Chap. 7. Discussion and conclusion

Nervous system:

The results obtained from the study of *Heterometrus xanthopus* (Pocock) (Scorpionidae) and *Orthochirus bicolor* (Pocock) (Buthidae) together with the research carried out on the nervous system of other scorpion species permit to identify the series of structures those should be included in a basic plan of order Scorpiones. There are homologous structures occupying highly similar position as well as possessing the same specific features as other species studied by Millot and Vachon, 1949; Awati and Tembe (1952); Sasira Babu (1965); Hjelle, 1990; Polis, 1990 and Horn and Achaval, 2002.

Scorpions are one of the early land arthropods, have believed to preserved an ancient nervous system organization, but one that is functionally capable of integrating highly sophisticated senses, and producing coordinated movements in locomotion, burrowing, and attacking prey. Compared with other invertebrates, arachnids generally have highly cephalized nervous systems representing the fusion of many segmental ganglia (Polis, 1990).

The dorsal portion of the cephalothoracic mass is more noticeably equivalent to the “Brain” of other arthropods, but comparisons of its histological structure with those of the protocerebrum, deuterocerebrum, and tritocerebrum of other arthropods are not precise. Moreover, since arachnids lack antennae, a deuterocerebrum is not defined in the adult. As a result, many different names have been given to divisions of the cephalothoracic mass as dorsal “Brain” including protocerebrum and tritocerebrum and ventral sub-oesophageal ganglion (Bullock & Horridge, 1965); dorsal supra-oesophageal ganglion and ventral sub-oesophageal ganglion (Sasira Babu, 1965); dorsal cerebral ganglion and ventral sub-oesophageal ganglion (Henry 1949); dorsal cephalic or dorso-pharyngeal ganglion and ventral thoracic or ventro-pharyngeal ganglion (Birula 1917). A microscopic examination of microtome sections of the brain shows four parts i.e. the dorsal one being the fore-brain followed by the midbrain, hind brain and accessory brain (Awati and Tembe, 1952). The most logical divisions, based upon histological information, seem to be as follows: the supra-oesophageal ganglion is equivalent to the protocerebrum, the circum oesophageal connectives are derived from the tritocerebrum and the sub-oesophageal ganglion, representing the fusion of several ganglia, contains neural centers controlling segments of the cephalothoracic region (Polis, 1990).
The scorpions have morphologically primitive nervous system in which the ancestral organization has been preserved (Polis 1990; Root, 1990). The basic layout consist of an anterior cephalothoracic mass divided into a dorsal supra-oesophageal ganglion and ventral sub-oesophageal ganglion connected by oesophageal connective, as well as a long double ventral nerve cord that originates in this cephalothoracic mass and extends posteriorly. The ventral nerve cord composed of seven free ganglia joined longitudinally by connective and laterally by commissure (Millot and vachon, 1949, Hjelle 1990, Horn and Achaval, 2002). The central nervous system of both the scorpions *H. xanthopus* and *O. bicolor* consists of basic layout of central nervous system.

The central nervous system of scorpion is ensheathed by fibrous outer layer of connective tissue and cellular inner perineurium. The connective tissue sheath of scorpion is composed of collagen fibrils embedded in a mucopolysaccharide matrix. The perineurium acts as a blood brain barrier with numerous tight junctions between cells in a perineurium selectively restricting the passage of molecules into the central nervous system (Polis, 1990).

The protocerebrum of *O. bicolor* situated on antero-dorsal surface of the sub-oesophageal ganglion (Fig.5A.2 A, B). This location of protocerebrum in *O.bicolor* appears similar to the other scorpions such as to *Centruroides* sp. (Buthidae) (McClendon, 1904); *Uroctonus mordax* (Vejovidae) (Henry, 1949); *Tytius serrulatus* (Buthidae) (Lucas, et.al. 1965), *Bothriurus bonarisis* (C. L. KOCH, 1842) (Horn, A.C.M.and Achaval, M. 2002). While the protocerebrum of *H. xanthopus* is not situated exactly on the antero-dorsal surface but it is situated most anterior to the sub-oesophageal ganglion (Fig: 4A.2 A, B). Previous references do not explain this type of location of protocerebrum.

The median and lateral eyes nerves, rostral nerves, cheliceral nerves, pedipalpal nerves, ambulatory nerves, pectinal nerves, lateral nerves of first six ganglia and posterior nerves of the seventh ganglion show same specifications and innervations as in other species studied (McClendon, 1904; Henry, 1949; Sasira Babu, 1965; Lucas, et.al. 1965, Horn and Achaval, 2002).

The major nerves, tracts, and neural centers of the cephalothoracic mass have been described by Sasira Babu (1965). The protocerebrum of both the scorpions under study shows major nerves and neural centers as described by Sasira Babu (1965). The protocerebrum contains (Fig: 4A.5 and Fig: 5A.6) four bilateral optic nerve centers. Out of these four, paired median optic nerve centers, present at the base of median optic
nerve, while other two centers present at the base of lateral optic nerves. A single neuropile region is present at the base of each optic nerve center. Both median and lateral optic nerve centers send tracts to the central body through optic commissure. The central body receive inputs from other sensory and motor centers and therefore probably functions as a sensory-motor integration center (Sasira Babu K, 1965). The protocerebrum also consist of globuli cell regions. The globuli cell region are probable higher order association and integration centers (Sasira Babu K, 1965). It was also observed that the protocerebrum of specimens of *O. bicolor* is surrounded by large cell body of neurons containing secretory granules (Nsc). These cells are probably a neurosecretory cells (Habibulla M, 1961, and Sasira Babu K., 1965).

The area of brain that Awati and Tembe termed the midbrain was called the tritocerebrum by Bullock & Horridge (1965). The largest internal centers of the tritocerebrum are the cheliceral ganglia, located in the circum-oesophageal region at the bases of the paired cheliceral nerves, which control the movements of the mouthparts. It was also observed that peripheral portion of tritocerebrum and anterior portion of sub-oesophageal ganglion of young specimens of both species under study consist of large cell masses with secretory granules (Nsc). Such neurosecretory cells have also been marked and described by Habibulla M. (1961) in *Heterometrus swammerdami* and Sasira Babu K. (1965) in *Heterometrus fulvipes*.

Dorsally, the sub-oesophageal ganglion of *H. xanthopus* is slightly elongated in shape (Fig: 4A.2 A, B), but it is ovoid in *O. bicolor* (Fig: 5A.2 A, B). The shape of sub-oesophageal ganglion in *H. xanthopus* resembles with *Heterometrus fulvipes* (Sasira Babu, 1965) while shape of sub-oesophageal ganglion of *O. bicolor* correlates with *Centruroides* sp. (Buthidae) (McClendon, 1904) and *Tytius serrulatus* (Buthidae) (Lucas, et.al. 1965). The suboesophageal ganglion is a compound ganglion that supplies a pair of pedipalpal nerves, accessory pedipalpal nerves, four pairs of ambulatory nerves / pedal nerves, a pair of accessory ambulatory nerves, (Crural nerves; Henry 1949 also mentioned one small accessory pedal nerve to first, third and fourth legs and two accessory pedal nerves to second leg); and four pairs of vagus nerves, out of four pairs, the first pair goes to the pectines, the second pair to the genital operculi, the third and fourth pairs to the first two pairs of book lungs. All of these four pairs of vagus nerves common in Scorpionid and Buthid species under study. Henry (1949) said that the first pair goes to the genital operculi, the second are tegumentary nerves to the area around the pectines, the third innervates the pectines, and the fourth branches again, sending one branch to the area around the gonopore and a longer branch into the gonads. Sasira
Babu (1965) described an unpaired, dorsal ephemeral nerve, which innervates the endosternite and surrounding muscles; anterior and posterior genital nerves; large pectinal nerves; and third and fourth mesosomatic segmental nerves, which comprise dorsal branches to the dorsum of the respective segments and ventral branches to the book lungs and ventral muscles.

In this study, pair of pedipalpal nerves of *H. xanthopus* comparatively larger and arise from the anterior most portion of the sub-oesophageal ganglion, while in *O. bicolor* these seem to be comparatively smaller and arise from antero-lateral portions of the sub-oesophageal ganglion. In both the species, the first pair of ambulatory nerves present slightly frontward and enters in interior of first pair of legs. The second, third and fourth pairs of ambulatory nerves oriented in caudal direction and enter in interior of the respective legs (Fig: 4A.2 A, B and Fig.5A.2 A, B). It was also observed that the small pair of accessory ambulatory nerves arise from the dorsal and ventral region at the point of origin of main 1-4 ambulatory nerves and run parallel to them. The four pairs of mesosomal nerves (vagus nerves), the first pair goes to the pectines, the second pair to the genital operculi, and the third and fourth pairs to the first two pairs of book lungs.

Histologically, the sub-oesophageal ganglion of both species *H. xanthopus* (Fig: 4A.7) and *O. bicolor* (Fig: 5A.7) consist of major nerve centers includes major pedipalpal nerve centres, ambulatory nerve centres, and vagus nerve centers and ventral nerve cord nerve centers. Sasira Babu (1965) and Polis G.A. (1990) describe pedipalp ganglia, leg ganglia and group of cells at the base of vagus nerves. They describe separate large nerves and cell groups for pedipalp and leg centers. Root (1980) confirms motoneurons are located in the leg ganglia. Sasira Babu (1965) describes groups of giant cells located in the cheliceral, pedipalp, and leg ganglia of *Heterometrus*. Bowerman and Burrows (1980) in *Paruroctonus mesaensis* stained motoneurons ambulatory/ leg nerve centers of the sub-oesophageal ganglion, innervated eight different leg muscles. These are located in the ventral cortex of the sub-oesophageal ganglion. The central ganglion lie along the midline, near the ventral surface. It receives connections from sensory tracts arising in the pedipalps and legs and connects with many of the major incoming and outgoing tracts in the sub-oesophageal ganglion (Polis G.A., 1990).

The ventral nerve cord is a group of nerve fibers arising from the posterior region of sub-oesophageal ganglion and extends in mesosoma, metasoma and also gives innervations to the telson. There are three mesosomal ganglia, four metasomal ganglia. Each ganglion is a fusion of two symmetrical hemiganglia, internally connected by
connectives. Two pairs of nerves arise from each of the first six ganglia. In each hemiganglion, there is an antero-dorsal nerve and postero-ventral nerve respectively. The dorsal nerves from each ganglion supply branches to the muscles, receptors and dorsal body wall structures, while the ventral nerves supply to the corresponding ventral side. First, second and third mesosomal ganglia of *H. xanthopus* are located in posterior end of third mesosomal segment, fifth mesosomal segment and posterior half of seventh mesosomal segment respectively, while in *O. bicolor* the first, second and third mesosomal ganglia are located in the anterior margin of the fourth mesosomal segment, slight posterior to the fifth mesosomal segment and posterior half of sixth mesosomal segment respectively. Fourth metasomal, fifth metasomal and sixth metasomal ganglia of both the species are located in first, second and third metasomal segments respectively. According to Millot and Vachon (1949), their exact position of ganglia varies between families.

The last or seventh ganglion of *H. xanthopus* is located in most anterior margin of fourth metasomal segment and it is slightly larger than other ganglia (Fig: 4A.1), while in *O. bicolor* it is located in the anterior half of fourth metasomal segment (Fig. 5A.1). It is the ganglion from which a five pairs of nerves arise in both the species. Yellamma et al. (1983), found that this last metasomal ganglion consists of a larger number of cells compared to other ganglia and hypothesized a role in the stinging reflex.

Histologically, the first six ganglia of both the species are similarly organized, bilateral symmetrical group of nerve cells are observed. It indicates the double organisation of the ganglia and ventral nerve cord. The first six ganglia are structurally similar but seventh ganglion is more complex. In the first six ganglia, the major nerve fiber tracts run the central neuropile of each ganglion, where as ganglion cells are located in the distal half of the ganglion (Fig. 4A.8 & Fig: 5A.8). These cell bodies are connected to centrally located neuropile and also to each other via commissural and longitudinal tracts. The central neuropile contains a large associated center of receiving and sending fibers from and to the sensory and motor cell groups and adjacent ganglion (Sasira Babu, 1961). The longitudinal section of seventh ganglion (Fig: 4A.10 & Fig: 5A.9) also consists of bilaterally symmetrical groups of nerve cells, nerve tracts and in addition it also contains the large nerve fibers originate from posterior portion of ganglion and extend in fifth metasomal segment. The cell bodies of these fibers are located in the distal border of the 7th ganglion (Fig. 4A.9 & Fig: 5A.10). The cell body
of these cells contain large centrally placed nucleus. This system of giant fibers integrates quick movement and stinging behaviour of scorpion (Sasira Babu, 1961).

Sasira Babu (1965) described major nerve tracts with schematic diagram of first and seventh ganglia. He described the centro-lateral tracts, central tracts, dorso-lateral tracts, ventro-lateral tracts, latero-segmental tracts, mid-central tracts, mid-dorsal tracts, mid-ventral tracts as well as dorsal and ventral commissures. The seventh ganglion along nerve tracts also shows major cell masses located in the cortex of the ganglion. He also indicated the fifth segmental nerves and telsonic nerves in schematic diagram of 7th ganglion.

In both scorpions the ventral and dorsal pairs of nerves arise from the posterior region of the 7th ganglion, ventral pair supply branches to the fifth metasoma called fifth metasomal nerve, and other dorsal pair called telsonic nerves supply branches to telson. In H. xanthopus, the pair of telsonic nerves supplies branches to telson where it directly penetrates into respective venom glands and corresponding muscles. In O. bicolor, at the posterior half of the fifth metasoma the paired dorsal telsonic nerves cross over each other without contact and again run parallel posteriorly, to enter in to venom glands and corresponding muscles in opposite side of the origin. At the base of vesicle, around a dorso-ventral muscular strand, the telsonic nerves form thick ramification by dividing into fine branches. These fine branches concentrate in the muscles present at the posterior end of venom glands; appear more or less as a band or a collar. It supplies their branches to dorsal, lateral and ventral muscles to form a ring like structure around the base of telsonic muscles, continue and penetrate both the venom glands, where it supplies fine branches to venom glands and musculature present around it. In Bothriurus bonarinsis (C. L. KOCH, 1842) Horn,A.C.M.and Achaval, M. (2002) described that, at the division to fourth and fifth metasomal segments, these telsonic nerves adhere to each other for a short distance, forming a ring around the alimentary channel, from this point onward, turn ventrally and transverse the abdomen in the direction of the central nervous system, until opening at the anus. In the one third posterior of the fifth metasomal segment, the pair of nerves once again separates, forming a second ring around a dorso-ventral muscular strand located at the insertion point of the telson.

The double ventral nerve cord of both the scorpion species surrounded by perineurium (Fig. 4A.11 & Fig: 5A.11). It encloses group of nerve fibers, forming many large longitudinal nerve tracts. Sasira Babu (1961) and Babu and Venkatachari (1966) reported numerous “giant fibers” in the ventral nerve cord of H. fulvipes and H. swam-
merdami, the fibers range from approximately 15 to 40 µm in diameter. Giant fibers seem to mediate quick behaviours, such as escape, by virtue of their large diameters and their consequently fast conduction velocities. The giant fibers typically have much greater diameter axons than other nerve fibers. In these scorpions, giant fibers were observed in all the major longitudinal tracts of ventral nerve cord. The fibers range from approximately 12-18 µm in diameter in *H. xanthopus* and 10-12 µm in diameter in *O. bicolor*; the largest of these innervate the fifth metasomal segment and the telson. The cell bodies of these fibers are located in the distal border of the 7th ganglion.

**Male reproductive system:**

The account of the reproductive organs of the scorpions available in the literature is neither precise nor complete. There are many points that still need explanation. Here, the efforts have been made to provide additional description of reproductive systems of *H. xanthopus* and *O. bicolor*.

The male reproductive system of scorpion, in general, has been described by many authors such as *Buthus australis* L and *Scorpio maurus* L (Pavlowsky 1915, 1921); Male reproductive system, embryology and parturition of scorpions reviewed by Werner F. (1935); *Buthus tamulus* Fabr (Awati and Tembe, 1952), *Buthus quinquestriatus* (Abd-el-Wahab, 1957).

The male reproductive organs of *H. xanthopus* (Fig.4B.1) and *O. bicolor* (Fig: 5B.1) consists of paired testes, each of which is formed by two longitudinal tubes united by four transverse tubes, thus forming three loops in each testis. In *H. xanthopus* three paired loops of testes extending from 3rd to 6th mesosomal segment while in *O. bicolor* (Fig: 5B. 1) three paired loops of testes extending from 3rd to 5th mesosomal segment. Millot and Vachon (1949) have described the male reproductive system as having two longitudinal tubules but always shown four tubes in their illustration. In *Scorpio maurus* (Pavlowsky 1921), each testis is made up of only a single longitudinal tubule provided with four blind diverticula projecting medially, although the first one or two diverticula may partially unite with one another.

The histological structures of the testes of scorpion studied by Awati and Tembe (1952) and Abd-el-Wahab (1957). Histologically, the testes of both the species of scorpions show outer extremely thin layer of circular muscles covers basement membrane and germinal epithelium. The germinal epithelium rest on basement membrane. The cells of germinal epithelium are flat and each with large distinct nucleus; Germinal epithelium is divided by septa into different lobules. There is a lumen
in the centre of testes. The lobules consist of various stages of development such as spermatogonia, spermatids and spermatozoa and mature sperms found in bunches.

The antero-lateral loop of each testis of both the scorpions under study gives rise to a vas deferens, into which the vesicula seminalis opens. In *O. bicolor* additional accessory glands are also found. There is considerable confusion in the literature about the terminology to be applied to the accessory glands and no definite knowledge about their functions. Several authors have described two pairs of accessory glands: the cylindrical glands and the oval glands (described as seminal receptacles in Millot and Vachon 1949). Birula (1917) described the oval gland (ovate vesicle) is posterior to the cylindrical gland, whereas Millot and Vachon (1949) and Abd-el-Wahab (1957) showed the reverse situation in their illustrations. In this study in *O. bicolor* cylindrical glands are found and oval glands are not seen in both the species. The hypothesized function of the accessory glands is the secretion of the various components of the hemispermatophore or the material to glue the two halves together (Francke 1979a and Polis, 1990). It was observed that the wall of cylindrical gland of *O. bicolor* (Fig: 5B. 5) consists of cylindrical epithelium with secretory granules. The cytoplasmic granules of epithelial cells are stained by haematoxylin. In addition, Abd-el-Wahab (1957) noted pair of dorsal and ventral annex glands of unknown functions. The wall of dorsal and ventral annex gland of *O. bicolor* (Fig: 5B. 6 & Fig: 5B. 7) consists of columnar epithelial cells. The epithelial cells consist of cytoplasm with granules stained by haematoxylin. The lumen of glands contains secretory fluid.

Various authors referred to paired ejaculatory sacs, ejaculatory organs, or a penis, and inferred that these structures formed tubular organs for transmitting sperms. It is now considered that the ejaculatory sacs (elongate, hollow tubules behind the seminal vesicles), the seminal vesicles, and the accessory glands constitute the paired paraxial organs (terminology after Pavlowsky 1917). Histologically, the wall of ejaculatory sac of *H. xanthopus* is covered by thin smooth muscle layers enclosing a connective tissue capsule (Fig.4B.10 B-E). The inner of columnar epithelium continue throughout the inner border of ejaculatory sac. The basal plate consists of inward foldings of epithelium. The trunk region consists of thick layer of epithelium, enclosing a lumen with nutritive fluid. The lumen of capsular region consists of sperm packets along with nutritive fluid. The chitinous supporting stalk extends dorsally from the base of posterior border of capsular region to the distal portion of lamella. The ejaculatory sac of *O. bicolor* is also covered by thin smooth muscle layer and connective tissue capsule, internally lined by columnar epithelium rest on the basement membrane (Fig:}
The lining of epithelium is thin in basal plate, while thick in trunk portion. In the lumen of capsular region sperm packets were observed along with nutritive fluid. The chitinous supporting stalk extends from proximal portion of capsular region to flagellum. Pavlowsky (1921) mentioned the chitinous nature of the posterior process of the supporting shaft in *Scorpio mauros*.

Each of the paraxial organ stores sperms with seminal fluid from vas deference and seminal vesicle respectively. During courtship the two halves of the hemispermatophores are extruded and fused together to form the spermatophore, which is glued to a substratum. Each paraxial organ produces half the spermatophore, called the hemispermatophore (Polis, 1990). The right paraxial organ produces the right hemispermatophore and the mirror image, on the left, is produced by the left paraxial organ (Bastawade, 1994).

The sperms enter into the capsule of the hemispermatophore by the seminal vesicle; the accessory glands produce components of the hemispermatophore or the material to glue the two halves together, or both and the ejaculatory sac serves to push the two halves out of the gonopore, forming a complete bilaterally symmetrical spermatophore. Mature male ejects a complete spermatophore at a time during nuptial dance (Francke 1979a).

The report on the glued post insemination spermatophore of Indian species *Mesobuthus tamulus tamulus* (Fabr.) (Buthidae) are available by Bastawade (1992) (Fig.5B.1B, Left figure) and *Heterometrus scaber* (Pocock) by Mathew (1957). Indian scorpion fauna comprises of five families namely Buthidae, Chaerilidae, Vaejovidae, Ischnuridae and Scorpionidae. Amongst these only Buthidae possesses a flagelliform spermatophore, consists of Basal plate (Bp), Trunk (Tr), Capsular region (Cap), Flagella (Fl) and remaining families possess the lamelliform spermatophore (Bastawade 1994) (Fig.4B.1B, Left figure) with Basal plate (Bp), Trunk (Tr), Capsular region (Cap) and Lamella (Lam). The spermatophore in post-insemination state in *Mesobuthus tamulus tamulus* (Fabr.) (Buthidae) is pinkish-brown, much darker on capsular region, which is chitinious and pale, transparent on the stem and almost whitish on the flagellum. It is flagelliform and measures about 15 to 18 mm in total length (Bastawade1992). The phylogenetic relationship of some Chactoids has been discussed by Francke and Soleglad (1981) on the basis of hemispermatophores along with other characters. Francke (1979a) suggested that the lamelliform spermatophore is homologous to the spermatophores of atenmid pseudo-scorpions. Maury (1980) indicated, it is not always a
simple matter to obtain pre insemination spermatophores for study, but it is probable that these will have the greatest significance for taxonomic purposes.

The capsular region of spermatophore contains the sperm packets (Sp) (Fig.5B.1B, Left figure, Bastawade 1992). Alberti (1983) reviewed the structure of scorpion spermatozoa. Structurally the mature sperm of *H. xanthopus* and *O. bicolor* consists of head, middle piece and tail. The head of sperm is elongated. It is broader at the anterior 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 mature sperm of *H. xanthopus* (Fig.4B.7B) measure about 47µm in length while the mature sperms of *O. bicolor* (Fig. 5B. 4) comparatively small and measure about 35.7µm in length.

**Female reproductive system:**

Female reproductive system, embryology, and parturition of scorpions reviewed by Werner F. (1935). According to Birula 1917a, Millot and Vachon 1949, Mathew 1956 and Francke 1979a family Buthidae have five transverse ovariuterus tubes, whereas all other families have a four transverse ovariuterus tubes. Female reproductive system of *H. xanthopus* consists of three longitudinal tubes, interconnected by four transverse tubules. It forms six loops, three on each side. These constitute ovariuterus of scorpion *H. xanthopus*. The loops of reproductive system are present in 3rd mesosoma to 6th mesosoma. While female reproductive system of *O. bicolor* consists of three longitudinal tubes, interconnected by five transverse tubules. It forms eight loops, four on each side. These constitute ovariuterus of scorpion *O. bicolor*. The loops of reproductive system are present in 3rd mesosoma to 5th mesosoma. Matthiesen (1970) however, showed that *Tityus cambridgei* and *T. stigmurus* as Buthids do not have five transverse tubes; only the anterior and posterior transverse ovarian tubes are present, forming a two-celled ovariuterus. In both the species under study, from the anterior angle of each lateral longitudinal ovariuterus tube, the oviducts proceed anteriorly, forming the dilated receptacle seminalis. The receptacle seminalis open into an genital chamber, which opens to the exterior through the gonopore (genital aperture), which is externally covered by the genital operculi.

The ultrastructural study of ovariuterus and oocytes maturation has been studied in *Euscorpius carpathicus* (L.) (Euscorpiidae) (Soranzo (L. et. al. 2000). The present studies of ovariuterus of both the species (Fig: 4C.13 and Fig.5C.5) are formed by two layers of cells surrounding irregular lumen. The outer layer consists of
irregularly polygonal cells with rounded nuclei. The inner layer surrounds lumen of the tube. It is formed of very long and thin columnar cells. These cells are with distinct oval nuclei present at the base with faintly granular cytoplasm. This is the part of ovarian tube called germinal epithelium. It undergoes meiotic division to form ova and its follicle. The development of oocyte is similar in their early stages.

Further development of embryos has been studied by many authors (Laurie, 1890, 1896a, b; Pavlowsky, 1924b, 1925; Mathew, 1948 and 1956, 1957). According to embryonic development, the scorpions are grouped into apoikogenic and katoikogenic. In Buthidae, Bothriuridae, Chactidae, Chaerilidae, luridae, and Vaejovidae the oocytes are located in follicles that are in direct contact with the ovariuterus. The developmental type of these six families belong is referred to as apoikogenic (Laurie 1896a, b). The ova of apoikogenic scorpion have variable amount of yolk (Pavlowsky, 1924b, 1925), while the Diplocentridae, Ischnuridae, and Scorpionidae differ considerably from the other families. The oocytes are located within numerous lateral diverticula arising from the branches of the ovariuterus with one embryo developing in each diverticulum. This type of development is referred to as katoikogenic (Laurie 1896a, b). The ova of katoikogenic scorpions are yolkless (Mathew, 1948 and 1956).

*H. xanthopus* is katoikogenic scorpion, the oocyte develop in specialized diverticulum that branch from the female ovariuterus. The ova of *H. xanthopus* measures about 28 µm in diameter and alecithal. The size of the oocytes of some species is known in *H. fulvipes*, ova measure 0.12 mm (Laurie 1891) and in *H. scaber*, 0.04 mm (Mathew 1956). The zygote nucleus of *Heterometrus* undergoes holoblastic, equal cleavage through the second division. Cleavage is then unequal, and the result is a coeloblastula (Mathew 1956). In this study the zygote nucleus of *H. xanthopus* undergoes holoblastic cleavage, results in formation of blastula. Cleavage leads to formation of multicellular body called morula. The developing cells situated at the tip of diverticuli undergo gastrulation, the embryo in diverticulum attains elongated shape. The rounded structure of cephalic region seems to be developing towards appendix and remaining posterior portion becomes elongated and extend towards ovariuterus. The head region presumed to be in the phase of organogenesis while posterior portion undergoes segmentation. In later stages of development, the oral feeding mechanism developed in embryo. It may help the embryo to absorb maternal nutrient through an appendix embedded in hepatopancreatic mass. This feeding apparatus is called “bottle and teat”. It is a connection between the stomodeum of embryo and appendix. The specialized feeding apparatus is present in katoikogenic scorpions (Mathew 1957). The
appendix is very closely associated with the hepatopancreas of the mother; it receives and transports nutrients from the hepatopancreas to the embryo.

The unknown cellular structure (UCS) (Fig: 4C.25) attached to the inner lining of diverticulum adjacent to the mouth of embryo. It was noticed that this structure appear to develop during embryonic development in the month of December and remains persistent up to the month of April and disappear in late stage of development before parturition. This type of structure along with embryo has not been mentioned by previous authors.

During development, the basic pattern of anterior to posterior development is maintained in *H. xanthopus*, the embryos develop a pronounced flexure in the mesosomal region (Fig: 4C.7). The body is divided into three regions, a prosomal rudiment, a mesosomal rudiment, and a growth zone of metasoma. The mesosoma develop more rapidly than the prosoma, producing its seven segments. The mesosomal segments possess dorsolateral protrusions; apparently serve as exchange surfaces between the embryo and mother (Mathew 1956, Anderson 1973). The embryos develop with formation of chelicerae, pedipalps and legs. The venom glands develop as transparent, white, bilobed structure (Fig: 4C.8). The slit like mouth portion appears in the anterior portion and the gap between appendix and mouth seems connected by thick tubular teat conveying food for the developing embryos (Fig: 4C.31).

The gestation period of *H. xanthopus* is June to April (about 11 months). It is notable that some Scorpionid exhibit long gestation period (7-18 months), such as *P. imperator* (C. L. Koch) (7 months), *Heterometrus longimanus* (Herbst) (12 months), *Scorpio maurus* L. (14-15 months), *Urodacus manicatus* (Thorell) (16 months), *U. yaschenkoi* (Birula) (18 months). Such long period of gestation appear to have no parallel among other arachnids (Savory 1977) and are longer than those of many vertebrates.

The embryonic development of *O. bicolor* is comparatively different than that of *H. xanthopus*. The *O. bicolor* is apoikogenic scorpions. The oocyte of *O. bicolor* (measures about 14.28 µm in diameter) consists of large amount of yolk. The ova of apoikogenic scorpion have variable amount of yolk (Pavlowsky, 1924b, 1925). These studies have been shown that the Buthid scorpions have heavily yolked eggs.

The oocytes of Buthid scorpions develop in ovarian follicles, attached directly to the ovariuterus. These oocytes are variable in size and yolk content (Laurie 1890). The oocytes of *O. bicolor* develop in ovarian follicles, as the ovum increases in size, however, it pushes its way, towards the outside (Fig.5C.6) and appears as an initial
outgrowth on ovariuterus. The outer layer of ovariuterus tube become very thin and remains as a membrane covering on the developing ovum (Fig.5C.7). By the time, the ovum is very small and passed completely through the outer layer and is visible as a small protuberance on the surface of the ovariuterus. It remains connected to the inner layer of the ovariuterus tube by columnar epithelial cells. These cells also grow around the ovum, so as to form a follicle. The cells of these follicles rapidly become flattened and remain clustered at the base of ovum (Fig.5C.8).

The other apoikogenic families have large ova rich in yolk such as, the ova of *Centruroides vittatus*, (Francke 1982); *Euscorpius italicus*, (Werner 1935); *Lychas tricarinatus* (has alecithal ova) (Mathew 1960). Ova of apoikogenic scorpions are alecithal, isolecithal, or telolecithal (Yoshikura 1975). The ova of *O. bicolor* are isolecithal. The yolk is distributed equally in ova. After fertilization, the zygote of buthid scorpion undergoes cleavage. The cleavage of the yolky ovum is discoid (Laurie, 1890), with first division producing two flattened equal cells and subsequent division producing a germ disc, or blastoderm, which spreads over the yolk. The germ disc eventually differentiates into an epiblast (ectoderm rudiment) and a hypoblast (endoderm rudiment). The hypoblast produces the mesoblast (mesoderm rudiment), and the two apparently separate through delamination (Laurie 1890).

The cleavage of the yolky ovum of *O. bicolor* is discoid. The first division producing two flattened, equal cells, followed by subsequent divisions producing a multicellular body (Fig.5C.9 to Fig.5C.12). These cells spread around the yolk at one end and form a thick layer called germ disc or the blastoderm (fig.5C.13). The blastoderm differentiates into an epiblast and hypoblast (Fig.5C.14). The hypoblast produces a mesoblast (Fig.5C.15). The extra embryonic membranes are formed from these germ layers. The epiblast of the germ band produces a second membrane called the amnion (Fig.5C.15). As the three germ layers are forming, so are the extraembryonic membranes. The serosa forms first from the blastoderm. The epiblast of the germ band produces a second membrane, the amnion, underneath the serosa, enclosing an amniotic cavity (Anderson 1973). The germ disc undergoes further development. It attains an elongated structure and grows further (Fig.5C.16). The embryo grows around the yolk and become 10 segmented (Fig.5C.17) and development of coelomic cavity at the site of mesoderm (Laurie 1890). The hepatopancreas are formed below the developing alimentary canal. The ventral blood vessel is also developed below the hepatopancreatic mass. The preliminary neural groove developed at the ventral side (Fig. 5C.18). The growth zones of developing embryo proliferate and
The gestation period of *O.bicolor* is about June to November (5-6 months). The gestation period of buthid scorpion is much shorter than other families. The average gestation period of buthid is 5.2 ±2.3 months and for non-buthid 11.4±3.56 months (Polis G. A., 1990).

**Venom gland:**

The venom glands of scorpions were extensively studied by Pavlowsky (1913, 1924a, 1925); Werner F. (1935); Joseph E. Mazurkiewicz & Eldridge M. Bertke (1972); Halse, Prideaux, Cockson & Zwicky (1980); Francke & Soleglad (1981); Sissom (1990) and Farley (1999b).

Pavlowsky (1913, 1924b, 1925) was among the first author to survey important anatomical systems of scorpions across many scorpion genera and families, with an unusually representative selection of scorpion genera. He specifically paid attention to the phylogenetic importance of anatomical features.

The cuticle of venom glands of both the species made up of three layers. Joseph E. Mazurkiewicz & Eldridge M. Bertke (1972) named these as outermost layer epicuticle, a waxy layer. The middle homogeneous layer is exocuticle and innermost thick layer is endocuticle. In *Bothriurus vittatus* (Bothriuridae) the transverse section of poison gland shown the chitin of poison vesicle corresponding to the dorsal concavity, sometimes thicker than remaining surface of the ampulla. The hypoderm forms a special organ in the form of many longitudinal folds of the cuboidal epithelium (Pavlowsky, 1924c). The cuboidal epithelial cells are present in between the cuticle and secretory
epithelium of venom gland of both species under study. These cuboidal epithelial cells consist of irregularly shaped centrally placed nucleus. The cytoplasm of these cell is dark granular.

The venom glands of both the species are surrounded by striated muscle fibers. Joseph E. Mazurkiewicz & Eldridge M. Bertke (1972) reported that the muscle fibers are attached to the cuticle by means of tendon cells. The muscle fibers surround gland from all sides and also extended in median fissure to separate both the glands. It keeps the glands away from each other. Thick musculature surrounds the venom glands of *H. xanthopus*, it is densely arranged in dorsal side. Comparatively the musculature surrounding venom glands of *O. bicolor* appear thin and comparatively thick in medial fissure along with connective tissue to form a wide gap.

Pavlowsky (1913) discovered two types of glands: one with simple, smooth epithelium (Type I), and another, with folded epithelium (Type II). Type I glands were found by Pavlowsky (1913, 1924b) in families Chactidae, Euscorpiidae, Iuridae (Calchas), and Liochelidae. Type II glands were found in Bothriuridae, Buthidae, Caraboctonidae, Iuridae (Iurus), Liochelidae, Scorpionidae, and Urodacidae. Type I gland was considered to be the primitive (or embryonic type) condition due to the fact that Type II folded gland is derived from Type I during embryogenesis in both *Scorpio maurus* (Scorpionidae) and *Androctonus crassicauda* (Buthidae); this developmental feature was later confirmed by Probst (1972). The venom glands of *H. xanthopus* and *O. bicolor* are included in type II, because the venom glands of both the cases encloses a lumen surrounded by folded epithelium. The venom gland of *H. xanthopus* consists of one large fold and four small folds (Fig: 4D.2), while the venom gland of *O. bicolor* consists of four major folds (Fig. 5D.3).

In *Bothriurus vittatus* (Bothriuridae) the epithelial cells are much longer with rounded nuclei containing fairly granular network of chromatin (Pavlowsky 1924c). The venom apparatus of scorpion *Centruroides sculpturatus* (Ewing) consist of paired glands, lined by secretary epithelium with columnar cells. The secretary products are either membrane bound or unbound vesicles released in the lumen of the gland (Joseph E. Mazurkiewicz & Eldridge M. Bertke, 1972). The venom gland of scorpion *Urodactus novaehollandiae* Peters (Scorpionidae) is complex and contains two types of secretary epithelial cells: goblet and columnar cells. The secretary compound contains proteins, indole compounds, PAS-positive and acidic mucoid substances (Halse S.A., Prideaux P.L., Cockson A. & Zwicky K.T., 1980). The venom glands of both the species, encloses a lumen, surrounded by extensive folding of secretary epithelium. The
epithelial cells arranged on basement membrane and connective tissue layers. There are two types of epithelial cells. One type is goblet cells and other columnar epithelial cells. These goblet cells and columnar cells are numerous in *H. xanthopus* and are compactly arranged on the both the sides of connective tissue. In *O. bicolor* the venom glands consist of very few goblet cells and columnar cells.