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THE INCIDENCE OF THE PARASITIC MITE UNIONICOLA ANODONTAISE INSIDE THE MOLLUSCAN HOST ANODONTA RUBENS

(With 2 Tables, 5 Plates and 6 Figures)

By

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تواجد الحلم المتطفل يونيكولا أنودونتايز داخل العائل الرخوى أنودونتا روبنز

حسنى أبو الدهب ، محمد حسين ، سميه رمضان

لقد أثبتت الدراسة أن حلم الماء العذب Unionicola anodontaise n. sp المتطفل على العائل الرخوى Anodonta rubens قد سجل نسبة أصابة ٩٨ر٥٪ فى ٦٠٠ عائل رخوى. ولقد بينت الدراسة أن الطفيل بمراحله المختلفة يتواجد بكثرة داخل العائل الذى يتراوح طول صدفته ما بين ١٣ سم الى ١٧ سم ويكون أقل تواجدا فى العائل الأقل والأكثر طولا من ذلك. :كما بينت الدراسة أن تواجد الطفيل داخل أنسجة العائل أحدثت تغييرات هستوباثولوجية من تهتك للأنسجة الضامة وظهور فجوات بها ووجود تجمعات دموية تحت الغشاء القاعدى للخلايا الطلائية التى تحد الخيوط الخيشومية.

SUMMARY

The most common numbers of different stages of the parasite are restricted between 13 and 17 cm of the host shell length, but are rarely found at shell length below 13 cm and above 17 cm. The infection percent in the host population by the mite is very high (98.5%). The parasite-host relationship between the two parteners causes pathogenic severe changes which may lead

to reducion of fecundity or death of the host. A distinct damage to the tissue of the host was recorded as a result of the presence of the mite inside the gills of the molluscan host.

INTRODUCTION

Unlike other assemblages of class Arachnida, many representative groups of the Acari are not predators. Some are exclusively phytophagous, while others include external and internal parasites of both vertebrates and invertebrates (Krantz, 1970).

Molluscans are an interesting group of invertebrates, that are parasitized by a variety of organisms, ranging from viruses to arthropods. Accordingly, a number of pathological changes are induced by the foreign organisms inside the body of the molluscan hosts, ranging from extremely severe to minor alterations. Now, it is well documented that the larvae of some parasites reduces the fecundity of the host, make host's cellular reactions and find passive pathologic alterations (Malek & Cheng, 1974).

The majority of water mites parasite on the water organisms either plants or animals. Members of the genus *Unionicola* have parasitic associations with molluscan or poriferan hosts. The present species is found to be parasitic on the molluscan host *Anodonta rubens.*

No available literature on the effect of this parasite on the bivalved molluscan host has been found. So, the present study is a trial to investigate the incidence of the parasite and its pathogenic effects on the host.

MATERIAL and METHODS

The samples were collected from the eastern bank of the River Nile, far about 5 km north-east of Sohag city. Monthly sampling was carried out for the molluscan host *Anodonta rubens* from the studied area during a period of two years; from October, 1992 till September, 1994. The length of mussels was measured and the collected mites from them were counted and preserved in 70% ethylalcohol. Statistical analysis and regressions were performed by a computer program. For histological preparations, alive mussels were collected from the River Nile. They were opened to remove small pieces of infected and non infected gill tissues and fixed in Carnoy's fluid, dehydrated in a graded series of alcohols, embedded in paraffin wax and sectioned at 6-8 um. Some sections were stained for ordinary studies with Haematoxylin and Eosin combination, others were stained with

Massion's trichrome stain to detect collagen (Gabe, 1976). The stained sections were examined under research microscope and then photocopied.

RESULTS

Unionicola anodontaise develops a parasitic association with the molluscan host *Anodonta rubens*. The adult forms and the nymphs, inhabiting the mantle cavity, are closely associated with the surfaces of the foot, mantle lobes and the gills of the host (Pl. 1A). while, the prehatching and posthatching embryos are parasitic in the gill tissues (Pl. 1B).

The present part of the study aims to demonstrate the relationship between the host and the parasite, showing the incidence of the parasite and the pathological changes in the gill tissues of the host.

I- Incidence of the parasite inside the host:

The parasite has two phases, with respect to its activity inside the host; active phase including the adults (females & males) and nymphs, and inactive one including the prehatching and posthatching embryos (Smith and Oliver, 1986). The incidence of the monthly distribution and infection percentage was studied as follows:

A- Relationship between host shell length and the number of different stages of the parasite.

The frequency distribution histograms for the prehatching, posthatching and nymphal stages are shown in Fig. (1A-C). It illustrates the relationship between the shel length and the number of the different stages in 600 host. It is interesting to note that the most common number of each stage is restricted in hosts of shell length between 13 and 17 cm, but the parasite stages are rarely distributed in mussels of shell length below 13 cm and above 17 cm.

Fig. (2A) indicates that the frequency number of the adult forms (females & males) inside 600 host mussels is restricted in shells lengths of 7 cm or more. The two patterns of the frequency number for females and males are nearly similar and consent with that of the grouped values of the two adult forms (fig. 2B, C). These results are in accordance with those of embryonic forms. It is likely to assume that, all stages of the parasite prefer mussels at the same range of shell length (13-17 cm). It is interesting to note that, the parasite is absent in mussels with shell length below 7 cm, and a few number of parasites were recorded with the mussels at shell length 7-12 cm and 18-20 cm. This phenomenon is difficult to explain. But, the incidence of the parasites inside the host may depend on the physiological state of the host.

B-Monthly distribution of different stages of the parasite.

Monthly variations in the mean density of early embryos indicate four peaks within the period of study, in January and May 1993, January, May 1994 (fig 3A). The maximum and minimum values of the mean density are 58.2 and 6.4 recorded in May and August 1993, respectively.

Monthly fluctuations in the mean density for larvae (posthatching) illustrate four peaks; in June, 1993. September, December 1993 and June 1994 (fig. 3B). The minimum and maximum means of pooled values for the previous stages, are 7.7 and 84.7, recorded in October and December 1993, respectively.

Monthly variations in mean density for the nymphs are shown in Fig. (3C), with two peaks during June 1993 and 1994. The minimum and maximum means for nymphs are 1 and 8.31, recorded in February and June, 1993, respectively. Monthly variations in the mean densities for the two adult forms (females & males) are shown in Fig. (4A), with two sharp peaks during July 1993 and 1994. The minimum and maximum pooled values of the mean distribution are 5.7 and 45.1, recorded in June 1993 and July 1994, respectively (fig. 4A). The minimum and maximum values of mean distribution of females are 2.76 and 24.32 recorded in June 1993 and July 1994, respectively (fig. 4B). While, those of males are 2.8 and 19.28 recorded in June 1993 and July 1994, respectively (Fig. 4C).

II- The prevalence of different forms of the parasite inside the host.

The percentage for each of the different forms of the parasite (early embryos, larvae, nymphs and adults) inside the host shown in Fig. (5). The highest value of percent for the parasite population was recorded for larval stage (posthatching), while the lowest one was recorded for the nymph stage. It is likely to assume that, the expected values for different forms of the parasite population are, in descending orders, as follows: early embryos, larvae, nymphs and adults. But, the percentages of early embryos and nymphs are smaller than the predicted ones. So, the percentage values of nymph is the lowest value, while the corresponding value of early embryos is lower than that of the larval stage only. The low of early embryos is due to that the females brood some of early embryos in their bodies (Aboul-Dahab, 1996 in press). While, the small number, or even the absence of nymphs, may be due to their migration from the molluscan host into another aquatic insect host (Proctor and Pritchard, 1990).

III- Infection percent within the host population.

Table (1) illustrates the infection percentage of the host with the different stages of the parasite recorded at the collecting site from October, 1992 to

September, 1994. Generally, the pooled data show that the levels of infection in the host population ranged from 92 to 100% during the two years of collection. These results show that the majority of the mussels were infected throughout the two years of investigation, with a mean value of 98.5%.

The infection percentage of 600 mussels with different forms of the parasite are 61, 84.3, 17.3, 94.8 and 92.8% recorded for the early embryos, larvae, nymphs, females and males, respectively. Fig. (6) shows the monthly infection percentages for the host population by different stages of the parasite. The values ranged from 32-88, 64-100, 0-76, 88-100, for early embryos, larvae, nymphs and adults, respectively. The previous data show that the adult forms have the highest value of infection, while the nymphs have the lowest one.

Regression analysis:

The relationship between the shell length of the host and the number of active stages (adults and nymphs) of the parasite was examined, using Ordinary Least Square (OLS) regression analysis for the data of October, 1992 and June, 1994 as an example. It is represented by three models; Linear, logarithmic and exponential (Table 2).

The regression coefficient (b) of the linear equation (1) implies that for every 1 cm increase in the shell length of the host, there is an increase in number of adult mites of 5.63. While, the degree of association (correlation between the shell length of the host and the number of the adult parasite was 50%. The value of b (2.61) of the exponential show that the number of adults increases proportionally to the shell length with the exponent (2.61), which is nearly area relationship.

On the other hand, the regression coefficient (b) of the linear equation (4) implies that for every 1 cm increase in the shell length of the host, there is an incrrease in number of nymphs of 0.496. Also, the degree of association (correlation) between the shell length of the host and the number of nymphs was 21%. The value of b (1,27) of the exponential equation (6) show that the number of nymphs increases proportionally to the shell length, with the exponent (1.27) which is nearly a linear relationship.

IV- Damage of the host tissue by different stages of the parasite.

A- Inactive forms:

Large number of different stages of embryos either prehatching or posthatching, were observed immersed inside the gill tissues of the host (Pl. 1B). The prehatching stages are surrounded with fertilization membranes and placed in gaps inside the gill tissues (pl. 1C). There is no evidence for the present stages to feed on the host tissue, since they are surrounded with

fertilization membranes and chorions, and contain a large quantity of yolk materials enough for developing of embryos (Pl. 2A). Also, there is no connection between the embryos and the gill tissues. While, the posthatching ones are found in gaps and surrounded directly with the connective tissue of the gills (Pl. 2B), their pedipalps are immersed in the connective tissue showing that they may be used in feeding (Pl. 2C). The connective tissue around the embryos of the two stages is completely destroyed and forming gaps. Many of these gaps were left empty inside the connective tissue of the host, after the parasite left them and migrated to the mantle cavity (P1. 1C). In heavy infection, the epithelial tissue of the gill filament shows a sign of inflammation with aggregation of proliferative cells in the filament connective tissue core (Pl. 3A). The size of parasitic embryos increases the connecctive tissue containing them becomes swollen and projecting into the interlamellar spaces of the gills (Pl. 1B).

In sections stained with Haematoxylin and Eosin combination, there are large numbers of deep yellowish or brownish nucleated bodies or globules scattered inside the connective tissue (Pl. 3B). They acquired green colour with Masson's trichrome stain (Pl. 3C). The yellowish brown bodies are types of blood cells. They are designated as yellowish nucleated bodies because of the presence of yellowish brown pigment globules in their cytoplasm. These cells measure from 3 to 16 um in greatest diameter and occur as free cells in tissues. They have been considered by Malek & Cheng (1974) as modified leucocytes.

B-Active forms:

The active forms of the parasite include the adults (females & males) and the nymphs. They live in the mantle cavity and are closely attached to the foot, gills and mantle lobes (Pl. 1A). In the present work, the behaviour of the active forms in feeding is not studied, and these data need further investigations. But, there is evidence showing that, at least, one or both of the previous stages, were feeding on the gill tissues of the host. In addition, the females lay their eggs which develop and hatch inside the gill tissues. Accordingly, the mechanical insertion and withdrawal of the gnathosoma by the adults or nymphs, and that of ovipositors by adult females make damage for the gill tissues of the host (Pl. 5A). Plate (4A) shows that the last 2 segments of the pedipalp of the adult mite are found deeply embedded in the tissue of the gills. The presence of the curved pedipalps inside the tissues indictes that they may act as feeding processes and cause a damage to the host cells.

The gills of alive non-infected mussels are yellowish in colour, while those of infected ones are dark yellowish in colour. Each demibranch has an outer folded surface which forms the epithelium of the gill filaments. Also, it has an inner smooth surface connected to that of the adjacent lamella by transverse interlamellar connections. The bulk of the gill lamellae has a fibrous connective tissue (Pl. 4B, C). The demibranches of infected mussels are swelled, with a concomitant expansion of the gill filaments and edema of the leuocytic infiltrated connective tissue bulk. (Pl. 5A, B).

DISCUSSION

Water mites are an interesting group of Acari developing parasitic association with Mollusca. While, the Mollusca is the most convenient group of invertebrates that harbours parasites, ranging from viruses to arthropods. The parasite-host relationship between the two groups causes a pathogenic severe changes which may reduce fecundity or cause death of the host. In the present investigation, the parasite *U. anodontaise* inhabits the mantle cavity and the gills of the host. It benefits from finding food supply, incubation area and shelter.

Specimens of the molluscan host *A. rubens* were found heavily infected by different stages of the unionicolid parasite *U. anodontaise*. Levels of infection in populations of A. rubens during collecting months were high (98.5%). Mitchell (1965) recorded levels of 90% infection by the parasitic mite U. fossulata. Gordon et al. (1979) found that infection percentages of the mussels, A. cataracta by females and males of the parasitic mite U. formosa were 49.8 and 52%, respectively. Baker (1987) recorded that the infection of the mussel *A. anatina* was 95% by females and 76% by males of the parasitic mite *U. formosa.*

In the present study, the incidence of parasitism increases with host shell length. The largest sized groups (shell length>13 cm) showed high levels of infection. Young specimens (smaller than 7 cm) were not parasitized. Dimock (1985) found that the infection of *A. imbecillis* by the parasite *U. formosa.* Increases with the increase of shell length While, Mithell (1965) did not detect any correlation between host size and any parameters of the population biology of *U. fossulata.* Jones & Baxter (1978) and Aboul-Dahab (1990) did not detect parasites in small sized groups of the molluscan hosts Repidochitona cinereus and Lasaea rubra. Aboul-Dahab (1990) suggested that the lack of infection in the small sized classes of the previous hosts is unlikely to be related to the size of the potential host, but is more likely to be

a function of host metabolism, and possibly related to the state of maturity of the host.

In the present study, there are sharp seasonal peaks in the distribution of total number for all stages of U. anodontaise. These results disagree with the observations of Mitchell (1965) and Gordon et al. (1979), where they did not record seasonal differences in the distribution of U. formosa in its host.

The pedipalps of the present parasite were found to be deeply embedded inside the gill tissues. Baker (1976) suggested that the pedipalps in Acari are essentially sensory appendages, but may be modified for grasping food and other functions in certain species. The same author (1977) concluded that, the pedipalps are means of attachment and cause erosion to the gill tissues. Subsequently, an inflamentory infiltration of blood cells draws out into the infected area; causing edema of gill filaments and underlying tissues. Baker (1977) reported that *U. intermedia* feeds on the mucus as principal food and host cells by drawing out haemocytes into the infected area and mixing them with its own salivary secretion.

In the present study, a number of pathogenic signs was observed resulting from the presence of the parasite inside the host. Among of these, there are a bulge of aggregated cells below the surface of the swelling epithelium of the gill, many of yellowish brown cells scattered in different parts of the connective tissue which were coloured green with Masson's trichrome in connective tissue. Epithelium of the gill filaments became edemous and swollen. The same findings were recorded by Baker (1976) for the host *A. anatina* infected by *U. intermedia*. He concluded that, these changes in the host tissues represented the cellular response due to the presence of the parasite inside the host tissues.

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LIST OF PHOTOS

Plate. 1: A- Photograph of the mussel with two shell valves opened from ventral aspect to show the parasitic mite *Unionicola anodontaise* n. sp. on its soft parts.

B- Photomicrograph of a section through the gill tissues of the host showing the prehatching and posthatching embryos (em) and the swelling connective (sct).

C- Photomicrograph of a section through the gill tissues of the host showing the prehatching embryo (em) and gap (gs).

Plate. 2: A- Photomicrograph of a section through the gill tissues of the host showing yolk materials of the prehatching embryos (yg).

B- Photomicrograph of a section through the gill tissues of the host showing the posthatching embryo inside the gap (em).

C- Photomicrograph of a section through the gill tissues of the host showing the pedipalp of posthatching embryo (p).

Plate. 3: A- Photomicrograph of a section through the gill tissues of the host showing the aggregation of proliferative cells (pr).

B- Photomicrograph of a section through the gill tissues of the host showing the yellowish nucleated bodies inside the connective tissue (ynb).

C- Photomicrograph of a section through the gill tissues of the host showing the green nucleated bodies inside the connective tissue (gnb) stained with Masson's trichrome.

Plat. 4: A- Photomicrograph of a section through the gill tissues of the host showing the last 2 segments of the pedipalp (p) of the adult mite. X 1300.

B- Photomicrograph of a transverse section through the non-infected demibranch showing its structure; gill filaments (fs), inner smooth surface (ss) of the gill lamella and the interlamellar connection (tt).

C- Photomicrograph of an enlarged part of (B) showing the epithelium of the gill dilament (epi).

Plate. 5: A- Photomicrograph of a transverse section through the infected demibranch showing the damage of gill filament (fs) and bulky connective tissue (bct).

B- Photomicrograph of an enlarged part of (A) showing swelling of the epithelial cells of the gill filament (epi).

LIST OF ABBREVIATIONS

at = attachment area
bct = bulky connective tissue
cho = chorions
em = embryos (prehatching or posthatching)
epi =epithelial cells gill filament gills green nucleated bodies
fs = gill filament
g = gills
gnb = green uncleated bodies
gs = gap (gaps)
m = mantly cavity
p = palp (palpi)
pm = parasitic mite
pr = proliferative cells
sct = swelling connective tissue
ss = smooth surface of the gill lamella
tt = interlamella connection of the demibranch
yg = yolk granules or yolk materials
ynb = yellowish nucleated bodies

Table. 1: The percent of infection in the host population. N= 25 mussels each month.

Months	Infected	%	Non-infected	%	Months	Infected	%	Non-infected	%
Oct, 92	25	100	0	0					
Nov	25	100	0	0					
Dec	25	100	0	0					
Jan,93	25	100	0	0	Jan, 94	25	100	0	0
Feb	24	96	1	4	Feb	24	96	1	4
Mar	25	100	0	0	Mar	25	100	0	0
Apr	24	96	1	4	Apr	24	96	1	4
May	25	100	0	0	May	25	100	0	0
Jun	23	92	2	8	Jun	23	92	2	8
Jul	25	100	0	0	Jul	25	100	0	0
Aug	24	96	1	4	Aug	25	100	0	0
Sep	24	96	1	4	Sep	25	100	0	0
Oct	24	96	1	4					
Nov	25	100	0	0					
Dec	25	100	0	0					

Total % of infected mussels = 98.5
Total % of Non-infected mussels = 1.5

Table. 2: Regression models of the relationship between numbers of adult forms (NA), nymphs (NN) of the parasite and the shell length of the host (L).

	Months	Equations	Type	R	b	t	f	correl
1-	Oct.92	NA= -62.6+ 5.63L	Lin.	18.9	5.63	2.57	6.58	0.5
2-		NA= -4.24+ 2.61L	Log	5.7	2.61	1.57	2.46	
3-		NA= 0.0237399L	Exp		2.61			
4-	Jun,94	NN= 12.1- 0.496L	Lin	0.1	0.496	-1.0	1.03	-0.21
5-		NN= 4.93- 1.27L	Log	0.0	1.27	-1.0	0.98	
6-		1.27 NN= 0.0065722L	Exp		1.27			

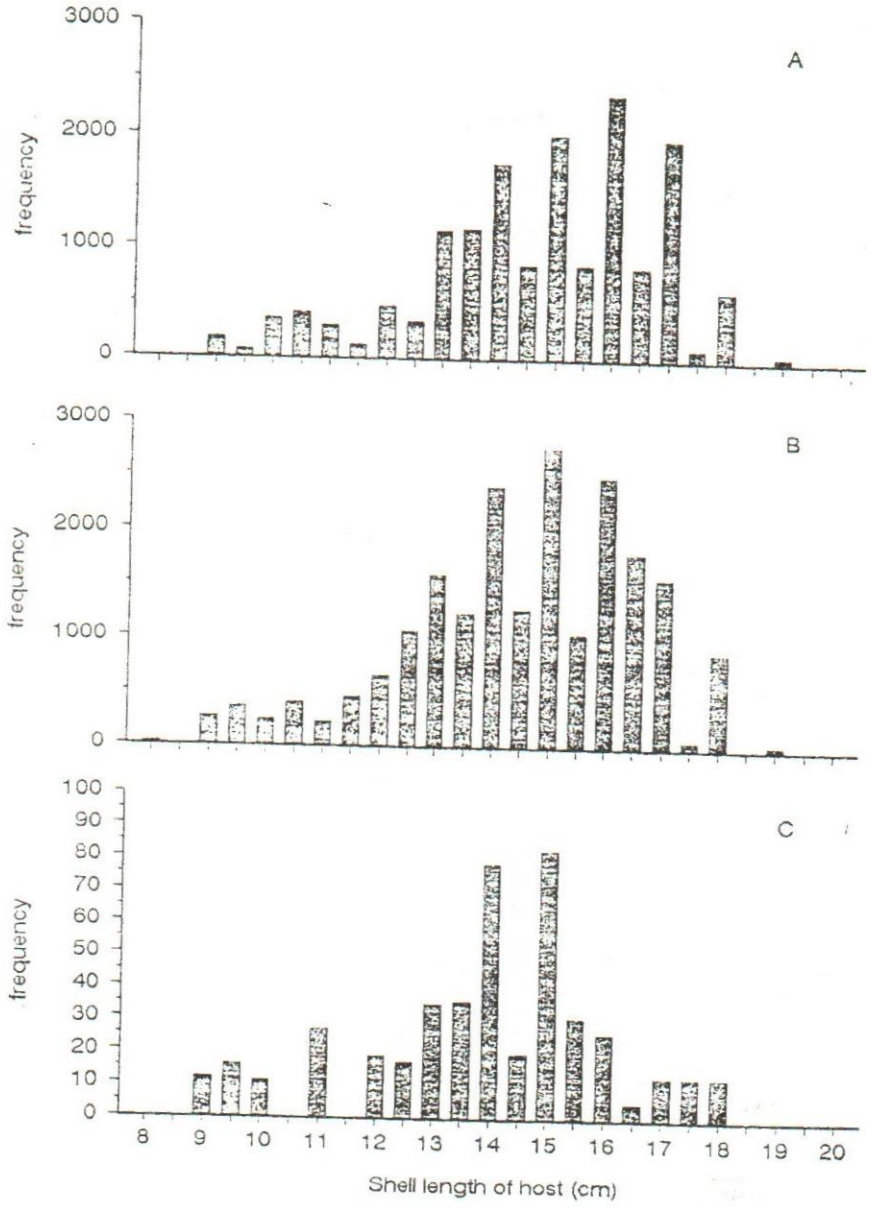


Fig. I . Relationship between the shell length (cm) of the host and the frequency of prehatching embryos (A), larvae (B) and nymphs (C) of *Unionicola anodontaise*.

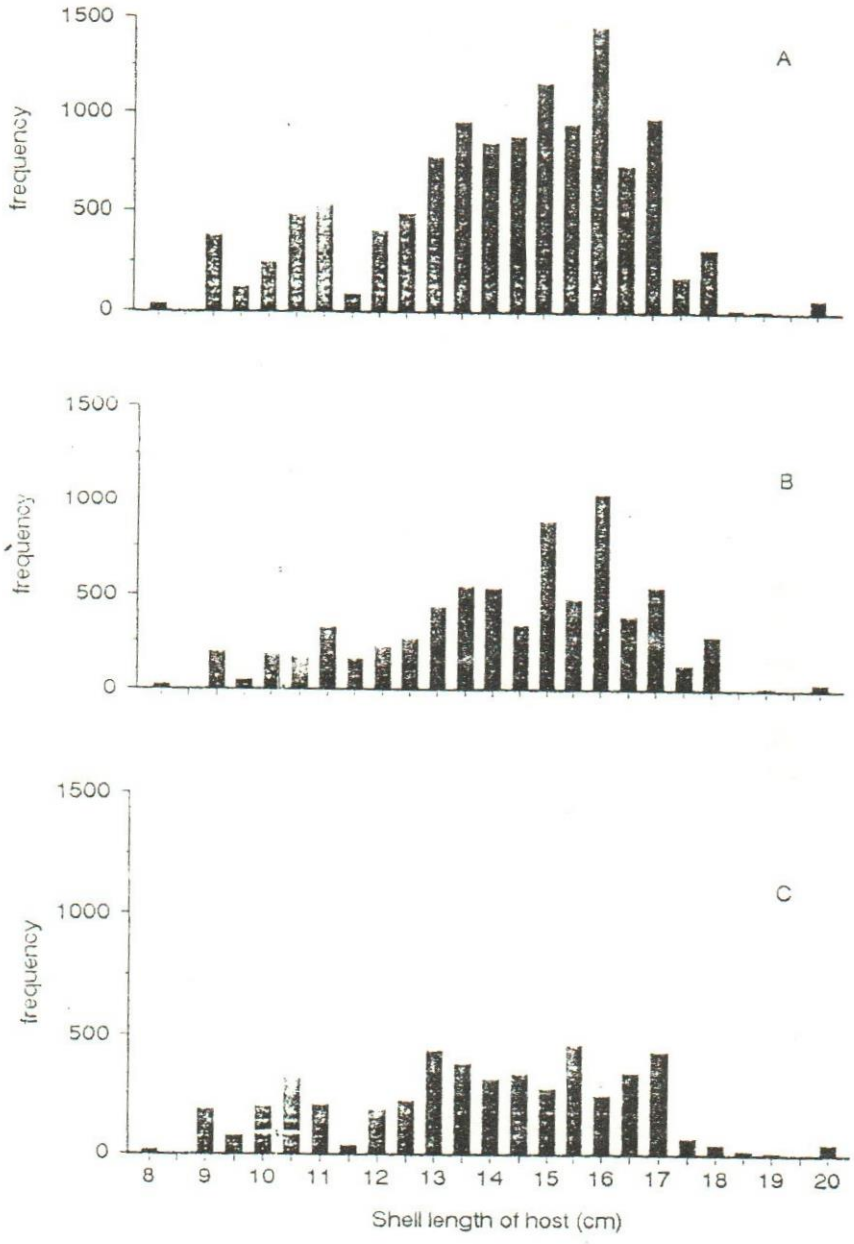


Fig. 2. Relationship between the shell length (cm) of the host and the frequency of two adult forms (A), females (B) and males (C) of *Unionicola anodontaise*.

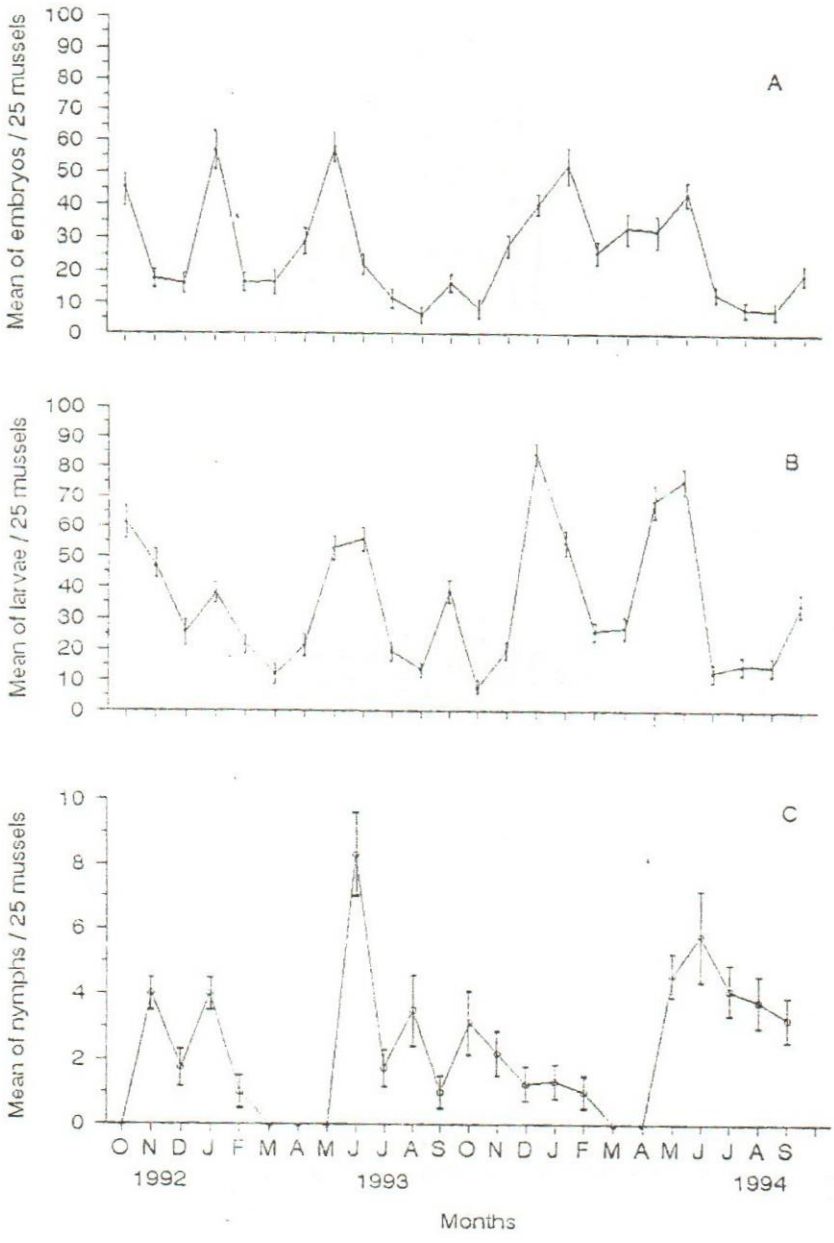


Fig. 3 . The distribution of prehatching embryos (A), posthatching larvae (B) and nymphs (C) of *Unionicola anodontaise* among *Anodonta rubens* over two years period. Error bars ±2 SE.

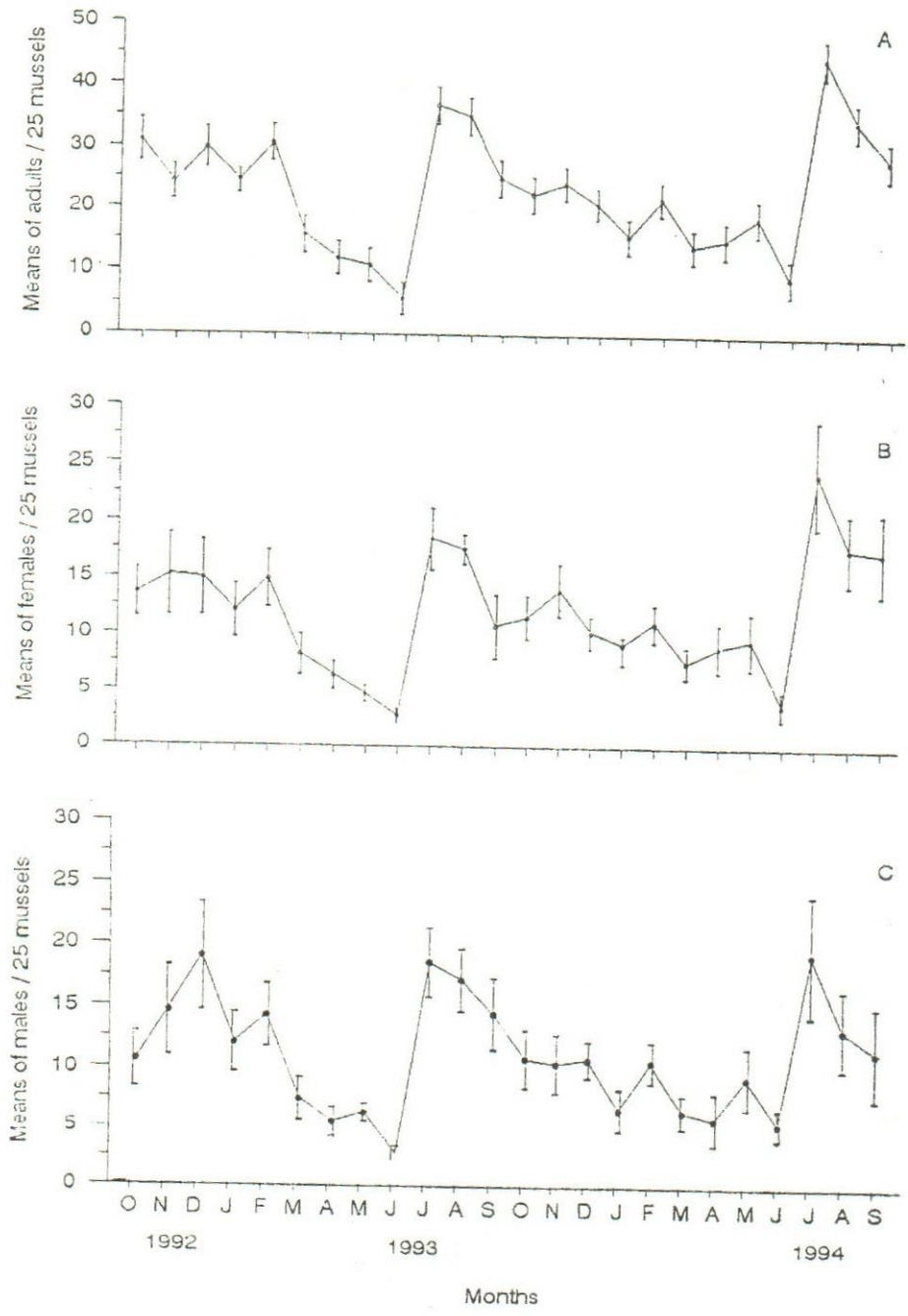


Fig. 4 . The distribution of adult forms(A), females (B) and males(C) of *Unionicola anodontaise* among *Anodonta rubens* over two years period. Error bars ± 2 SE.

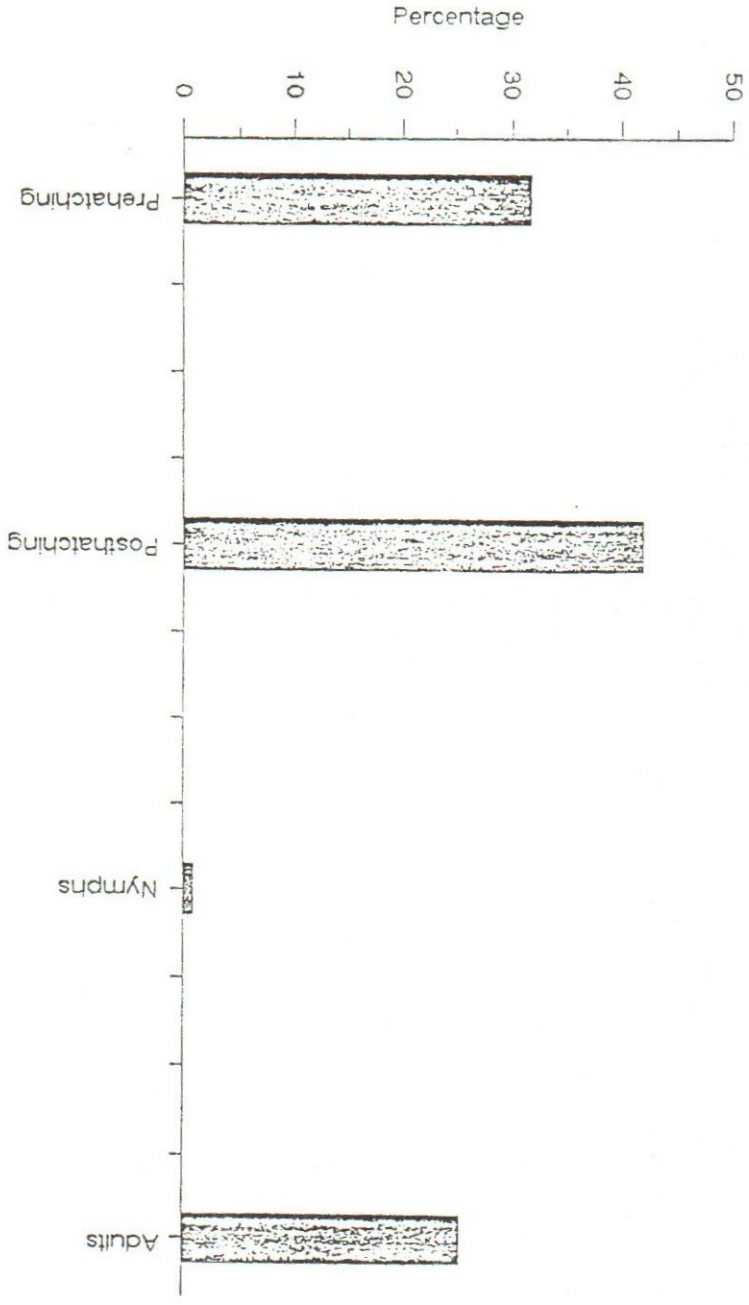


Fig. 5. Prevalance of different forms of the parasite inside the host.

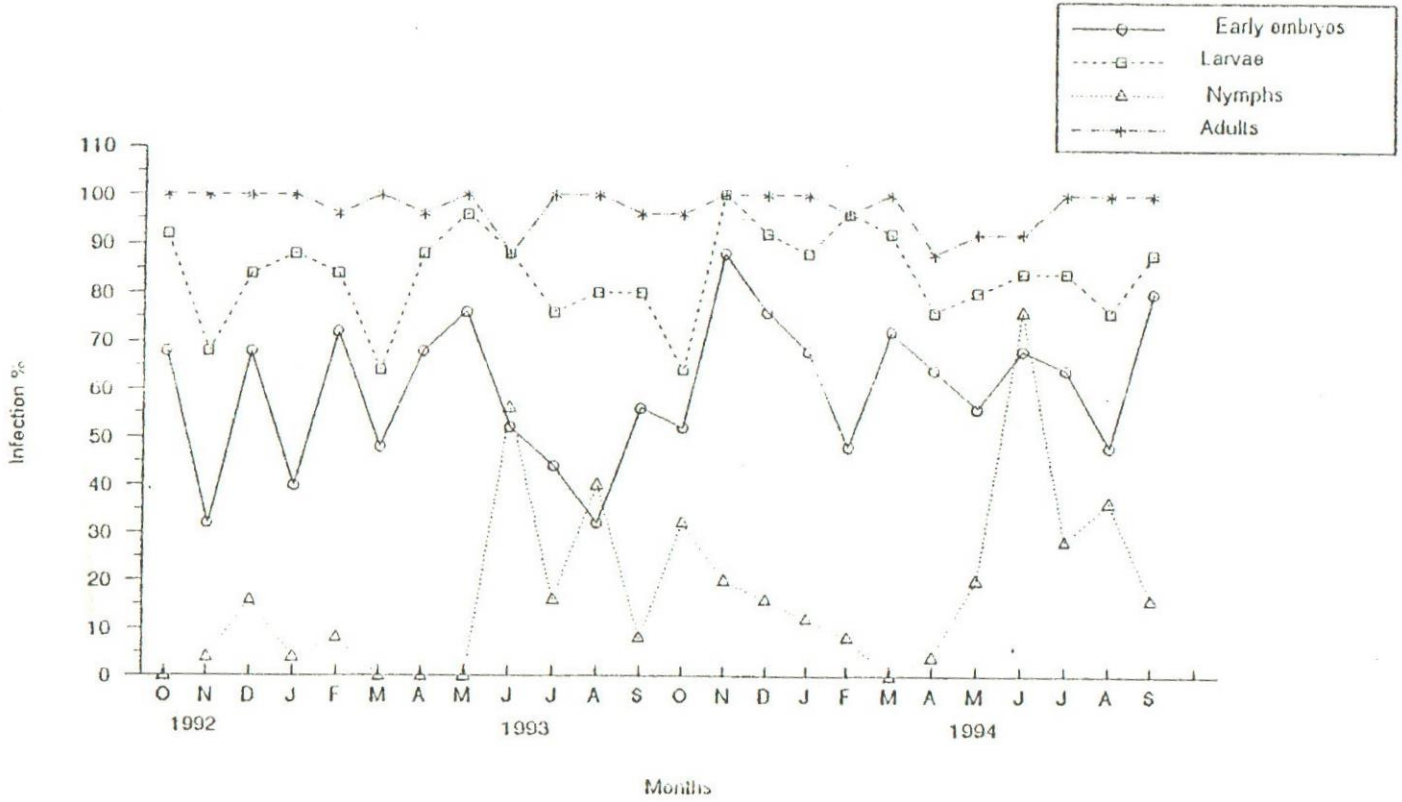
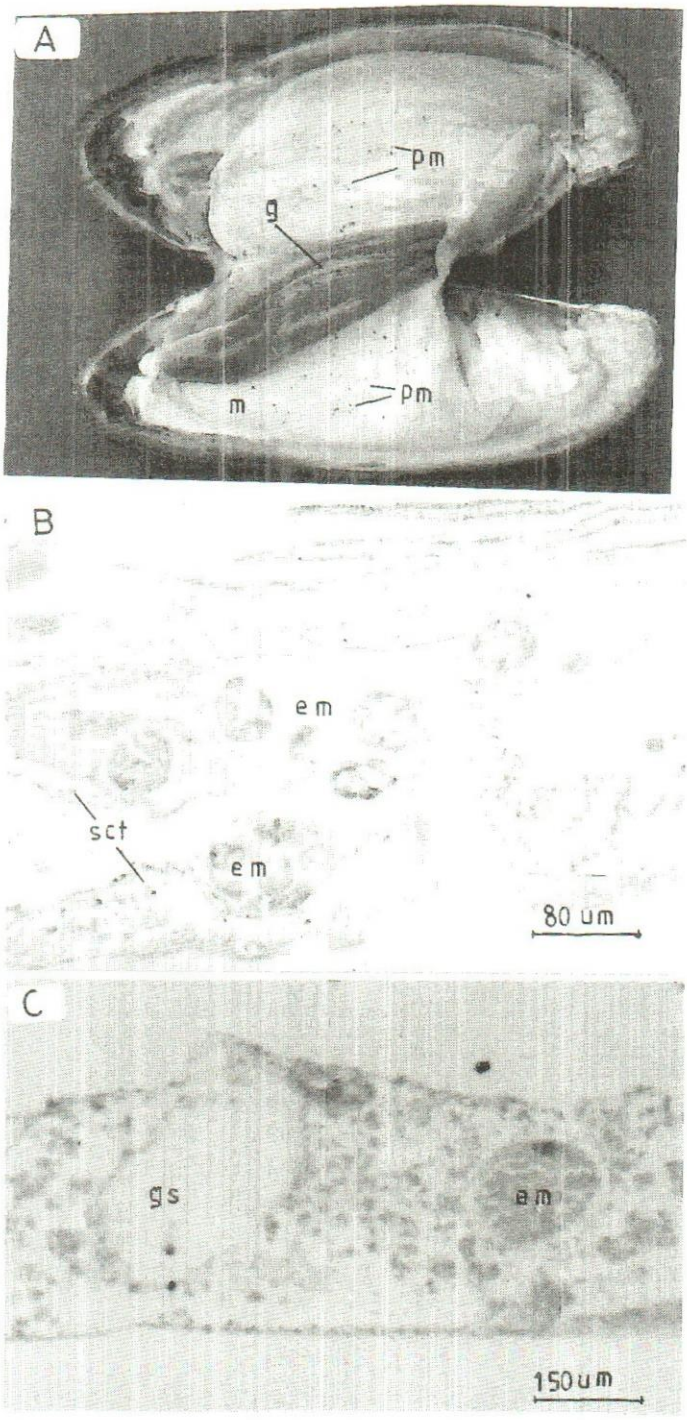
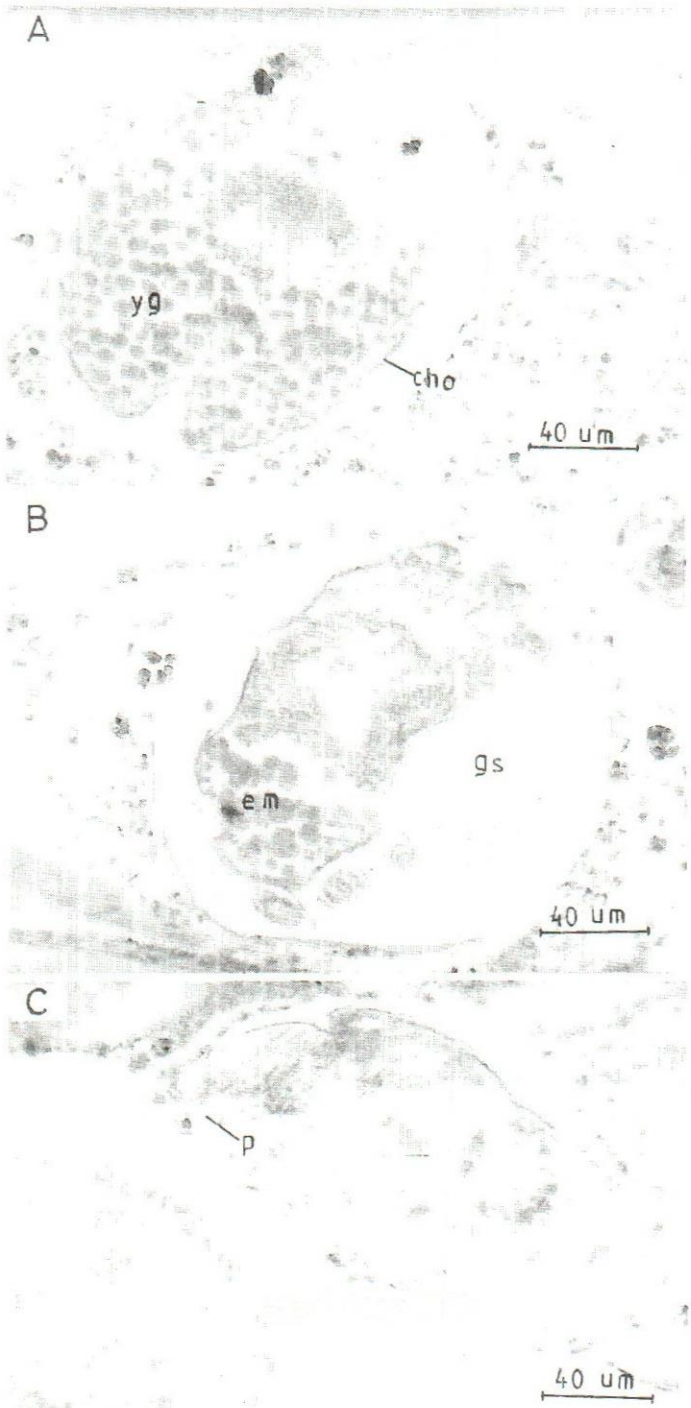


Fig. 6 . Monthly infection percentage for host population by different stages of the parasite.

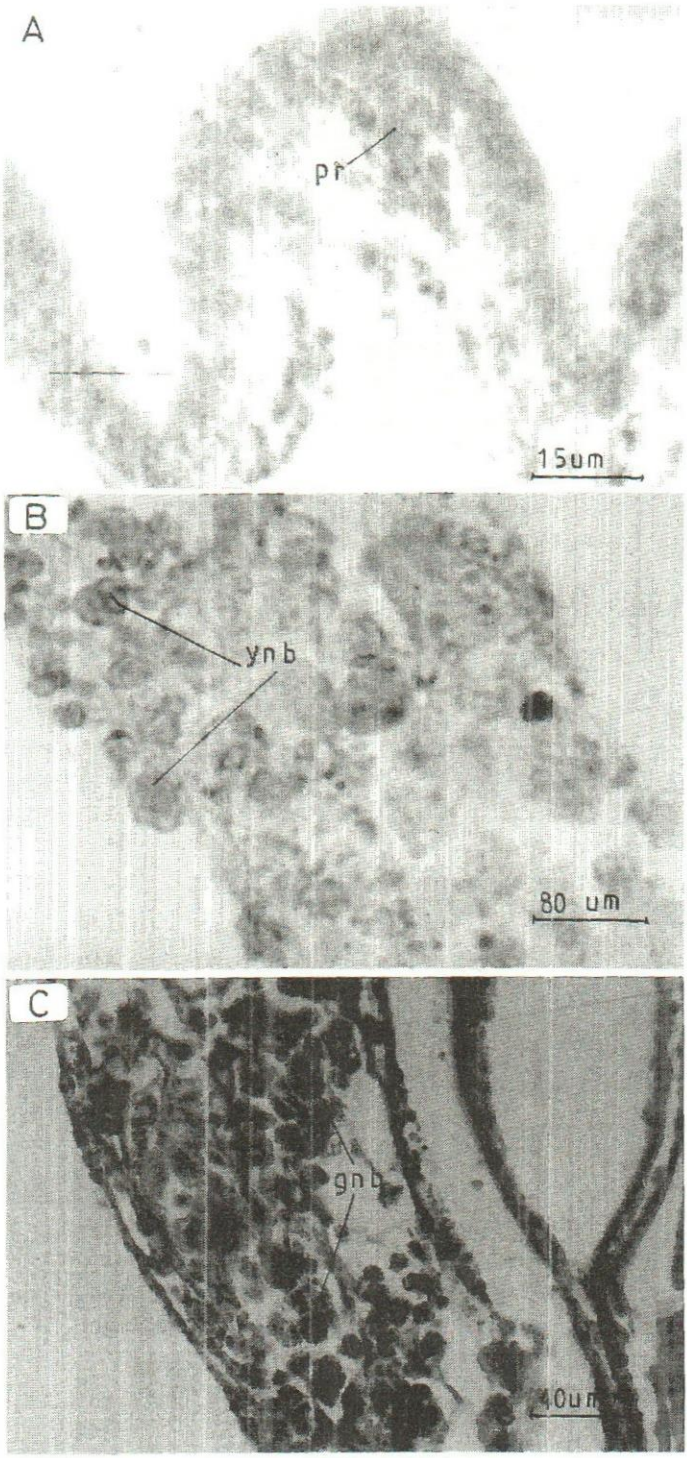
Pl.1



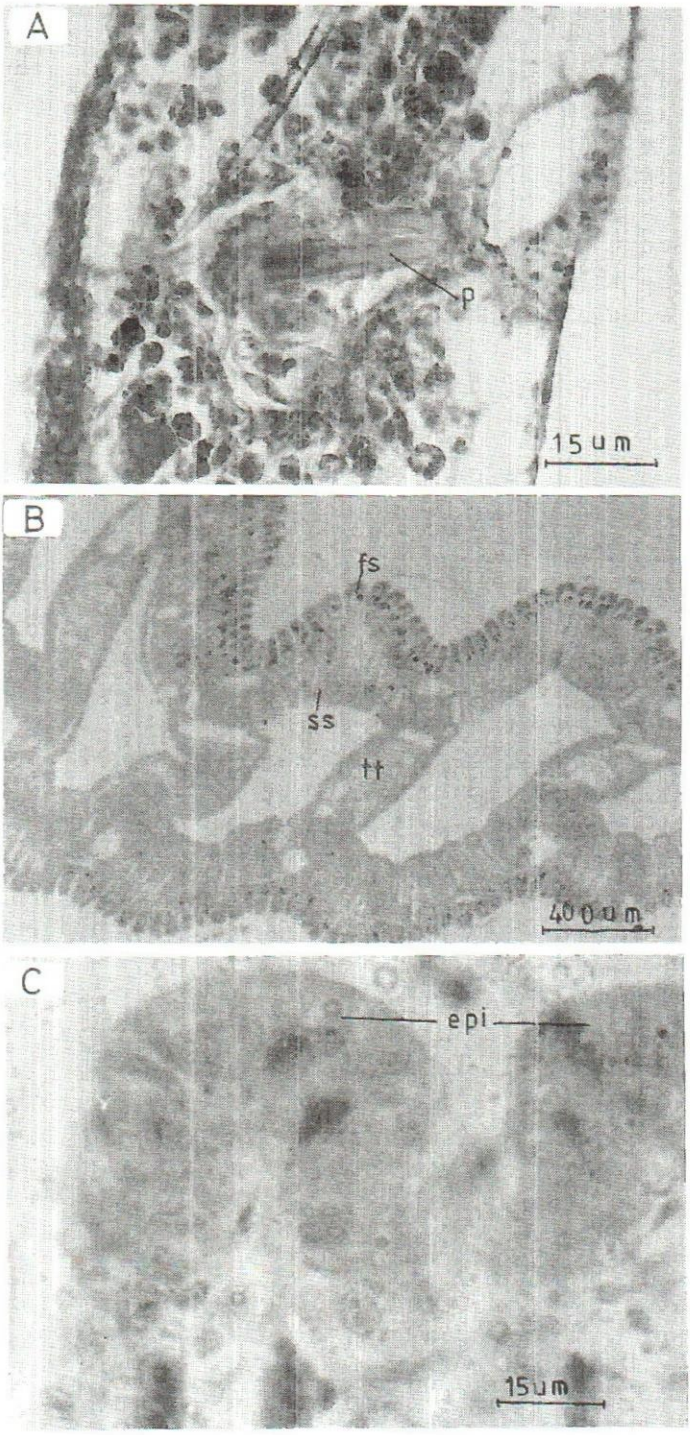
Pl. 2



Pl.3



Pl. 4



Pl. 5