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GROSS ANATOMY OF MALE REPRODUCTIVE SYSTEM AND HISTOLOGY OF TESTIS AND VAS DEFERENS IN FRESHWATER CRAB *BARYTELPHUSA CUNICULARIS* (WESTWOOD 1836) (DEACAPODA: CRUSTACEA)

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ABSTRACT
This study provides the description of the gross anatomy and histology of the male reproductive system of *B. cunicularis*. Results of present probe showed that the male reproductive system consists of a pair of testes, a pair of vas deferens ending into ejaculatory ducts and a pair of penises. Two pairs of gonopods (modified pleopods) are also present which are known to serve as copulatory organs which transfer the spermatophores into female gonopores. The macroscopic anatomy of the testes showed a similar pattern to that described in other brachyuran species: the paired testes, H like appearance, presence of gonopods, etc. Histologically, the testes consist of large number of testicular lobes containing spermatogonial cells that differentiate into spermatocytes and a collecting duct. Each testis as a whole is covered by a connective tissue sheath, the tunica. Vas deferens is almost circular in cross section and has a fibrous sheath of connective tissue and muscle fibres. Is lined internally by a layer of glandular epithelium and its lumen contain large number of spermatophores that are being transported towards the penises.

INTRODUCTION

The freshwater crab *Barytelphusa cunicularis* is a commonly distributed freshwater crab in Maharashtra occurring in freshwater habitats such as rivers, ponds, lakes, wells, dams, running water streams, etc. It is abundantly available in freshwater bodies of Marathwada and other parts of Maharashtra, states of Southern India and Sri Lanka (Ng and Tay, 2001; Srivastava, 2005). The *B. cunicularis* is an edible freshwater crab with commercial value and sold in large numbers in weekly markets in cities, towns and villages in Maharashtra. The studies on reproductive biology especially the morphology and histology of the reproductive system of *B. cunicularis* are scanty and insufficient. Some aspects of reproductive system of *B. cunicularis* have been studied earlier by Diwan (1971) and Sutar (2002). However, a detailed account of macroscopic and microscopic anatomy of male reproductive system of this crab is still wanting.

The present paper deals with the anatomical and histological organization of male reproductive system in freshwater crab *B. cunicularis*.

MATERIALS AND METHODS

Collection and maintenance

The adult specimens of *B. cunicularis* were bought from local weekly market and brought to the laboratory. Alternatively, the animals were also supplied by Zoology department animal suppliers. In both the cases the ultimate sources of animals were local freshwater bodies in the vicinity of Aurangabad such as ponds, lakes, Kham river, dams, water streams, etc. Upon bringing the animals to the laboratory, they were maintained in plastic water tubs of approximately 10 litre capacity. Some of the crabs die in first two to three days which are ultimately removed and disposed off. The survived crabs are maintained in aerated condition and are fed regularly with wheat grains and earthworms. The water is changed every week and replaced with dechlorinated tap water. Fecal material and debris, if any, is also removed as and when necessary.

Gross anatomy

Healthy adult male *B. cunicularis* (carapace length between 4 to 6cm) individuals were selected for dissection. Before dissection, crabs were anaesthetized by cold treatment by maintaining them at 4ºC for 20 – 30 minutes. The crabs were then weighed using digital balance and carapace length and width were measured with vernier calliper. Subsequently, the crabs were dissected under dissecting lens so as to expose the reproductive system. The colour and location of the different parts of reproductive system was recorded by visual observation. Thereafter, the system was removed from the

body, weighed in wet condition and placed in a clean petridish. The size of different parts of reproductive system was then estimated with the help of graph paper and vernier calliper.

Histology

The reproductive systems dissected out in above manner were fixed in aquatic Bouin's fluid for about 24 hr. Subsequently the tissues were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. The tissue blocks were cut at the thickness of 7μm on a rotary microtome. The sections obtained were then double stained with haematoxylin and eosin stains to prepare permanent histological slides. Microphotographs of properly stained tissues were taken under compound light microscope using digital camera of Samsung company (Model no.: ES55).

RESULTS

The reproductive system of male freshwater crabs *B. cunicularis* consists of a paired testes, vasa deferentia, penises (papillae), gonopods-1 and gonopods-2. The testes and vasa deferentia join together in such a way as to have H-shaped appearance (Plate I Fig. A and B).

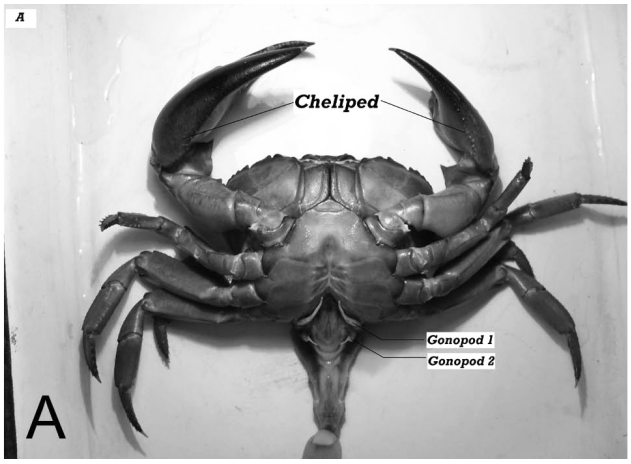
Testes: The testes lie in the cephalothorax on top of the hepatopancreas just below carapace. They have creamy white colour and soft texture. Each testis is elongated and lobulated structure and extends anterodorsally on the cephalothorax and continues laterally to the stomach. The width and diameter of each testis is not uniform along its length. In general, the width of each testis along its length lies between 2 to 6 mm. Total length of the testis ranges between 20 to 30 mm. However, the overall length and width of right testis is slightly more than that of the left testis. The distal ends of both testes and anterior ends of both vasa deferentia are joined together to form a commissure or cross bridge so as to give 'H' like shape to the system (Plate I: Fig. B). They produce spermatozoa that are carried away in paired ducts (the vasa deferentia) which open ventrally.

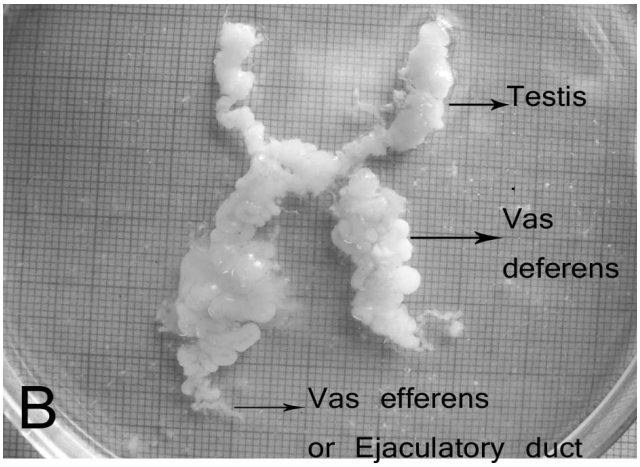
Histologically, each testis may be circular, oval or little elongated in cross section. The microscopic architecture of testis of *B. cunicularis* consists of several testicular lobules (also known as seminiferous tubules or testicular acini) and a central collecting duct, which is the general pattern described for decapods (Krol *et al*., 1992; Taketomi *et al*., 1996). Entire testis is enveloped by a fibrous layer made up of collagen fibres. Internally, each testis contains large number of oval lobules or follicles. Each testicular follicle is lined by a single layer of germinal epithelial that gives rise to spermatogonial cells (Plate I: Fig. C and D). The cells in each testicular lobule seem to in a single stage of spermatogenesis; however, cells in different lobes may be in the different stages of spermatogenesis. The non-germinal cells called as Sertoli cells (also referred to as accessory cells, sustentacular cells, interstitial cells, nurse cells or nutritive cells in literature) are also found interspersed in between. These cells are considered to be nutritive in function helping the process of spermatogenesis and spermiogenesis.

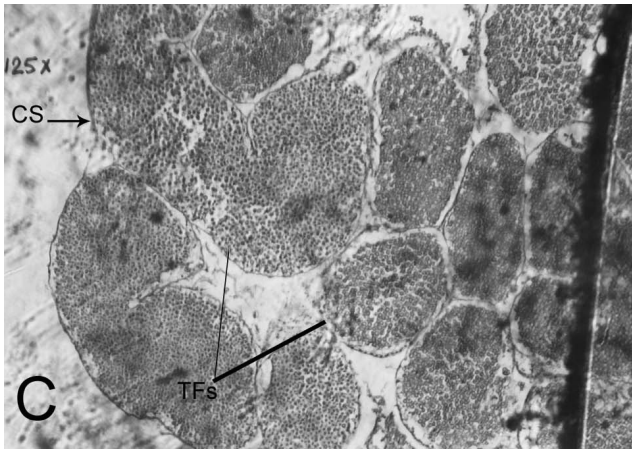
Vasa deferentia: A pair of vasa deferentia arise from the point of commissure (where posterior ends of two testes are joined together) and extend posteriorly in the form of extensively coiled tubule. Each vas deferens is creamy white coloured thin, extensively coiled tube ultimately ending into thin ejaculatory duct to open at the base of fifth pleopods in the form of small papilla or so-called penis. However, because of coiling and folding of the tube, it forms a wide and elongated structure entangled in mass of fibrous and muscular tissues. Because of coils the overall structure formed from vas deferens becomes much wider and lobulated about 5 to 7 mm in the anterior, 10 to 12 mm in the middle and 2 to 4 mm in the distal part of the vas deferens. The diameter of each tubule of vas deferens is roughly 1 mm. From distal end of each vas deferens arise a thin tubule called ejaculatory duct. Each ejaculatory duct is transparent whitish in colour and about 10 to 15 mm long tubule with diameter of less than a half mm (Plate I: Fig. B). It leads into penis of respective sides and release spermatophores into penis during act of copulation. Histologically, each vas deferens is almost circular in cross section. The vas deferens is covered by a thick muscular sheath stained reddish and it is immediately followed by a layer of glandular epithelium that lines the lumen of vas deferens. Glandular epithelial cells secrete a kind of fluid that serves in the making of spermatophores and as a transporting medium for transport of spermatophores. Both the epithelium and spermatophores are stained deep violet with haematoxylin-eosin. The lumen of vas deferens contains number of spermatophores containing numerous spermatozoa. Spermatozoa from spermatophores are to be carried to the penises during the act of copulation. Thus, the main function of vas deferens is the transfer of male gametes, *i.e.,* spermatozoa, in the form of spermatophores to the external openings of the male reproductive system. In intimate contact of vas deferens, towards the posterior end of vas deferens, there is an endocrine structure termed as androgenic gland. This gland is known to be responsible for development of secondary sexual characters in male crabs (Charniaux-Cotton, 1960; Charniaux-Cotton and Payen, 1985).

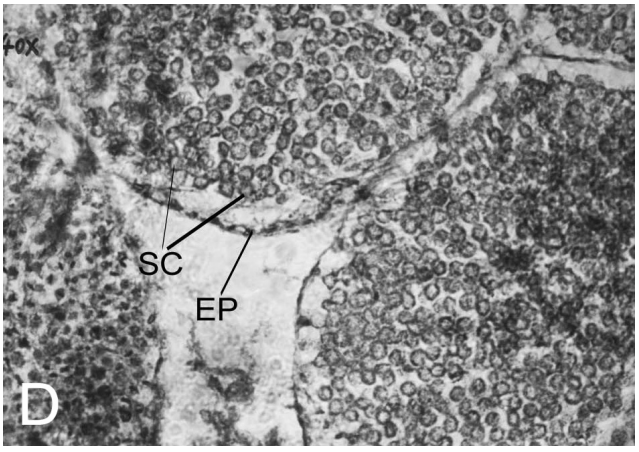
Penises: Each vas deferens thins towards its posterior end to form ejaculatory duct of smaller diameter. Ejaculatory duct of each side opens at the base of coxae of fifth pleopods of respective side in the form of small papilla called penis. Each penis contains a gonopore for the release of sperms during copulation. The penis of papilla is a short, soft, flexible, membranous and transparent tube-like structure. The penises do not pass through the sternum in this species as is the case with other of freshwater crabs. In freshwater crabs, the penises are not main copulatory organs. Instead, the spermatophores from penises are transferred to female gonopores with the help of male gonopods.

Gonopods: Gonopods 1- and 2 are paired abdominal appendages (pleopods) that are modified to function as copulatory organs. The gonopods-1 of freshwater crabs is a three or four-part hollow tubular organ with an apical opening in the terminal part. The first gonopod, in particular, is unusually long and has a special morphology, ending in a long tube. It is about 2 cm in length with broad base which gradually tapers distally until it ends in a slighltly blunt end. The gonopod-2 is shorter than gonopods-1; it has a short but broad base and a long thin but sharp thread- like end (Plate I: Fig. A). Both gonopods and their different parts are suggested









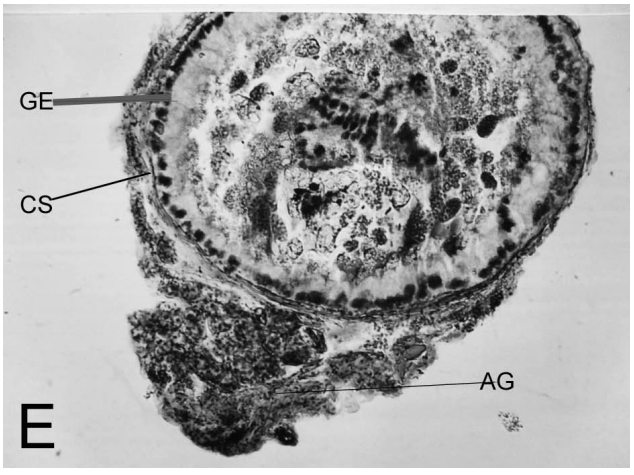


Plate 1: Fig. A Ventral side of male crab with the gonopods exposed. Fig. B. Dissected out male reprodutive system. Fig. C: T. S of testis. Fig.D: T. S. of testis with spermatocytes in testicular follicles. Fig. E: T. S. through vas deferens

Abbreviations: AG = Androgenic gland, CS = Connective tissue sheath, EP = Epithelium, GE = Germinal epithelium, SC = Spermatocytes and TFs = Testicular follicles or lobules. Magnification Fig. C" 100X, Fig. D: 400X, Fig. E: 400X. Stain Heamatoxylin - eosin.

by many workers to play a role in copulation and spermatophore transfer (Brandis *et al.,* 1999; Cumberlidge, 1999; Moriyasu *et al*., 2002).The gonopods-2 fits tightly into the subterminal segment of gonopods-1 and leaves a lateral basal opening into which the penis extends. Male and female freshwater crabs copulate in the normal brachyuran way; by lying head-to-head and sternum-to-sternum, with their abdomens relaxed so that the abdomen of the female overlaps that of the male. This brings the female openings into contact with the gonopods which swing out away from the sternum when the male abdomen is relaxed. The terminal articles of gonopods-1 connect with, and are inserted into, the paired vulvae of the female sited on sternite 5. The spermatozoa (in spermatophores), together with the secretions of the vas deferens, are ejected through the penis into the subterminal gonopod chamber between the bases of the subterminal segments of the two gonopods. The spermatophores are pumped out of the apical opening of the terminal article of gonopods-1 into the spermathecae that lie just deep to the female sexual openings, where they are stored until the eggs are laid (c.f. Cumberlidge, 1999). In the present investigation, we have not studied the microscopic anatomy of penises and

gonopods. Hence, further study needs to be done in this regard.

DISCUSSION

The present study expands the previous works of Diwan (1971) and Sutar (2002) on the reproductive system of *B. cunicularis*. The general layout of reproductive system of *B. cunicularis, i.e.* paired testes and vas deferens was similar to those found in other decapods (Cronin, 1947; Ryan, 1967; Joshi and Khanna, 1982; Krol *et al.,* 1992; Cumberlidge, 1999; Garcia and Silva, 2006; Castilho *et al.*, 2008). Thus, the sytem consists of a pair each of testes, vasa deferentia, penises and gonopods-1 and gonopods-2. The system shows the bilateral symmetry and H shape, characteristic of many brachyuran crabs, formed due to joining of posterior ends of testes. This arrangement is found in many crabs and crayfishes (Krol *et al*., 1992; Cumberlidge, 1999; Lopez Greco *et al*., 2007 and Castilho *et al.,* 2008). The testis of right side is often slightly larger in size than that of left side. Testicular lobules are filled with large number of spermatocytes in different stages of development as described by Mota-Alves (1975) and Castilho *et al*. (2008). In addition, the testes are also known to undergo seasonal changes in morphology and physiology (Joshi and Khanna, 1982). In many crustaceans, the vas deferens is reported to be consists of proximal, middle and distal portions on the basis of macroscopic and microscopic features (Lopez Greco *et al*., 2007; Castilho *et al*., 2008), however, we could not ascertain the same in case of this crab during the present investigation. Testes were also reported, in some cases, to be consisted of anterior, intermediate and posterior regions based on histological observation (Garcia and Silva, 2006). Perhaps, further studies in this regard solve this problem. The presence of androgenic gland, or structures similar in functions to those carried out by the androgenic gland, along the vas deferens has been reported in many crustaceans (Charniaux-Cotton, 1960; Garcia and Silva, 2006, Castilho *et al*., 2008). In the presence study, the androgenic gland was clearly noted along the vas deferens. This gland is known to produce some hormones that regulate the aspects of male reproduction.

Similar to many decapod crustacean species, the first two pleopods of *B.cunicularis* males are modified to serve as gonopods in the insemination of females. Gonopods are located just behind the gonopores. Histology of gonopods was not investigated in the present study; however, experimental studies in other crustacean species have demonstrated a role of gonopods in the transfer of an adhesive spermatophore during a brief copulation (Cumberlidge, 1999). In several decapod species, complex male gonopods and copulation with a relatively long duration (several minutes to hours) are associated. In a few such species, the direct role of the gonopods in transferring sperm to the female has been described and verified (Ryan, 1967; Hartnoll, 1969, 1975; Bauer, 1976, 1986; Berg and Sandifer, 1984). However, Bauer (1996) in his argument pointed out that the gonopods may serve (a) to temporarily connect male and female genitalia during copulation, and (b) to adjust the position of a male genital papilla relative to the aperture of a spermatheca or to stimulate the female and as sensory devices providing information to the male. The variability of reproductive morphology and products constitutes a good source of characters that can help to solve some of the existing taxonomic and help to establish Phylogenetic relationships among many groups of crustaceans. Recently, comparisons of the functional morphology of genitalia and subsequent sperm transfer and storage mechanisms, and the structure of spermatozoa and spermatophores have been carried out among crustacean taxa and provide useful information on phylogenetic relationships and evolutionary divergence, especially in the Decapoda (Bauer, 1986, 1991; Tudge, 1997; Kronenberger *et al.*, 2004 *for review*). We hope that the present study will help to understand the reproductive biology of *B. cunicularis* and other freshwater crabs of economical importance so that effective strategies can be formulated for management of hatcheries and to enhance the production of crabs.

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