REPRODUCTIVE ANATOMY AND GONADAL DEVELOPMENT OF THE TURRID *Gemmula speciosa* (REEVE, 1843)

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ABSTRACT

The reproductive anatomy and development of the gonads of the turrid *Gemmula speciosa* (Reeve, 1843) were studied histologically. Specimens collected from September 2004 to June 2007 from waters off Cavite and Batangas, Philippines exhibited gonochorism, with an average male:female sex ratio of 1.5:1. Females were slightly larger than males, and sexually mature at about 47 mm shell length. The Maturity and Gonad Indices showed that *G. speciosa* had an annual reproductive cycle with a peak during the fourth quarter. Spawning was synchronous, although males had continuous gonad development, while females exhibited a long resting phase after spawning.

Key words: gastropod, *Gemmula speciosa*, gonad development, Gonad Index, Maturity Index, reproductive anatomy, toxoglossate, turrid

INTRODUCTION

The turrid *Gemmula speciosa* belongs to the Order Neogastropoda, and has a spindle-shaped shell with a typical siphonal canal. This species is strictly marine, carnivorous (Taylor et al., 1980), and secretes venom. It is a nocturnal soft-bottom dweller. *G. speciosa* has been collected locally from 54 to 73 m depth (Heralde et al., 2010), but turrid species are widespread, occurring from high to low latitudes, and from the shallow intertidal to abyssal depths (Sysoev, 1988, cited in Tucker, 2004).

Toxoglossate gastropods of the families Conidae, Terebridae and Turridae are emerging as an important pharmacological resource. The recent growing interest in the turrid *G. speciosa* stems from scientific research on another toxoglossate group, the Family Conidae (Powell, 1964). Over 20 years of research on the component of the cone venom led to the importance of the *Conus* venom peptides as basic tools in neuroscience, as diagnostic agents and as therapeutic drugs. These studies also revealed that there are still about 50,000 different conotoxins in the cone venom that need to be investigated. Cone shells may have evolved from the turrids, which may exhibit similar mechanisms of envenomation (Terlau et al., 1996; Espiritu et al., 2001; Espiritu et al., 2002; and Jimenez et al., 2003). Turritoxins are now gaining ground in terms of research (Lopez-Vera et al., 2004).

G. speciosa has been collected from waters off Badian Island (western Samar), Mantaguin Island (eastern Palawan) (Powell, 1964), Bataan, Cavite, Batangas, Cebu, and Bohol (Heralde et al. 2010). Beyond Philippine waters, it is generally found in the Indo-Pacific ocean and the Red Sea (Olivera 2005), particularly in the Arabian Sea, Beibu Gulf, South China Sea,

Nansha Islands, Japan (Baoquan and Xinzheng, 2008), and Yule Island (Gulf of Papua) (Hinton, 1972).

Previous literature on turrids has been on its systematics (Powell, 1964; Springsteen and Leobrera, 1986; Tucker, 2004; Puillandre et al., 2008). Ecological information about turrids, especially *G. speciosa*, is just emerging (Heralde et al., 2010). There is a dearth of information regarding this species' reproductive biology.

This study provides basic knowledge on the reproductive biology of *Gemmula speciosa* (Reeve, 1843) (Family Turridae, Subfamily Turrinae) collected from the waters off Cavite and Batangas (Philippines) between 2004 to 2007. Specifically, this work describes the reproductive anatomy and gonadal development of *G. speciosa* histologically. Specimens in storage were utilized; hence, no data on physico-chemical and biological parameters of the collection site at sampling times were available.

MATERIALS AND METHODS

Live specimens of *G. speciosa* were purchased from commercial divers/collectors. Fig. 1 shows empty shells of *G. speciosa*. Such purchase was justified as the divers' had better knowledge of the collection methods and collection areas. The collection sites were in waters off Barangays Patongan (Cavite), Papaya and Calaya (Batangas) (Fig 2). The specimens were obtained at depths at about 23 m, initially using a trawl, then with later collections by hookah diving and sieving loose substrate. Collection of snails was aborted when waters were rough. There were gaps in sampling during the collection period from September 2004 to June 2007 because the rough waters made the collection site inaccessible. Nonetheless, about 20-30 specimens were purchased from available monthly collections, and when bimonthly (i.e. twice a month) samplings could be made, specimens were also acquired. Samples closest to the new moon phase were utilized in this study.

Live samples were brought to the laboratory, and each animal removed from its shell by manually cracking the shell. Whole soft tissues were preserved individually in 10% seawater-buffered formalin (Winsor, 1984), for histological processing (hematoxylin-eosin stain: Luna, 1968; Winsor, 1984). The shell length and wet body weight of all specimens were taken using a vernier caliper ($\pm$ 0.01 mm) and analytical balance ($\pm$ 0.001g), respectively.

Male snails were sexed macroscopically by the presence of a penis located near the base of the right eye tentacle (Fig. 3). Sexing was based on primary and secondary characteristics, specifically for males, presence of spermatogonia and of the penis, respectively; for females, only primary characteristics, i.e. presence of oogonia. Specimens that possessed a penis were then histologically examined for presence of spermatogonia, and confirmed to be male, except for one (shell length=64.3 cm). All specimens considered female for lack of a penis were similarly examined histologically for presence of oogonia, and confirmed to be female.

Six to nine snail specimens per month over 20 sampling months were used for histology. Each snail was divided into cephalic and large posterior coil regions. For the cephalic region, histological slides were prepared from five sections. For the large posterior coil where the gonad and viscera were located, histological slides were prepared from three sections: proximal,

middle, and distal tip of large posterior coil. Four slides were prepared per section of the cephalic region and per section of the posterior coil (Fig. 3C).

Gonadal development was determined either as developing, ripe, or spawning, based on the predominant stage (i.e. at least 50% are of that stage) (Llana & Aprieto, 1980; Libutaque 1988). The developing stage I of males had tubules with spermatogonia, spermatocytes, and spermatozoa, from the periphery to the lumina's center. The ripe stage II had abundant spermatozoa in the lumina of the seminiferous tubules. The spawning stage III had few spermatozoa in the tubules' lumina (Fig. 4A-C) (Libutaque, 1988). Histological analyses to determine male reproductive stages focused on the middle region of the large posterior coil, as the testis was observed to be located at the distal to the middle regions.

For females, the developing stage I had small oogonia, and oocytes with limited yolk in the ovarian follicles. The ripe stage II contained oocytes laden with yolk. The spawning stage III had few yolky oocytes and/or empty follicles (Fig. 4D-F) (Libutaque, 1988).

Maturity Index (MI) (Yoshida, 1952, cited in Libutaque, 1988) and Gonad Index (GI) (Kennedy, 1977; Green, 1978) were used and compared to quantify the reproductive stage of a particular batch of samples. MI is a measure of the specific stage of gonad development, determined by assigning different numerical values per stage, and uses the formula (Yoshida, 1952, cited in Libutaque, 1988):

$$MI = [(\text{stageIi} \times 1) + (\text{stageIIi} \times 2) + (\text{stage IIIi} \times 3)] / N$$

where, Stage I is Developing = 1

Stage II is Ripe = 2

Stage III is Spawning = 3

i is number of individuals per stage

N = is total number of individuals staged for that month

MI was determined per month, and values for male and female tabulated.

GI is a gauge for reproductive activity of a population using individual gonad condition. In this study, reproductive stages were determined by examining and assigning stages to histological sections of the gonad (Kennedy 1977). It was noted that GI has also been used by other workers (Helm & Bourne, 2004) as a gonadosomatic index (reported as GSI or GI), which is based on weight or volume of the gonad relative to whole body weight. Application of such parameters for the gonadosomatic index is useful only if the gonad is a discrete organ and is easily separated from other organs (Giese, 1959; Lopez & Gomez, 1982).

As used by Libutaque (1988), the GI is computed using the formula:

$$GI = [(\text{stageIi} \times 1) + (\text{stageIIi} \times 2) + (\text{stage IIIi} \times 1)] / N$$

where, Stage I is Developing = 1

Stage II is Ripe = 2

Stage III is Spawning = 1

i is number of individuals per stage

N = is total number of individuals staged for that month

In this study, GI was determined per month, and values for male and female were tabulated. Increasing GI values, or values close to 2, suggest active gonad development. On the other hand, decreasing GI values, or values close to 1, suggest little gonad development or spawning phase (Lopez and Gomez, 1982; Toral-Barza and Gomez, 1986).

RESULTS AND DISCUSSION

Reproductive Anatomy

Gemmula speciosa has a dextral shell (i.e. the aperture is right-handed). From shell measurements, females were slightly larger than males, with average lengths ± standard deviation (s.d.) of 48.29 ± 5.22 mm (N=339), and 45.90 ± 5.22 mm (N=298), respectively. Parts of the male reproductive system (from posterior to anterior) that were identified were the testis, seminal vesicle, prostate gland, and penis. (Fig. 5A-E). The male gross reproductive anatomy is diagrammed in Fig. 6A. The male *G. speciosa* had a single testis, with seminiferous tubules containing developing spermatogonia. From the posterior end to the middle region, sections of the large posterior coil showed dense darkly-stained spermatocytes and lighter-stained spermatozoa within the seminiferous tubules of the testis (Fig. 5B). The seminal vesicle at the proximal coil region was filled with spermatozoa (Fig.5C).

The seminal vesicle secretes the seminal fluid that transports the sperm via the penis to the female's bursa copulatrix (Catalan et al. 2002; Castillo and Brown, 2008). From the proximal region of the large posterior coil, the seminal vesicle leads anteriorly to the prostate gland located in the cephalic region. The prostate gland has an inner zone of secretory columnar epithelia, as well as an outer moderate layer of muscular stroma, which enclosed a lumen (Fig. 5D). The penis was curved and had three layers of muscular sheets (Fig.5E).

Parts of the female reproductive system (from posterior to anterior) that were identified histologically were the ovary, oviduct, albumen gland, capsule gland, and bursa copulatrix (Fig. 7A-F). The female gross reproductive anatomy is diagrammed in Fig. 6B). The ovary was located from the distal to middle regions of the large posterior coil. During pre-reproductive activity, follicles were generally small (Fig. 7A), while in post-reproductive activity, follicles were either empty or with oogonial remnants (Fig. 4F). The ripe ovary occupied up to the large posterior coil's proximal region, consisting of large follicles full of oocytes (Fig. 7B). Oocytes were attached to gonadal septae in racemose arrangement.

The ovary narrowed into the oviduct, which consisted of two layers, the inner tunica mucosa and the outer tunica muscularis. Columnar epithelial cells comprised the tunica mucosa, which had both ciliated and non-ciliated cells (Fig. 7C). Ciliated cells serve to move oocytes from the ovary to the albumen gland, whereas non-ciliated cells may have a secretory function (Ownby, 2002). The oviduct had an outer tunica serosa, a relatively thin membrane consisting of connective tissue and simple cuboidal epithelium (Fig. 7C), possibly with a secretory function.

The oviduct leads to the albumen gland, located at the proximal region of the large posterior coil. The albumen gland appeared to be laterally-compressed, was composed of two lobes, and had numerous glandular epithelia lining the lumen (Fig. 7D).

In the cephalic region, the capsule gland consisted of a single lobe enclosing a lumen. The cells of the lobes of the capsule gland were highly glandular, with small secretory granules (Fig. 7E). The albumen gland and the capsule gland are both secretory glands, providing a protective envelope around fertilized oocytes, and nourishment to developing embryos (Ojeda & Chaparro 2004).

The bursa copulatrix, located further along the anterior cephalic region, consisted of two distinct layers, i.e. an inner epithelium enclosing a lumen, and an outer muscular zone. The inner epithelium was folded and consisted of columnar cells with granular cytoplasm. It borders the lumen which contained highly vacuolized epithelial cells. A thick outer layer of muscle lined the bursa copulatrix (Fig. 7F). The bursa copulatrix receives and stores the sperm. In other species, the bursa copulatrix has been reported to digest sperm (Koene and Schulenburg, 2005).

Gonad Development

The shell length of reproductively mature *G. speciosa* was based on histological examination of the gonad was about 47.6 mm for males, and 46.7 mm for females. Seventy-five percent of 2005-2006 samples were males, with an average male to female ratio of 1.5:1 (s.d. = 0.8).

The computed Maturity Index (MI) for male was tabulated (Table 1). Some seasonality trend was observed in 2006, when samples were available for most months of the year. Male MI values were almost always greater than 1.0, implying that males were continuously in a state of reproductive activity (Lopez & Gomez, 1982). For females, the ovary was in various stages of reproductive development, so that the female reproductive stage was based on the predominant stage (highest percentage) observed from histological samples. Based on 2006 data, in contrast to male MI, female MI values indicated three reproductive stage categories, showing that females went into a spawning phase from November to March (i.e. cold dry months), proceeding to resting phase by June to September (i.e. warm wet months). This is contrary to the general trend that most marine invertebrates spawn during the warm months (Giese 1959). Basic metabolic requirements must be met so that growth and reproductive development can proceed (Bayne, 1975). Energy requirements for gametogenic development and spawning during cold months (Tong, 1988) may be met if *G. speciosa* feeds intensely during the earlier quarters. *G. speciosa* reportedly feeds on tube-dwelling terebellid polychaetes (Heralde et al., 2010). Knowledge on feeding habits of *G. speciosa* are only now being uncovered, and needs further study.

The observed spawning periodicity in *G. speciosa*, i.e. spawning during the cold dry months, was unusual but not unique, as this has also been observed in the whelk *Neptunea antique,* a temperate species which spawns during winter instead of summer (Pearce & Thorson, 1967).

The computed GI was tabulated (Table 2). Male and female GI values reflected some synchrony in gonad development, manifested by coinciding peak values of GI during the latter part of each year (October to January) (Table 2). Unlike females, males appeared to be almost in a state of gonad development with GI values between 1.33 to 2.0 for most of the histological samples. Female GI values showed peaks from October to January, proceeding to relatively long resting and development phases for other times of the year (March to September).

SUMMARY AND CONCLUSIONS

Gemmula speciosa possessed the basic organization of the reproductive anatomy found in most neogastropods (Richter et al., 2003). The presence of a penis in the male suggests that mating with a female involves copulation. The reproductive system's anatomical features indicate that fertilization is internal, with the possibility of sperm storage, and/or sperm selection by females. The presence of secretory organs and capsule glands in *G. speciosa* may imply a benthic developmental phase. This would follow the view of Powell (1964) who reports that other turrid species are known to lay egg cases. However, Olivera (2005) suggests that *G. speciosa* may have a planktonic developmental phase based on its polygyrate protoconch. More research needs to be conducted to confirm these inferences.

G. speciosa populations in waters off Cavite and Batangas had an annual reproductive cycle, with females spawning during the cold months of the fourth quarter of the year. This is most likely because the specimens were collected from relatively deep waters (about 23 m), where temperature fluctuations may be less of a driving force in gametogenic development. Rather, food availability needs to be considered regarding reproductive cycles of deep water species (Thorson, 1950, cited in Giese, 1959). In addition, environmental factors affecting food availability must also be considered. This needs further study.

Physiologically, faster rate of gametogenic development was observed in male *G. speciosa* than among females, in that males continuously had a new set of developing spermatogonia. It seemed to be always ready in case females were mature and ready to receive sperm. More information on mating behavior is needed to determine the significance of different rates gametogenic development, in this case, the near-absence of a resting phase in male *G. speciosa,* as opposed to the relatively long developmental and resting phases in the female.

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List of Figures

Fig. 1. Dorsal (A) and ventral (B) of shells of *Gemmula speciosa.*

Fig. 2. Map showing the collection sites along the Cavite/Batangas boundary.

Fig. 3. The male (A) and female (B) *G. speciosa*. C. Diagram of soft tissue showing sections made for histological studies. cr=cephalic region, lpc=large posterior coil, pe=penis.

Fig. 4. Gonad stages of *Gemmula speciosa*

Male gonad stages (A-C):

(A) Male gonad Stage I (magnification=100X), spg=spermatogonia, spc=spermatocyte, spz=spermatozoa

(B) Male gonad Stage II (magnification=100X), spz=spermatozoa

(C) Male gonad Stage III (magnification=100X), spz=spermatozoa

Female gonad stages (D-F):

(D) Female gonad Stage I (magnification=100X), oc=oocyte, og=oogonia

(E) Female gonad Stage II (magnification=100X), oc=oocyte

(F) Female gonad Stage III (magnification=100X), fo=follicle.

Fig. 5. Male reproductive system.

(A) Testis (magnification=100X), dg=digestive gland, st=seminiferous tubule, te=testis.

(B) Seminiferous tubules (magnification=100X), st=seminiferous tubule, spg=spermatogonia, spz=spermatozoa.

(C) Seminal vesicle (magnification=100X), spg=spermatogonia, spz=spermatozoa, sv=seminal vesicle.

(D) Prostate gland (magnification=100X), lu=lumen, pg=prostate gland, se=secretory epithelium.

(E) Penis (magnification=100X), pe=penis.

Fig. 6. Gross reproductive anatomy of *Gemmula speciosa* (A) male, (B) female. pe=penis, pg=prostate gland, sv=seminal, vesicle, te=testis, bc=bursa copulatrix, cg=capsule gland, ag=albumen gland, ov=oviduct, ovy=ovary.

Fig. 7. Female reproductive system

(A) Ovary with developing oocytes (magnification=200X), doc=developing oocytes.

(B) Ovary with ripe oocytes (magnification=200X), roc=ripe oocytes.

(C) Oviduct (magnification=100X), ov=oviduct, tma=tunica mucosa, tms=tunica muscularis, ts=tunica serosa.

(D) Albumen gland (magnification=100X), ag=albumen gland, lol=lower lobe, upl=upper lobe.

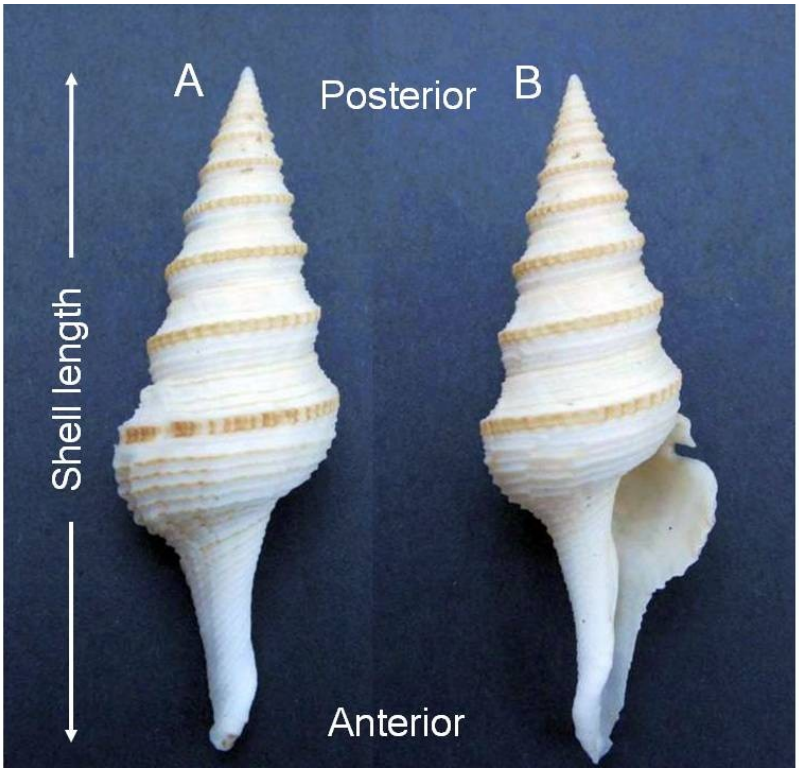
(E) Part of the capsule gland (magnification=100X).

(F) Bursa copulatrix (magnification=100X), bc=bursa copulatrix, epl=epithelium layer, muz=muscular zone.

List of Tables

Table 1. Maturity Indices (MI) of male and female *G. speciosa* for the period Sept 2004 to Jul 2007.

Table 2. Gonad Indices (GI) of male and female *G. speciosa* for the period Sept 2004 to Jul 2007.



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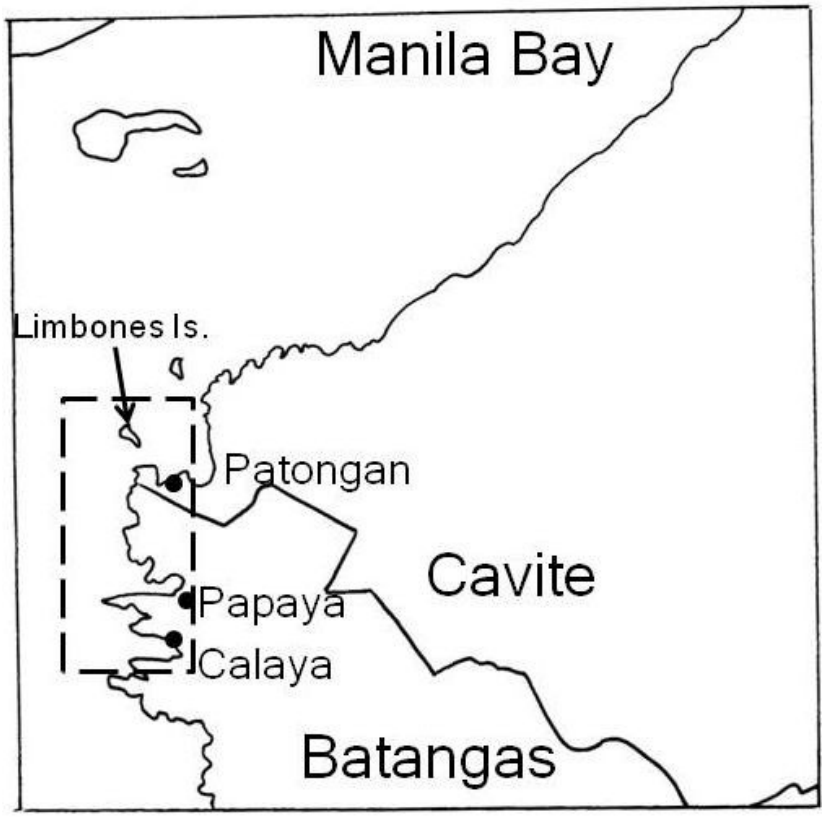

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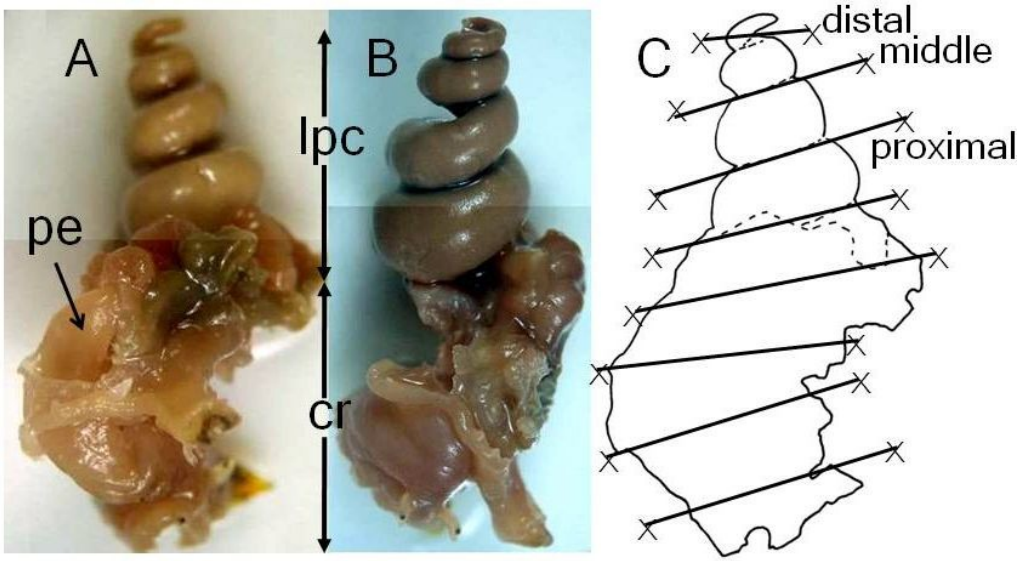

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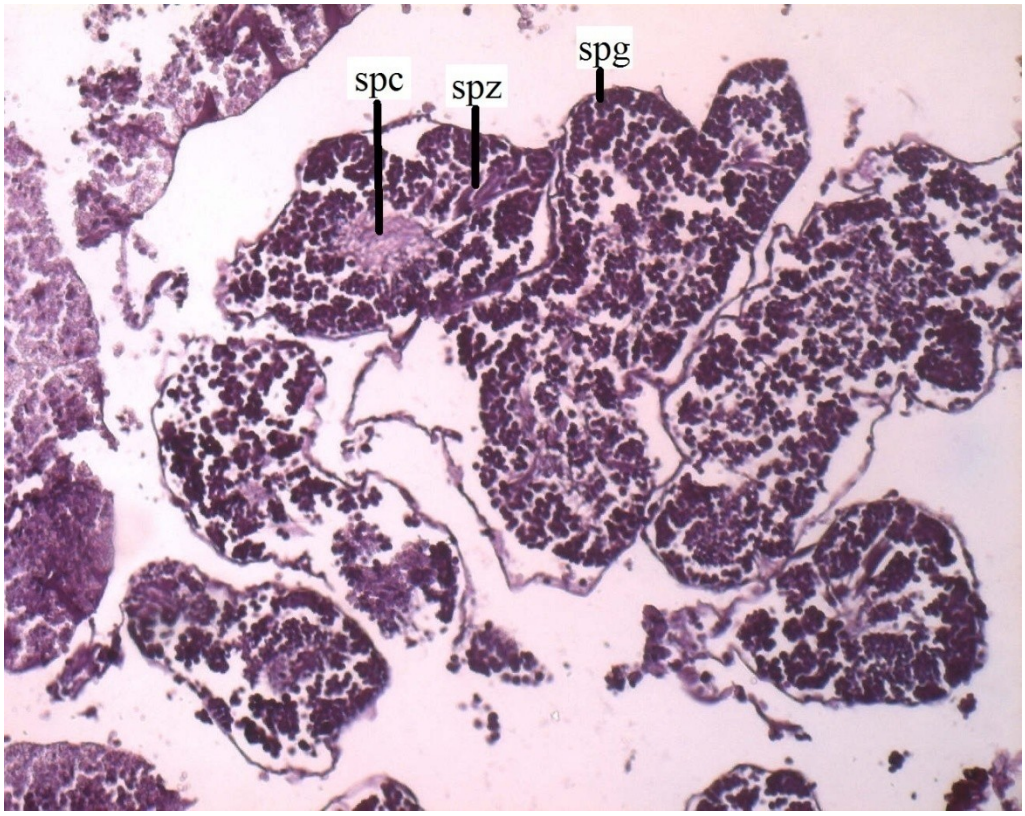

Fig. 4A. Male gonad Stage I (magnification=100X). spg=spermatogonia, spc=spermatocyte, spz=spermatozoa

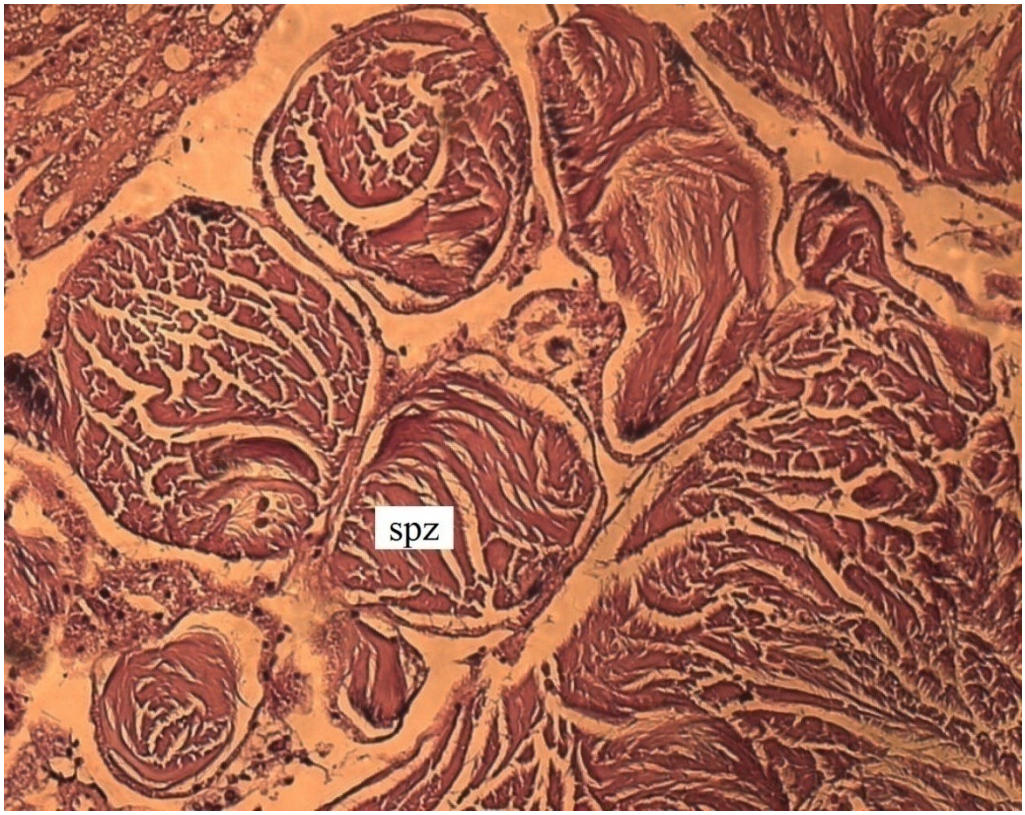

Fig. 4B. Male gonad Stage II (magnification=100X). spz=spermatozoa

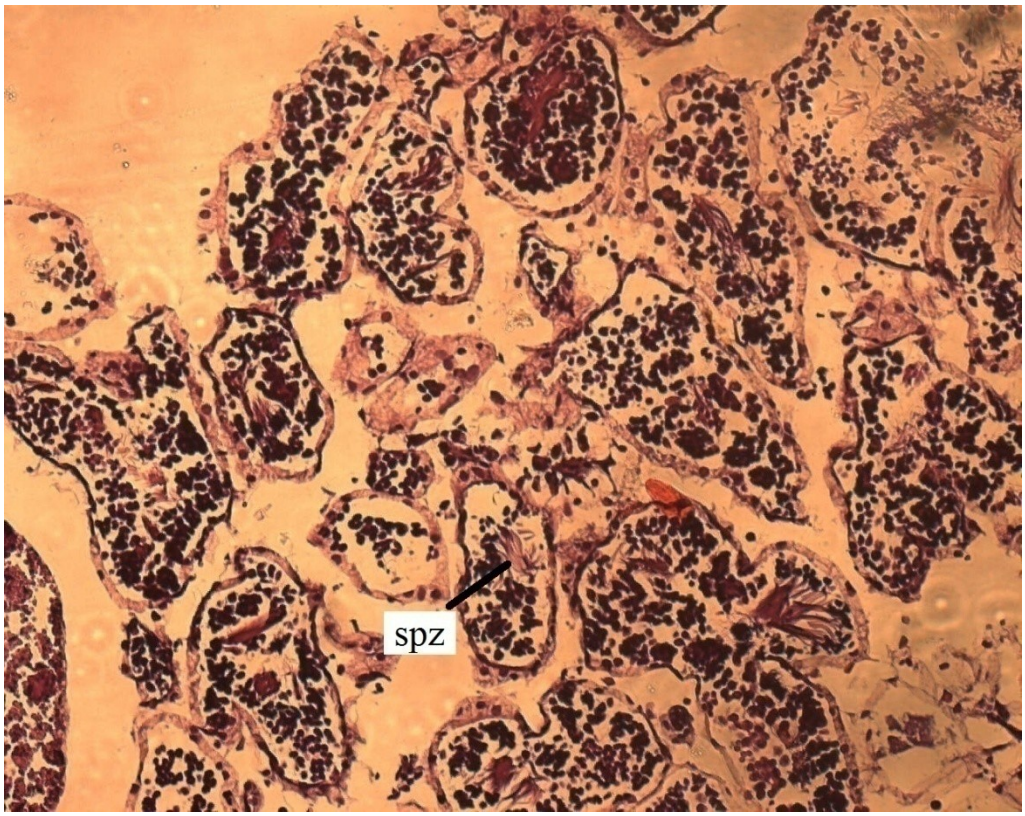

Fig. 4C. Male gonad Stage III (magnification=100X). spz=spermatozoa

Female gonad stages (D-F):

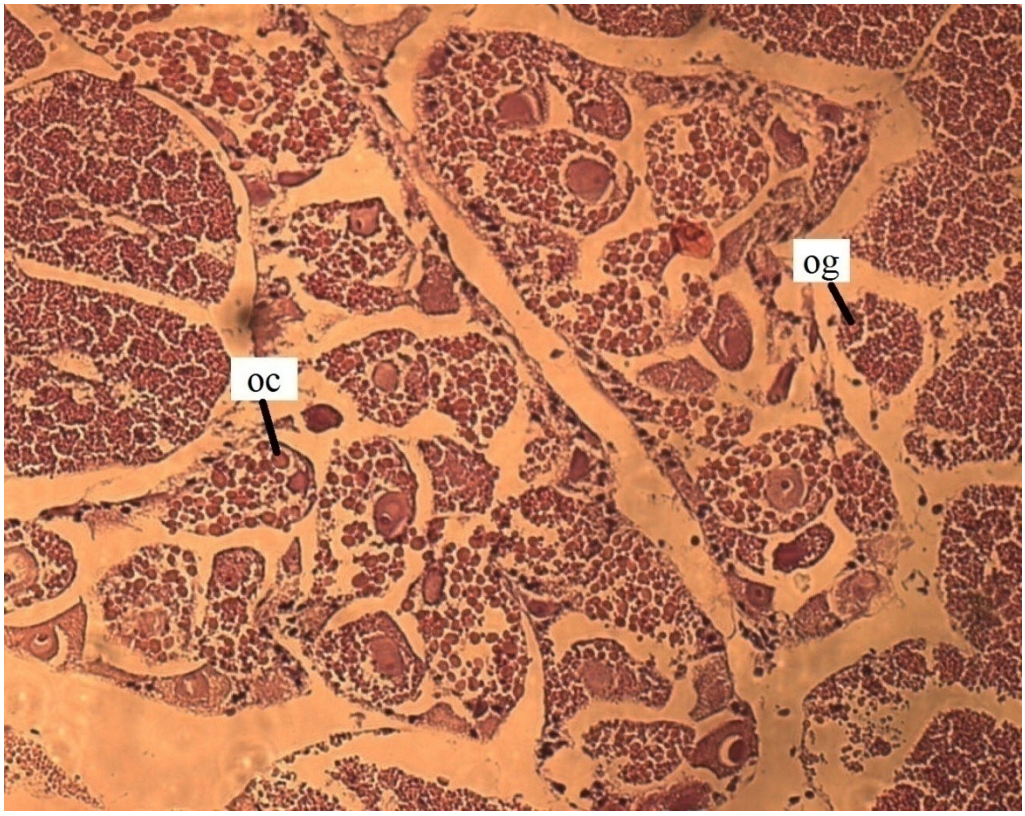

Fig. 4D. Female gonad Stage I (magnification=100X). oc = oocyte, og = oogonia

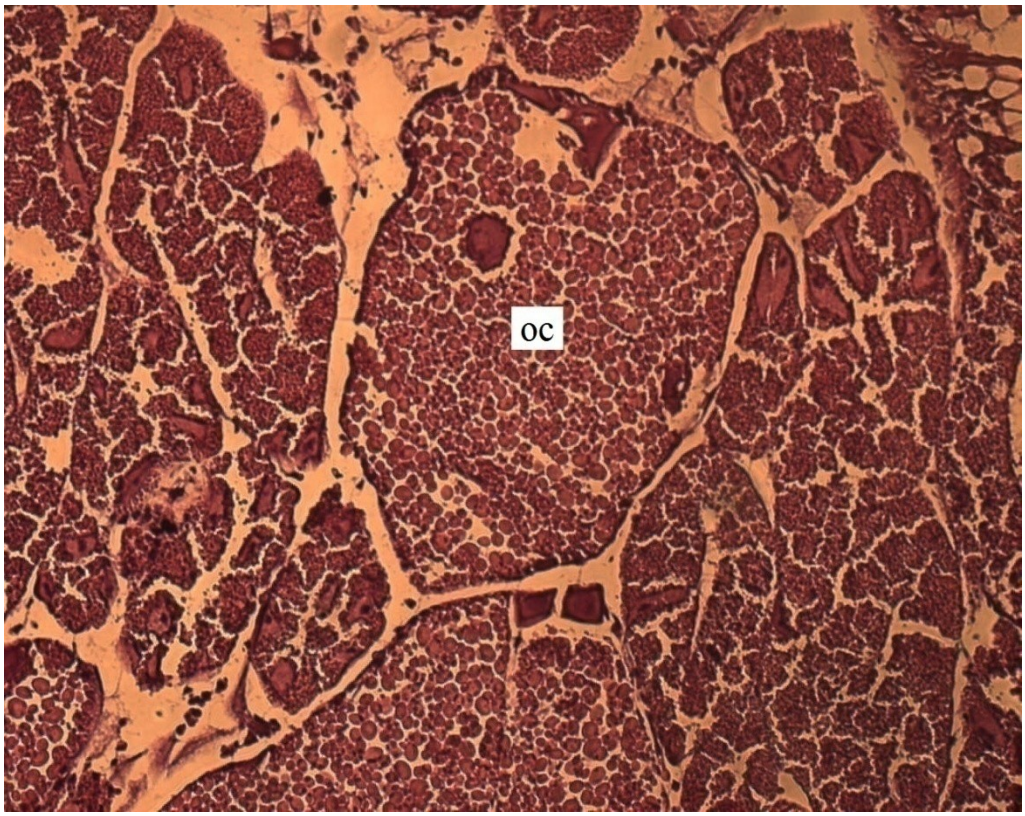

Figure 1. 4E. Female gonad Stage II (magnification=100X). oc = oocyte

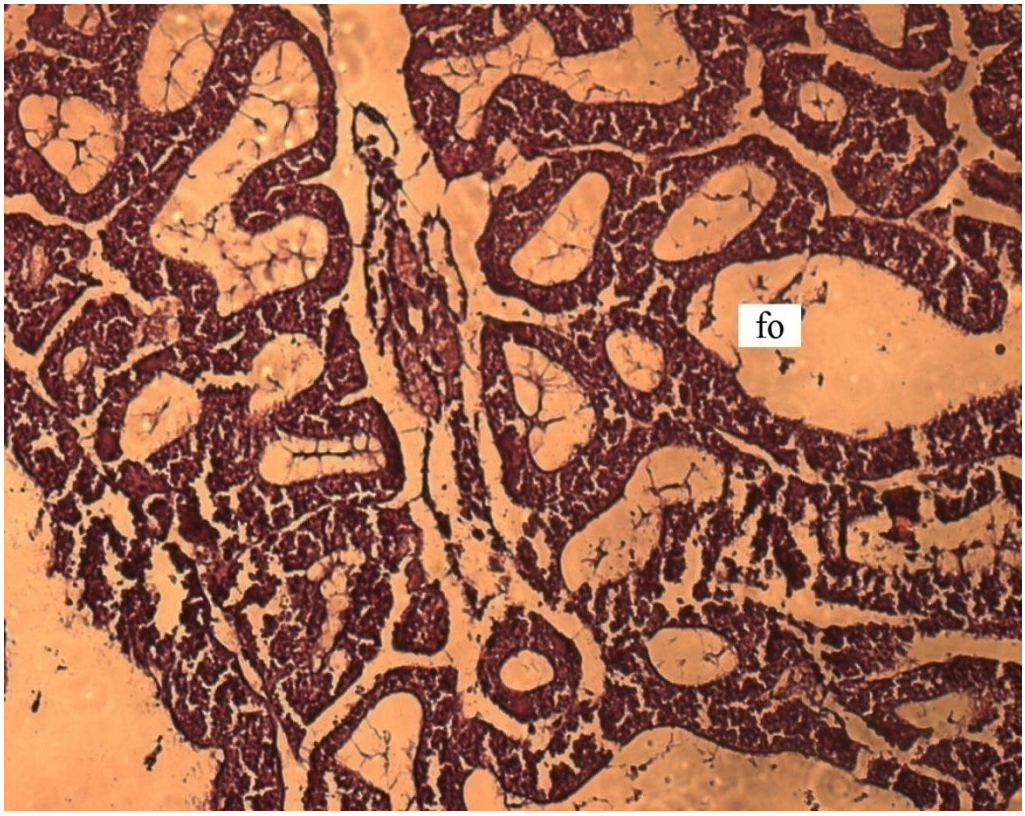

Fig. 4F. Female gonad Stage III (magnification=100X). fo = follicle

Fig. 5. Male reproductive system (A-E).

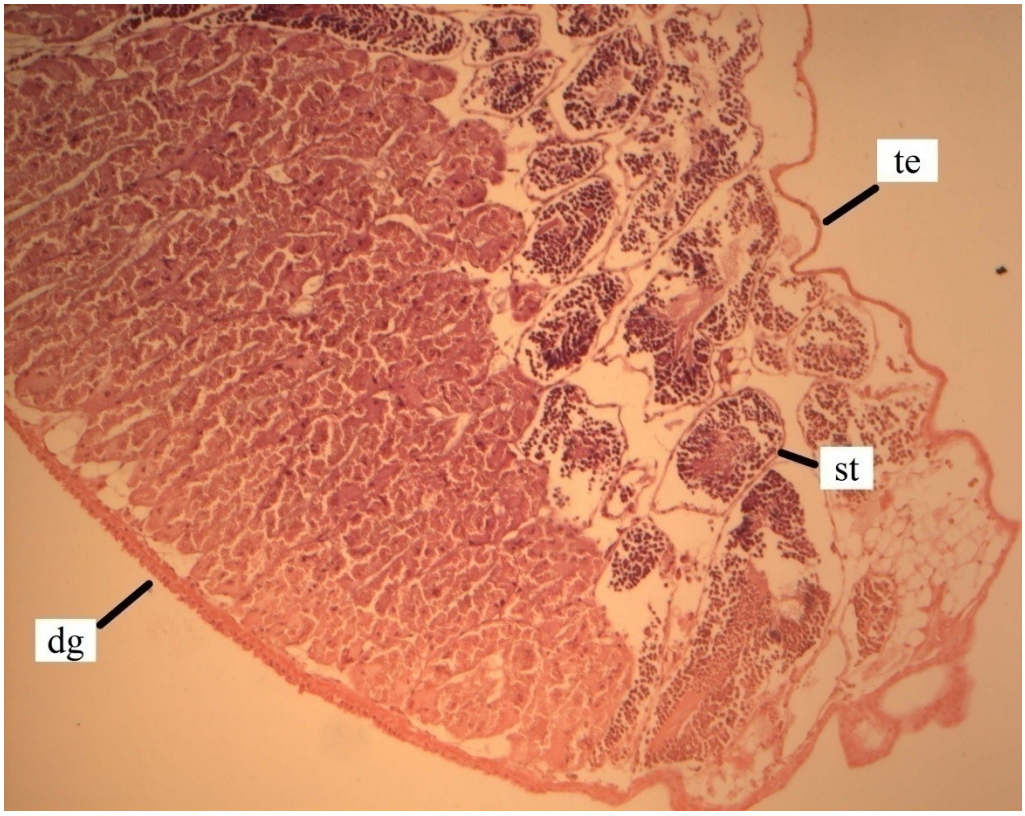

Fig. 5A. Testis (magnification=100X). dg = digestive gland, st = seminiferous tubule, te = testis

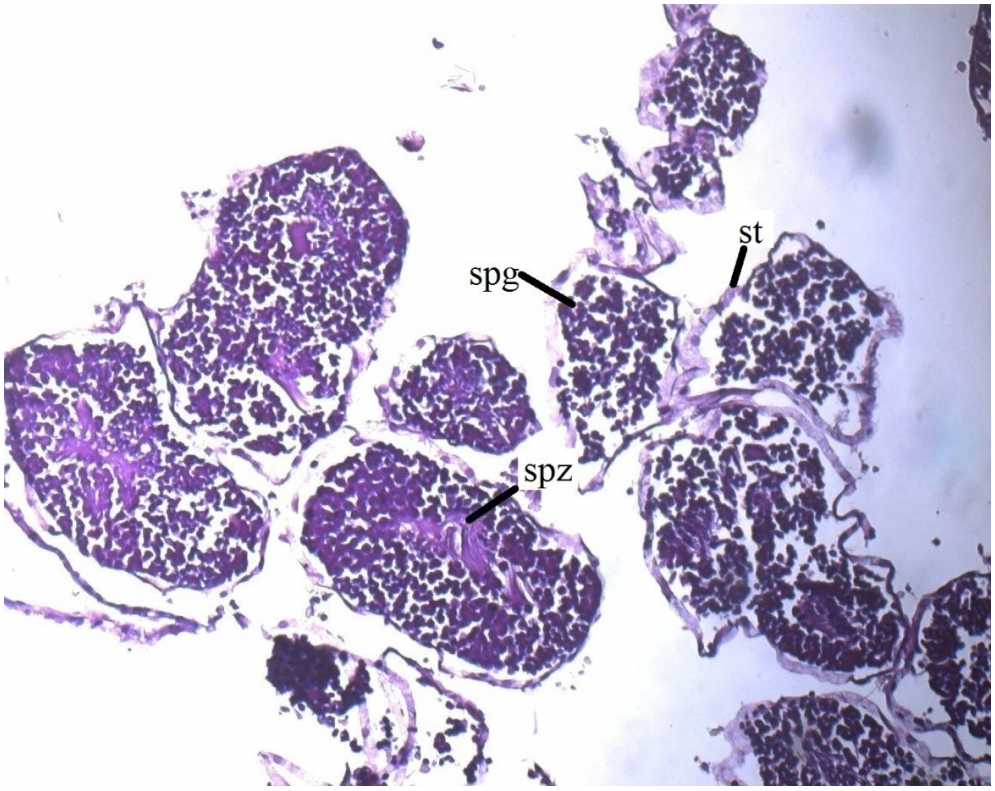

Fig. 5B. Seminiferous tubules (magnification=100X). st = seminiferous tubule, spg = spermatogonia, spz = spermatozoa

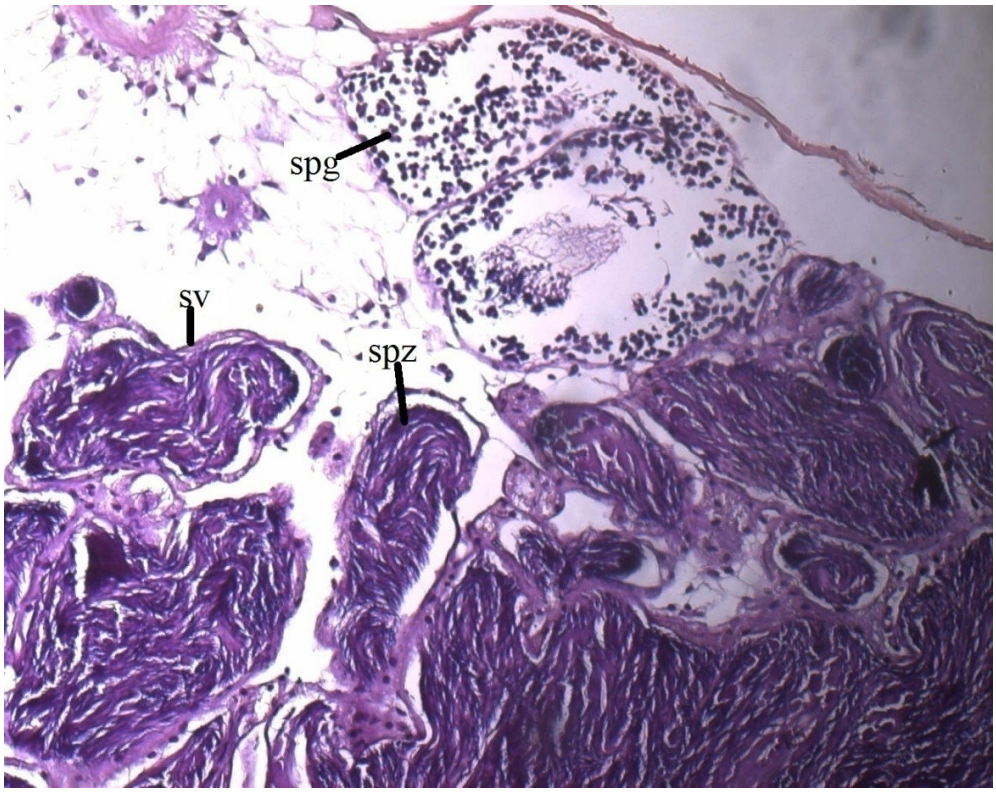

Fig. 5C. Seminal vesicle (magnification=100X). spg = spermatogonia, spz = spermatozoa, sv = seminal vesicle.

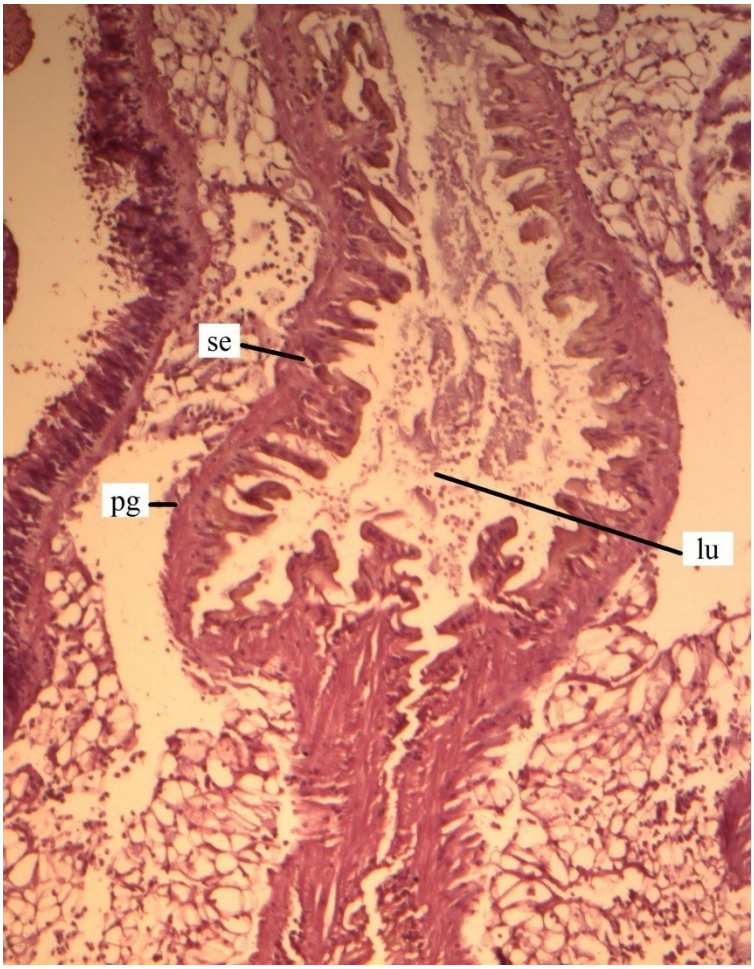

Fig. 5D. Prostate gland (magnification=100X). lu = lumen, pg = prostate gland, se = secretory epithelium.

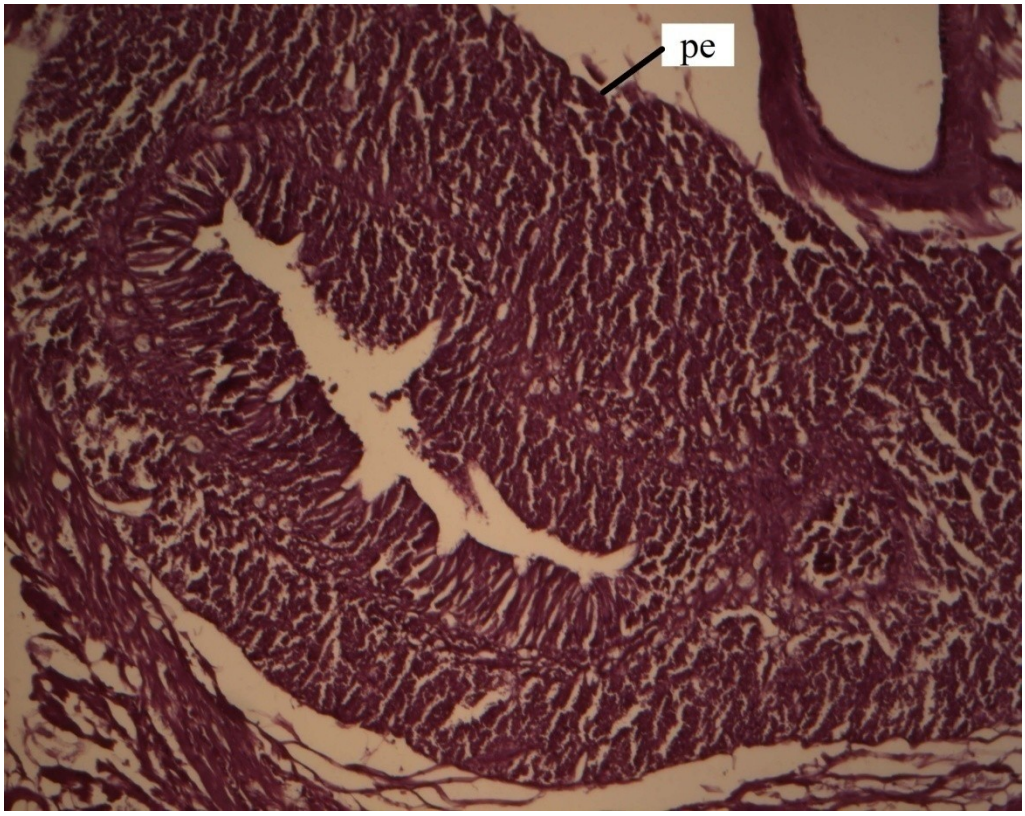

Fig. 5E. Penis (magnification=100X). pe = penis.

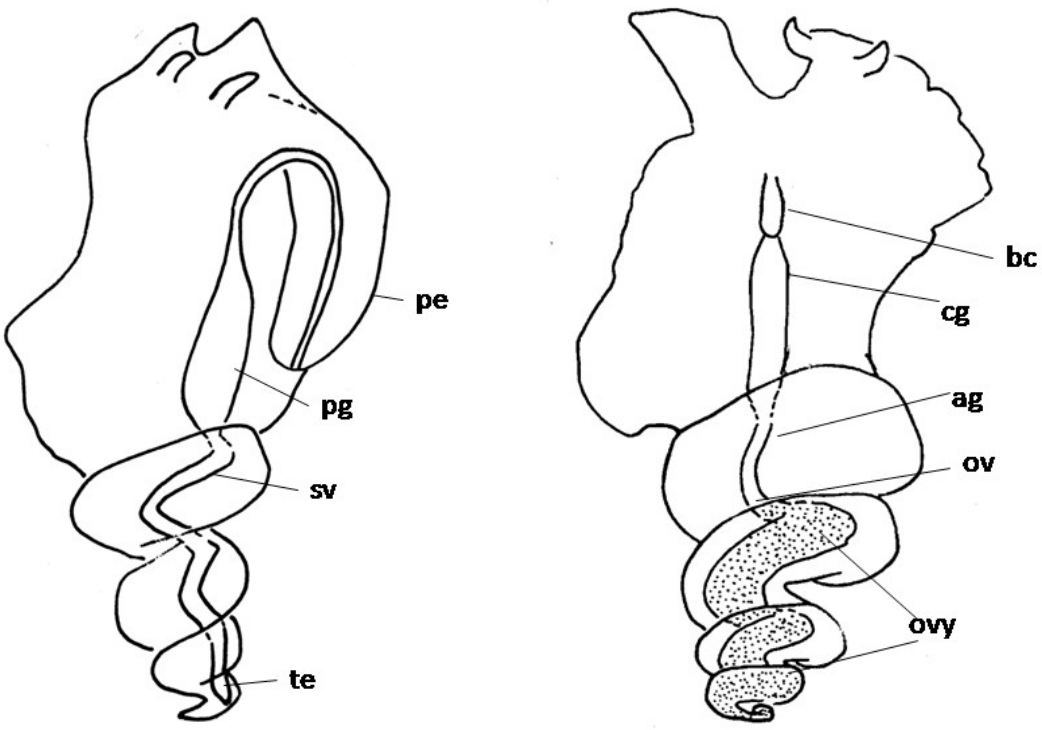

Fig. 6. Gross reproductive anatomy of *Gemmula speciosa* (A) male, (B) female. pe=penis, pg=prostate gland, sv=seminal, vesicle, te=testis, bc=bursa copulatrix, cg=capsule gland, ag=albumen gland, ov=oviduct, ovy=ovary.

Fig. 7. Female reproductive system (A-F).

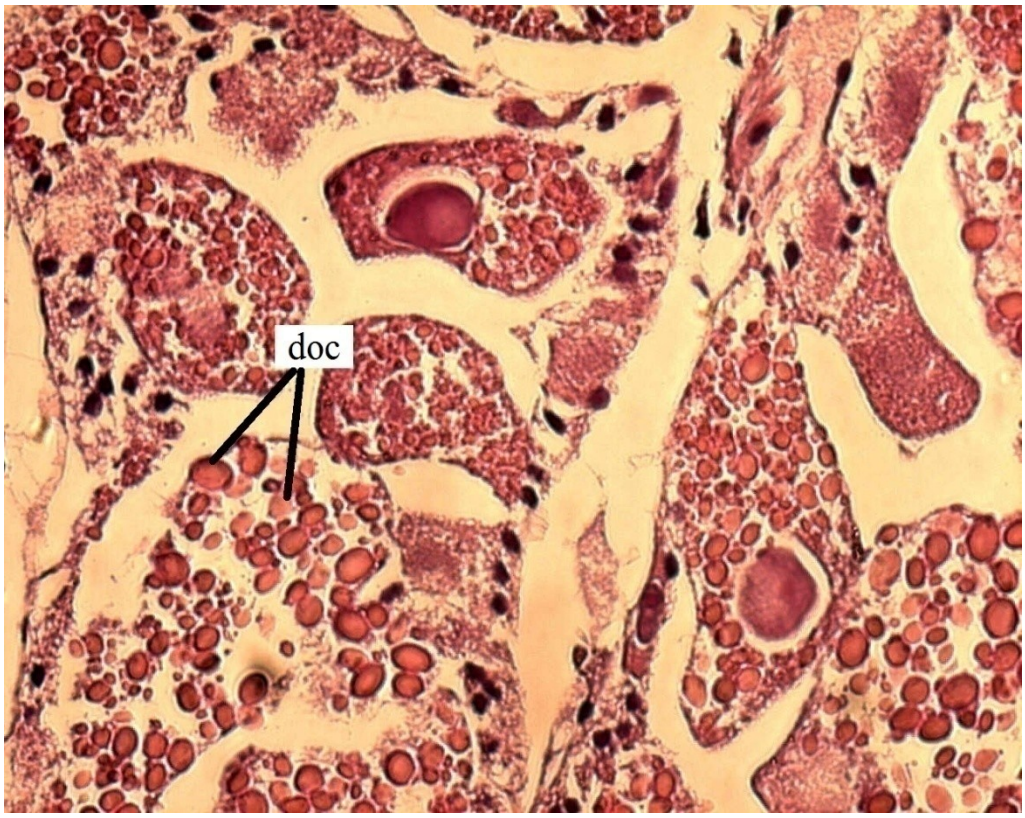

Fig. 7A. Ovary with developing oocytes (magnification=200X). doc = developing oocytes.

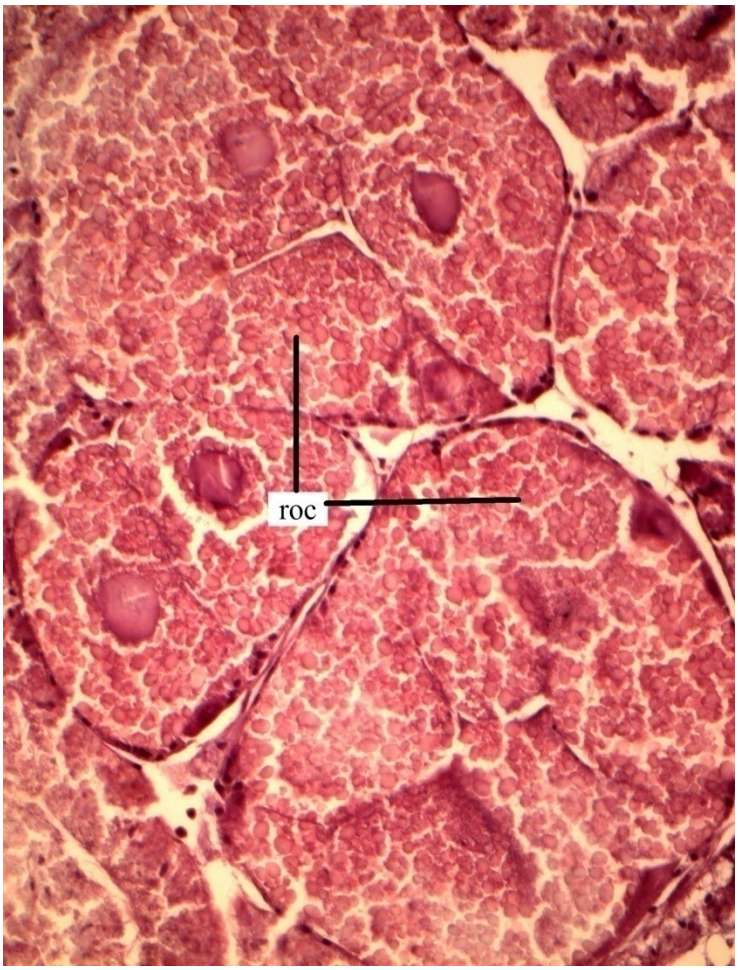

Fig. 7B. Ovary with ripe oocytes (magnification=200X). roc = ripe oocytes.

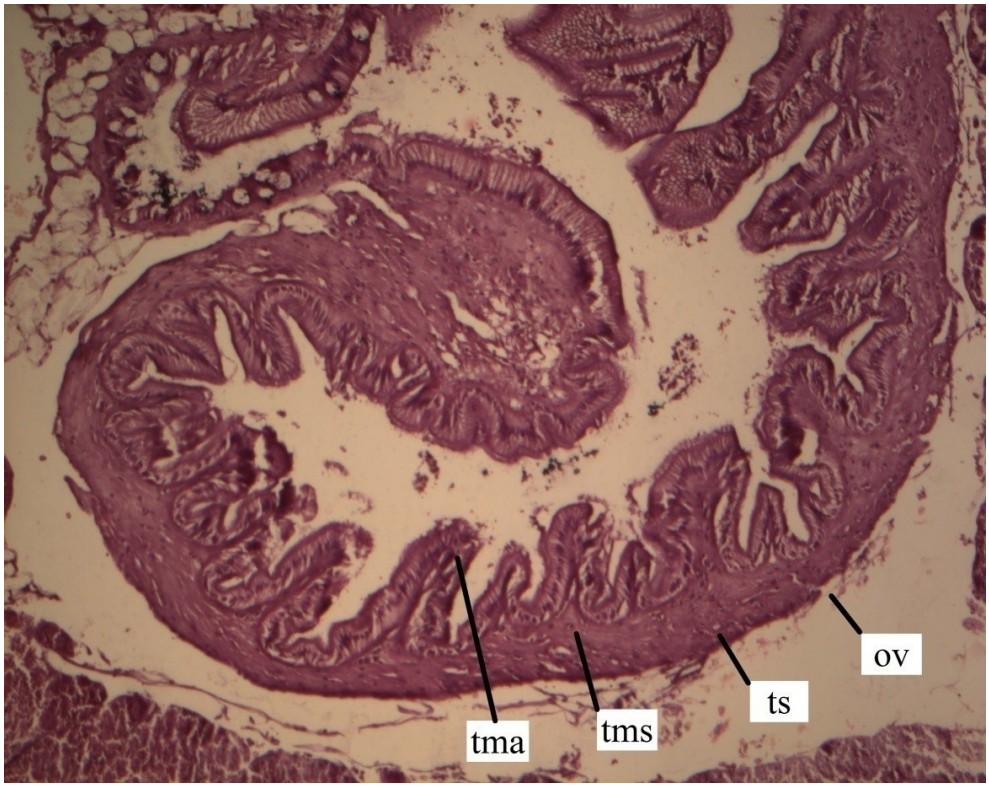

Fig. 7C. Oviduct (magnification=100X). ov = oviduct, tma = tunica mucosa, tms = tunica muscularis, ts = tunica serosa.

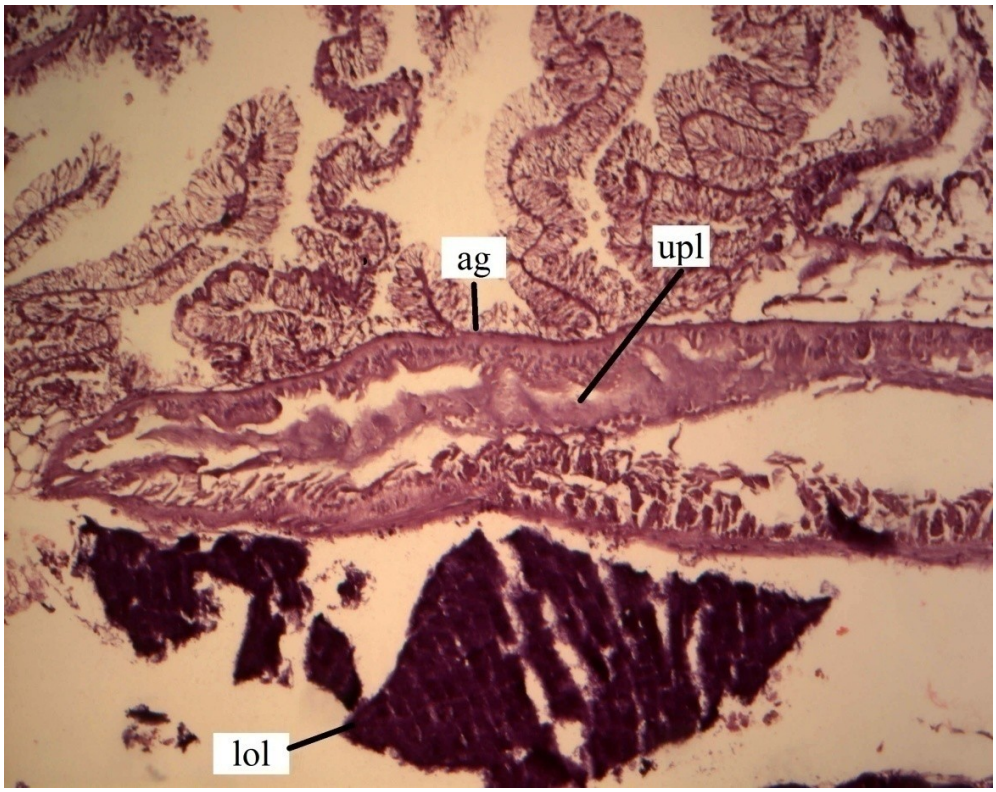

Fig. 7D. Albumen gland (magnification=100X). ag = albumen gland, lol = lower lobe, upl = upper lobe.

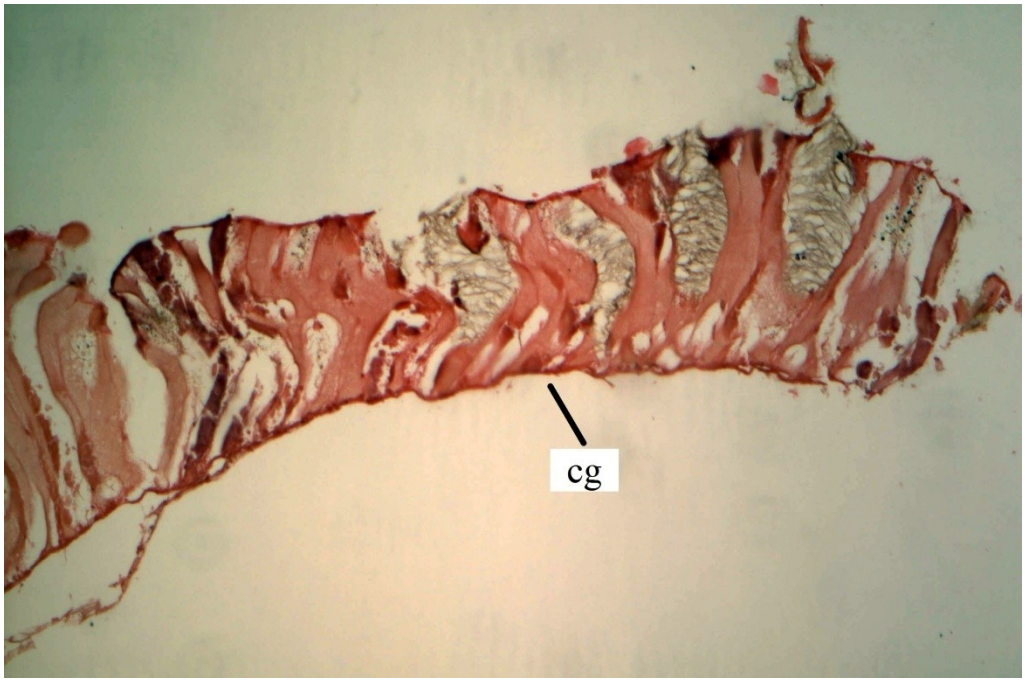

Fig. 7E. Part of the capsule gland (magnification=100X).

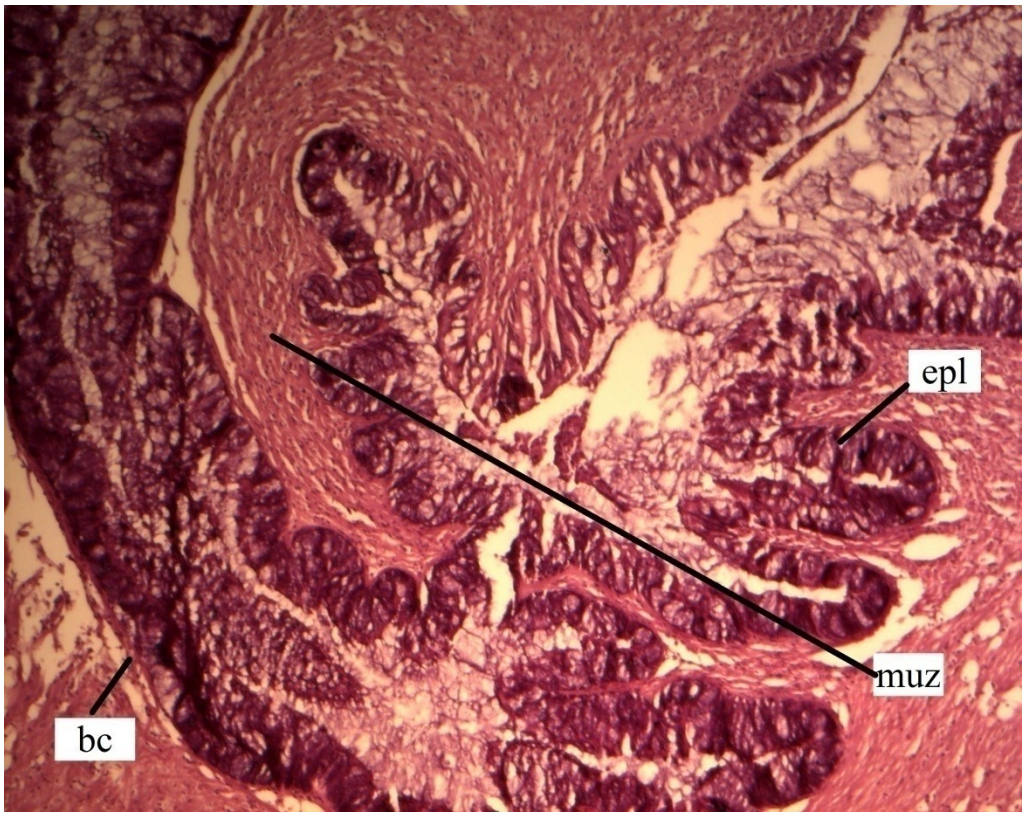

Fig. 7F. Bursa copulatrix (magnification=100X). bc = bursa copulatrix, epl = epithelium layer, muz = muscular zone.

Table 1. Maturity Indices (MI) of male and female *G. speciosa* for the period Sept 2004 to Jul 2007

Date of Sampling	Male MI	Female MI	Date of Sampling	Male MI	Female MI
9/13/2004	1.33	1.00	1/6/2006	1.33	3.00
11/19/2004	1.50	1.00	3/9/2006	1.33	3.00
12/17/2004	2.50	1.00	4/24/2006	1.67	2.00
1/18/2005	2.33	1.67	6/16/2006	1.33	1.00
7/25/2005	2.00	2.00	8/24/2006	1.33	1.00
8/12/2005	1.00	1.00	10/26/2006	1.67	1.67
9/13/2005	1.00	1.00	11/24/2006	2.33	2.33
10/12/2005	1.33	1.25	1/18/2007	2.33	3.00
11/11/2005	2.00	1.50	3/22/2007	2.00	1.67
12/15/2005	1.67	2.25	6/1/2007	2.33	1.67

Table 2. Gonad Indices (GI) of male and female *G. speciosa* for the period Sept 2004 to Jul 2007

Date of Sampling	Male GI	Female GI	Date of Sampling	Male GI	Female GI
9/13/2004	1.33	1.00	1/6/2006	1.33	1.00
11/19/2004	1.50	1.00	3/9/2006	1.33	1.00
12/17/2004	1.50	1.00	4/24/2006	1.00	1.00
1/18/2005	1.67	1.67	6/16/2006	1.33	1.00
7/25/2005	1.50	1.00	8/24/2006	1.33	1.00
8/12/2005	1.00	1.00	10/26/2006	1.67	1.67
9/13/2005	1.00	1.00	11/24/2006	1.00	1.67
10/12/2005	1.33	1.25	1/18/2007	1.00	1.00
11/11/2005	2.00	1.50	3/22/2007	1.33	1.00
12/15/2005	1.00	1.75	6/1/2007	1.00	1.00