also exposed for photography.

The most widely followed terminology is used in the following description of the male reproductive system of *Heterometrus xanthopus* (Pocock) (Scorpionidae): Pavlowsky 1924a, Tembe and Awati (1944), and for a histological discussion, Abd-el-Wahab (1957).

The general anatomical structure of male reproductive system of *H. xanthopus* bears three paired loops of testies extending from 3rd to 6th mesosomal segments. The testies anteriorly continue as pair of vasa deferentia and open into organ known as vesicula seminalis, attached to paraxial organ. In *H. xanthopus* the paraxial organ is short and lamelliform, opens in gonopore.

Each testis is composed of two longitudinal and four transverse tubes, interconnected together to form three loops (Fig.4B.1 A & B). One of the longitudinal tubes from each pair of testis is present along the peripheral body cavity and other along
the medial line of mesosomatic cavity. All the longitudinal and transverse tubes of testies are extremely delicate, flexible, transparent and white. They are comparatively narrow and uniformly calibrated throughout their whole length (Fig.4B.1 A & B). Each measures about 18-20 mm in length and 0.2-0.5 mm in diameter. These structures are embedded in hepatopancreatic mass.

There are two vasa deferentia, coming out from the outer angle of the anterior loops of the testies. Each vas deference runs forwards to join with the vesicula seminalis (Fig.4B.1 A & B). Each vas deference is composed of proximal (Pvd) and distal parts (Dvd). Its proximal part is more folded, slender and delicate. Its distal part is somewhat straight, dilated than proximal part. Each vas deference measures about 8-10 mm in length and 0.2-0.3 mm in diameter. Vas deference and seminal vesicle opens into middle portion of paraxial organ. The vesicula seminalis are paired, transparent, elongated, tubular structures. Each lies parallel to the vas deference and connected with the anterior end of vas deference. Vesicula seminalis measures about 4-5 mm in length and 0.3-0.6 mm in width. The anterior potion of vesicula seminalis is slightly slender and more delicate than distal.

In *H. xanthopus* each paraxial organ is elongated lamelliform and extended from genital opening to fourth mesosomal segment (Fig.4B.1 A & B & Fig.4B, 10: A.). Each paraxial organ consists of ejaculatory sac supported by supporting stalk. The ejaculatory sac is white and extremely elongated hollow structure. It extends longitudinally along the inner border of the body up to 4th segment. Each paraxial organ measure about 10-12 mm long. It is broader at the base as well as middle portion, while narrowed at posterior portion. The middle portion of this organ receives ducts from vas deference and receptacle seminalis.

**Histology**

For histological preparation of tissue, Bouin’s fluid prepared in Ringer’s solution is used as fixative, dehydrated with passing through alcohol grades. Benzene was used as clearing agent, and paraffin blocks were prepared. The transverse sections (T.S.) and longitudinal sections (L.S.) of vas deference, testis, glands and paraxial organ were taken. Histological slides were prepared as per the methods mentioned under chapter No.3. These permanent slides were studied, observed under higher magnifications.

A transverse section of the testis tube has a diameter about 393µm (Fig.4B.2), covered on the outside by a basement membrane surrounded by a thin layer of connective tissue and extremely thin layer of circular muscles. The germinal epithelium
rest on the basement membrane. The cells of germinal epithelium are flat with large
distinct nuclei. Germinal epithelium, basement membrane, connective tissue and
smooth muscle layer measure about 5.5 µm in thickness. Germinal epithelium is divided
by septa into different lobules. There is lumen in the centre of testis. A careful study of
the transverse section of testis of a mature specimen shows different stages of the
spermatogonia, spermatids and spermatozoa. The sperms are seen in bunches in the
lumen. The spermatozoa have an elongated head and long tail.

The germinal epithelial cells of testis undergo meiosis to form spermatogonia.
The dividing cells (DC) are arranged at the periphery of testis (Fig.4B.2). These cells
are larger than germinal epithelium (GE), individual spermatogonia (SG) and
spermatids (ST). The dividing cells are present in ovoid lobules measuring about 75.5
µm broad. These cells are arranged in groups. Each group consists of 12-14 dividing
cells. However the changes in chromosomal structure in dividing cells have not been
noted.

Spermatogonia (SG) are present in lobules and are arranged in groups
(Fig.4B.3). About 8-10 lobules of spermatogonia are located in periphery of testis or
present adjacent to dividing cells (Fig.4B.2). Each lobule measures about 72.5µm in
diameter (Fig.4B.3). Generally each lobule consists of either 20,24,28,32,36,or 40
spermatogonia. It forms loose masses of small, rounded similar cells containing a small
amount of cytoplasm with dense chromatic material. A thin wall encloses each group of
similar cells. The groups of cells are embedded in thick liquid material. It may be a
nutrient material supplied to developing cells.

The spermatogonia undergo phases of maturation to form small thick spermatids
(ST). These are the smaller cells with dark stained nuclei. These cells are also present in
ovoid lobules measuring about 72µm in diameter (Fig.4B.4). The cells are arranged in a
group, to form a bunch of cells inside the lobules. A bunch of cells measure about
59.2µm broad. Each group consists of either 20,24,28,32,36,or 40 cells as like
spermatogonia. There is 13-14µm irregular gap present between the wall of lobule and
bunch of cell.

The spermatids undergo maturation to form sperm heads (SH). Sperm heads are
arranged in a group around the nutrient (Fig.4B.5). It develops further to form head and
middle piece. Sperm tail develops later. An immature sperms remain attached to the
secretory nutritive fluid, where they aggregate, may take nutrients and develop into
spermatozoa. Thus, the spermatids develop into spermatozoa, set free into the lumen of
the testis, and form clusters of sperm called sperm packets (Fig.4B.6). From the lumen
of testis, along with nutrient they enter in vas deference to hemispermatophore along with seminal reservoirs.

A mature sperm (Fig.4B.7B & Fig.4B.7B) consists of head, middle piece and tail. The head of sperm is elongated. It is broader at the proximal end and narrower towards middle piece. The middle piece of sperm is much longer than head. There is no clear demarcation between head, middle piece and tail. The tail is the last portion of the sperm. It gets stained lightly than other parts. The mature sperm measure about 47µm in length.

Histologically, each vesicula seminalis is surrounded by thin wall of epithelial cells (Ep) (Fig.4B.8). The epithelial cell lining covers the internal spongy mass of secretory cells. These secretory cells are made of columnar cells, each with distinct nucleus and secretory granules (Gc). The vesicula seminalis encloses lumen, which is continues anteriorly and open in middle portion of paraxial organ.

The paraxial organ of *H. xanthopus* was removed from the base and kept in fixative for further histological study. The paraxial organ was washed in water and softened in hot 4% caustic potash (ABD Wahab, 1957). It was dehydrated passing through alcohol grades, kept in benzene for clearing and blocks were prepared. The longitudinal and transverse serial sections were taken. Stained with haematoxylin and eosin was used as counter stain.

In *H. xanthopus* each paraxial organ consists of ejaculatory sac supported by supporting stalk. The wall of ejaculatory sac of paraxial organ is externally covered with thin smooth muscle (Sm) layer enclosing a connective tissue (Ct) capsule (Fig.4B. 10: B-E). Internally it is lined by thick columnar epithelium (CE) resting on basement membrane. The lining of epithelium continues throughout the inner border of ejaculatory sac. The ejaculatory sac consists of four main parts viz, Basal plate (Bp), Trunk (Tr), Capsule (Cap) and Lamella (Lam). The basal plate is thin and flat. The basal plate consists of inward foldings of columnar epithelium (CE) (Fig.4B. 10: B). The lumen of basal plate contains nutritive fluid (Nf). At the junction of basal plate and trunk a slit like (Sl) opening is present. The inner lining of the trunk region consists of thick layer of epithelium, enclosing a lumen with nutritive fluid (Fig.4B. 10: B). The capsular region (Fig.4B. 10: C) shows quite complicated structure than other portion. The ventrolateral portion of this region consists of oblique slit like opening covered by membranous lid (Fig.4B.9). Internally the lumen of capsular region consists compartments, the chitinious supporting stalk forms ‘ T ’ shaped structure (Fig.4B.9). The epithelial lining forms a repetitive folding along with nutritive fluid containing
sperm packets (Fig. 4B. 10 C). The lamellar portion is internally lined by thin epithelial lining enclosing a lumen filled with nutritive fluid (Fig. 4B. 10: D). The chitinous supporting stalk extends dorsally from the base of posterior border of capsular region to the distal portion of lamella (Fig. 4B. 10: E).