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# RESEARCH ARTICLES

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## COMPARATIVE STUDIES ON REPRODUCTIVE SYSTEMS OF *ACHATINA FULICA*, *HEMIPLECTA DISTINCTA* AND *CYCLOPHORUS AURANTIACUS*

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### ABSTRACT

The reproductive systems of three species of land snails, *Achatina fulica*, *Hemiplecta distincta* and *Cyclophorus aurantiacus* were comparatively studied in terms of anatomical, histological and functional organization.

*Cyclophorus aurantiacus* is dioecious. The female organs are the ovary, oviduct, pallial oviduct (uterus), bursa copulatrix and vagina. The male organs are the testis, vas deferens, pallial vas deferens and penis.

*Achatina fulica* and *Hemiplecta distincta* are monoecious. The ovotestis produces sperm and ova. The hermaphroditic duct is divided into an apical portion, a seminal vesicle and a basal portion bearing a talon, and ending in a carrefour. Spermatozoa are found stored in the seminal vesicle. The albumen gland is large and communicates with the carrefour through a narrow, small duct.

The spermoviduct is fairly long, and all along its length, the narrow sperm groove is incompletely separated from the wide uterus. The vas deferens is continuous with the sperm groove and ends in the penis, while the uterus continues as the oviduct ending in the vagina. Both the penis and vagina open into the genital atrium.

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## INTRODUCTION

Mollusks are the second largest group of the animal kingdom after insects, and they are important invertebrates of the world. The mollusks can be divided into 6 classes: Amphineura (Aplacophora, Polyplacophora), Monoplacophora, Gastropoda, Scaphopoda, Pelecypoda and Cephalopoda. The members of some classes, such as Gastropoda, Pelecypoda and Cephalopoda are widely consumed all over the world. Land snails belong to the class Gastropoda, which can be divided into 3 subclasses, Prosobranchia, Opisthobranchia and Pulmonata. *Achatina fulica* and *Hemiplecta distincta* belong to the subclass Pulmonata, whereas *Cyclophorus aurantiacus* belong to the subclass Prosobranchia. They are edible and can be considered to be another source of high-protein food.

Pulmonate land snails are a very large group of more than 60 families.<sup>1</sup> The members of this order, Stylommatophora, are fully terrestrial and successfully adapted to terrestrial environment.

The classification of *A. fulica*, *H. distincta* and *C. aurantiacus* can be put as follows:

### Phylum Mollusca

Class Gastropoda

Subclass Prosobranchia	Subclass Pulmonata
Order Mesogastropoda	Order Stylommatophora
Family Cyclophoridae	Family Helicarionidae
( <i>Cyclophorus</i>	( <i>Hemiplecta distincta</i> )
<i>aurantiacus</i> )	Family Achatinidae
	( <i>Achatina fulica</i> )

The genera *A. fulica*, *H. distincta* and *C. aurantiacus* may be characterized as follows:

### Genus *Achatina fulica*

The shell consists of 7 to 9 convex whorls, with a moderately swollen body-whorl and a narrowed sharply conical spire. The outer lip is sharp, thin, convex, and inserted on the body whorl at a sharp, open angle. The columella is concave. The snail has no gills and operculum, but has two pairs of retractile tentacles with eyes at the tip of the posterior tentacles. The mantle cavity serves as the lung.

### Genus *Hemiplecta distincta*

The shell is rounded or turbinate, with a wide aperture, and has low conical spires of 5 to 7 whorls. The foot is broad and flat. The head bears two pairs of tentacles, a short anterior pair and a longer posterior pair with eyes. The mantle cavity serves as the lung.

### Genus *Cyclophorus aurantiacus*

The shell is umbilicate, depressed conical and solid, consisting of 4 to 5 convex whorls, the last whorl not descending. The umbilicus is moderate and deep. The aperture is slightly oblique, broadly ovate, with an operculum. The sexes are separate. In the female, the digestive gland reaches the apex of the visceral mass, surrounding the ovary for approximately the first whorl, except on the columellar side. In the male, the digestive gland begins at the lower edge of the testis. The stomach is embedded in the outer side of the digestive gland.

*C. aurantiacus* and *H. distincta* are tropical land snails which are distributed in the northeastern part of Thailand and are consumed by the local people.<sup>2</sup>

*A. fulica* can be found everywhere in the country, and in urban areas they are considered as garden pests. *A. fulica* has not been a popular diet among the Thai people, but it has been very popular in other countries such as Taiwan, China, Japan, Europe and the Western countries. In fact, it has been one of the important export items of Taiwan, in both frozen and canned forms, since 1974. A systematic study of growth, reproduction and mass cultivation of these three species of land snails will provide not only an additional protein source for the local people, but also methods for increased and continuous production of the animals for the purposes of export.

In order to understand the cellular level of physiological processes, such as the process of hormone synthesis, the function of growth hormones and reproductive hormones of these snails in increasing the reproduction and growth rate, it is important to study the morphological and physiological aspects of the neuroendocrine and reproductive system of these land snails. In this particular study, the general anatomy and histology of reproductive system of the three land snails; *A. fulica*, *H. distincta* and *C. aurantiacus* were investigated.

### MATERIALS AND METHODS

*Achatina fulica*, *Hemiplecta distincta* and *Cyclophorus aurantiacus*, were collected from the field and maintained in the laboratory for systematic cultivation.<sup>3</sup>

For anatomical study, specimens of *A. fulica*, *H. distincta* and *C. aurantiacus* used for dissection were first relaxed with menthol crystals. A few crystals of menthol were floated on the surface of water containing the snails, and within 24 h, they were relaxed in an extended position.

After the snails were relaxed, their shells were removed with the aid of a hammer. Dissections were made by placing the snails in enamel pans, into each of which a layer of paraffin had been poured. Specimens were secured to this base with entomologists' needles. The dissecting instruments were watchmakers' forceps, steel sharpened to a fine point, and good quality steel sewing needles embedded in short wooden rod handles. Most of the dissections were made under a stereoscopic binocular microscope, with the specimen in 70% alcohol. Anatomical drawings were made with the aid of a camera lucida.

For histological studies, the following procedures were employed: after relaxation, the ovotestis and other reproductive organs of the three species of land snails were fixed in Bouin's fluid for 8 to 12 h. depending on the size of the specimens. Then they were washed in several changes of 50% alcohol for 4 to 6 h, dehydrated in a graded series of alcohol (50%, 70%, 85%, 90%, 100%), and embedded in paraplast. Sections were cut at 5 to 7 microns thick, stained in Harris's hematoxylin and eosin, viewed and photographed with an Olympus BH-2 transmission compound microscope.

## RESULTS

### *Achatina fulica*

In common with the other pulmonates, *Achatina fulica* is hermaphroditic and has elements of both male and female systems in its reproductive tract (Fig. 1A). *A. fulica* possesses a complex reproductive system containing a hermaphroditic gonad which is a lobed ovotestis or hermaphroditic gland embedded in the digestive gland (liver) and a number of ducts with their associated glands. The ovotestis produces oocytes and spermatozoa simultaneously in close proximity.<sup>4</sup> Some parts of the reproductive system are involved in both male and female functions, but some are involved only in one or the other. The common elements include the ovotestis and hermaphroditic duct. The male organs are the prostate gland, vas deferens and penial complex. The enlargement of the hermaphroditic duct which serves as the seminal vesicle should probably be included here. The female portion of the reproductive system is comprised of the albumen gland, the uterus, the vagina, and the seminal receptacle or spermatheca.

Figure 2 shows the reproductive system of *A. fulica*. The ovotestis is a roughly hemispherical gland, yellowish in colour and composed of follicles, the lobes of which are embedded in the digestive gland. An ovotestis lobe is made up of numerous small follicles or acini, each lobe with 1,000 to 3,000 acini.<sup>5</sup> These acini are separated from each other by a thin membrane inner to which lies the follicular germinal epithelium which produces the gametes (Fig.3A).

Within these acini of the ovotestis, all of the stages of spermatogenesis and oogenesis are observable, but details of these processes will not be elaborated here. Clusters of maturing sperm cells can be observed gathering around the Sertoli cell near the upper portion of the gland (Fig.3B). Developing eggs are seen in the uppermost part of the ovotestis (Fig.3C).

Each acinus of the ovotestis is drained by a small duct, and the ducts draining the acini of a lobe all unite and open into the main hermaphroditic duct through which the gametes move to the more distal part of the system.

The hermaphroditic duct leads out from the ventral anterior face of the ovotestis. The lumen of the duct is lined by an epithelium, partly ciliated, outer to which lie circular muscle and connective tissue, in that order (Fig.4).

The hermaphroditic duct is divided into three distinct regions which differ in the size of the duct lumen, the form of the epithelial cells lining this lumen, as well as in the thickness of the layers of circular muscle and connective tissue surrounding the epithelium. The first or the apical hermaphroditic duct is narrow, rounded, almost straight, covered externally only with a thin membrane. This region lies adjacent to the ovotestis and its characteristic histological features are a very small lumen lined by ciliated cuboidal epithelial cells with a large prominent nucleus, and a thin layer of circular muscle composed of only a few strands (Fig. 4A). The epithelium becomes slightly folded closer to the second region.

The second region or the seminal vesicle has a diameter of about three to four times that of the first part, and is always loaded with spermatozoa. It is usually dark cream to light brown in colour. It is folded upon itself, the short folds being tightly held together by a thin sheet of connective tissue which gives the whole structure a broad, short, compact appearance (Fig. 4B).

The characteristic histology of the second region is as follows : the lumen, which is the largest of the three parts, is incompletely divided into two channels-a larger upper and a smaller lower channel-by two inwardly projecting folds of the epithelium (Fig. 4B). The cells of the epithelium are of two types-columnar, non-ciliated and apparently glandular cells, and narrow columnar ciliated cells. The former constitute the major portion of the epithelium while the latter are confined to the folds and the lower channel. Around the epithelium is a thick layer of circular muscle followed by a band of connective tissue.

The third region or the basal hermaphroditic duct is the longest, and has the shape of a narrow and round tube. The dorsal and ventral surfaces of the duct are placed against the columellar muscle. The duct beyond the talon is about 4 to 5 mm and enters the albumen gland where it becomes slightly curved and ends in a conspicuous carrefour.

The histology of the third region is similar to that of the second region. However, distally, the columnar nonciliated cells of the epithelium decrease in number with a concomitant increase in the number of ciliated cells, and as the duct approaches the albumen gland, the narrow ciliated cells increase in number to eventually constitute the entire epithelium. The epithelium gradually becomes folded and the duct, close to the albumen gland, is lined with a completely ciliated and much folded epithelium. A thick band of circular muscle and an outer layer of connective tissue form the jacket investing the epithelium (Fig. 4C).

Lying closely parallel to the third region of the hermaphroditic duct and opening into it is a sac-like structure called the talon (Fig. 2).

The talon has a wall of circular muscle enclosing a number of branched tubules which end blindly and proximally, and whose lumens unite before opening into the hermaphroditic duct. Each tubule has a central lumen lined by an epithelium of columnar ciliated cells, whose cilia appear to be longer than those of the epithelial cells lining the

lumen of the adjacent hermaphroditic duct (Fig. 5A). Around the epithelium is a thin coat of circular muscle. Spermatozoa pack the narrow lumens of these tubules, lying in very close contact with the epithelial cells.

Both the talon and the hermaphroditic duct share a common coat of circular muscle where they lie in close proximity to each other. In cross sections, one can readily distinguish between the talon and the adjacent hermaphroditic duct because of the wider diameter, numerous tubules and longer epithelial ciliation of the former.

The talon and the hermaphroditic duct unite to form a common duct which at first appears circular in cross section, with a central lumen lined by a folded epithelium, but gradually changes to a flat duct, oval in cross section, with an even, unfolded epithelium. The basic histology of the duct, however, remains the same throughout its length - a central lumen lined by a ciliated epithelium, outer to which is a layer of circular muscle.

At its distal end, the hermaphroditic duct opens into a small, irregularly shaped chamber. This area may be called the carrefour. A duct from albumen gland opens into this chamber, as do the ducts from the prostate gland and the uterus. The carrefour, which has been found only in the hermaphroditic snails, had earlier been described and considered to be the site of fertilization of the ova in pulmonates.

### *The Female Part of the System*

The albumen gland in *A. fulica* is whitish in colour, with a rather oblong shape. The function of the albumen gland is the production of albumen, a major constituent of which is galactogen. It has a duct opening at the carrefour. The eggs receive the secretion of the albumen gland after fertilization.

The gland is bounded externally by a thin epithelium of flattened cells whose boundaries are not clear although their nuclei are visible. It is a compound gland made up of a number of secretory follicles held together by thin strands of connective tissue (Fig. 5B).

Each follicle is circular in cross section and consists of a number of glandular cells arranged radially in a single layer around a small central lumen. Each cell has a broad basal region in which a granular nucleus is located, and tapers towards the lumen. The cytoplasm of the cell is filled with globular secretory material. Small oval nuclei are observed close to the central lumen of the follicle.

Situated centrally in the albumen gland is the albumen canal, into which the secretion of the gland cells is emptied (Fig. 5B). The canal is lined by an epithelium of ciliated columnar cells outer to which is a thin coat of circular muscle. The albumen is stored temporarily in the albumen canal, from where it is driven to the apical uterus by the beating movement of the cilia present both in the albumen canal and duct and in the common exit duct. With the increase in the size of the gland during the breeding season, its canal also

enlarges and the storing capacity is increased.

The uterus is an elongated sac, extremely glandular, and forms the perivitelline membrane and shell of the egg (Fig. 6A). It can be divided into an apical and a basal part of almost equal lengths. The apical uterus is large, cream-white, much-folded in the form of a loop. The basal uterus is elongated, dull-brown, widest at the middle and narrower at both ends. This corresponds to the female part of the spermoviduct and the eggs are formed and stored here prior to being deposited in the soil.

The uterine epithelium is composed of short cells with little cytoplasm, prominent nuclei and short cilia. This epithelium is folded, resulting in the formation of numerous crypts. The outermost layers are composed of circular and longitudinal muscles.

Connecting with the uterus at its distal end is the vagina. The vagina serves to connect the uterus with the common genital opening. It is a muscular tube, bears a rounded swelling anteriorly, dividing it into a long and narrow apical vagina, and a short and basal vagina ending in the genital atrium (Fig. 2). Both regions of the vagina have prominent longitudinal, circular and radial muscles (Fig. 6B). The lumen is lined by strongly ciliated and much-folded epithelium.

At some distance from the genital opening, the vagina bifurcates: one arm passes up and forward to the uterus, and the second branch, the spermatheca ducts, lead to the spermatheca or seminal receptacle, whose function is thought to be the storage of foreign sperms.

The spermatheca is an elongated-oval, thin-walled sac with a narrow duct. It is attached to the anterior end of the basal uterus on the left side by strands of connective tissue.

The spacious lumen of the spermatheca sac is lined by a folded epithelium of elongated glandular cells with prominent basal nuclei and granules of secretion in the cytoplasm (Fig. 7A). Connective tissue with a few strands of circular muscle constitute the wall of the spermatheca.

The spermatheca opens into the proximal end of the vagina through a slender spermatheca duct which differs from the spermatheca in its histological organization. Its lumen is much narrower and it is lined by an epithelium which is thrown into long slender folds unlike those of the sac epithelium. The epithelium cells are columnar and ciliated but apparently non-glandular. The coat of the duct consists of connective tissue and a prominent band of circular muscle.

The oviduct is a distal continuation of the uterus (Fig. 2). It has a branched lumen which is lined by a folded epithelium of narrow, columnar ciliated cells with their nuclei lying closer to the luminal rather than the basal end of the cell. The epithelium is surrounded by connective tissue and an outer jacket of circular, longitudinal and oblique muscles (Fig. 7B).

It is interesting to note that the sperm groove, with its characteristic ciliation on the two flaps, is retained for a short distance in the proximal oviduct.

### *The Male Part of the System*

Following through the male part of the system from its junction with the hermaphroditic duct at the carrefour, the only gland encountered is the large, folded prostate gland (Fig. 2). It is generally assumed to provide fluid in which the sperms are transferred during copulation. This gland consists of several lobes folded over upon themselves.

The lobes are composed of numerous acini arranged in several rows. An acinus is tubular with a few glandular cells arranged around a central lumen into which the prostatic secretion is discharged (Fig. 8A).

The secretion is produced by the acini, then flows into a duct lying in the center of each follicle from where it is discharged into the sperm groove which is really a continuation of the hermaphroditic duct, through a prostatic canal. The prostatic canal is in continuous open communication with the sperm groove along its entire length. In *A. fulica* the prostate gland secretes fluids during egg laying.

The sperm groove communicates with the vas deferens by way of a transition region which opens proximally into the prostatic canal (where the prostatic acini decrease in number and eventually disappear) and distally into the vas deferens (Fig. 2). This region appears externally as a dark band, and in section it has the form of a much-branched tubular structure whose lumen is lined by a characteristic epithelium of tall glandular cells, with basal nuclei and granules of secretion in the cytoplasm. These granules may also be observed in the lumen of the tubules.

The vas deferens is a narrow tube with muscular walls. They originate from the anterior end of the transition region and run forward to end in the penis (Fig. 2).

The vas deferens has a thick muscular coat composed of longitudinal and oblique muscles, and a narrow but highly branched lumen lined by an epithelium of short, apparently non-glandular cells with prominent nuclei (Fig. 8B). These cells are readily distinguishable from the epithelial cells lining the tubules of the transition region referred to above.

The tubules of the transition region join up with the branches of the lumen of the vas deferens, and we envisage the path taken by the spermatozoa to reach the penis, to be as follows: hermaphroditic duct → sperm groove → prostatic canal → tubules of transition region → branches of the lumen of the vas deferens and central lumen of the vas deferens → penis.

The penis is a specialized extension of the vas deferens where the lumen of the vas deferens is continued as the seminal canal and the muscular wall of the vas deferens as the muscular penis sheath. The slightly bent penis is muscular, broad at the middle and slightly narrow at both ends.



The seminal canal has a slightly folded epithelium of non-ciliated cells surrounded by longitudinal muscles. The muscular wall of the canal is much thickened at this point in the form of a bulge, and the prominent retractor muscle is attached to the penis here.

The genital atrium is a small but stout muscular structure with a narrow lumen serving as the common terminal passage for both the male and female conduits. The lumen of the genital atrium opens to the exterior by a round genital aperture placed at the center of a whitish rounded area, the genital area.

### ***Hemiplecta distincta***

*Hemiplecta distincta* is hermaphroditic (Fig. 1B), with the reproductive system consisting of the hermaphroditic organ, male genitalia and female genitalia (Fig. 9). The male organs are the prostate gland, vas deferens, calcium gland, epiphallus, penis and dart sac. The female organs are the albumen gland, uterus, vagina and spermatheca. The ovotestis is the organ that produces both sperm and ova. It consists of creamish - white lobes embedded in the digestive gland, each lobe extending to almost the entire height of the digestive gland.

In the adult, the ovotestis may consist of many lobes, each lobe consisting of numerous smaller units called acini. The acinus is rounded and closed at the basal end but opens into an efferent ductule at the other end, all the efferent ductules combining together to form the little hermaphroditic duct.

The histology of the ovotestis and the stages of maturation of the reproductive tract are similar to that of *A. fulica*. Figure 10A shows the ovotestis which is composed of numerous acini. Within the acini of the ovotestis, all the stages of spermatogenesis and oogenesis are observable.

Figure 10B shows spermatozoa arranged in clumps around Sertoli cells. The ova are the largest cells, usually located at the edge of the acinus (Fig. 10C).

The hermaphroditic duct is clear and unpigmented. The duct leads out from the ventral anterior face of the ovotestis. It is narrow, rounded and typically divided into three parts. The first two parts are visible externally but the third is often embedded and hidden in the albumen gland and opens into the carrefour.

The first region of the hermaphroditic duct lies adjacent to the ovotestis, and its characteristic histological features are a very small lumen lined by ciliated columnar epithelial cells, and a thin layer of circular muscle composed of only a few strands (Fig. 11A). The second region has a ciliated ridge, and the whole epithelium rests on a basement membrane surrounded by circular muscle. The sperms lie immobile in fluid inside the lumen (Fig. 11B). The third region is often embedded and hidden in the albumen gland and opens into the carrefour. The histology of the third region is similar to that of the second region (Fig. 11C).

The proximal end of the hermaphroditic duct dilates to form the seminal vesicle, which is about the same diameter as the hermaphroditic duct itself. The seminal vesicle

is normally densely packed with mature spermatozoa.

The more distal region of the hermaphroditic duct is slender and lies within a slight depression of the albumen gland and connects to the talon (Fig. 9). The talon has a central lumen lined by an epithelium of columnar ciliated cells. Spermatozoa are packed in the narrow lumens of the central lumen, with the heads of the sperms lying in very close contact with the epithelial cells.

The hermaphroditic duct opens tangentially into the carrefour opposite the opening of the duct of the albumen gland. The structure of the carrefour cannot be distinguished from that of the duct of the albumen gland.

#### *The female part of the system*

The albumen gland in *H. distincta* is light yellow in colour, smooth-surfaced with a rather oblong shape (Fig. 9). It is composed of a large number of follicles which all open into the slitlike central lumen. Each follicle consists of a layer of secretory epithelium surrounding a central lumen (Fig. 12A). The simple epithelium of the duct consists of low columnar cells covered with long dense cilia.

The uterus is a flattened tube which is very long in length. Distally, the uterus leads into the free oviduct, which leads into the vagina bulb (dark coloured due to ingested material), spermatheca duct, vagina, then into the genital atrium (Fig. 9).

The uterus is of a large diameter with folded walls (Fig. 12B). The lumen is bounded by a ciliated epithelium outside of which is a high columnar epithelium which probably secretes the mucous envelopes for the eggs. The distal uterus or oviduct consists of three layers : an inner ciliated epithelium, an elaborate middle glandular layer, and an outer connective tissue capsule.

The spermatheca is short or bulbous and is attached to the uterus by connective tissue. The short duct of the spermatheca is similar histologically to the spermatheca of *A. fulica*. The heavily ciliated, simple columnar epithelium of the duct forms several longitudinal folds and rests on a relatively thick basement membrane (Fig. 13A). The epithelial cells apparently possess secretory function. The spermatozoa in the spermatheca may occasionally lose their structure completely and appear degenerate.

The vagina starts at the origin of the spermatheca duct and leads to the genital atrium. The lumen of the vagina usually appears occluded by the cilia and the epithelial folds (Fig. 13B). The outer connective tissue sheath is fused with the surrounding tissue of the neck and contains abundant small mucous cells.

#### *The male part of the system*

The prostate gland is a long, thin tubular gland connected to the spermoviduct. It is generally assumed to provide fluid for autosperm during mating.

The lumen of the prostate gland invariably lies at an angle to that of the uterus from which it is partially separated by two folds or ridges of tissue. The lumen of the prostate gland is lined with a ciliated epithelium continuous with that of the uterus (Fig. 12B).

Continuing with the male system of *H. distincta*, the vas deferens passes out of the area of the prostate gland, and joins the penis (Fig. 9).

The vas deferens has a thick muscular coat composed of longitudinal and circular muscles. The lumen is lined by a folded epithelium of elongated glandular cells with prominent basal nuclei and granules of secretion in the cytoplasm (Fig. 14A). The lumen contains the cilia to convey the sperms to the penis at copulation. A thin connective tissue sheath covers the outer surface of the vas deferens.

The penis is a highly muscular organ that is everted at copulation and is typically inserted into the genital atrium and vagina of the mate. The penis is often actually a penial sheath. The penis retractor is attached to the inner wall of the body cavity beneath the anterior end of the heart.

The penis contains the spermatophore. The inner surface of the penis is lined with a layer of low cuboidal cells containing cilia. Beneath the basement membrane are two layers of muscle cells, an inner circular and an outer longitudinal cell layer. A thin connective tissue sheath covers the outer surface (Fig. 14B).

Structures which serve to form parts of the spermatophore are the dart sac, epiphallus and calcium gland (Fig. 9). The dart sac, comparatively speaking, is of great length, evenly cylindrical, having a retractor muscle at its somewhat blunt end. The epiphallus may also have a calcium gland attached to it. The calcium gland and epiphallus are tightly bound by connective tissue to the penis. The epiphallus is simply a swollen tube equal in diameter to that of the penis, with the vas deferens entering laterally onto it.

### ***Cyclophorus aurantiacus***

The family Cyclophoridae is characterized by having the penis located on the side of the head behind the right tentacle.

The uppermost part of the visceral hump is occupied entirely by the testis in the male. In the female, the ovary lies on the columellar side and the digestive gland on the outer side. The middle portion of the visceral hump is occupied entirely by the digestive system in both sexes. The lowest part of the visceral mass in both sexes has reproductive organs on the columellar side and the digestive system on the outer side.

The majority of *C. aurantiacus* are dioecious (Figs. 1C, 1D). The female organs are the ovary, oviduct, seminal receptacle, bursa copulatrix, uterus (pallial oviduct) and vagina (Fig. 15). The male organs are the testis, vas deferens, seminal vesicle, prostate gland (pallial vas deferens) and penis (Fig. 16).

### *Female reproductive system*

Figure 15 shows the female reproductive system of *C. aurantiacus*. The ovary of *C. aurantiacus* is bright orange in colour. It is embedded in the digestive gland and is found mostly on the columellar side or the right side of the visceral mass.

The ovary is composed of a large number of follicles. The wall of the follicles of the ovary is composed of thin connective tissue and germinal epithelium (Fig. 17A).

The ovary consists of a solid mass of cell and is lined by a layer of basal epithelial cells which appear darker than the ovarian cells. These cells are sometimes difficult to identify because they are compressed over most of the area of the ovary.

The ovary contains many cell types which can be divided into three groups: oogonia, oocytes and ova. Oogonia are the smallest cells and are located close to the edge of the germinal epithelium (Fig. 17A). Oocytes are covered by a thin layer of cells which forms a follicle. The oocytes vary in size, but a few are very large. The oocyte size increases with distance from the epithelium. The ova are the largest cells which are usually located at the edge of the follicle and are easily found (Fig. 17B).

The oviduct leads out from the ventral anterior face of the ovary. It lies on the columellar side of body, lateral to the esophagus and ventral to the stomach and digestive gland.

The oviduct is narrow and very thin. However, it is very thick when it passes forward towards the mantle and reaches the posterior end of the uterus located in the right mantle. The epithelium of the oviduct has a ciliated ridge situated on a basement membrane surrounded by circular muscle (Fig. 18A).

At its distal end, the oviduct opens into a small knob-like structure. This area may be called the seminal receptacle (Fig. 15). The seminal receptacle is bright orange in colour. It acts as a reservoir for spermatozoa during the entire breeding season.

The seminal receptacle has a central lumen lined by an epithelium of ciliated columnar cells. The heads of the sperms all lie in very close contact with the epithelial cells (Fig. 18B).

The bursa copulatrix lies partly on the kidney. It is a very long slender duct, black in colour and thin-walled (Fig. 15). The lumen is lined by much-folded ciliated epithelium. The duct from the bursa copulatrix opens directly into the lateral side of the pallial oviduct (uterus) (Fig. 15). The uterus is a long broad, glandular structure, light yellow in colour. It lies along the right wall of the mantle, from the posterior end almost to the anterior mantle margin. It runs parallel to the rectum.

There are two portions of the uterus, one anterior and the other posterior. The anterior uterus is thicker and is separated into two parts, one dorsal and the other ventral. The opening of the anterior uterus is dorsal to the bursa copulatrix.

The female has an open-vaginal slit that extends almost the entire length of the uterus and is continuous with the uterine lumen. The uterus leads to the genital aperture. The genital aperture can be seen at the anterior margin of the mantle wall above the right side of the neck.

The uterus has a much branched lumen lined with glandular cells (Fig. 18C). The epithelium is folded and composed of ciliated cells. The folded wall of the uterus permits the necessary expansion for the storage of eggs.

#### *Male reproductive system*

Figure 16 shows the male reproductive system of *C. aurantiacus*. The testis is the organ that produces sperm. It is one of the largest organs in these snails and is embedded in the apex on the right side. The testis is brown in colour.

On the surface, the testis is lobate and composed of numerous branched tubular follicles. The walls of these follicles are composed of thin connective tissue. These walls contain germinal epithelium.

The follicles always contain spermatozoa and all the stages of spermatogenesis (Fig. 19A). The stages in sperm maturation are similar to those of *A. fulica* and *H. distincta*.

The vas deferens runs forward as the seminal vesicle on the columellar side of the visceral mass. In the central visceral section, it lies lateral to the esophagus and ventral to the stomach.

The vas deferens is thick and muscular for the function of bringing sperms from the testis to the prostate gland (pallial vas deferens) and penis. The lumen of the duct is narrow and lined by a much-folded epithelium which is partly ciliated, outer to which lie the circular muscle and connective tissue (Fig. 19B).

The terminal portion of the vas deferens which expands near the open genital tract is called the seminal vesicle. The seminal vesicle runs down on the columellar side of the body and enters the prostate gland directly (Fig. 16). It has a much branched lumen lined by much-folded epithelium and ciliated cells (Fig. 19C).

The prostate gland runs to the genital opening near the anus and contains the sperm groove to the penis. It is visible through the integument on the columellar side of the body. The structure of the prostate gland resembles a sandwich, folded in half longitudinally. This structure gives a cross-section with two layers of bread (prostatic tissue) in the middle surrounded by the sandwich filling (lumen of the prostate).

The prostate gland enters the pallial cavity on the right side, within the mantle wall, below the rectum and at an angle formed by the junction of the mantle and the body. The epithelial lining of the inner wall of the prostate gland has numerous folds creating pockets (Fig. 20A). The prostate gland has a much branched lumen lined by glandular ciliated cells (Fig. 20B).

The penis of *C. aurantiacus* is typically situated on the head behind the right tentacle and has a sperm groove extending from the base to the tip. The base of the penis is bulbous and the distal end is slender.

## DISCUSSION

### Anatomical Study

In this study, the anatomy of the reproductive systems of three species of land snails are compared. Two species, *Achatina fulica* and *Hemiplecta distincta*, are monoecious. Some parts of the reproductive system are involved in both male and female functions, but some are involved only in one or the other.

The ovotestis is a coiled organ located in the sunken spire of the shell. The product of the ovotestis is conveyed by a hermaphroditic duct which is provided with a seminal vesicle near the junction of the duct with the gland. The carrefour has been found only in hermaphroditic snails. The albumen gland and uterus of the female system and the prostate gland of the male system show characteristic structure differences from other forms.

The spermatophore and dart sac are present in *H. distincta* but absent in *A. fulica*. The formation of the spermatophore, the special structure used to stimulate the partner during the elaborate copulatory behaviour, is characteristic of *H. distincta*. The dart sac of *H. distincta*, is similar to that of *Helix* sp., and the amatorial organ of *Ariophanta ligulata*, armed with a needle, is capable of injecting fluid.<sup>6</sup>

The prostate gland of *A. fulica* was found in the form of follicles (acini), but that of *H. distincta* was found joined to and running along the small sperm groove parallel to the uterus. In addition, the prostate gland of *C. aurantiacus* was found in the form of the pallial vas deferens resembling a sandwich. Hubendick<sup>7</sup> made a special study of the prostate in the Basommatophora, and concluded that the structure was responsible for the development of glandular formation which forms part of the vas deferens.

The reproductive system of *H. distincta* contains the calcium gland, epiphallus and dart sac, but these structures are absent in *A. fulica*. The calcium gland, the enlarged portion of the vas deferens, secretes calcium for the sperm coat. The epiphallus, a modification of the vas deferens or penis for spermatophore formation, may also have a calcareous gland which serves in the formation of parts of the spermatophore. In dioecious prosobranchs, both the male and the female genital ducts are divided into a thin-walled apical part (the vas deferens and seminal vesicle in the male; the oviduct and seminal receptacle in the female) and a thick-walled glandular part (the prostate gland and penis in the male; the uterus, bursa copulatrix and vagina in the female).

The majority of *C. aurantiacus* are dioecious, while the pulmonate land snails are monoecious. The penis of *C. aurantiacus* is found outside the body, while that of the pulmonate land snails is present inside the body of the snails. In the family Cyclophoridae,

characterized by possessing a common duct for the copulatory bursa, oviduct and seminal receptacle, the male snails have the penis located on the nape or behind the right tentacle.<sup>8</sup>

In the present study, the female reproductive structure of *C. aurantiacus* is found to be very similar to that of *Aperostoma mexicana salleana*, the other species of prosobranch land snail of the family Cyclophoridae, with only some minor differences.<sup>9</sup> *C. aurantiacus* lacks the albumen gland while this structure is present in *A. mexicana salleana*. In addition, the bursa copulatrix is present in *C. aurantiacus*, but absent in *A. mexicana salleana*.

The male reproductive system of *C. aurantiacus* shows a small triangular penis on the side of the head behind the right tentacle. Sperms are transferred through the seminal groove, extending from the prostate gland along the side of the nape to the tip of the penis. In *A. mexicana salleana*, sperms are transferred through the prostate gland along the seminal duct to the penis.<sup>9</sup>

### Histological Study

A histological study of the three species of land snails have been carried out as a necessary requisite for subsequent investigations on the snails.

In *A. fulica* and *H. distincta*, the acini of the ovotestis are lined with the germinal epithelial cells. The germinal epithelium is delivered via the hermaphroditic duct to the carrefour where they are separated into the respective genital tracts.

The histology of the reproductive system of *H. distincta* was found to be similar to that of *A. fulica* in the general structure of hermaphroditic duct, albumen gland, uterus, spermatheca, oviduct and vas deferens.

The hermaphroditic duct of *A. fulica* is similar to that of *H. distincta* and *Helix pomatia*. They are divided into three distinct regions. The third regions of the hermaphroditic ducts of *H. distincta* and *H. pomatia* are often embedded in the albumen glands. The second regions of the hermaphroditic ducts (seminal vesicles) of *A. fulica*, *H. distincta* and *H. pomatia* are covered by pigmented connective tissues. In each species the epithelium of the seminal vesicle often has a ciliated ridge, and the whole epithelium rests on a basement membrane surrounded by circular muscle. The sperms lie immobile in fluids inside the lumen.<sup>1</sup>

The albumen glands of *A. fulica* and *H. distincta*, consist of a number of secretory follicles held together by thin strands of connective tissue. Each follicle is circular in cross section and consists of a number of glandular cells arranged radially in a single layer around a small central lumen. The canal is lined with an epithelium of ciliated columnar cells. The histology of the albumen gland of *H. pomatia* has been described as consisting of acini surrounded by a thin layer of connective tissue,<sup>10</sup> each acinus consisting of a layer of secretory epithelium, one cell thick, surrounding a central lumen.

In the uterus of subadult specimens of *A. fulica* and *H. distincta*, a thick layer of connective tissue is present between the mucous membrane and the circular layer of the

apical uterus, but in the basal uterus the connective tissue is less thick and bears many intercellular spaces.

The prostate gland of *A. fulica* is similar to that of *Helix aspersa*.<sup>11</sup> The prostate consists of numerous acini separated by delicate strands of connective tissue. Each acinus usually has a small central lumen surrounded by a columnar epithelium. During the breeding season, the acini increase in size, but reduce considerably after egg-laying.<sup>12</sup> In *H. distincta*, the prostate gland is a thin, long, tubular gland. The lumen of the prostate gland is lined with ciliated epithelial cells.

The penis of *A. fulica* consists of a verge inside. The wall of the verge is less thick than that of the vas deferens, and is formed from circular muscle bands made of branched fibers. Narrow spaces are present among the bundles.<sup>12</sup> The penis of *H. distincta* contains spermatophore for transferring sperms into the vagina of the partner.

## ACKNOWLEDGEMENT

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## บทคัดย่อ

การศึกษาระบบสืบพันธุ์ของหอยทาก 3 ชนิด คือ *Achatina fulica* (หอยทากยักษ์), *Hemiplecta distincta* (หอยเคือ) และ *Cyclophorus aurantiacus* (หอยหอม) เป็นการศึกษาเปรียบเทียบในด้านกายวิภาค ลักษณะตำแหน่ง และชนิดต่าง ๆ ของเซลล์ในแต่ละส่วนของระบบสืบพันธุ์ รวมทั้งการทำงานของแต่ละระบบสืบพันธุ์

หอยหอม จัดอยู่ใน Family Cyclophoridae และ Subclass Prosobranchia เป็นหอยที่มีฝาปิด เพศผู้และเพศเมียอยู่กันคนละตัว ในหอยเพศเมีย ส่วนของรังไข่จะผลิตไข่ ส่งไปตามท่อรังไข่เพื่อจะไปผสมกับสเปิร์มของหอยเพศผู้ที่เก็บไว้ในส่วนเก็บสเปิร์มของหอยเพศเมีย ไข่ที่ผสมแล้วจะถูกเก็บไว้ในส่วนของยูเทรัสซึ่งจะผลิตสารมาหุ้มไข่ไว้ในหอยตัวผู้ส่วนของอวัยวะสืบพันธุ์เพศผู้จะสร้างสเปิร์มส่งไปตามท่อนำสเปิร์ม ผ่านต่อมโพรสเทต และออกมาทางเพนิสซึ่งเป็นอวัยวะที่ใช้ในการผสมพันธุ์

สำหรับหอยทากยักษ์ (Family Achatinidae) และหอยเคือ (Family Helicarionidae) จัดอยู่ใน Subclass Pulmonata ซึ่งไม่แยกเพศ อวัยวะสร้างเซลล์สืบพันธุ์ของทั้งสองเพศอยู่ในหอยตัวเดียวกัน ซึ่งจะผลิตทั้งไข่และสเปิร์มส่งไปตามท่อสืบพันธุ์ร่วม ซึ่งแบ่งเป็น 3 ส่วน ส่วนแรกมีขนาดเล็ก ส่วนที่สองมีขนาดใหญ่ขึ้นเป็นที่เก็บสเปิร์มก่อนที่จะส่งออกเพื่อผสมพันธุ์ ส่วนที่สามเชื่อมกับเทลอน และต่อมอัลบิวมิน สเปิร์มจะถูกส่งออกไปภายนอกเพื่อผสมโดยผ่านต่อมโพรสเทต ท่อนำสเปิร์ม เพนิส และช่องเปิดของอวัยวะสืบพันธุ์เข้าผสมกับหอยอีกตัว โดยสอดใส่เพนิสเข้าไปในท่อสืบพันธุ์เพศเมีย สเปิร์มจะถูกนำไปที่สเปิร์มมาทิกา สเปิร์มบางส่วนถูกย่อยบริเวณนี้โดยเอนไซม์ในสเปิร์มมาทิกา สเปิร์มส่วนที่เหลือจะผ่านทางท่อรังไข่ ยูเทรัสและผสมกับไข่ที่ส่วนของแคร์ฟอร์ ไข่ที่ผสมแล้วจะได้รับอาหารจากต่อมอัลบิวมิน และส่งไปเก็บไว้ในส่วนของยูเทรัส ซึ่งจะผลิตเปลือกไข่จนกว่าจะวางไข่ออกสู่ภายนอกต่อไป

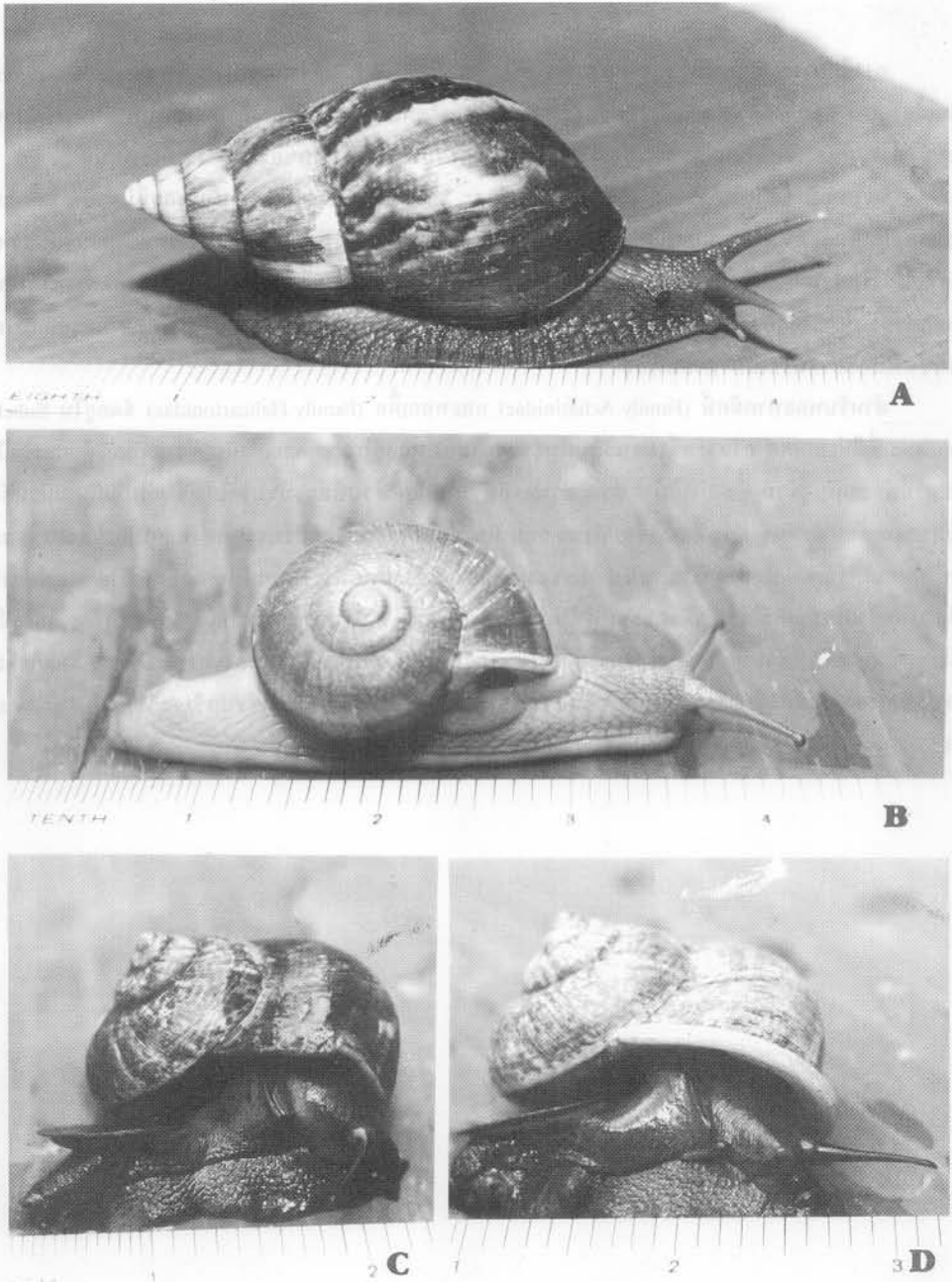
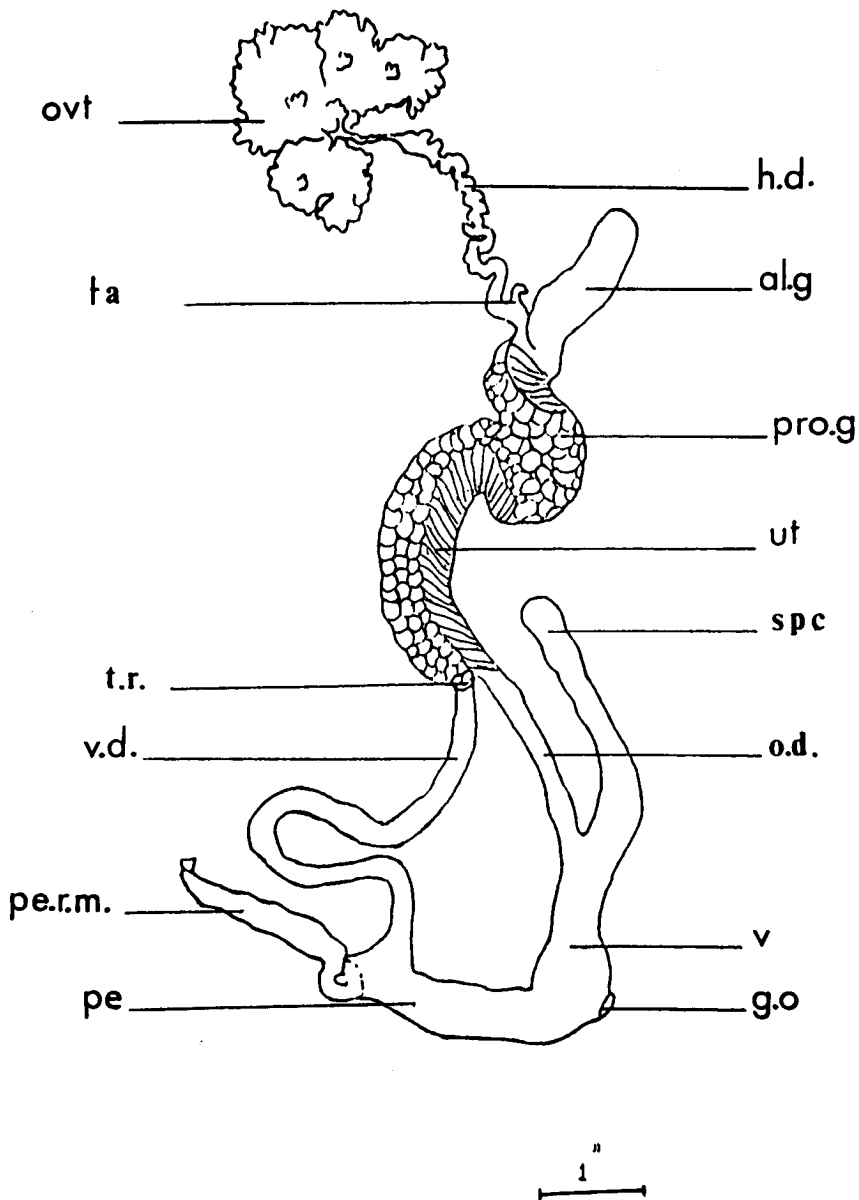


Fig. 1 External morphology of snails.

- A. *Achatina fulica*
- B. *Hemiplecta distincta*
- C. *Cyclophorus aurantiacus*, female
- D. *Cyclophorus aurantiacus*, male



**Fig. 2** Reproductive system of *Achatina fulica*.

al.g = albumen gland, g.o = genital opening,

h.d = hermaphroditic duct, o.d. = oviduct,

ovt = ovotestis, pe = penis, pe.r.m = penial retractor muscle, pro.g = prostate gland, spc = spermatheca,

ta = talon, tr = transition region,

ut = uterus, v = vagina, v.d. = vas deferens

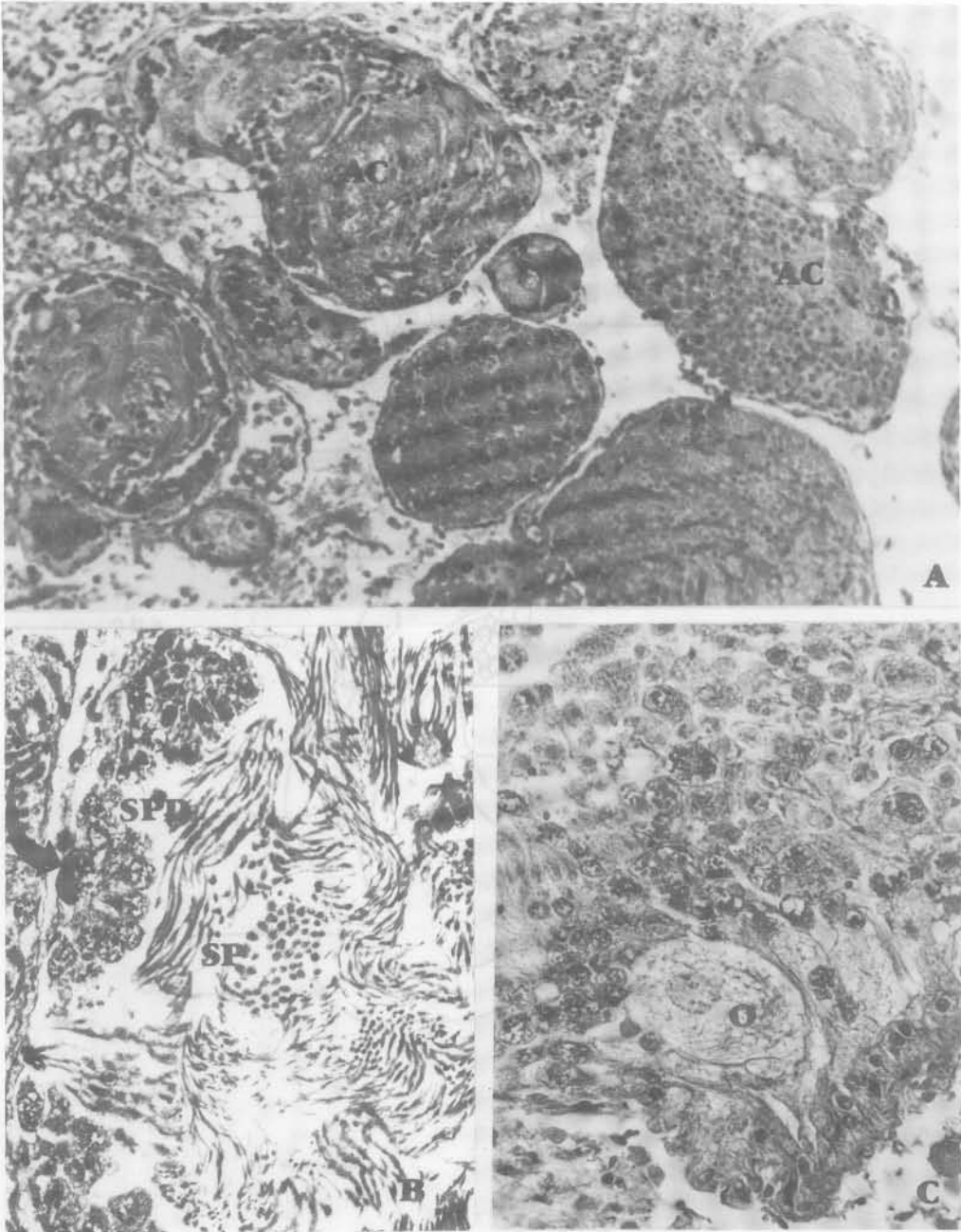
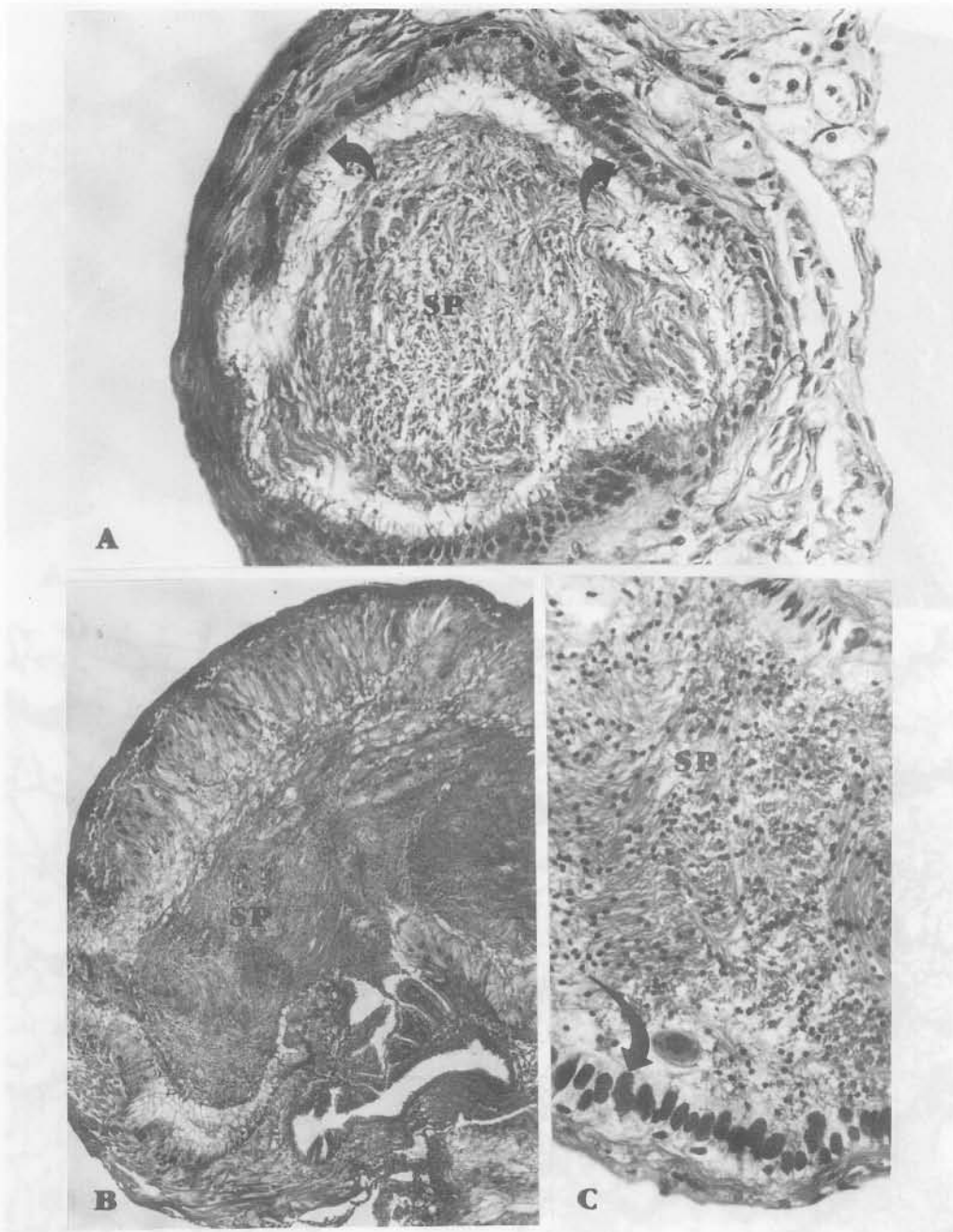


Fig. 3 A transverse section of ovotestis of *Achatina fulica*.

- A. A transverse section of ovotestis of *A. fulica*, showing numerous follicles or acini (AC) containing gametes. X16.
- B. Showing acinus with spermatids (SPD) arranged in clumps around Sertoli cell (arrow) on acinus wall, and spermatozoa (SP) liberated into the lumen. X34.
- C. Showing tip of acinus with ovum (O) as the largest and most distinguishable cell. X200.



**Fig. 4** A transverse section of hermaphroditic duct of *Achatina fulica*.

- A. First region of hermaphroditic duct with small lumen lined with ciliated cuboidal epithelium (arrows) and a thin layer of circular muscle. Spermatozoa (SP) can be seen in the lumen. X200.
- B. Second region of hermaphroditic duct with the largest lumen dividing into upper and smaller channels. The larger channel is always packed with spermatozoa (SP). X34.
- C. Third region of hermaphroditic duct with the largest tube lined with narrow ciliated columnar epithelium (arrow) and covered with circular muscle and connective tissue. SP = spermatozoa. X200.

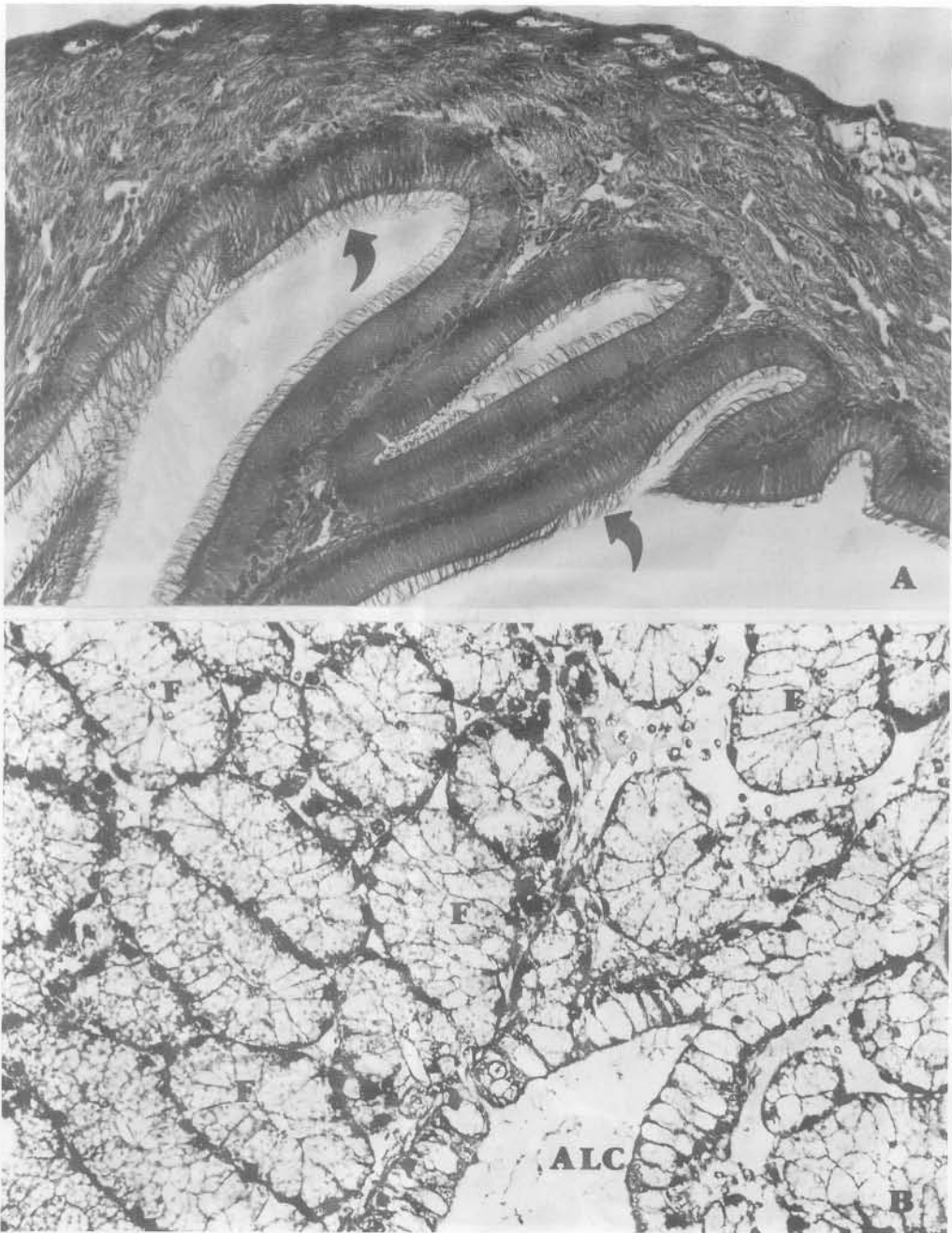
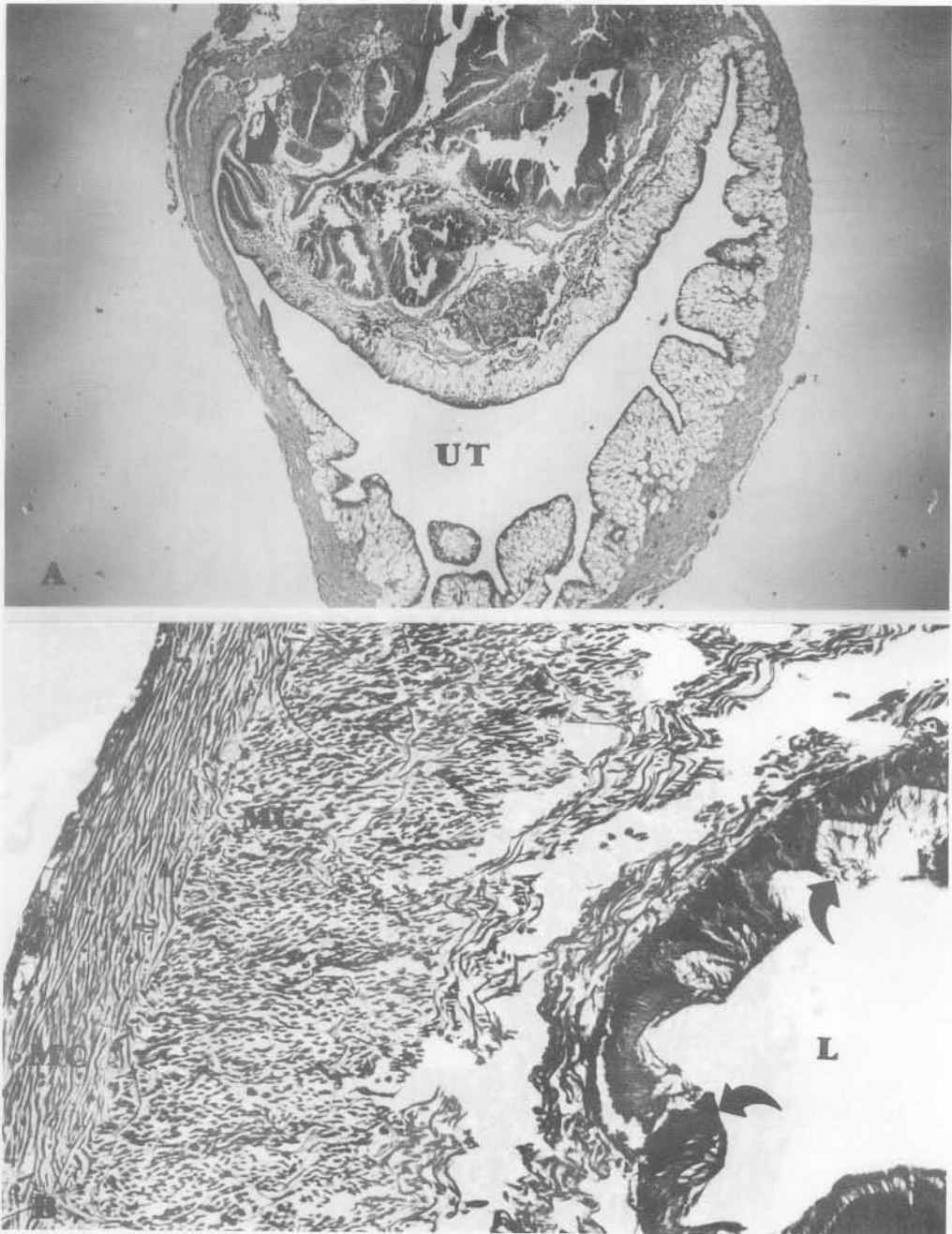


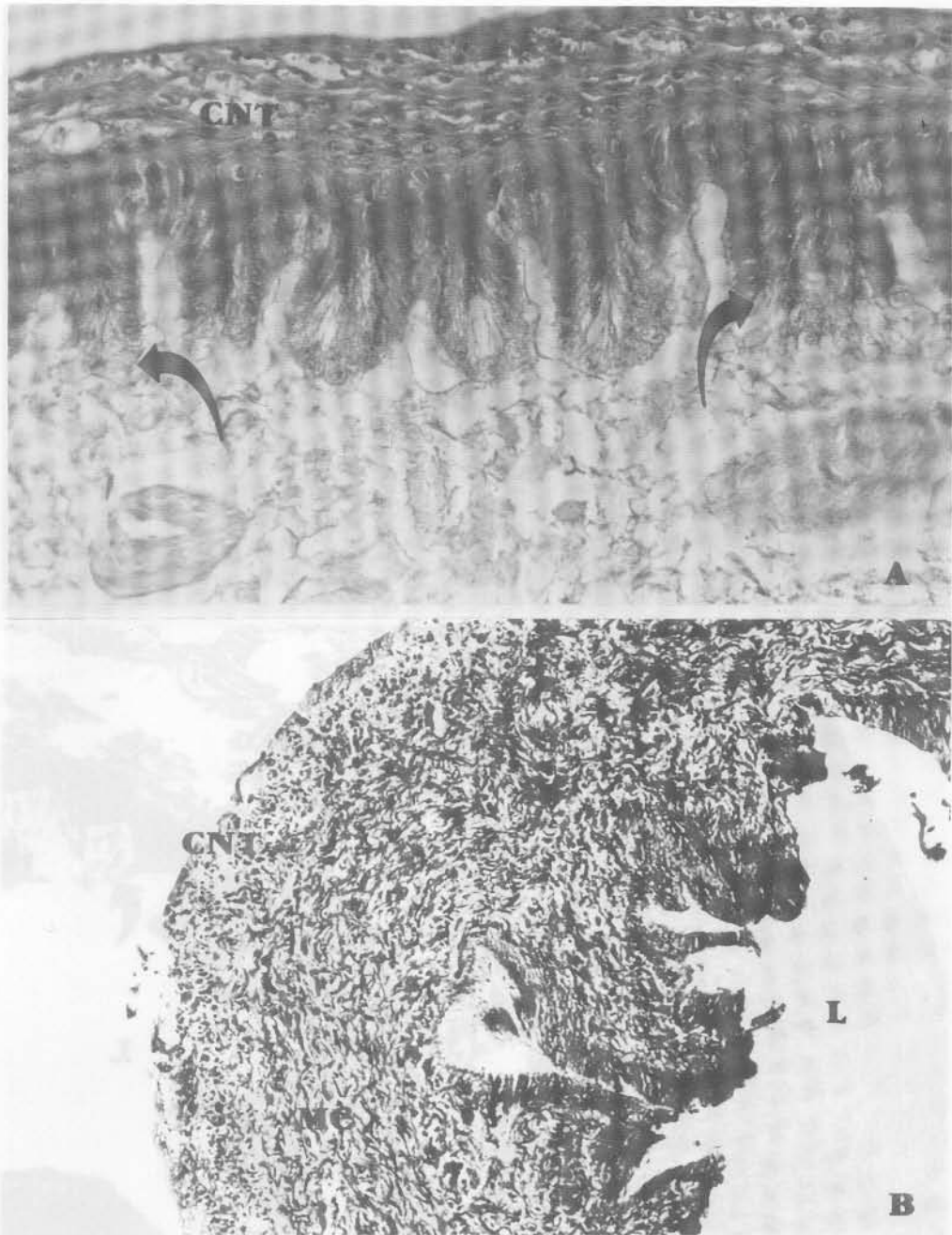
Fig. 5 Transverse sections of talon and albumen gland of *Achatina fulica*.

- A. Branched tubule of talon lined with ciliated columnar epithelium with very long cilia (arrows). X74.
- B. A transverse section of albumen gland showing follicles (F) and albumen canal (ALC) lined with ciliated columnar epithelium. X74.



**Fig. 6** Transverse sections of uterus and vagina of *Achatina fulica*.  
A. A low-power micrograph of uterus (UT) and sperm groove (arrow). X16.  
B. A transverse section through lumen (L) of vagina lined with ciliated columnar epithelium (arrows) with prominent muscular coat (MC). X74.



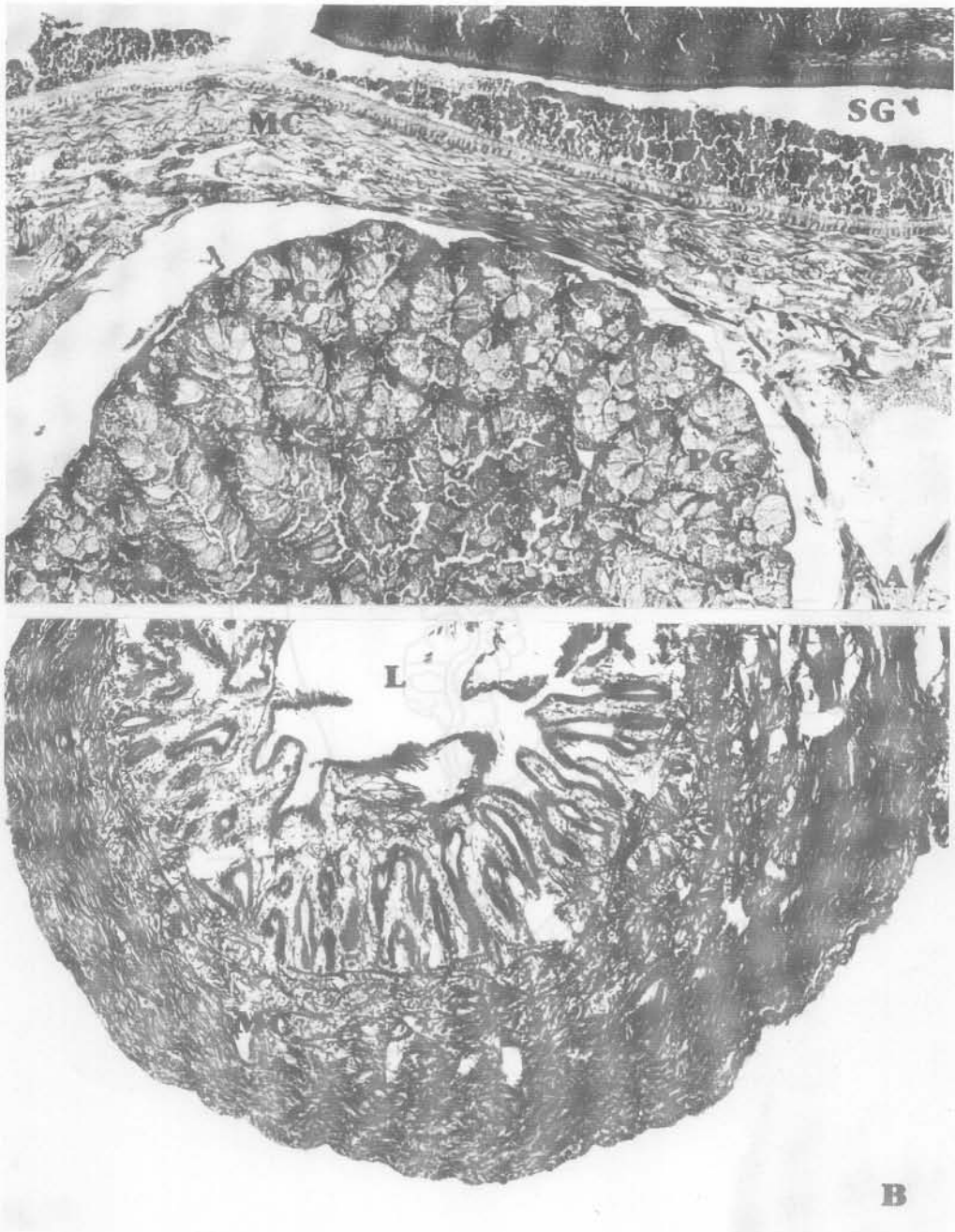


**Fig. 7** Transverse sections of spermatheca and oviduct of *Achatina fulica*.

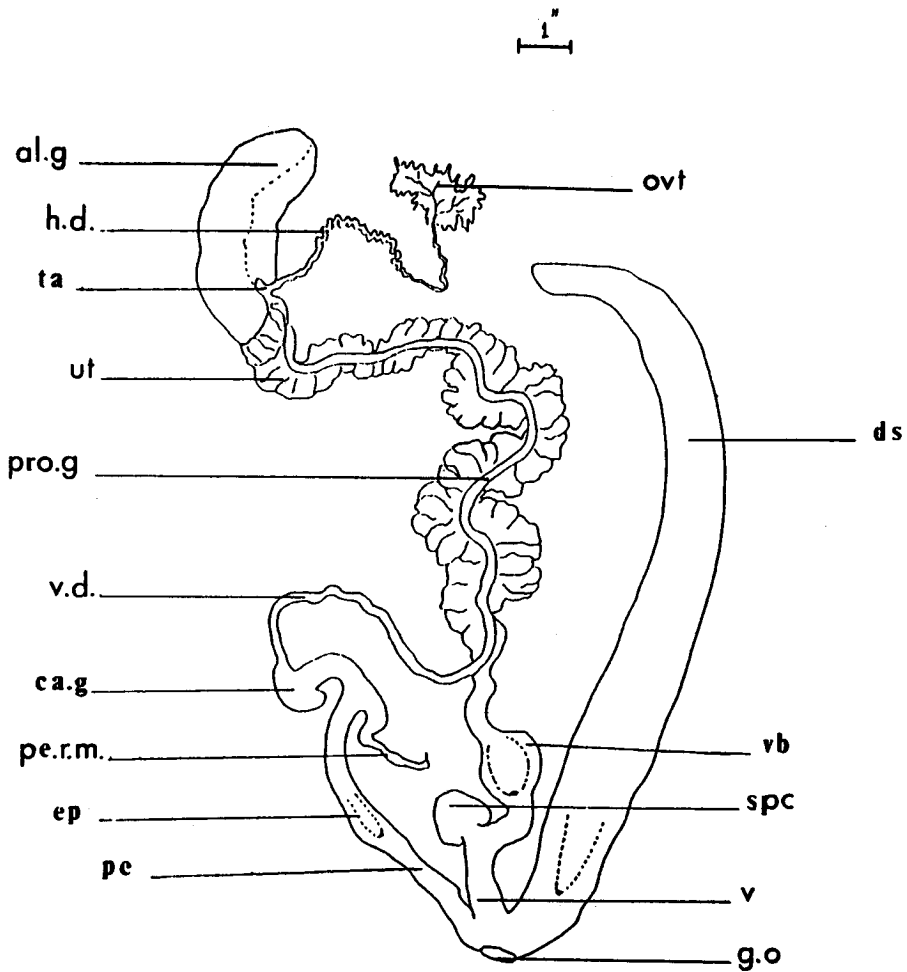
A. A transverse section of spermatheca sac lined with a folded epithelium of elongate glandular cells (arrows). CNT = connective tissue. X200.

B. A transverse section of vas deferens showing thick muscular coat (MC) and narrow, highly branched lumen (L) lined by short non-glandular epithelium. X34.





**Fig. 8** A longitudinal section of prostate gland and a transverse section of vas deferens of *Achatina fulica*.  
A. A longitudinal section through sperm groove (SG) and prostate gland (PG). MC = muscular coat . X34.  
B. A transverse section of vas deferens thick muscular coat (MC) and narrow, highly branched lumen (L) lined by short non-glandular epithelium. X34.



**Fig. 9** The reproductive system of *Hemiplecta distincta*.

al.g = albumen gland, ca.g = calcium gland,  
 d.s = dart sac, ep = epiphallus, g.o = genital opening, h.d. = hermaphroditic duct, ovt = ovotestis,  
 pe.r.m. = penial retractor muscle, pe = penis, pro.g = prostate gland, spc = spermatheca,  
 ta = talon, ut = uterus, v = vagina, vb = vagina bulb, v.d. = vas deferens

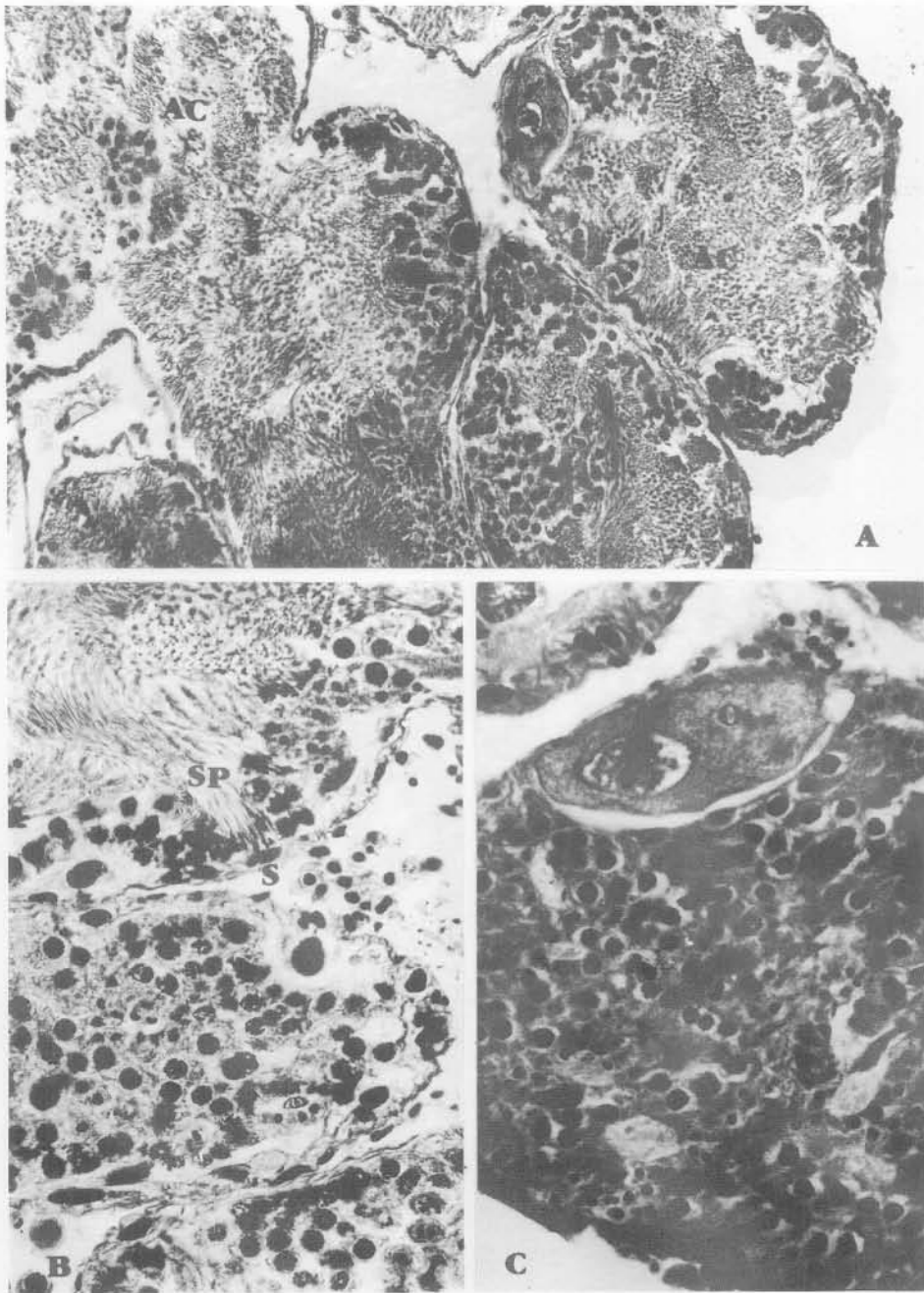
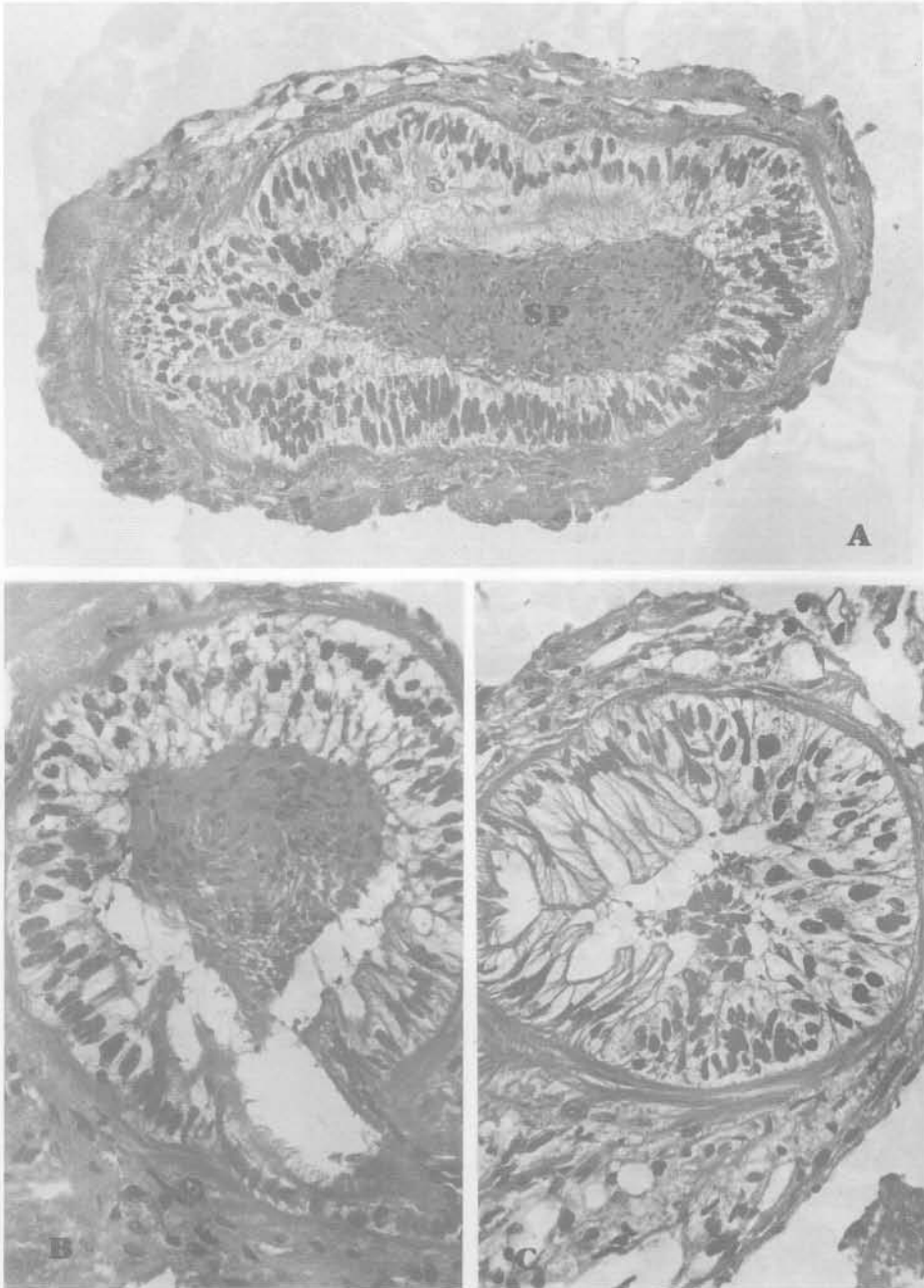


Fig. 10 A transverse section of ovotestis of *Hemiplecta distincta*.

A. A low-power micrograph of a transverse section of ovotestis which is composed of numerous small acini (AC). X16.

B. Within an acinus, mature spermatozoa (SP) are arranged in clumps around Sertoli cells (S). X200.

C. Showing mature ovum (O), the largest cell, usually located at the edge of acinus. X200.



**Fig. 11** A transverse section of hermaphroditic duct of *Hemiplecta distincta*, which is divided into three regions.  
 A. First region with very small lumen lined with ciliated columnar epithelium and filled with spermatozoa (SP). X280.  
 B. Second region with a ciliated ridge, with immobile spermatozoa (SP) in fluid inside lumen. X200.  
 C. Third region with similar histology to first region. X200.

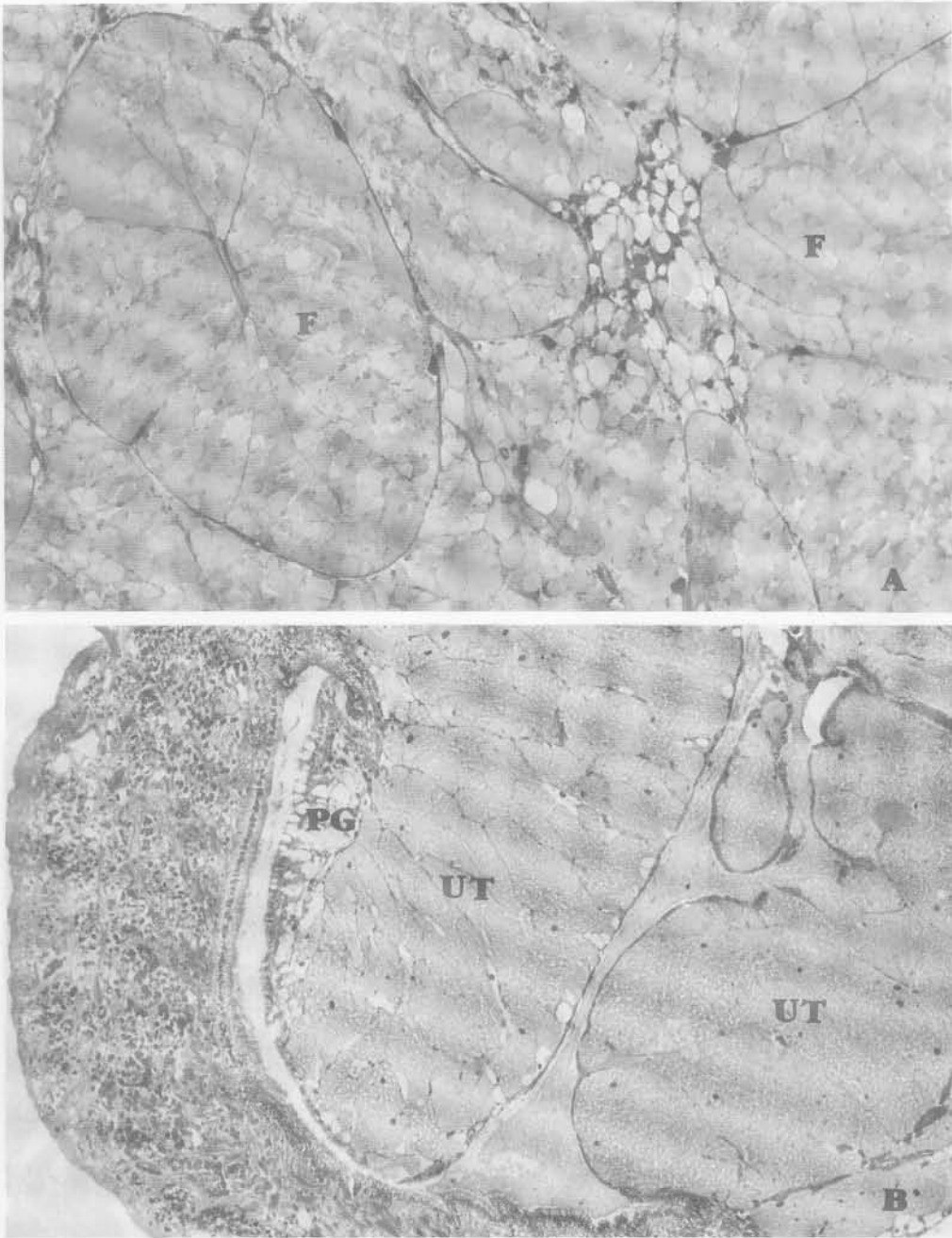


Fig. 12 Transverse sections through albumen gland and uterus of *Hemiplecta distincta*.

- A. A high magnification of follicles (F) of albumen gland with secretion. X88.
- B. A transverse section through apical uterus (UT) with densely packed glandular cells. Enclosed within the uterus is prostate gland (PG). X34.

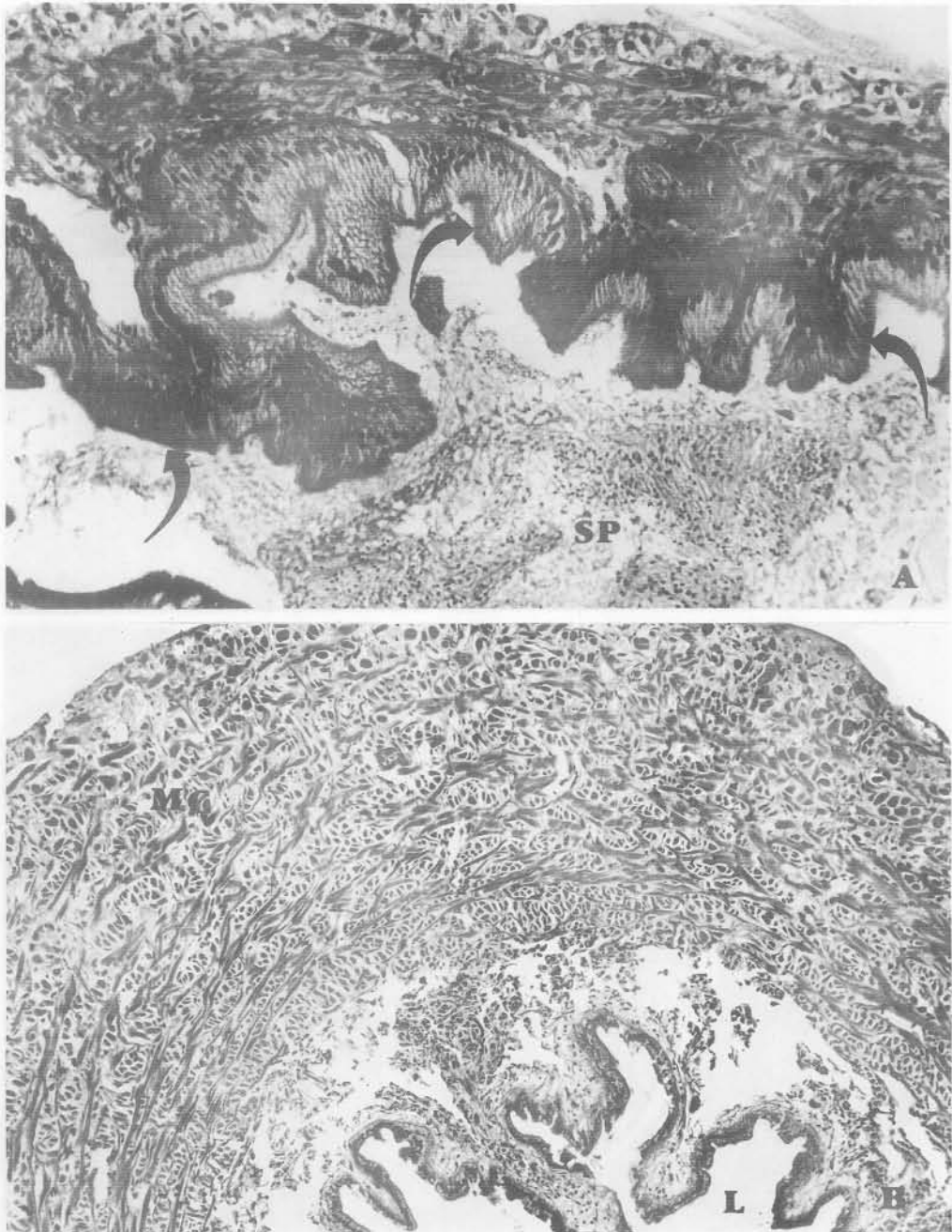
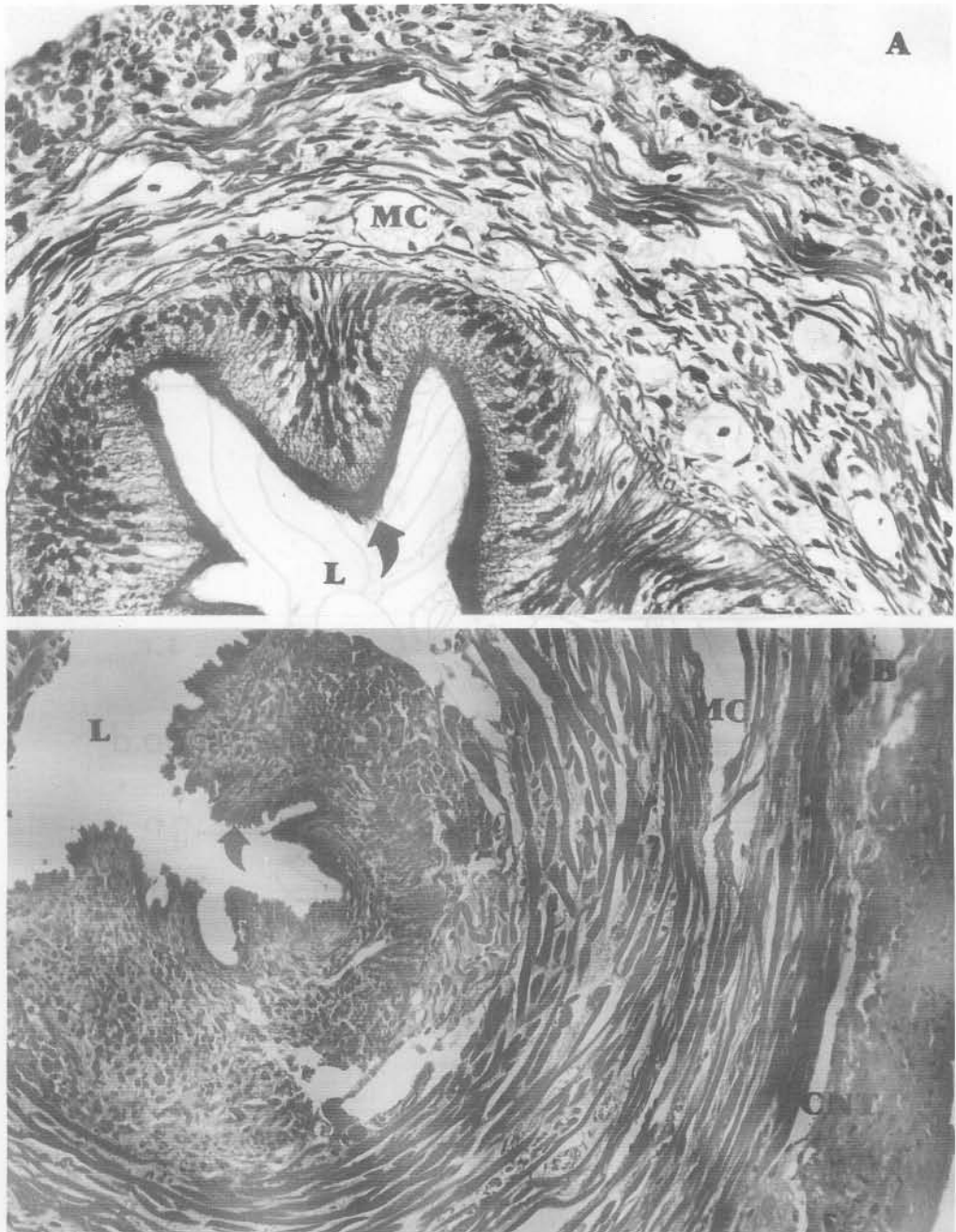


Fig. 13 Transverse sections of spermatheca and vagina of *Hemiplecta distincta*.

- A. A high magnification showing spermatheca duct lined with heavily ciliated simple columnar epithelium (arrows). Degenerating spermatozoa (SP) can be seen inside. X200.
- B. A high magnification of vagina showing outer jacket of muscular coat (MC). L = lumen. X34.





**Fig. 14** Transverse sections of vas deferens and penis of *Hemiplecta distincta*

A. A transverse section of vas deferens showing thick muscular coat (MC), and lumen (L) lined by glandular columnar epithelium (arrows). X200.

B. A transverse section of penis with inner circular and outer longitudinal muscles and thin connective tissue (CNT). Lumen (L) is lined by ciliated cuboidal epithelium (arrows). MC = muscular coat. X74.

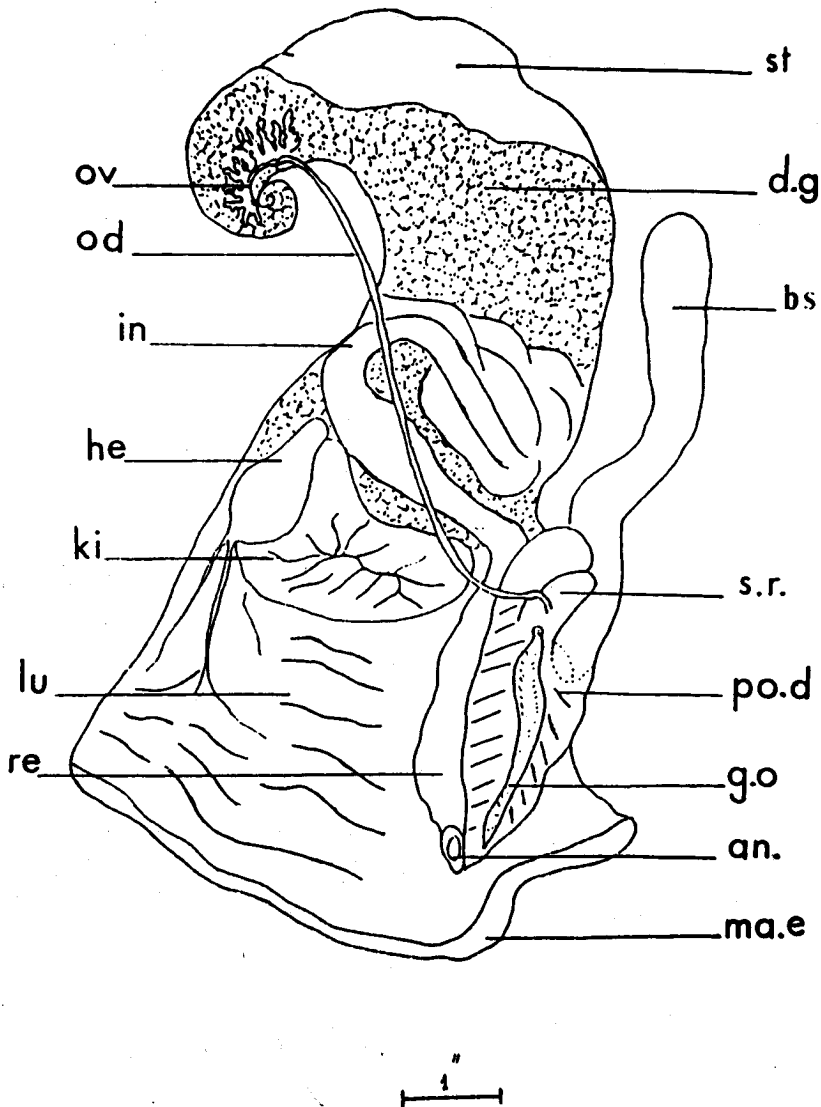
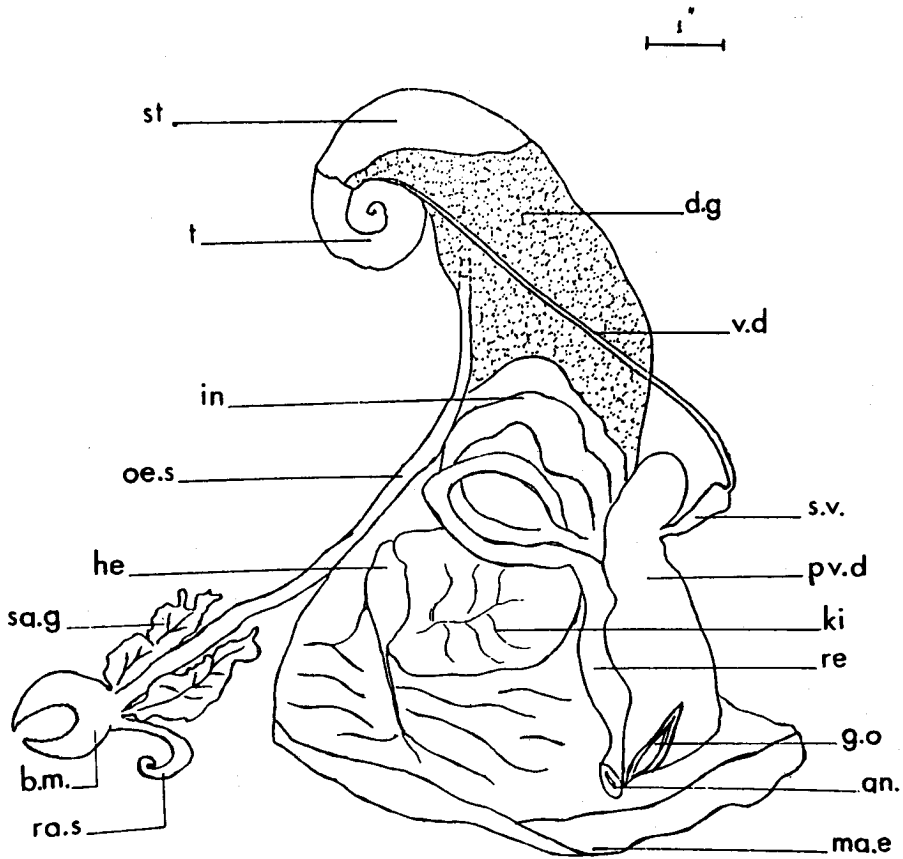


Fig. 15 The parts of female reproductive organ of *Cyclophorus aurantiacus*.

an = anus, bs = bursa copulatrix, d.g. = digestive gland, g.o = genital opening, he = heart, in = intestine, ki = kidney, lu = lung, ma.e = mantle edge, od = oviduct, ov = ovary, po.d = pallial oviduct, re = rectum, st = stomach, s.r. = seminal receptacle.





**Fig. 16** The internal organ systems of male *Cyclophorus aurantiacus*, showing digestive and reproductive systems.  
 an = anus, b.m. = buccal mass, d.g. = digestive gland, g.o = genital opening, he = heart, in = intestine,  
 ki = kidney, ma.e = mantle edge, oe.s = oesophagus, pv.d = pallial vas deferens, ra.s = radular  
 sac, re = rectum, sa.g = salivary gland, st = stomach, s.v. = seminal vesicle, t = testis, v.d. = vas  
 deferens.

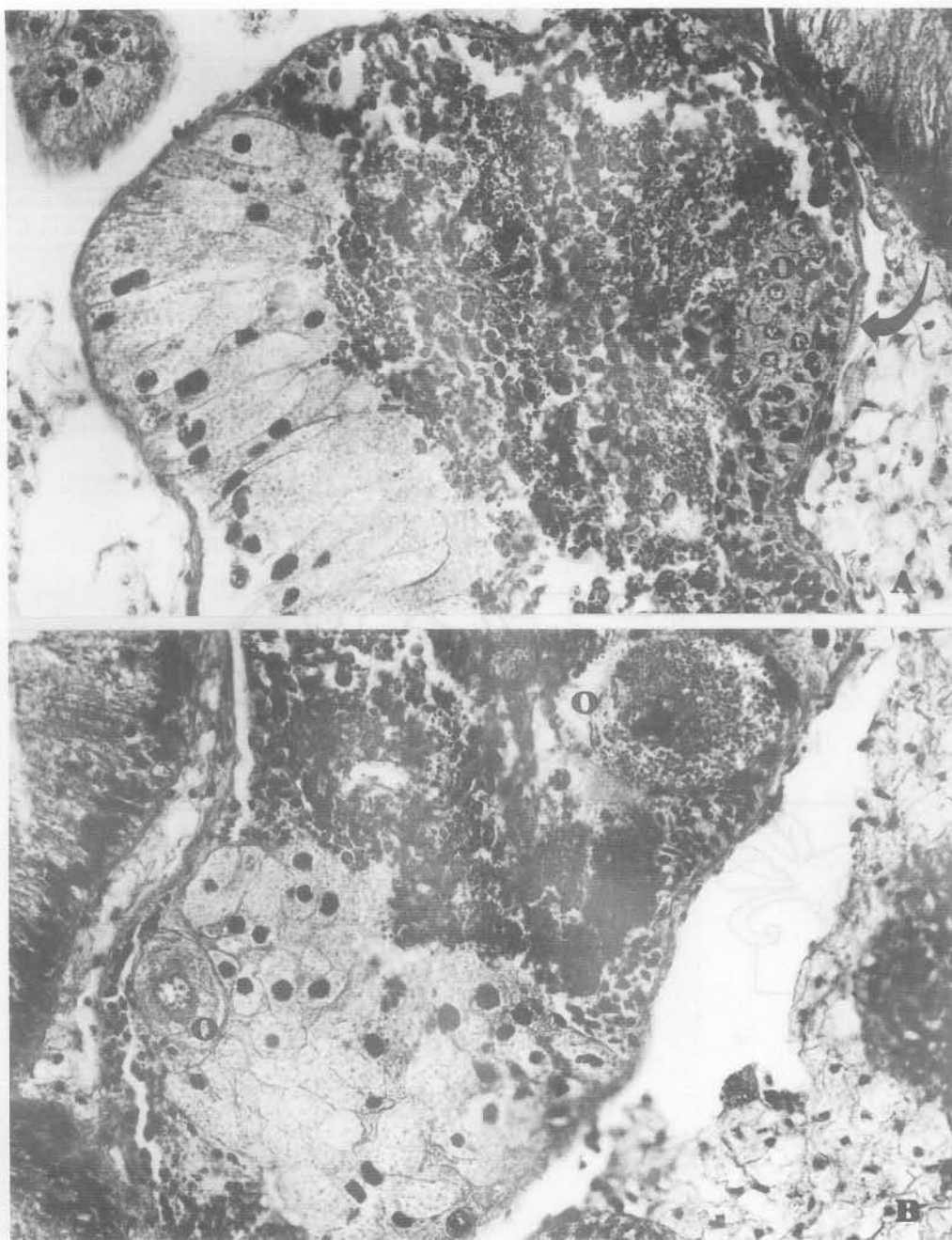
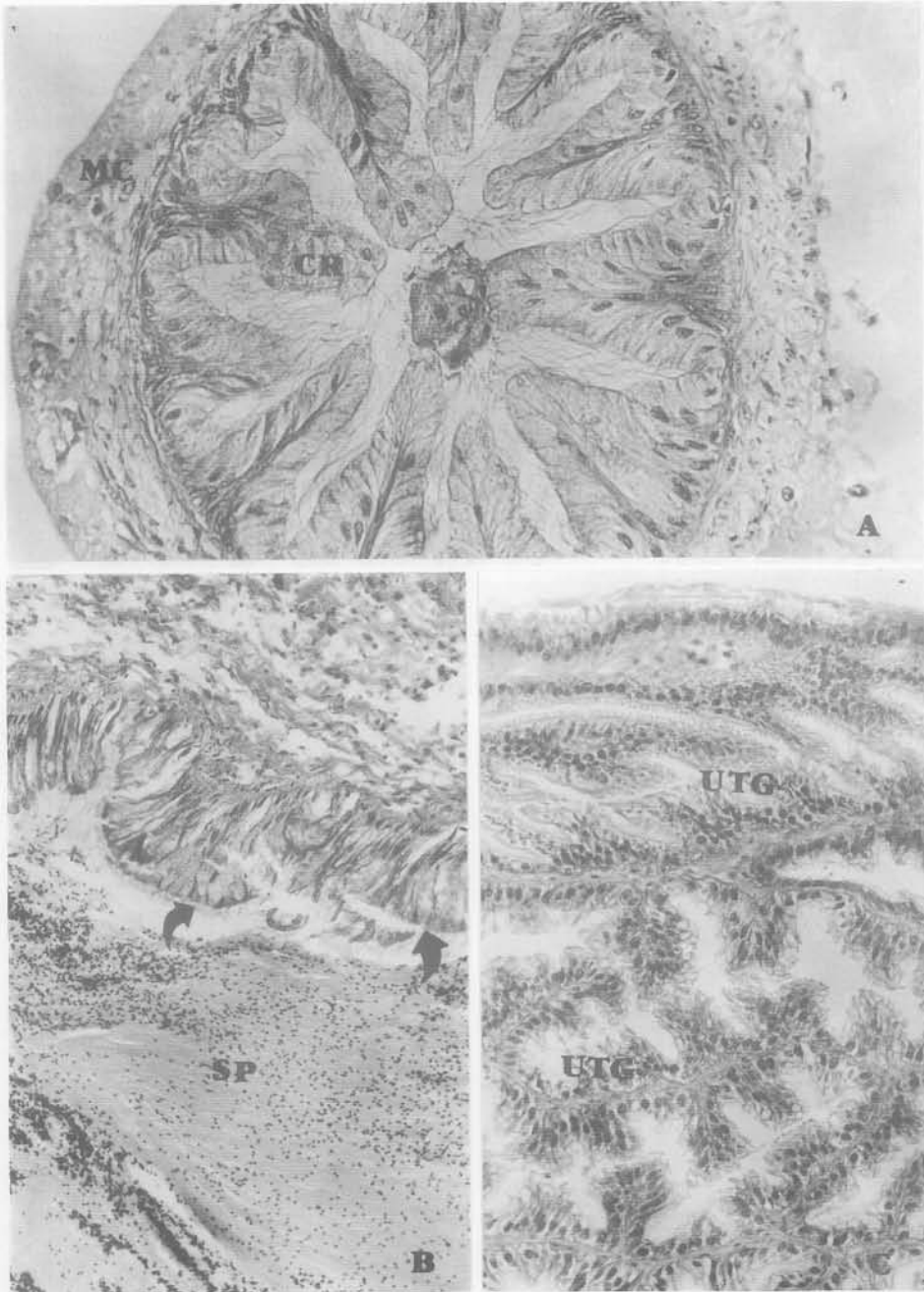
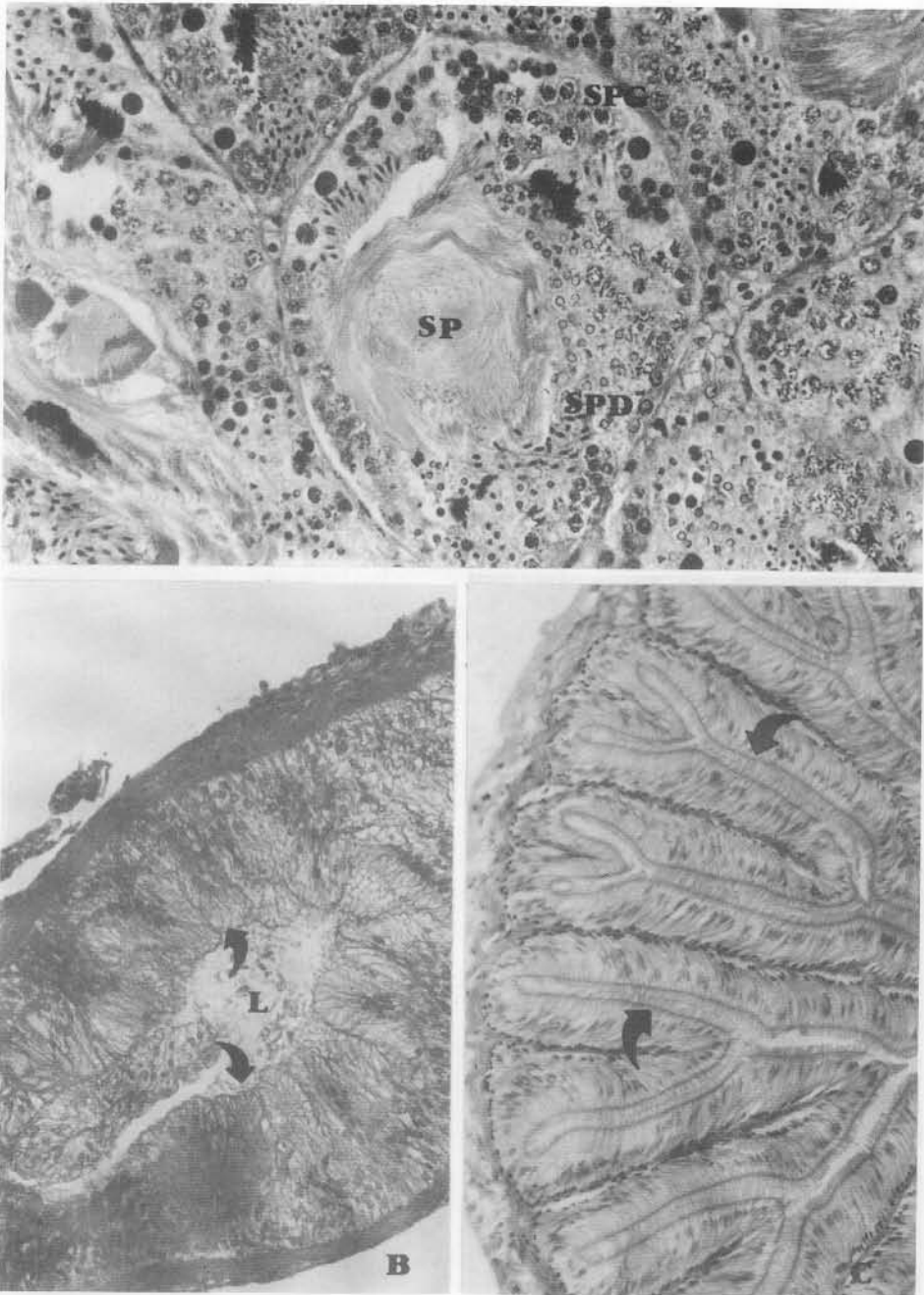


Fig. 17 A transverse section through a follicle of ovary in *Cyclophorus aurantiacus*.

- A. Showing oögonia (OG), the smallest cells, located close to the edge of germinal epithelium (arrows). X74.
- B. Showing mature ovum (O), the largest and most conspicuous cell in the ovary. X74.



**Fig. 18** Transverse sections of oviduct, seminal receptacle and uterus of female *Cyclophorus aurantiacus*.  
 A. A transverse section of oviduct showing epithelium with ciliated ridge (CR), surrounded by circular muscle (MC). X200.  
 B. A transverse section of seminal receptacle showing sperms (SP) with their heads lying in very close contact with epithelium (arrows). X74.  
 C. A high magnification of uterus showing a much branched lumen lined by glandular cells (UTG). X74.



**Fig. 19** Transverse sections of testis, vas deferens and seminal vesicle of male *Cyclophorus aurantiacus*.

- A. A transverse section of testis showing follicle (F) with various stages of spermatogenesis. SP = spermatozoa, SPG = spermatogonia, SPD = spermatid. X200.
- B. A transverse section of vas deferens showing narrow lumen (L) lined by much-folded and partly ciliated epithelium (arrows). X74.
- C. A transverse section of seminal vesicle, lined with ridges of ciliated epithelium (arrows). X74.

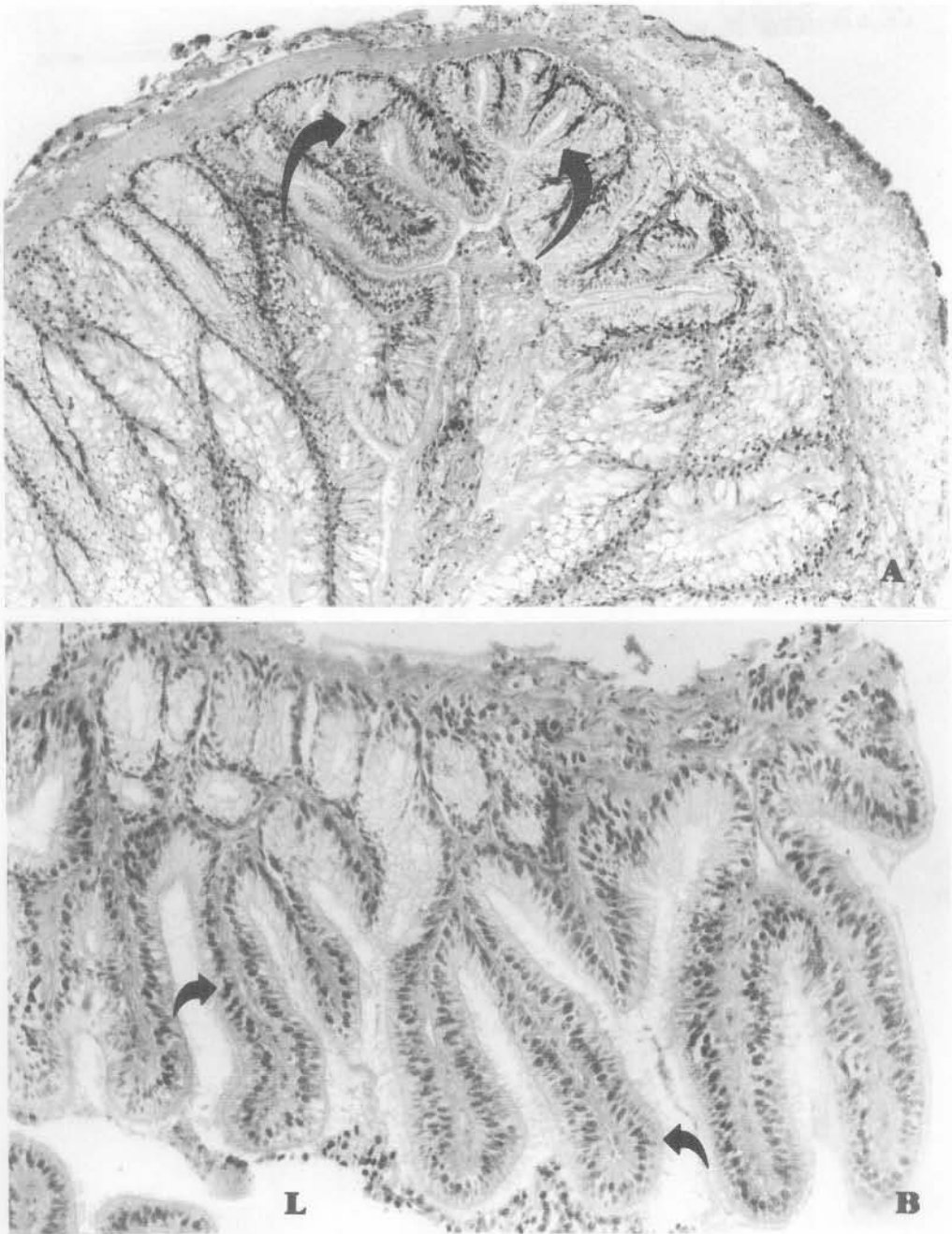


Fig. 20 A transverse section of prostate gland.

- A. A transverse section of prostate gland showing inner wall with numerous folds creating pockets (arrows). X34.
- B. A higher magnification of prostate lumen (L) to show lining by glandular ciliated epithelium (arrows). X74.