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Digenea of clams (Bivalvia: Veneridae) in Lake Timsah (Suez Canal- Egypt)

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ABSTRACT

In order to determine a causal relation between infection and death of some clams, seven species of clams (Bivalvia: Veneridae) collected from Lake Timsah (Suez Canal) were examined histopathologically. Two species of digenean larvae; *Cercaria lata* Lespés, 1857 (Faustulidae) and an unknown species were encountered in three venerid species. The infection level in the venerid tissues ranges from light to heavy. In light and moderate infection, larvae were restricted to male and female gonads, while in heavy infection; they were found around the visceral mass and inside the digestive gland. In light infection, the gonad's structure was not affected while in moderate infection, the gonads lost some parts of their structure and in heavy infection, complete castration of gonads occurred. The prevalence of infection increased with increasing of clam size and reached maximum values in spring.

Keywords: Bivalvia; Veneridae; Digenea; Histopathology; Suez Canal; Lake Timsah.

INTRODUCTION

Biological pathogens, chemical contaminants and physical factors have been associated with weak productivity and mortality of bivalves (Lee *et al.*, 2001). Protozoan parasites have been involved in such mortality (Haskin & Andrews, 1988; Perkins, 1993; Sparks, 1993; Choi & Park, 2001 and Park & Choi, 2001). Discharges of anthropogenic by-products, a varity of organic or inorganic chemicals and physical stressors have been studied for possible direct and indirect causes for bivalve death (Sinderman, 1990; Chenge, 1993 and Pipe & Coles, 1995). In spite of all these studies, the exact pathological mechanism causing eventual death of bivalves is still not understood.

Marine bivalves often serve as a first intermediate host for Digenea. A high level of parasitism often results in slow growth and reduced fecundity of host organisms (Lauckner, 1983; Taskinen & Valtonen, 1995 and Taskinen, 1998). Khamdan (1998) reported that *Bucephalus* sp. destroyed female gonads of the pearl oyster *Pinctada radiata,* resulting in reproductive failure. Lee *et al.* (2001) revealed that, sporocysts and cercariae of the digenean parasite infecting the gonads and digestive gland of *Tapes philippinarum* disturb the reproductive processes of the clam and cause atrophic changes of the digestive gland epithelium which eventually lead to weakness and consequently massive mortality of clams. Ngo & Choi (2004) also observed that parasitism impacts clam reproduction, at least during part of the annual reproductive cycle.

The intensity of parasites in marine bivalves is often categorized from histological preparations of infected tissues (Ford, 1985; Ford & Figueras, 1988 and Barber *et al.,* 1988). Ellis *et al.* (1998) developed histology-based numerical scales as measures of the intensity of *Bucephalus* infection in mussels. Similarly, Ngo *et al.* (2003) developed a numerical scale for diagnosis of *Marteilioides chungmuensis* infection in the Pacific oyster, *Crassostrea gigas.* Ngo & Choi (2004) revealed that, although these scales are not truly quantitative, they are affordable and sensitive enough to differentiate the effects of different levels of infection on the hosts.

Lake Timsah lies in a half-way of the Suez Canal. Although its water is salty, yet fresh water, coming from the Nile through Ismailia Canal reduces its salinity and its pH value allowing higher fertility of the lake and consequently phytoplankton and zooplankton flourish. Such plankton-rich water results into fouling flourishing not only in Lake Timsah as well as the whole area bordering it. Moreover, the effect of pollution on this lake plays an essential role in the appearance of some filter feeding bivalves (Emara & Belal, 2004) which are considered as first intermediate host for Digenea.

Venerid clams are one of the favorite seafoods found in abundance in Lake Timsah. The present work aims to study the monthly pathological effects of digenean larvae on the venerid clams of this area.

Materials and methods

1- Venerid samples collection:

Venerid samples were collected monthly in 2006 from Lake Timsah (Suez Canal) and transported to the laboratory in buckets of sea water. In the laboratory, all specimens were identified morphologically according to Tillier and Bavay (1905), Moazzo (1939), Riedl (1970), Barash and

Danin (1973), Sharabati (1984), Mienis (1999) and Fishelson (2000).

2- Histopathological study:

Twenty-five male and Twenty-five female clams of different venerid species were examined for the presence of digenean larvae. Shells were separated from the soft bodies and different tissues were examined under the dissecting microscope (10x). Normal and infected tissues were fixed in 10% formalin. All tissue blocks were dehydrated with graded ethanol, cleared with xylene and embedded in paraffin wax. 5 µm transverse sections from each embedded block were cut and stained with hematoxylin-eosin. For digenean larvae separation, a number of infected venerid gonads were fixed in 10% formalin. Digenean larvae were settled down in the fixative. Formalin supernatant was removed then samples were stained on slide glasses under slight cover pressure by alum carmine, cleared in terpineol and mounted in Canada balsam. All drawings were made with the aid of camera Lucida.

Results

1- Venerid species:

According to the morphological characterstics (Figure 1), the collected venerid specimens belongs to seven species, *Callista florida* Lamarck, 1818; *Dosinia radiata* Reeve, 1850; *Gafrarium pectinatum* Linnaeus, 1758; *Paphia undulata* Born, 1778; *Tapes decussatus* Linnaeus, 1758; *Venerupis aureus* Gmelin, 1791 and *Venerupis pullastra* Montagu, 1803.

2- Parasitic infection:

Tapes decussatus was found to be infected with *Cercaria lata* Lespés, 1857 (Digenea: Faustulidae) as described morphologically by Ben Abdallah *et al.* (2009); *Venerupis pullastra* was infected with unknown digenean sp. Larvae, while *Venerupis aureus* was infected with both digenean species. The other four venerid species were uninfected.

2-1- Description of digenean larval stages inside the clam:

2-1-1- *Cercaria lata* Lespés, 1857 sporocyst (Figure 2A):

Live sporocysts were colorless, thick-walled, sausage-shaped, measuring 1.66–4.11 mm in length and 0.28–0.68 mm in width. Each sporocyst harbored 20–26 immature rediae and numerous germinal balls. No birth pore was observed.

2-1-2- Unknown digenean sporocyst (Figure 2B):

Sporocysts resemble the previous type and measuring 1.67–4.05 mm in length and 0.31–0.83 mm in width. Each sporocyst contains 24–32 immature rediae and numerous germinal balls. No birth pore was observed.

2-1-3- *Cercaria lata* Lespés, 1857 redia (Figure 2C):

Live specimens were colorless, thick-walled, sausage-shaped, 2.20–4.92 mm in length and 0.38–0.82 mm in width. Each redia is filled with germinal balls and developing cercariae. The number of cercariae within each redia varies according to the degree of development of the redia. Small redia contains 4–8 cercariae while large redia contains 11–17 cercariae. The mouth is antero-terminal, leading to a muscular pharynx that measure 0.09–0.20 mm in length and 0.05–0.11 mm in width and is followed by a short saccular gut which terminates at the level of the birth pore. The latter is located at about 0.46–1.03 mm from the anterior end.

2-1-4- Unknown digenean redia (Figure 2D):

Rediae resemble the previous type and 2.50–5.31 mm in length, 0.37–0.80 mm in width and contain numerous germinal balls and developing cercariae. Small redia contains 6–8 cercariae and large redia contains 16–22 cercariae. The mouth is antero-terminal, leading to a muscular pharynx; measuring 0.08–0.18 mm in length and 0.06–0.11 mm in width and is followed by a short saccular gut which terminates at the level of the birth pore. The latter is located at about 0.57–1.32 mm from the anterior end.

2-1-5- *Cercaria lata* Lespés, 1857 (Figure 3A):

A fully developed cercaria released from a redia, possess an oval, dorso-ventrally flattened, colorless body, measuring 0.19–0.34 mm in length and 0.11–0.23 mm in width. The tegument is covered by minute spines. The oral sucker is spherical, sub-terminal and measures 0.03–0.07 mm in diameter. The ventral sucker is globular, located approximately at the middle region of the body, and measures 0.02–0.05 mm in diameter. The mouth leads into a round muscular pharynx measures 0.03–0.06 mm in diameter. The oesophagus is narrow measures 0.02–0.05 mm in length and bifurcates anterior to the ventral sucker into two intestinal caecae that extends backwards to terminate blindly near the posterior end of the body. Genital primordium and cystogenous cells are present. Penetration glands are absent. The excretory vesicle is Y-shaped with long arms and the excretory ducts extend anteriorly to reach the intestinal bifurcation. Two lateral collecting ducts open at the anterior extremity of each excretory canal. Flame cells formula is 2 [(2 + 2) + (2 + 2)] = 16. The tail is unforked, slender, with annular folded plumose membrane, which carries 24–27 pairs of setae arranged in bundles laterally along the tail. Tail lacking flame cells and measures 0.25–0.55 mm in length and 0.03–0.07 mm in diameter. The structural features of this reported cercaria allow it to be identfied as tricocercus cercaria.

2-1-6- Unknown digenean cercaria (Figure 3B):

Live cercaria are pear-shaped, colorless, elongated, dorso-ventrally flattened and measuring 0.23–0.34

mm in length and 0.06–0.10 mm in width at the middle region. A pair of rounded black eyes lies anteriorly behind the oral sucker. The latter is spherical, subterminal and measures 0.035–0.063 mm in diameter. The ventral sucker is globular, located at the third part of the body between the two intestinal caecae. Pharynx well developed and measures 0.011–0.045 mm in diameter. Oesophagus is long, narrow, 0.09–0.19 mm in length and bifurcates after the middle of the body into two intestinal caecae that extend backwards to terminate blindly at the posterior end of the body. Genital primordium and cystogenous cells are present. Excretory vesicle is spherical with two excretory canals. Flame cells formula is 2 [(2 + 2) + (2 + 2)] = 16. The tail is unforked, long, slender, and unplumose and measures 0.46–0.80 mm in length and 0.02– 0.04 mm in diameter.

2-2- Prevalence of infection:

The prevalence of infection was higher in *Tapes decussatus* (27.3%) than in *Venerupis pullastra* (19.5%) and *Venerupis aureus* (5.0%). The prevalence of infection in the three infected clams followed almost the same pattern. No infection was observed in August, and December, however the prevalence of infection reached its higher level in May followed by a decline in June and July (Table1 and Figure 4).

In the three infected clams, the prevalence of infection increased with increasing of clam length and weight and no infection was detected in clams with shell length < 2.5 cm, also it was higher in females than males (Table 1). In *Tapes decussatus* the prevalence reached 29.5% in females and 25% in males, while in *Venerupis pullastra* reached 21.7% in females and 17.3% in males and in *Venerupis aureus* reached 5.7% in females and 4.3% in males.

3- Histopathological investigations:

The infection level by digenean larvae in the clam tissues ranges from light to heavy. In light and moderate infection, larvae were restricted to male and female gonads. In heavy infection, they were found around the visceral mass and inside the digestive gland. The level of infection by the two collected digenean larvae was similar in the venerid hosts.

A- Gonads:

- Male:

The normal testis of *Tapes decussatus* consists of acini with developing spermatocytes, spermatids and sperms organized in a rosette formation. Interacinar connective and muscular tissues surround these acini (Figure 5A, B and C). In light infection, larvae were found around the testis, few were found between the acini, leaving the gonad intact. In moderate infection, the number of larvae increased inside the testis between the acini causing degeneration of the connective and muscular tissues. Acini shrinkage occurred with decreasing in sperm number and degeneration of spermatocytes in some acini. In heavy infection, acini and muscular as well as connective tissues were replaced by the parasite larvae resulting in testis castration (Figure 5A, B, C, D, E, F, G & H).

- Female:

The normal ovary of *Tapes decussatus* consists of follicles with developing and mature oocytes. The interfollicular connective and muscular tissues are surround follicles. In light infection, most of theparasite larvae were found around the ovary and few were present between the gonadal follicles keeping the follicles and the enclosed oocytes intact. In moderate infection, the larvae increased in number and the interfollicular connective and muscular tissues were degenerated in the sites of infection.

Heavy infection by digenean larvae resulted in ovary castration, where the larvae invade the ovary in a dense mass causing the deterioration of follicles, muscular and connective tissues (Figure 6A, B, C, D, E & F).

B- Digestive gland:

The normal digestive gland of *Tapes decussatus* is large, tubulo-acinar consisting of primary and secondary tubules and is covered by squamous epithelium. The primary tubules are lined with simple epithelium consisting of two main cell types, digestive and basophilic cells. The digestive cells are cube-shaped or columnar, rest on a very thin basement membrane, and their nuclei are basal, usually oval, but sometimes spheroid or irregular. The cell body exhibits various degrees of vaculation. The basophilic cells are present in much smaller number than the digestive cells. They are shorter; pyramidal, cone-shaped or columnar; and appear wedged in between groups of the digestive cells. In heavy infection, the digenean larvae were found around the digestive gland and between the tubules. Intense autolysis was observed in all digestive cells with loss of basophilic cells and increased vacuolization. Despite these pathological alterations of the cells, basal membranes were intact (Figure 7A, B, C & D).

Discussion

The trichocercous cercaria obtained from *Tapes decussatus* and *Venerupis aureus* was identified by morphological examination as *Cercaria lata* Lespés (1857). *C. lata* was the first to reported and named from *Tapes decussatus* collected in Arcachon. It was also reported from the same host in Tunisia by Ben Abdallah *et al.* (2009). Ramon *et al.* (1999) identified the same cercaria from clam *Donax trunculus* in the Western Mediterranean as *Bacciger bacciger* (Fellodistomidae) depending on the description of Palombi (1933a, b & 1934). Hall *et al.* (1999) promoted the Baccigerinae from the Fellodistomidae to Faustulidae after using the sequence of gene (18S rRNA gene). Hanafy *et al.* (1997) found the same cercaria in the gonad of *Venerupis decussata* in Lake Timsah and identify it to family level (Lepocreadiidae).

The other digenean cercaria found in *Venerupis pullastra* and *Venerupis aureus* was not identified in this study due to the lack of morphological informations about this parasite.

The presence of parasites in clams collected from Lake Timsah may be due to heavily exposed to different types of organic contamination; agriculture drainage, domestic wastes and shipping activities as stated by Hanafy *et al* (1997), or to the presence of second and/or final hosts in this area.

Lumb *et al.* (1993) postulated that digenean of families Lepocreadiidae and Faustulidae are common parasites of shallow and deep-water marine fishes (the main host), so three species of collected clams; *Tapes decussatus, Venerupis pullastra* and *Venerupis aureus* were infected by the digenean larvae related to the deep water habitat of these infected species (intermediate host), while the other species; *Callista florida, Dosinia radiata, Gafrarium pectinatum and Paphia undulata* were habit the surface sediment.

There is a difference in infection prevalence between clam species and between months, this may be related to the clam size and/or the availability of second or final host as suggested by Ngo & Choi (2004). The prevalence of infection increased with the increase of the clam size. Heasman *et al*_(1996) postulated that this increase could be caused by one or a combination of the following factors: (1) a cumulative effect, as larger and older clams have been exposed longer to the chance of miracidial infection; (2) the increased chance of miracidial contact because larger clams filter greater volumes of water; or (3) decreasing resistance to infection with advanced age. Similar finding have been reported by Heasman *et al.* (1996) and Roman *et al.* (1999).

The absence of larval infection in clams < 2.5 cm suggests that individuals attained this size before the invasive stage appeared, although clams of this size were found throughout the year in the population (Ramon, 1993). However the presence of larvae may depend on the sexual maturity of the bivalve. Roman *et al* (1999) postulated that, clam reach the sexual maturity at shell length 1.9 cm.

The present study revealed that, the gonads were the first organs to be infected. Histopathological study demonstrated that high level of digenean infection may cause gonad castration. Since the gonads of infected clams collected during the spawning season (spring) were completely occupied by digenean larvae. Several studies have reported the detrimental effects of digenean parasitism on reproduction in marine molluscs. Khamdan (1998) reported that *Bucephalus* destroyed the gonads of pearl oysters and that heavily infected oysters could not initiate gametogenesis. Ngo and Choi (2004) revealed that the presence of *Cercaria tapidis* in high levels in *Ruditapes philippinarum* disturbed the reproductive processes, possibly retarding gonadal development or destroying gametes. Hassanine (2006) reported gonadal castration in female *Crassostrea cucullata* caused by *Diploproctodaeum arothroni.* This may result from the fact that the larvae utilize the storage substances (glycogen) of the intertubullar tissue, which lead to arrest of the gametogenic cycle as suggested by Roman et al. (1999). The higher infection prevalence and heavily infected females than males may be explained, where the higher content of glycogen in females allows the digenean larvae to grow faster and cause complete castration at higher rate than in case of males as suggested by Hanafy *et al.* (1997). Also the size of females was slightly greater than the size of males in this study.

The atrophy of the digestive epithelium of the digestive gland has been well referred to as response to a variety of stressors including starvation (Thompson *et al.* 1974), exposure to various environmental pollutants (Lowe *et al.*, 1981; Cajaraville *et al.*, 1992; Marigomez *et al.*, 2002; Pucheva *et al.*, 2006 and El-Shenawy *et al.*, 2009), low salinity (Winstead, 1995) and parasitic infection (Lee *et al.*, 2001) or combination of two or more of these factors as stated by Hamza (2010). In terms of their frequency, these atrophic lesions strongly suggest that, clams in Lake Timsah have been subjected to stressful environment. The finding that the epithelial atrophy was more frequently encountered in primary tubules than in secondary ones was also reported by Lee *et al.* (2001) who postulated that the epithelium of primary tubules may be more vulnerable to stressors than that of secondary tubules. They concluded that since the digestive epithelium is known to be important in digestion of foods and detoxification of contaminants, the atrophic changes of epithelium may lead to the weakness of clams and consequently massive mortality.

Sures (2008) referred to the contrasting effect of parasites on the health of their hosts. Whereas parasites might reduce pollutant levels in their hosts below a critical value and thus are capable of reducing adverse negative effects, they cause similarly pathological effects. On the other hands, parasites may also enhance toxic effects of pollutants by interfering with host's protection mechanism. Baudrimont *et al.* (2006) revealed that, parasite infection in cockles can modulate metallothionins synthesis that could consequently interfere with response of these protective proteins in case of metal contamination. These contrasting ideas may alter the common understanding and definition of parasites as being exclusively harmful to their hosts at least in light or moderate infection, while in heavy infection, the pathological effect is definite since castration of gonads leads to sterility of the clam, and atrophy of digestive gland leads to clam mortality.

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Table (1): Monthly prevalence of digenean larvae infection during sampling period of *T. decussatus, Venerupis pullastra* and *Venerupis aureus* collected from Lake Timsah.

Species	Sampling month	SL (cm)		WTW (g)		Cercaria infection			
						N		Prevalence (%)	
		Male	Female	Male	Female	Male	Female	Male	Female
T. decussatus	January	2.6 ± 0.14	2.8 ± 0.12	1.35 ± 0.01	1.61 ± 0.03	1	2	4	8
	February	3.1 ± 0.17	3.4 ± 0.16	1.55 ± 0.01	1.72± 0.03	4	5	16	20
	March	3.7 ± 0.18	3.8 ± 0.18	1.81 ± 0.02	1.90 ± 0.04	11	13	44	52
	April	4.0 ± 0.09	4.3 ± 0.12	2.32 ± 0.03	2.51 ± 0.01	14	17	56	68
	May	4.4 ± 0.08	4.6 ± 0.14	2.58 ± 0.03	2.80 ± 0.01	18	19	72	76
	June	4.5 ± 0.21	4.7 ± 0.16	2.22 ± 0.02	2.33 ± 0.02	15	17	60	68
	July	4.6 ± 0.19	4.7 ± 0.09	2.02 ± 0.03	2.15 ± 0.03	6	8	24	32
	August	3.8 ± 0.09	3.9 ± 0.08	1.58 ± 0.01	1.60 ± 0.03	0	0	0	0
	September	3.4 ± 0.09	3.6 ± 0.11	1.35 ± 0.05	1.43 ± 0.01	2	3	8	12
	October	3.1 ± 0.06	3.4 ± 0.12	1.12 ± 0.01	1.40 ± 0.03	3	3	12	12
	November	2.8 ± 0.14	3.0 ± 0.17	1.08 ± 0.03	1.12 ± 0.02	1	2	4	8
	December	2.4 ± 0.15	2.5 ± 0.08	1.01 ± 0.03	1.03 ± 0.01	0	0	0	0
V. pullastra	January	2.0 ± 0.13	2.2 ± 0.13	1.11 ± 0.01	1.32 ± 0.04	1	2	4	8
	February	2.8 ± 0.15	2.9 ± 0.10	1.23 ± 0.03	1.45 ± 0.03	2	3	8	12
	March	3.4 ± 0.15	3.5 ± 0.13	1.42 ± 0.03	1.68 ± 0.01	9	11	36	44
	April	3.8 ± 0.10	4.0 ± 0.19	2.01 ± 0.02	2.32 ± 0.05	11	13	44	52
	May	4.0 ± 0.21	4.2 ± 0.13	2.31 ± 0.04	2.61 ± 0.02	12	15	48	60
	June	4.1 ± 0.20	4.2 ± 0.12	1.95 ± 0.01	2.26 ± 0.03	10	11	40	44

	July	4.1 ± 0.12	4.1 ± 0.09	1.89 ± 0.02	2.03 ± 0.03	4	5	16	20
	August	3.6 ± 0.11	3.7 ± 0.08	1.41 ± 0.03	1.53 ± 0.01	0	0	0	0
	September	3.3 ± 0.18	3.4 ± 0.13	1.33 ± 0.04	1.32 ± 0.05	1	2	4	8
	October	3.0 ± 0.17	3.2 ± 0.18	1.01 ± 0.03	1.13 ± 0.02	1	2	4	8
	November	2.6 ± 0.16	2.8 ± 0.10	0.92 ± 0.03	1.01 ± 0.02	1	1	4	4
	December	1.8 ± 0.15	1.9 ± 0.07	0.71 ± 0.01	0.92 ± 0.01	0	0	0	0
V. aureus	**January**	1.6 ± 0.10	1.6 ± 0.08	0.42 ± 0.01	0.51 ± 0.03	0	0	0	0
	February	1.8 ± 0.08	1.9 ± 0.12	0.68 ± 0.01	0.69 ± 0.03	0	0	0	0
	March	2.1 ± 0.09	2.2 ± 0.15	0.81 ± 0.03	0.93 ± 0.09	2	3	8	12
	April	2.4 ± 0.12	2.4 ± 0.09	1.15 ± 0.01	1.29 ± 0.01	3	4	12	16
	May	2.6 ± 0.14	2.7 ± 0.05	1.26 ± 0.02	1.49 ± 0.09	4	5	16	20
	June	2.6 ± 0.09	2.6 ± 0.18	1.21 ± 0.04	1.24 ± 0.05	3	3	12	12
	July	2.5 ± 0.09	2.5 ± 0.17	1.01 ± 0.05	1.02 ± 0.03	1	2	4	8
	August	2.1 ± 0.08	2.2 ± 0.09	0.89 ± 0.01	0.95 ± 0.03	0	0	0	0
	September	2.0 ± 0.12	2.1 ± 0.09	0.81 ± 0.06	0.90 ± 0.01	0	0	0	0
	October	1.8 ± 0.05	1.9 ± 0.13	0.71 ± 0.07	0.83 ± 0.06	0	0	0	0
	November	1.6 ± 0.07	1.7 ± 0.03	0.51 ± 0.03	0.61 ± 0.03	0	0	0	0
	December	1.5 ± 0.03	1.5 ± 0.08	0.39 ± 0.02	0.50 ± 0.01	0	0	0	0

(Each mean ± standard error for 25 samples; **N**, number of infected clams; **SL**, shell length and **WTW**, wet tissue weight).

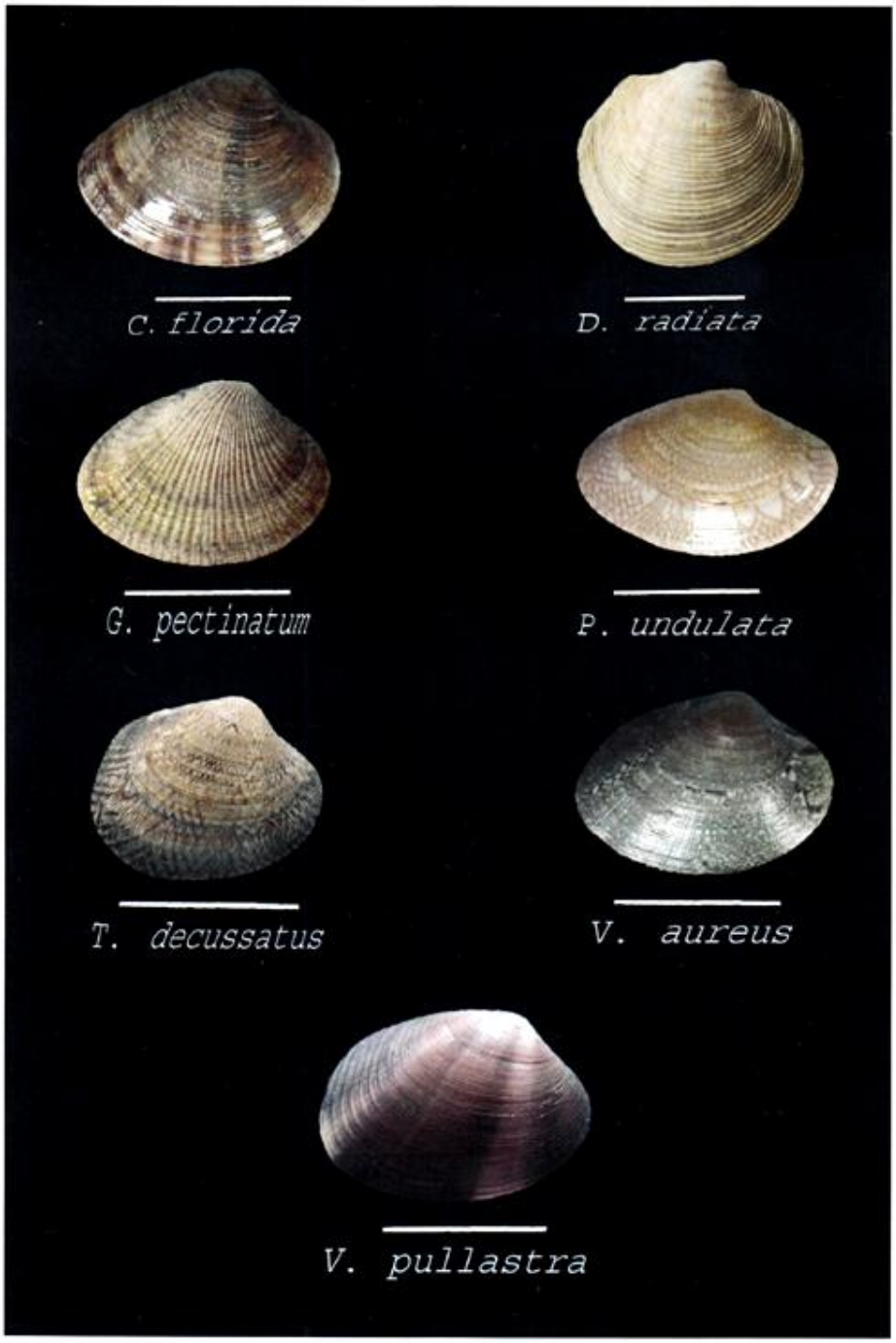

Figure (1): Exterior (right valve) view of the venerid clam shells, scale bar= 2 cm.

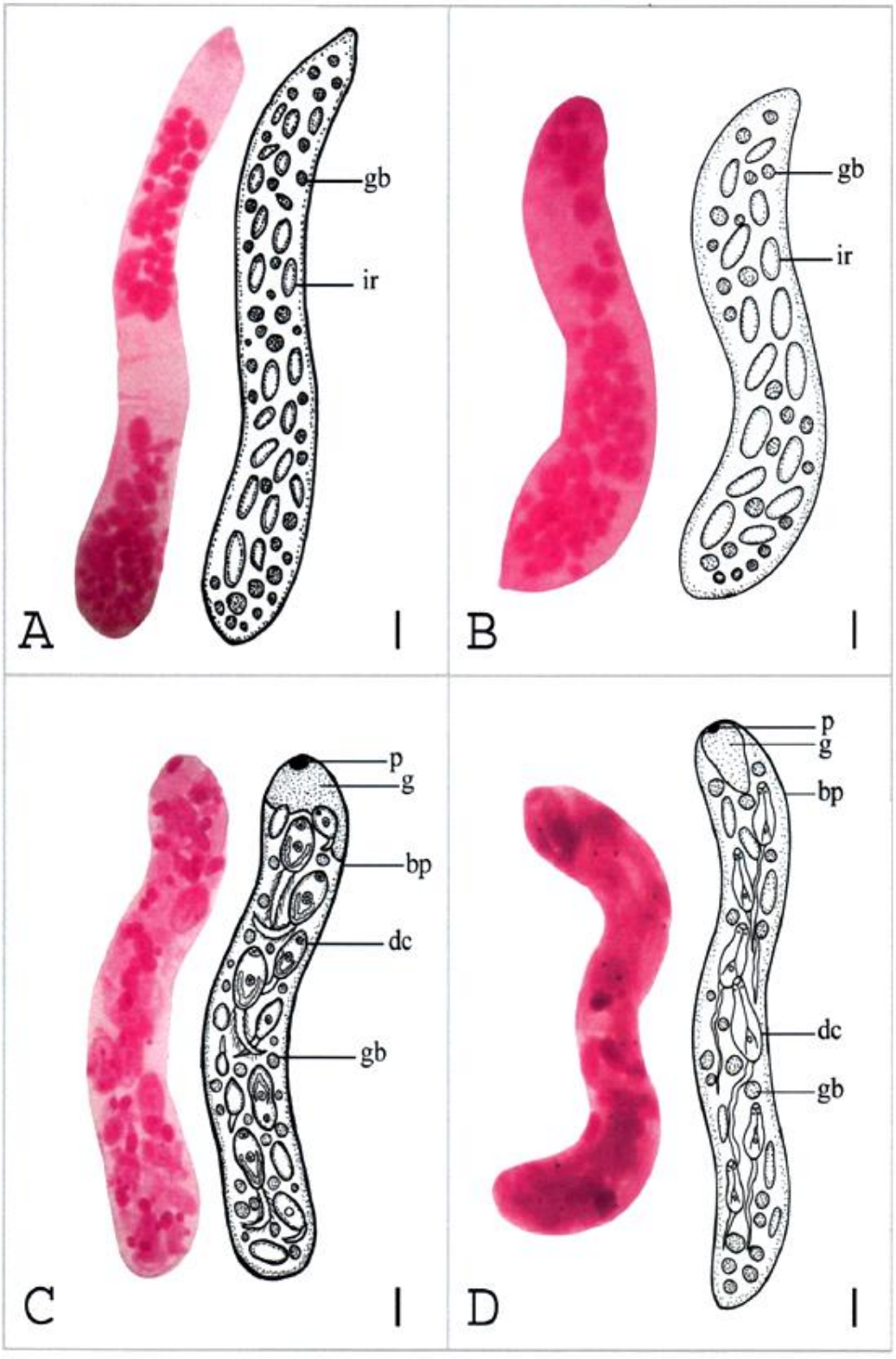

Figure (2): Photomicrographs of carmine stained and line drawings (A) Sporocyst of *Cercaria lata*, (B) Sporocyst of unknown Digenea, (C) Redia of *Cercaria lata* and (D) Redia of unknown Digenea. bp, birth pore; dc, developing cercaria; g, gut; gb, germinal ball; ir, immature redia; p, pharynx. Scale bar= 200 μm.

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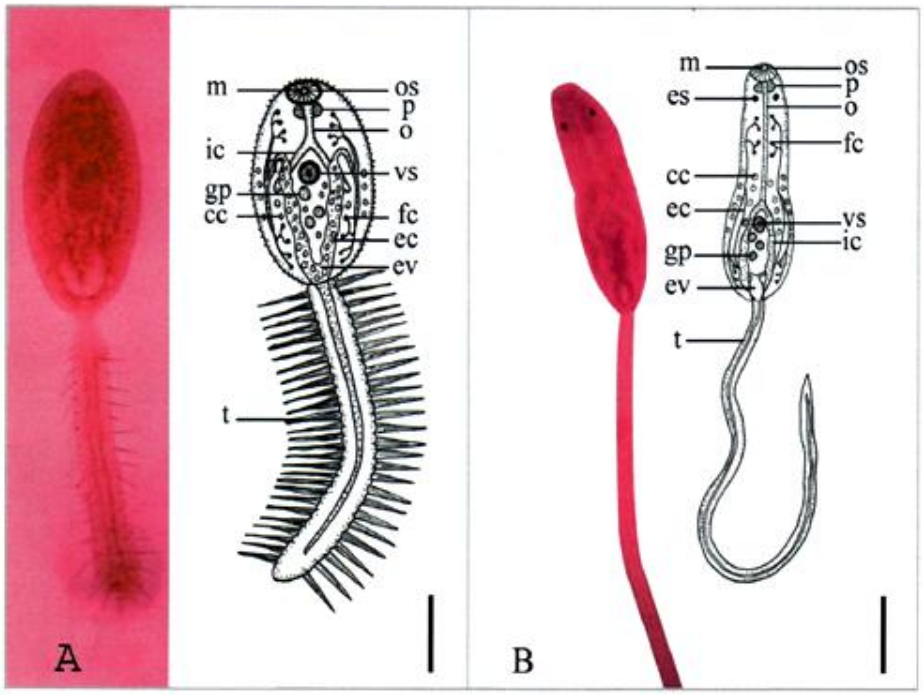

Figure (3): Photomicrographs of carmine stained and line drawings (A) *Cercaria lata* and (B) unknown digenean cercaria. cc, cystogenous cell; ec, excretory canal; es, eye spot; ev, excretory vesicle; fc, flame cell; gp, genital primordium; ic, intestinal caeca; m, mouth; o, oesophagus; os, oral sucker; p, pharynx; t, tail; vs, ventral sucker. Scale bar = 100 μm.

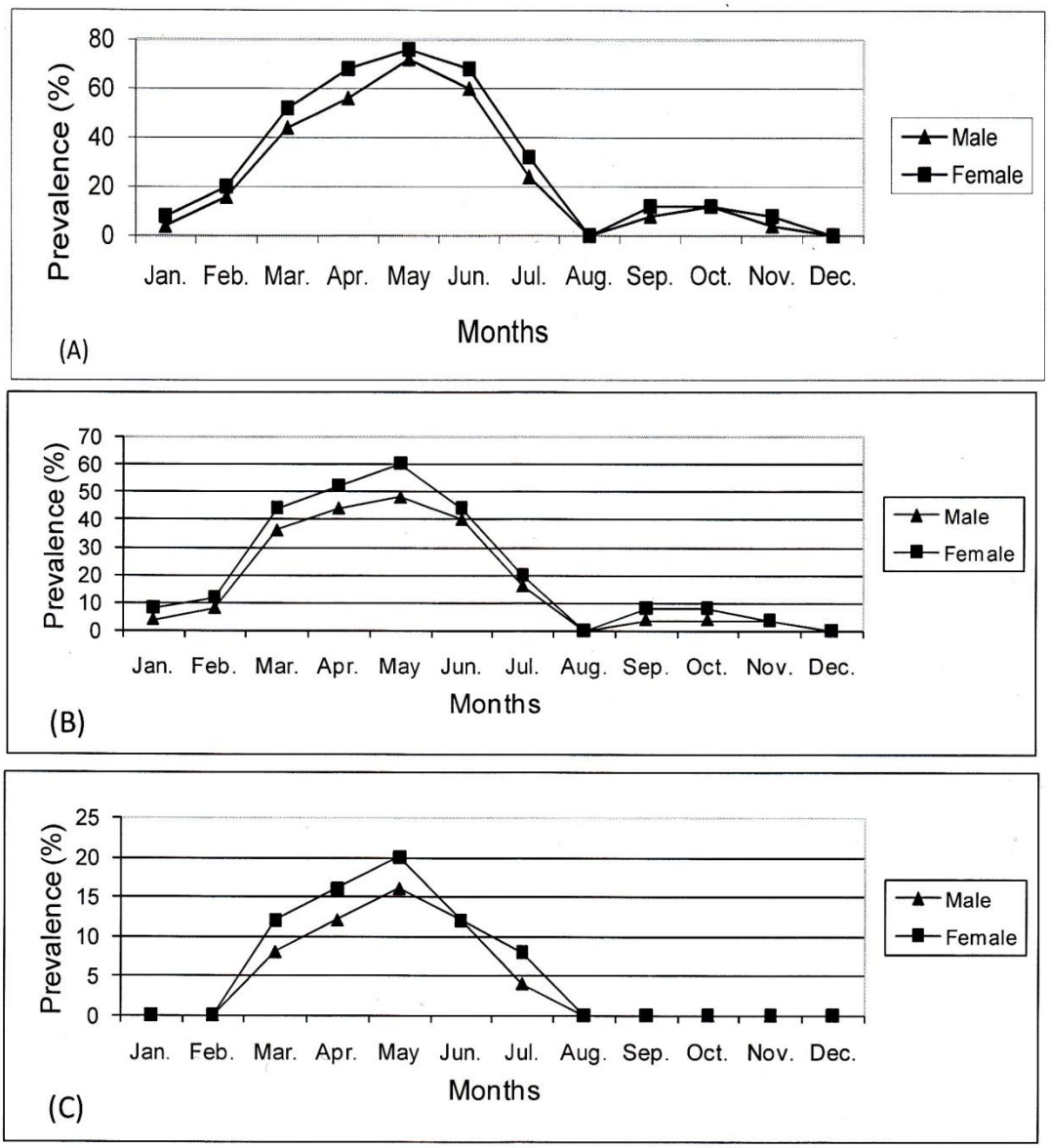

Figure (4): Monthly infection prevalence of digenean larvae in venerid clams. A. *Tapes decussatus*, B. *Venerupis pullastra* and C. *Venerupis aureus*.

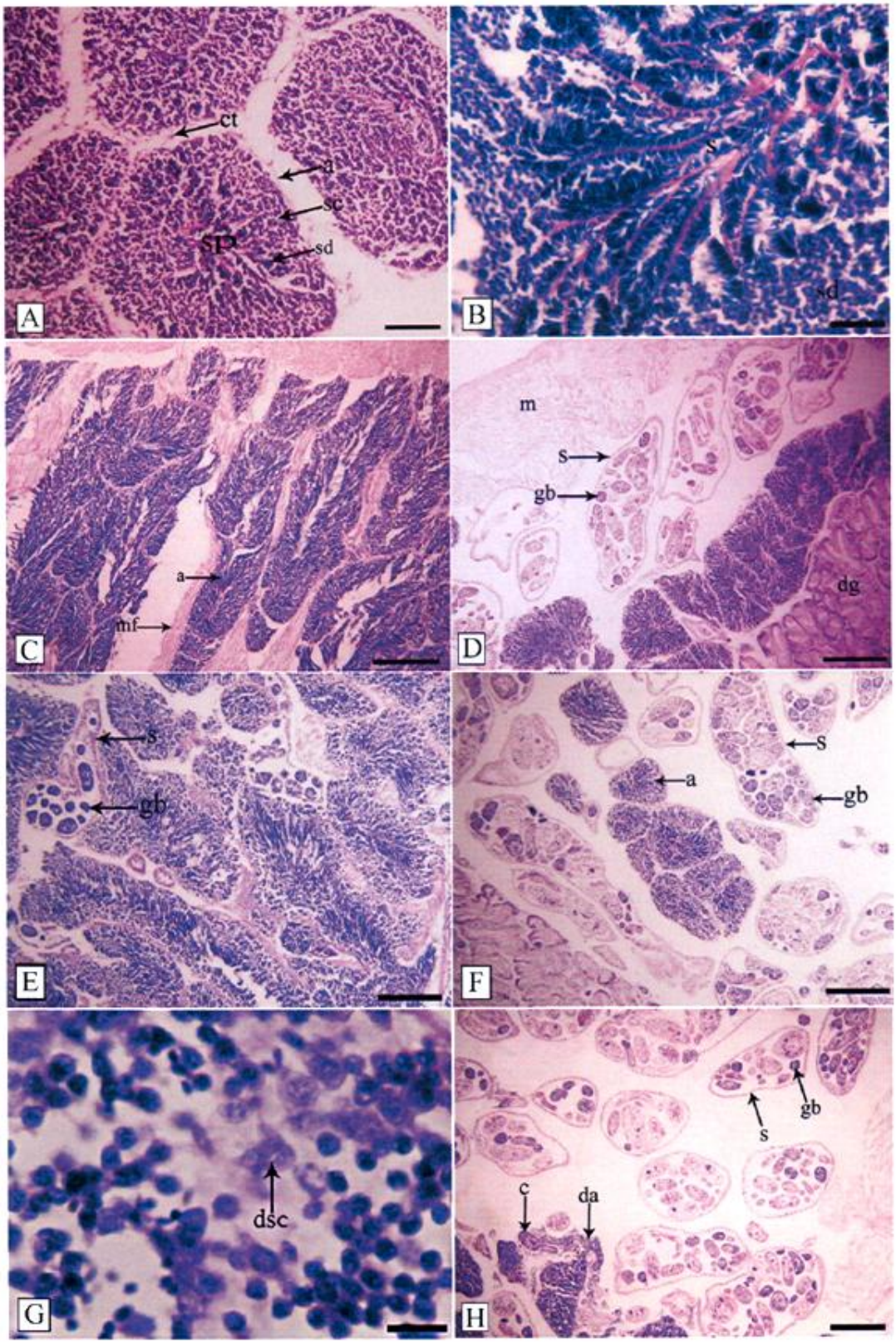

Figure (5): Photographs of transverse section through the testis of *Tapes decussatus*. (A), (B) & (C) normal, (D) & (E) light infected by *Cercaria lata* larvae, (F) & (G) moderate infection and (H) heavy infection. a, acinus; c, cercaria; ct, connective tissue; da, degenerated acinus; dg, digestive gland; dsc, degenerated spermatocyte; gb, germinal ball; m, mantle; mf, muscle fibres; s, sporocyst; sc, spermatocytes; sd, spermatids and sp, sperms. Scale bar = 100 μm in A, 25 μm in B, 400 μm in C, D, E, F & H and 5 μm in G.

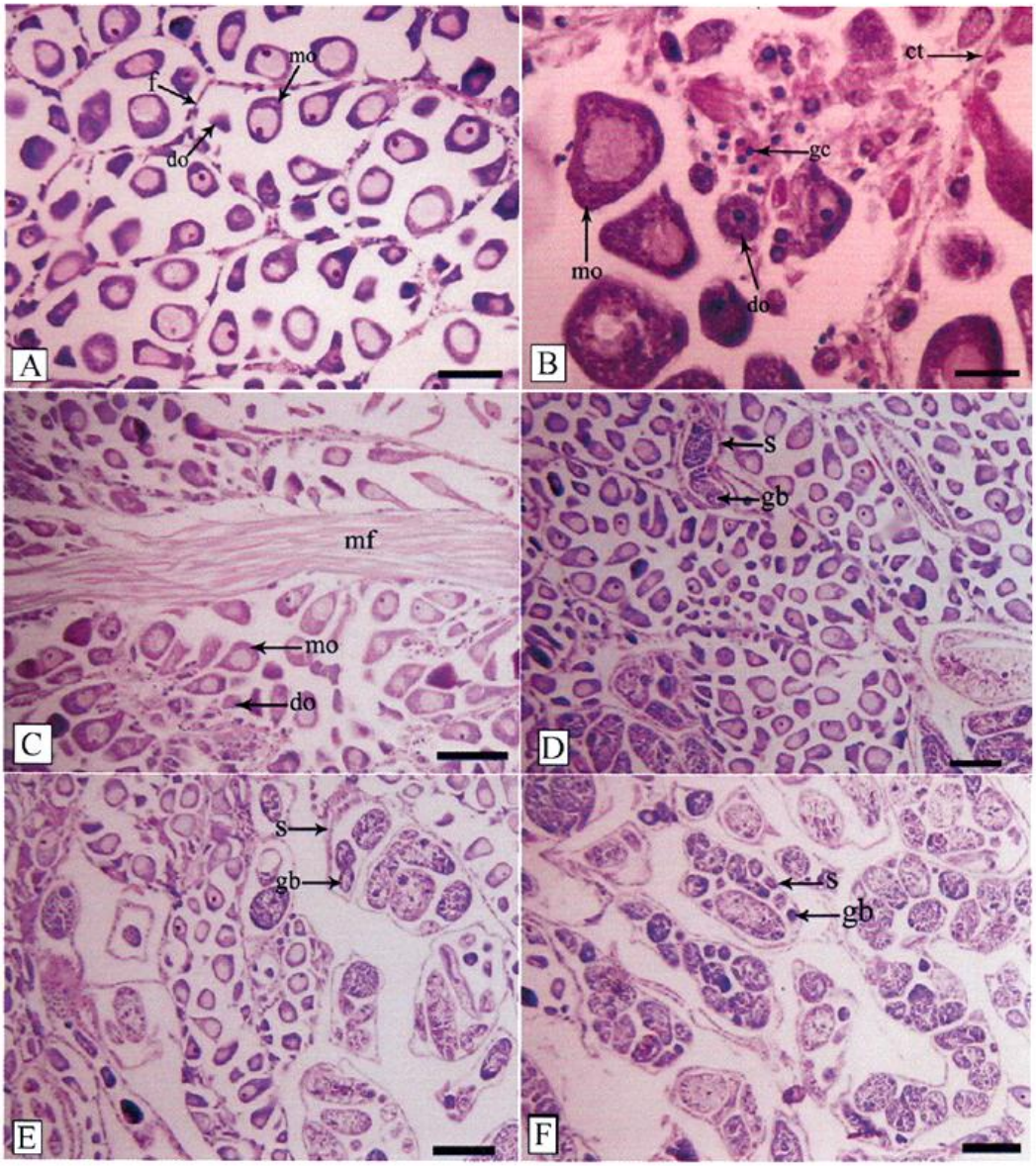

Figure (6): Photographs of transverse section through the ovary of *Tapes decussatus*. (A), (B) & (C) normal, (D) light infected by *Cercaria lata* larvae, (E) moderate infection and (F) heavy infection. ct, connective tissues; do, developing oocyte; f, follicle; gb, germinal ball; gc, germinal cell; mf, muscle fibres; mo, mature oocyte; s, sporocyst. Scale bar = 125 μm in (A), 50 μm in (B), 225 μm in (C) & (D) and 225 μm in (E) & (F) .