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Comparative Account on Histomorphological Study of Male Reproductive System in Bivoltine Races of Silk Worm Bombyx Mori

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Abstract: *The reproductions maintain the species existence on the earth. The silkworm B. mori is bisexual insect. The male and female gonads are similar in early stage of embryonic development. The larval stages going on, the morphological gonadal change had been differentiated. In larva, the male gonad was Kidney shaped with rudimentary duct and in adult it consists of pair of testes, pair of vas deference, seminal vesicles, pair of accessory glands, ejaculatory duct and Aedeagus.In present study, Histomorphological study of male reproductive system carried out in three bivoltine races (CSR2, CSR2X4 and CSR4) of Bombyx mori and made microphotographs for comparative account. The pure race CSR2 and cross breed CSR2X4 are more efficient than the Pure race CSR4. Knowledge of the basic reproductive morphology and histology can be providing key information for future research into reproductive behaviour, phylogeny and physiology in silkworm races.*

Keywords: Bivoltine Races, Male Reproductive System.

I. INTRODUCTION

The organism continuation from generation to generation through pre-existing one is known as reproduction. The maintenance of organism is also by the process of reproduction. The majority of insects reproduces bisexuallybut, in few species, reproduction is byparthenogenesisorunisexualreproduction (Matsuura, 2009).The insect is dominant group of animal kingdom on the earth at present. The huge diversityofinsect present in every environment fromAntarcticatotropicandwatertoair.An important factor in the success of the insect is their high reproductive capacity, the ability of single female to give rise to many offspring and a large proportion which may reach to sexual maturity under favourable environmental conditions. In majority of insects reproduction is almost always sexual. Insects being terrestrial animals the problem of bringing together sperms and eggs in absence of surrounding water has been resolved due to internal fertilization and egg is surrounded by water proof cover i.e., chorion (Gillot, 1980).The insects reproduce by sexual but insemination is external (Beers and Warner, 2012). The reproductive organs form and structure have variation in different insects studied by some workers such as Snodgrass (1935); Englemann, (1970); Richard and Davis (1977).

The internal reproductive organs differ from all other organs of the body in that these functions do not contribute primarily to the welfare of the individual of which they are part and their main function lies with the succeeding generation. On the other hand, many activities of the organisms are correlated with reproduction (Snodgrass, 1935). The formation of matured gametes is the chief functions of reproductive organs essential carry forwardly the generation and maintenance of race of given species.

II. MATERIAL AND METHODS

The Bombyx mori bivoltine races viz., CSR 2, CSR2×CSR4 and CSR4 were reared in the Department of Zoology, Shivaji University, Kolhapur by standard rearing technique (Krishnaswamiet,al 1978). The 5th instar larvae and adult of all races were used for histomorphological study. They were mild anestheslised by chloroform and dissection in chilled ringer's solution under stereoscopic dissecting binocular microscope. The tissues were used for morphological

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observation, measurements and photography. Then tissues were immediately kept in Bouin's fixatives for 24 hrs.The fixed tissues were washed with 50% and 70 % alcohol and followed standard procedure of microtechnique. The sections were stained by HE technique (Humason, 1962) the stained slide used for microphotograph and histomorphological study.

III. RESULTS

Male Reproductive System:

The male reproductive system consists pair of testes, pair of vas deference, seminal vesicles, pair of accessory gland, ejaculatory duct and Aedeagus.

Testis:

In fifth instars larvae of Bombyx mori well developed gonads showed in male and female larvae. The male gonad i.e., testis is paired, bean or kidney shaped situated ineither side of dorsal blood vessel to dorsal side in 6th abdominal segment. The testes have a rudimentary duct runs from its middle to posterior up to the 8th abdominal segment. Itscolour is creamy transparent and surrounded by fat bodies as well as full of tracheal network. It measured about 2 -3 mm in length and 1 to 2 mm in diameter in all the bivoltine races under study (**Plate no. 1 fig 1a&b, Plate no. 2 fig 1a&b, Plate no 3 fig 1a&b**). Histologically, the testis was externally covered by tunica externa and tunica interna. The tunica externa composed bycuboidal cells based on basement membrane. It enclosed entire follicles of a testis under a common envelope called as peritoneal sheath. Tunica interna made up of darkly stained cells as compared to the tunica externa. The entire bean shaped testis divided by three septa into the four testicular follicles (**Plate no. 1 fig 3&4, Plate no. 2 fig 3&4, Plate no 3 fig 3&4**).

The septum made up by double layer epithelial cell wall separated testicular follicles from each other and it extended towards the vase deference.The testicular follicle of larval testis is classified into the different zones of sexcells developing stages. These are as germarium, zone of growth, zone of maturation,zone of transformation. In the germarium, apical region contained apical cell complex and primordial germ cells. They were surrounded by 7 to 9 concentric rings of spermatogonial oval cells. The spermatogonia which were weaker located near the rings. In the zone of growth region of testicularfollicle showed few isolated spermatogonia. Each spermatogonial undergo repetitive celldivision and process of spermatogenesis. Each cyst divided and redivided six times successively and formed 32 small round spermatocytes cells. The membranous cyst wall isnon cellular type. In the zone of maturation and zone of transformation, the mature cystcontaining 32 spermatocytes cells were noticed in the testis of 5th instar larvae. In pupalstage, the 64 primary spermatocytes are observed in testicular follicle of testis.

The largersized cyst with spermatids in the zone of maturation has been noticed. In 7th day old pupa, transverse section of testis showed spermatids number increased in the testicular follicle and sperm bundles in the zone of maturation were marked. In 9th and 10th day old testisof pupa has fully matured testes and testicular follicle (**Plate no. 1 fig 4, Plate no. 2 fig 4, Plate no 3 fig 4**). In this stage, adult has been fully developed inside the pupal case. Thetesticular follicles of adult before emergence showed cyst with full of sperm bundles. Few sperms were migrated towards vas efference and vas deferens. Such condition alsoobserved in the adult testes. The apical cell complex was also observed in the L.S. of adult testis in all the races under study (**Plate no. 1 fig 4, Plate no. 2 fig 4, Plate no 3 fig 4**).The testis was covered by double membrane i.e., tunica externa and tunica interna. In adult, the sperm bundles were carried out by vas deference from testes to the seminal vesicle. It is short time storage organ of sperm. At the time of copulation, these sperms transferred through the ejaculatory duct into the genital tract of female. The ejaculatoryduct secretes a secretion during passage of sperm and coats them by the thin membrane soas to form spermatophores. These spermatophores are transferred in to the bursacopulatrix of female during mating. Later on, the sperm are stored into the spermathecae till fertilization of ovum.

Vas deference: (Plate no. 1 fig 4, Plate no. 2 fig 6, Plate no 3 fig 6)

The vas deference of adult male arises from each testis by short vasefference. The vas deferens were paired, long, narrow tube it measured about 2.8 ± 0.1 cm in CSR2, 2.9± 0.3 cm in CSR2XCSR4 and 2.4 ± 0.1 cm in CSR4 runs posterior and opens into a seminal vesicle. The longitudinal section of vas deference composed by folded epithelium

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based on basement membrane which surrounded by outer circular musculature. The sperm bundles along with the secretion also observed in the T.S. and L.S. of vas deference as well as in seminal vesicle. These sperm bundles are received fromthe testicular follicle of testis.

Seminal vesicle: (Plate no. 1 fig 6, Plate no. 2 fig 8, Plate no 3 fig 9)
The seminal vesicle is a single received the opening of vas differentia. It functions as storage of the sperms for short time. It measured 4 ± 0.1 mm all the bivoltine races ofB. mori under study. The seminal vesicle was yellowish in colour. Histologically it showed the cellular lining of the seminal vesicles was glandular type which provided nutrients to the sperm. The seminal vesicle externally covered by circular muscle layer.The lumen of seminal vesicle contained large number of sperms.

Accessory gland: (Plate no. 1 fig 5, Plate no. 2 fig 7, Plate no 3 fig7)
The accessory glands are paired, straight and narrow tube opens into the seminal vesicle. It is measured 1.6 ± 0.6 cm in CSR2, 2.4 ± 0.1 cm in CSR2XCSR4 and 1.3cm in CSR4. Histologically, it is made up by secretary epithelium with large central lumen and muscular sheath around. The accessory glands form membranous sac or spermatophores by its secretion.

Ejaculatory duct: (Plate no. 1 fig 7, Plate no. 2 fig 7, Plate no 3 fig 9)
The ejaculatory duct of male is single in number, straight long tube measured about 2.7± 0.2 cm in CSR2, 2.2 ± 0.5 cm in CSR2XCSR4 and 2.6 ± 0.2 cm in CSR4. The epithelium is made up by single layer. It is ectodermal in origin, lined with cuticle. Histologically, it showed that the epithelium was covered by thick muscular sheath. The ejaculatory duct was separated into the two parts called as muscular and glandular part.The anterior part of ejaculatory duct was made up by glandular epithelium and outer circular as well as inner longitudinal muscle layer. The folding of epithelium is absent and lumen showed its secretion. It contributes in the formation of the spermatophores.The muscular part composed by well-developed circular muscle to inner side and longitudinal muscle layer towards outer side. It showed epithelial fold with thick intima.

Aedeagus: (Plate no. 1 fig 9, Plate no. 2 fig 11, Plate no 3 fig 10)
Aedeagus was a brownish in colour, sclerotized, straight tube of male genitalia. Itis wider at anterior side. It measured about 2.6± 0.1 mm in CSR2 and 3mm inCSR2XCSR4and CSR4. The ostium bursae are connected to the aedeagus at the time ofsperm bundles transformation into the bursa copulatrix during mating. Histologically, it ismade up by chitinous layer bounded by muscular layer.

IV. DISCUSSION

The reproductive organs are developing in the post embryonic development. In bivoltine races of Bombyx mori under study does not show any defined or remarkable features in between male and female gonads during early instars. Based on shape, the gonads could not be differentiated into the testis of male and ovaries of the female larvae up to the 3rdinstars.
The adult male reproductive system made up by the pair of testes, pair of vas deference, single seminal vesicle, ejaculatory duct and aedeagus (Wigglesworth, 1972). In all races under study have similar anatomical structure with many Lepidopteran insects. The kidney shaped structure assumes the testis and triangular shape denoted ovaries. The colour of testis is creamy white coloured and is situated in the 6th abdominal segment. Each testis enclosed into the double coat of peritoneal sheath which made by outer tunica externa and inner tunica interna. The tunica interna internally extended and forms double walled three septa in between testicular follicle. Due to the presence of this septum the testicular follicles are divided into the four compartments in each testis. The testicular follicle is four in number observed in all bivoltine races. These observations are similar to the other lepiodpteran Jamieson et al., (1999); Mancini and Dolder,(2003);Gaikwad, (2007); Muniv, (2013). Spermatogenesis has been stared in 4th and 5th instar larvae of present races of Bombyx mori under study.

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The germarium of apical region in each follicle showed large apical cell complex. This cell complex is covered by 7 to 8 concentric rings of spherical cells called as spermatogonial cells. These spermatogonia are connected to the apical cell complex and the nutrient. It collects nutrition from the digestion of weak spermatogonia which are present in the concentric rings near to the apical cell. These nutrients were supplied to the potent spermatogonia through the cytoplasmic processes. The spermatogonia undergo the spermatogenesis. The sperms are located away from the concentric rings. This nutritional function of apical cell complex was reported by earlier workers in Lepidoptera (Sonoli S &Hool M 1992); hemiptera (Freitaset; al, 2010). Similar histological structure was reported in the larval testis of Lepidoptera by Srideviet al., (1989) in Spodopteralitura, and Gaikwad (2007) in Papiliopolytespolytes. In late age testis (9th and 10th old day) pupa, when almost adult is fully formed. The majority of testicular follicles were filled with the cyst containing sperm bundles. Their number are 256 which as like in another Lepidoptera. This number in each cyst was recorded by Japanese workers Suzuki et al., (1996).

The adult male reproductive system of bivoltine races under study showed paired testes, pair of vas deference, single seminal vesicle, an ejaculatory duct, paired accessory glands and aedeagus. Histologically adult and larval testis showed well distinct four testicular follicle separated by three septa and the other structure was similar in both. The testes of Plathypenascabrawere creamy white in colour. The testicular follicles are not differentiated because they are closely united (Buntin and Pedigo, 1983). In tobacco horn moth, Heliothisvirescense paired teste's structure was presented in the 5th abdominal segment (Meola and Loeb, 1995). In Ciulfinaklassihas reported paired yellow testes. They were located on either side of the digestive tract (Winnicket al., 2009). Each testicular follicle of larva and adult had divided into the spermatogonia in germarium, spermatocytes in zone of growth, spermatids in zone of maturation and sperm bundles in zone of transformation. These zones of testicular follicle were observed in insectHeliothisvirescens (Meola and Loeb, 1995). The testicular follicles are single present in insect species of the hebridae, hydrometridae, mesoveliidae, and veliidae.

The two testicular follicles reported in gerridae (Woodward 1950;Pendergrast 1956).The seven testicular follicles reported in gerridae Triatoma (Freitaset al., 2010) and in grasshoppers Chapman (1998) recoded over hundred testicular follicles insomeacrididae. The testicular organization of Leucopteracoffeella showed similar result to the moth and butterflies. It observed the follicles composed of cysts and a centripetal spermatogenesis. (Macini and Dolder, 2006). In embryogenesis insects get differentiated as early as in gastrula. Later on, these germ cells migrate inside the blastula. The germinal layer gets differentiated and organogenesis took place at that time these germ cells got lodged into the mesodermal testicular follicles of male embryo or embryonic ovarioles of the female embryo (Snodgrass, 1935; Gillot, 1988).

The vas deference was paired, long, narrow tube which terminated into the seminal vesicle in all bivoltine races. Histologically, seminal vesicle having folded epithelium composed by columnar cells with centrally placed nuclei. This layer was based on the basement membrane. Similar basic pattern of vas deference reported in all the insects (Wigglesworth, 1972; Imms, 1977; Gaikwad, 2007). Usually in the lepidopteron insect's seminal vesicles and the accessory glands at their proximal ends joins with the distal end of the ejaculatory duct. The accessory glands were present inside the seminal vesicle and lies towards the outer side. Similar type of arrangement was observed by the earlier worker in different insects and three bivoltine races. (Orr, 2002; Alves, 2006).The seminal vesicle was pair and whitish elongated tubes in Panorpidae. It is composed by single layer epithelium (Xie and Hua, 2010).

In the present aminals, seminal vesicle is also whitish in colour. It is enlarged posterior part of vas deferens and reported same histology. In Plathypenascrabaseminal duct was a simple without any additional structures (Buntin and Pedigo 1983). In ourinvestigation, histologically the seminal vesicle was made by folded secretory columnar cellstype of epithelium based on external basement membrane and muscularis surroundswas observed. The analogous results were reported in the Papiliopolytespolytes (Gaikwad, 2007). Histologically, it was made up of cuboidal cells surrounded by single layer of circular muscle showed in our result. Xie and Hua (2010) described the ultrastruture of seminal vesicle in panorpidae.

The median ejaculatory duct of insect is ectodermal in origin having internal chitinous intima stated by Snodgrass in 1935. In present study, whitish ejaculatory duct comes from seminal vesicle which opened posterior as an aedeagus. The epithelium of aedeagus was made from cuboidal cells based upon the basement membrane and provided with thick muscularis. The external and internal wall of lumen lined with soft cuticular intima. The detail structure and

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function of Aedeagus explained by Snodgrass (1935).The external cuticular covering is present. Histologically it showed the cuboidal epithelium enclosed by circular muscle layer and the lumen. They have sperm bundles. The male insects showed great variability in external genitalia with different insect. The detailed study was not carried out on genitalia. Krishnaswamiet al., (1973) studied detailed structure of male genitalia in silkmoth of Bombyx mori.

The accessory glands of present bivoltine races studied on comparative account. The accessory glands are bulbous at the base and branched at tip region. The accessory glands are different morphological types were studied in Ciulfinaklassi. In accessory gland, epithelium have large nuclei and the lumen was densely packed secretion while, another type showed smaller epithelial layer and lumen without secretion (Winnicket al.,2009). The accessory gland in all bivoltine races showed columnar epithelium with broad lumen. The accessory gland of male reproductive system secretes a proteinous typical type of secretion. It is mucoproteins and apocrine or mesocrine secretary cells and this type of study was done in many insects by various workers. The accessory gland of male studiedin cockroaches (vijayalekshmiet al., 1973; Roth and Dateo,1964; Destephano, et al., 1974). Spermatozoaininsectexitinthreepotentiallydifferentenvironmenttheseminalvesiclewheretheymaybestayforquitelongtime inaninactivestate,inthebursacopulatrix

wheretheyarefirstmixedwithotherconstituentsofthesemenandinthespermathecaofthefemalewheretheyarenourishedbythe secretionsofthespermathecalglandremainingviableforaslongasseveralyearsinthecaseofhoneybee (Taber and Blum, 1960).

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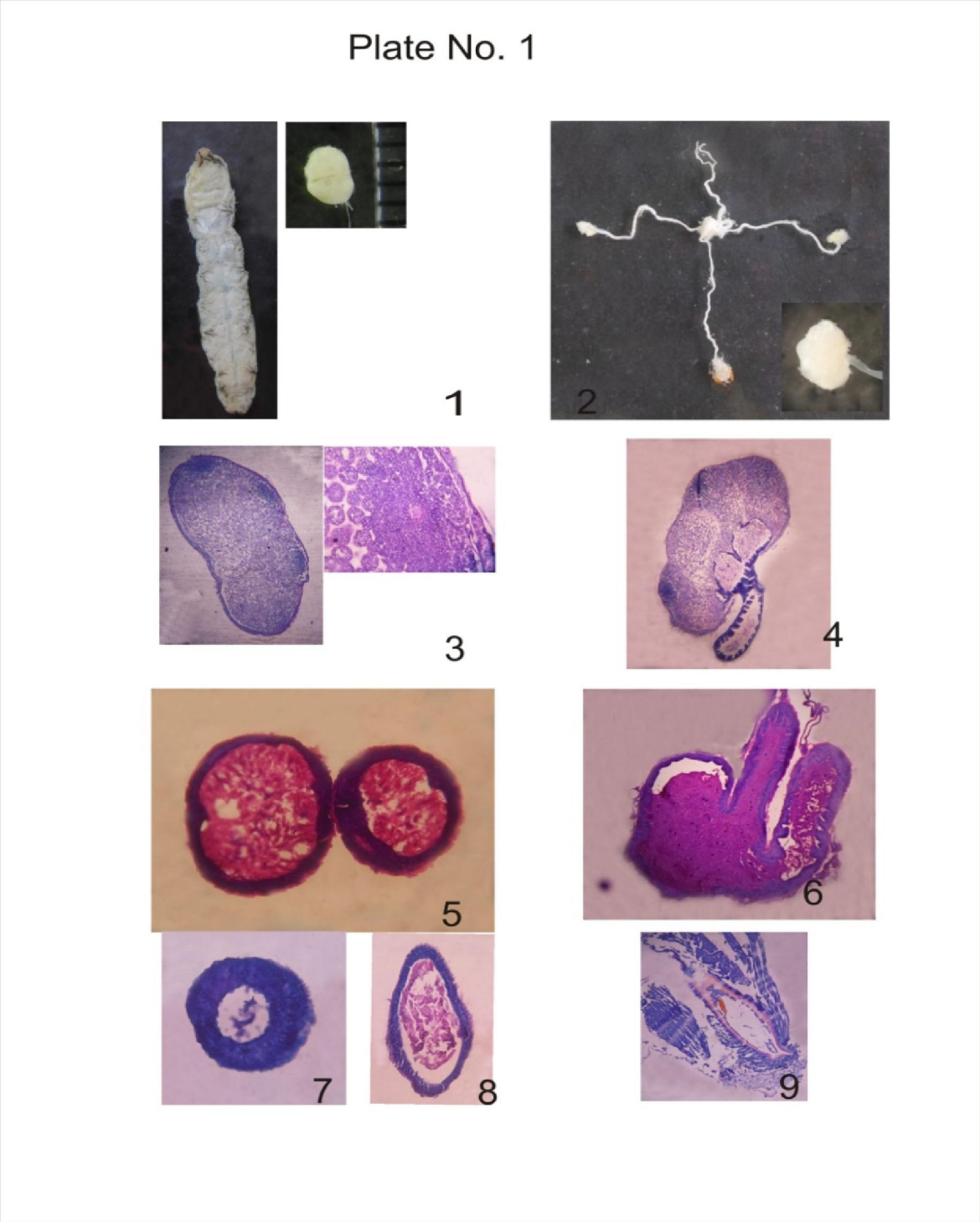

Plate No. 1: Silkworm Bombyx mori Race CSR2

Fig 1. Larval testis (insitu and exsitu); Fig 2. Reproductive system of adult; Fig 3. Histological section of larval testis with magnified view; Fig 4. Histological section of adult testis; Fig 5. Histological section of adultassesory glands; Fig 6. Histological section of Adult Seminal vesicle; Fig 7. Histological section of Adult Vas deference; Fig 8. Histological section of adult ejaculatory duct Fig 9. Histological section of Adult Adeagous.

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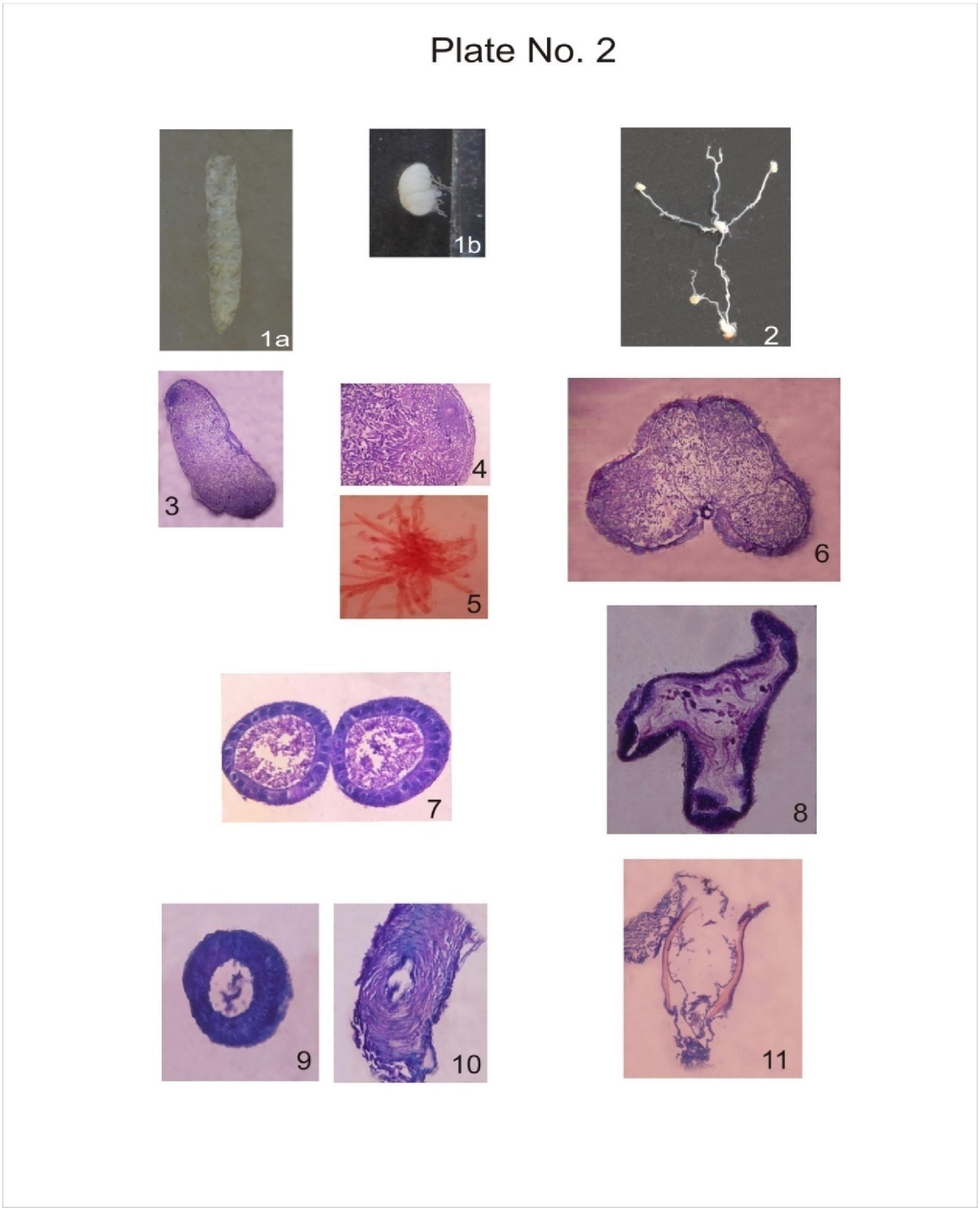

Plate No. 2: Silkworm Bombyx mori Race CSR4

Fig 1. Larval testis (insitu and exsitu); Fig 2. Reproductive system of adult; Fig 3 &4. Histological section of larval testis with magnified view; Fig5. Whole mount of testicular sperm bundles(Eosine stain); Fig 6. Histological section of Adult testis;Fig 7. Histological section of adult assesory glands; Fig 8. Histological section of Adult Seminal vesicle; Fig 9. Histological section of Adult Vas deference; Fig 10. Histological section of adult ejaculatory duct Fig 11. Histological section of Adult Adeagous.

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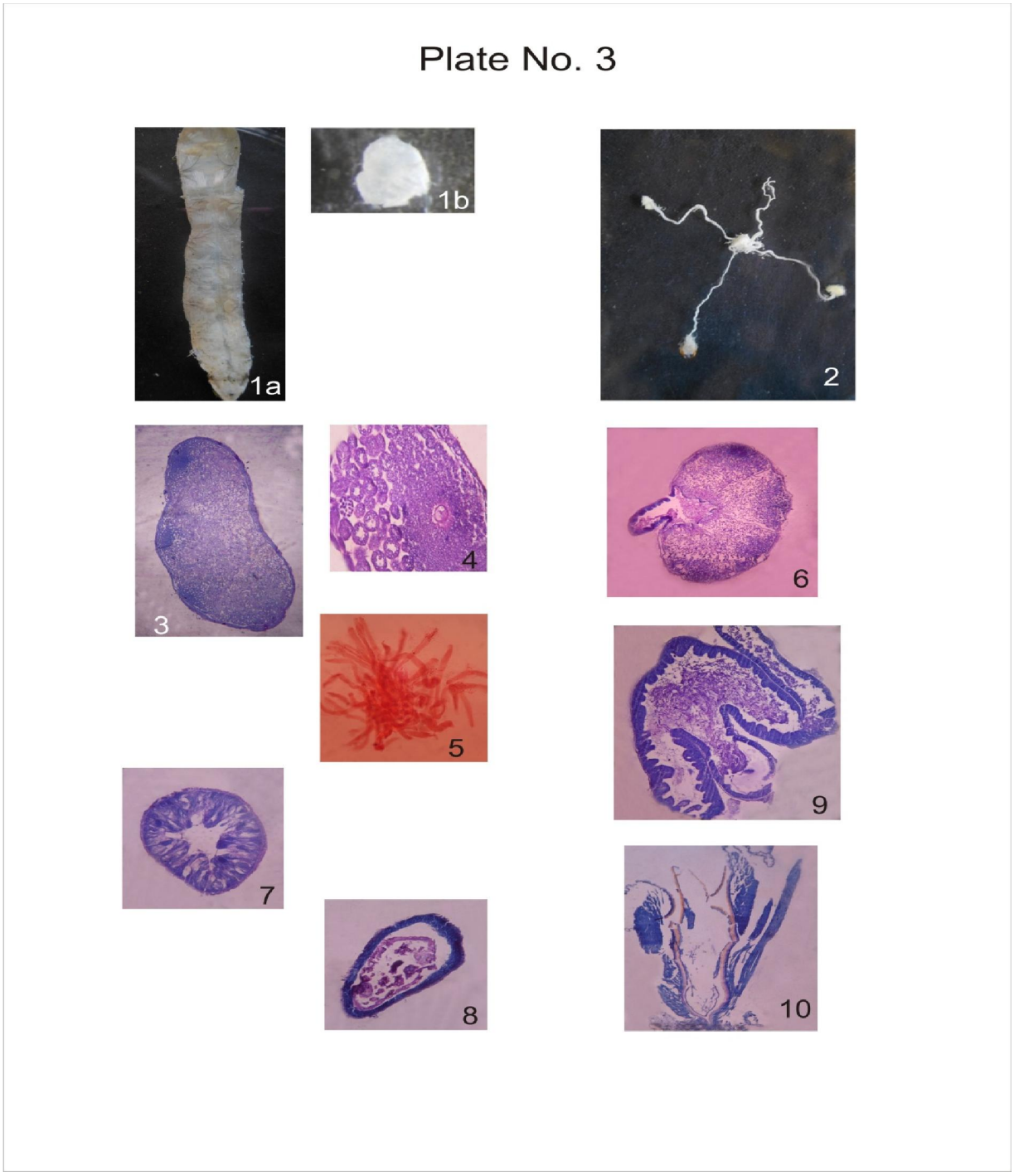

Plate No. 2: Silkworm Bombyx mori Race CSR2X CSR4

Fig 1(a & b). Larval testis (insitu and exsitu); Fig 2. Reproductive system of adult; Fig 3 &4. Histological section of larval testis with magnified view; Fig5. Whole mount of testicular sperm bundles(Eosine stain); Fig 6. Histological section of adult testis;Fig 7. Histological section of adult assesory glands; Fig8. Histological section of adult Vas deference;Fig 9. Histological section of Adult Seminal vesicle; Fig 10. Histological section of Adult Adeagous.