

# GROSS ANATOMY OF THE REPRODUCTIVE SYSTEM OF FRESHWATER PULMONATE SNAIL *LYMNAEA ACUMINATA* (GASTROPODA: PULMONATA)

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## ABSTRACT

The present research paper provides an account of gross anatomy of reproductive system in snail *L. acuminata*. Results obtained shows that the reproductive system of *L. acuminata* consists of three divisions: (1) The ovotestis or hermaphroditic gland and its duct i.e., the hermaphroditic duct, (2) The female genital tract and (3) The male genital tract. The female duct system consists of the oviduct, the uterus, the vagina and associated accessory glands which include the albumen gland, the muciparous gland and oothecal gland. The male duct system consists of the vas efferens, the prostate gland, vas deferens and the copulatory organ the Penial complex. We hope that the results obtained will be highly useful to understand reproductive anatomy and taxonomy of freshwater gastropods.

**Key Words:** *Lymnaea acuminata*, Anatomy, Reproductive system.

## INTRODUCTION

Freshwater pulmonate snail *Lymnaea acuminata* Lamarck, 1822 (Mollusca: Gastropoda: Pulmonata) is abundantly available in various parts of Indian subcontinent (Subba Rao, 1989). The reproductive system of freshwater gastropods varies greatly from one group to another and their reproductive strategies also vary greatly. A considerable diversity exists in the internal anatomy of the reproductive tracts of gastropods which is of taxonomic importance. The functional anatomy and histology of reproductive system of gastropods have been reported through many research publications in the past and considerable literature is available on the various aspects of the mollusc reproductive anatomy and biology (Morton, 1955; Duncan, 1958, 1975; Ghose, 1963; Quazi, 1974; Berry, 1977; Tompa, 1979; Dummalod, 1981; Harasewych, 1984; Nelson, 1984; Berezkina and Starobogatov, 1986; Pechenik, 1986; Houbriek, 1987; Haszprunar, 1988; Fretter and Graham, 1994; Glaubrecht, 1999; Dillon, 2000; Streit *et al.*, 1997; Wayne, 2001; Kohler *et al.*, 2004; Nakadera and Koene, 2013; Bocxlaer and Strong, 2016).

The extensive literature is available on the genital organization of many pulmonate families such as Otinidae (Morton, 1955a, b), Lymnaeidae (Holm, 1946; Quazi, 1974; Dama, 1977; Nelson, 1984) and also in many families of order Stylommatophora (Ghose, 1963). In addition, many published books on molluscs include discussions on various aspects of the reproduction of Basommatophora (Runham, 1983a,b,c; Geraerts and Joosse, 1984 and Hickman, 1992). However, there is paucity of literature regarding organization of reproductive system and various aspects of reproduction in *L. acuminata*. The anatomical details of reproductive system of snails are highly applicable in the taxonomy, phylogeny and reproductive biology of snails. Hence, present research work was undertaken to elucidate anatomy of reproductive system in *L. acuminata*.

## MATERIALS AND METHODS

Freshly collected, healthy, adult specimens of *L. acuminata* (shell length between 22 to 30 mm) were dissected and all observations were made on freshly dissected reproductive systems to study

gross anatomy and morphology of the reproductive system. All dissections were performed under stereoscopic dissection microscope (Meiji, zoom stereo, EMZ-2, Japan). Animals were dissected with the shell removed in snail Ringer solution (Composition: KCl 1.5 mM, NaCl 30 mM, MgCl<sub>2</sub> mM, CaCl<sub>2</sub> 4 mM, Na<sub>2</sub>HPO<sub>4</sub> 0.25 mM and NaHCO<sub>3</sub> 18 mM.) Prior to dissection, animals were narcotized by injecting one ml solution of 0.3 M Magnesium Chloride (MgCl<sub>2</sub>) through the foot into body cavity. Colour of each reproductive organ was visually recorded. Dissected out system was photographed. Measurements about length, width, diameter of various reproductive organs were recorded using ocular micrometer wherever necessary.

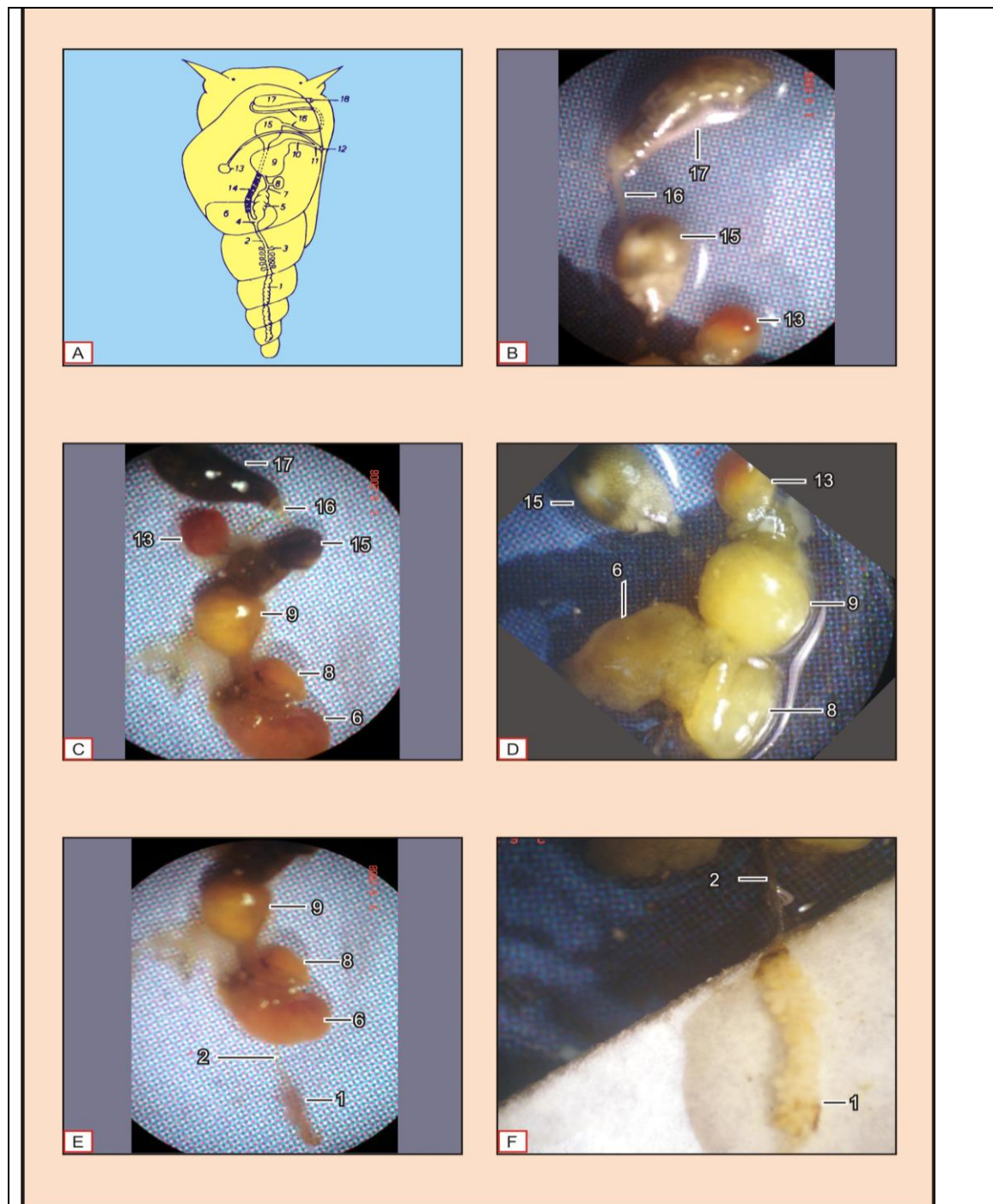
## RESULTS

Results obtained shows that the reproductive system of *L. acuminata* like that of other hermaphroditic pond snails is consists of: (1) *The ovotestis or hermaphroditic gland* and its duct. (2) *The female genital tract*, and (3) *The male genital tract*. The female duct system consists of the oviduct, the uterus, the vagina and associated accessory glands. Accessory organs are the albumen gland, a fertilization pocket, muciparous gland, pars contortis, oothecal gland and the seminal receptacle or bursa copulatrix. The male duct system consists of the vas efferens, the prostate gland, vas deferens and the Penial complex. In the body of snail, the male and female systems are closely apposed to one another along their length except at their external outlets near anterior end of the snail. In general, it may be said that the male system lies on the left side of the body cavity and female duct system lies on the right of the male system. Like most of the pulmonates *L. acuminata* is hermaphrodite snail in which male and female reproductive organs are present in one and the same individual. Although these snails are hermaphroditic, they do not self fertilize. They copulate reciprocally with exchange of sperms which results in cross fertilization.

### A. THE OVOTESTIS

The ovotestis or hermaphroditic gland is single, irregular, unbranched, and lobular or acinous gland located in pneultimate whorl of the shell embedded in hepatopancreatic mass [**Plate I: Fig. A and F**] and its size depends upon the season and age. It is granular in appearance and creamy white in colour. The ovotestis is made up of numerous round lobules or hermaphroditic follicles. The ovotestis is main reproductive organ and produces both eggs and sperms as reported by studies in the past (Nelson DJ, 1984). The sperms and ova are simultaneously developed, hence both types of cells could be observed in the ovotestis as reported earlier (Nelson, 1984).

## PLATE-I



**PLATE-I: Reproductive system of *L. acuminata*:** A: Diagram of reproductive system; B, C,D,E, F: Photographs of various parts of reproductive system

1 = Ovotestis, 2 = Hermaphrodite duct, 3= seminal vesicles ,4= Carrefour,5 = Pars contortis,6 = Albumen gland, 7= Pars recta, 8 = Muciparous gland, 9= Oothecal gland,10= Uterus, 11= Vagina, 12= Female gonopore,13 = Bursa copulatrix, 14= Sperm duct/ vas efferens, 15 = Prostate gland.,16 = Vas deferens, 17 = Penial complex,1= Male gonopore.

**Hermaphrodite duct:** It is a coiled thread-like duct that arises from ovotestis. The middle portion of the hermaphrodite duct is constricted and bears a number of small diverticula called as seminal vesicles which store sperms. The main role of the hermaphroditic duct is to carry both eggs and sperms up to the point where male and female systems separate [Plate I: Fig. A, F].

**Carrefour:** The carrefour is the junction of the hermaphrodite duct with the male and female tracts [Plate I: Fig. A]. The hermaphroditic duct carry sperms and eggs up to the point of carrefour, thereafter the sperms follow the male duct system and eggs follow the female duct system. The mechanism by which sperms and eggs are sorted and targeted into their respective individual passages

is still not known. The duct from albumen gland also opens at the point of carrefour. The detailed arrangement varies in different genera.

## B. FEMALE GENITAL TRACT

As mentioned earlier, the female genital tract include following structures:

**The Albumen gland:** Albumen gland is kidney-shaped, yellowish or orange, curved structure [Plate I: Fig. D & E] located in the body whorl below the hepatopancreatic mass and above the stomach. It looks bulgy in breeding season because of increased secretory activity. During post-breeding season, it looks flat, thin due to contribution of albumen fluid to the eggs and ceased secretory activity. The gland meets the hermaphrodite duct at carrefour. It is known to store albumen and apply a coat of albumin to egg while it travels through hermaphroditic duct.

**Fertilization pocket:** It is a small vesicular structure of about 1 mm diameter located in the concavity of the albumen gland. It is connected to the carrefour by a very short duct. The endogenous eggs are fertilized within the fertilization pocket using sperms from copulatory partner.

**Oviduct:** The oviduct is a separate duct that arises from the carrefour. [Plate I: Fig. A]. It runs anteriorly up to the uterus. Histological studies made on *L. stagnalis* (Holm, 1946) showed that the oviduct is thicker, coiled tube and are composed of ciliated cuboidal cells; and a well developed basement membrane covers these epithelial cells externally. During the course of the oviduct, it develops two different glandular structures: muciparous gland and oothecal gland.

**Pars contortis:** Pars contortis is white, translucent, coiled glandular expansion of oviduct. It partially covers the upper portion of the prostate gland. Its function is not known.

**Muciparous gland:** At the distal of the pars contortis, is a large spherical diverticulum named as muciparous gland [Plate I: Fig. D & E]. It arises as a blind outpocketing of female duct. It is creamy white to yellowish in colour. This gland was referred to as "mass membrane gland" by Crabb (1927). This gland contributes the mucous matrix within the egg mass.

**Oothecal gland:** The female duct system immediately anterior to the muciparous gland is composed of two regions: (1) a cylindrical translucent tube which is continuous with the pars contortis and (2) a dilated pear-shaped organ [Plate I: Fig. D & E] called as oothecal gland. Diagonal striations are seen on the surface of oothecal gland which represent internal glandular folds. Histologically, the oothecal gland is shown to have highly folded elastic walls (Holm, 1946; Nelson, 1984) which are continuous with the folds of the vagina. Because of these folds the lumen of this gland becomes reduced and irregular. Studies on other lymnaeids (Holm, 1946; Dama, 1977) have reported the presence of a ciliated groove in the oothecal gland that is continuous with the uterus. Prior to the vaginal opening, the uterus bears a stalked, sac-like structure known as capsular gland also known as spermathecal gland or seminal receptacle.

**Uterus and vagina:** From the base of the oothecal gland extends a narrow, elongated chamber called as uterus. It is wide at the beginning but gradually narrows down into vagina. It should be noted that the uterus is the part strictly between the anterior end of the oothecal gland and the point of origin of the duct of seminal receptacle. The part of duct after this point upto the female gonopore is the vagina. Thus uterus and vagina are different regions of the same duct demarcated by point of origin of duct of seminal receptacle. The vagina continues upto the base of left optic tentacle to open to the exterior through the vaginal opening or female gonopore. Studies on other snails (Holm, 1946; Nelson, 1984) has shown that the walls of the vagina are highly folded and made up of the columnar cell layer resting on the basement membrane.

**Spermatheca (Seminal receptacle):** There is a long narrow tube that comes off from the end of the vagina. This tube passes across the bulbous region of the prostate gland to the left side of the snail and dilates there to form a spherical structure- the seminal receptacle; which is also known as spermatheca [Plate I: Fig. A, B, C & D]. The spermatheca is stalked, oval, sac-like and thin walled structure having a large cavity. The spermatheca is attached to the vagina with a long stalk. The bulbous portion of the gland looks red brown in colour. The colour intensity depends upon the reproductive activity of the snail. It becomes larger and becomes dark in colour during breeding season. The lumen of spermatheca has a lining ciliated epithelial cells similar to that in the vagina. Mucous secreting cells occur in the epithelial lining of the spermatheca as noted earlier (Holm, 1946; Nelson, 1984).

### C. THE MALE GENITAL TRACT

The male genital tract include following structures:

**Vas efferens (Sperm duct):** It is a thin coiled tube arising from carrefour and it carries endogenous sperms to the prostate gland [Plate I: Fig. A]. Vas efferens is not clearly visible as it is overlapped along its most part by parts of the female reproductive tract.

**Prostate gland:** At the Carrefour, male and female tracts bifurcate. The male reproductive duct begins as a thin vas efferens and continues into the posterior end of the prostate gland. The prostate gland consists of two portions: the apical region and basal region. The apical part is continuous with the vas efferens and looks as a long flattened structure, more or less oval in outline. In living snails, it is pale yellow, brown to blackish in colour [Plate I: Fig. A, B, C & D] and its colour intensity changes from individual to individual, according to seasons and physiological conditions of the snail. The basal region of the prostate gland is bulbous; it may be quite dark to pale yellow in colour. The basal region has smooth surface. The common prostatic duct remains attached to the vas deferens by its anterior end. The prostate gland passes its secretions into vas deferens during the process of copulation as reported in other pulmonate snails.

**Vas deferens:** It is transparent long, twining, filiform and very delicate tube that originates from within the bulbous portion of the prostate gland. It starts as a comparatively wide tube gradually becoming smaller in diameter as it runs away from prostate gland. [Plate I: Fig. A, B, & C]. Vas deferens is about eight times the length of the preputium. It pursues a tortuous course, passes between male and female genital openings, then emerges into the body cavity finally joining the penis sheath. The vas deferens observed to undergo continuous writhing and peristaltic movements within body cavity of live snail. These movements were also observed in the whole intact reproductive system kept in snail Ringer solution.

**Penial complex:** The penis and its sheath, preputium and associated muscles are together referred to as 'the penial complex' (Duncan, 1975). The penis sheath is short (about  $\frac{1}{4}$ <sup>th</sup> the length of preputium) highly muscular, cylindrical in shape, with diameter little more than that of the vas deferens. The penis is the muscular structure that lies within the penis sheath, measuring moderate in length, about  $\frac{2}{3}$  of the length of the penis sheath.

The preputium is cylindrical muscular structure and tends to lie at an angle to the longitudinal axis to the body. It opens to the exterior through the male genital pore lying immediately behind the base of right tentacle. The preputium is attached to the body wall and columellar muscle by a short muscle band. It is attached to set of retractor and protractor muscle which are helpful in eversion of penis during copulation. [Plate I: Fig. A, B, & C]. In *L. acuminata* the average number of protractors is five to eight, with one to three retractors and a single muscle for penis sheath. During copulation, the preputium is everted, forcing penis outside the body through male gonopore; the penis then enters into the female gonopore so as to transfer the sperms to the copulating partner. The penis morphology has taxonomic value as its morphology varies from species to species (Taylor, 2003).

## DISCUSSION

The reproductive system of the Basommatophora is quite an elaborate structure characterized by the complexity of the reproductive organs adding to the difficulty of elucidating the functional morphology. The reproductive organs of *L. acuminata*, like those of many pulmonates conform to a common basic plan in which spermatozoa and ova are produced in an ovotestis and, when released, both pass anteriorly along the little hermaphrodite duct. Thereafter male and female gametes follow separate. The reproductive system of *L. acuminata* is organized on same structural plan as observed in most of the pulmonates path (Duncan, 1975; Geraerts and Joosse, 1984). As in other pulmonates, reproductive system of *L. acuminata* has a hermaphrodite gland with a convoluted hermaphrodite duct which later gets separated into male and female ducts. The male duct opens to exterior through

penis, while the female duct ends with a female gonopore. Several accessory glands are associated with reproductive tract viz., albumen gland, prostate gland, muciparous gland, oothecal gland etc. The similar organization of reproductive system has also been observed in many other gastropods. For example, *Cryptozonia semirugata* (Mantale, 1970), *Indoplanorbis exustus* (Chintawar, 1976; Mantale, 2008), *L. auricularia* (Dama, 1977) are found to be having same plan of the reproductive system as described above for *L. acuminata*, thus hinting at the close evolutionary relationship among all above gastropods. *L. acuminata*, like other pulmonate gastropods is hermaphroditic and is either protandrous or gynandrous owing to differential maturation periods of gonads and cross fertilization is of common occurrence. The ovotestis of *L. acuminata* consists of variable number of follicles or acini, the lobes of which are embedded in the digestive gland. Because it is embedded in digestive gland, researchers in the past (Quazi, 1974 and Nelson, 1984) took the present albumen gland for ovotestis and ovotestis remained either unnoticed or considered some nonessential structure related to digestive system. All three workers mentioned above have sketched the reproductive system diagram wrongly showing the albumen gland as ovotestis and treating oothecal gland as albumen gland. The reason for this is that in snails male and female parts are so intricately entwined that it is really very challenging task, specially for new researchers, to correctly identify female/male parts in the reproductive tract. Identification of anatomical parts of reproductive system in *Lymanea* are available in Crabb (1927) and Holm (1946). In the present study, correct identification of various reprofuctive parts by referring published works by Holm (1946), Duncan (1975) and Runham (1983a, b). Albumen gland of *L. acuminata* is kidney shaped structure as found in many other cogenerics such as *L. stagnalis* (Holm, 1946), *L. auricularia* (Dama, 1977). But it is bilobed in Ellobiids *Ovatella* and *Carychium* (Morton, 1955c), formed of a series of lobules in *Otina* (Morton, 1955a) and *Ellobium* (Berry et al., 1967). The penial complex of *L. acuminata* shows considerable similarity with that of *L. stagnalis* and *L. auricularia* (Holm, 1946; Dama, 1977). The histology of pulmonate ovotestis has been studied by many workers (Dama, 1977; Dummalod, 1981; Pande, 2008) and the histological details of the ovotestis of *L. acuminata* shows considerable uniformity in structure to those of the other pulmonates. The terminology on the morphology of the basommatophoran reproductive tract is often confusing (Duncan, 1975; Runham, 1983a, b). In the present investigation, terminology is followed from Holm (1946), Duncan (1975) and Geraerts and Joosse (1984).

The histological study on various parts of reproductive system will reveal many more details regarding internal organization and physiology of the system. The results presented here will hopefully be useful to conduct future studies on reprofuctive biology and taxonomy of freshwater molluscs.

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