# OBSERVATIONS ON THE EMBRYONIC DEVELOPMENT OF FRESHWATER PULMONATE SNAIL *LYMNAEA ACUMINATA* (LAMARCK, 1822) (GASTROPODA: MOLLUSCA)

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**ABSTRACT**
The freshwater pulmonate snails lay transparent gelatinous strips of eggs called egg masses or capsules. In the present study, a gross observation of embryonic development of *L. acuminata* was done. Early cleavages and subsequent various embryonic stages and time of appearance of different embryonic stages were recorded. The study revealed that the cleavage is spiral and holoblastic. After early cleavages followed the appearance of blastula, gastrula, trochophore stage, veliger stage, hippo stage ultimately resulting into hatching of fully developed young one on eleventh day. Many developmental characteristics observed are unique in molluscs. Various other characteristics of embryonic development in molluscs are also discussed.

## INTRODUCTION

Molluscs have played important role in strengthening our knowledge of fundamental processes in developmental biology, with the common pond snail *Lymnaea stagnalis* contributing greatly to this body of literature for nearly a century (Gatenby, 1919; Sturtevant, 1923; Canton and Sloof, 1977). The morphological, morphometric and behavioral aspects of development of *L. stagnalis* have been well documented (Cumin, 1972; Morill, 1982; Marois, 1989; Mescheryakov, 1990; Marois and Croll, 1991; Voronezhskaya *et al.*, 1999; Nagy and Elekes, 2000). The freshwater snails of family Lymnaeidae develop through what corresponds to trochophore and veliger stages within transparent egg capsules (Morill, 1982; Mescheryakov, 1990). These capsules while conveniently permitting observations of growing embryos, acts as diffusion barrier and prevent passage of large molecules. The fluid in the eggs capsules has been reported to fulfil nutritional requirements of the embryos. Molluscs are model experimental animals because of simplicity in organization of various organ systems. They have been used as model animals in various types of studies in many laboratories all over the world (Canton and Sloof, 1977; Browder, 1980; Victor and Balamban, 2001). They are also animals of choice in studying embryonic development. A number of research workers preferred molluscs over other animal groups for embryological experimentation (DeWitt, 1954; Barraud, 1957;

Bayne, 1968; Suliman *et al.*, 1987; Cumming, 1993; Rondelaud and Dreyfuss, 1996; Naoaki and Yamakawa, 1999; Okusu, 2002).

Despite many investigations on embryology of number of snail species, the detailed work on embryonic development of *L. acuminata* is either lacking or insufficient. Earlier, Quazi (1974), Borale (2002) and Sarker *et al.* (2007) have attempted to study some aspects of embryonic development of *L. acuminata*. However, studies made by them lack in many details and need to be elaborated further. Present investigation is an extension of the work carried by these workers and it, we hope, will add newer details to the existing embryonic developmental aspects of snail *L. acuminata*. The present investigation is an attempt to uncover various aspects of embryology of *L. acuminata* based on microphotographs of various developmental stages.

## MATERIALS AND METHODS

Specimens were collected from cement tanks in the botanical garden in the university campus, made for culture and maintenance of various aquatic plants. The animals can also be had from Kham river near Aurangabad, Maharashtra. After bringing animals to the laboratory, animals were kept in plastic troughs containing sufficient water. Snails were allowed to acclimatize to the laboratory conditions for two days. The active snails ranging from 20-30 mm in shell length were se-

lected for the experiment. They were fed regularly on spinach leaves. The swollen petioles and leaves containing eggs strings were separated carefully with the help of scalpel and kept in Petri dishes containing filtered tap water. Finally, the egg masses showing different developmental stages were sorted out in different Petri dishes according to stages of development. Out of these, one or two eggs strings in the earliest stages of development were selected for observing further development and the rest were also observed at the intervals. The time taken for each of them for hatching was noted. As the eggs were transparent and surrounded by mucus string, changes taking place upto the time of hatching could be observed directly. The water temperature during period of investigation was $25.6 \pm 2.3$°C. Various developmental stages were photographed using digital camera of Sony company

## RESULTS

**Egg string:** Eggs were laid in triple layer in a gelatinous matrix which was ribbon shaped and known as egg mass or egg string (Plate 1, Fig. A) which measures from 0.5 to 3.7 cm in length and 0.5 to 1 cm in width. All the eggs in same egg mass were in the same stage of the development suggesting that they were not laid in batches. Eggs masses were laid generally in the night or early in the morning. The number of eggs varied in each egg mass, but on an average there were 50 to 100 eggs per string. The egg is oval in shape, the average dimension being 1180 $\mu m \times 640$ $\mu m$. The eggs were transparent so that the embryonic development could be observed under light microscope directly. The fertilized eggs are having their own vitelline membrane. Each egg receives a coat of albumen from albumen gland and also internal and external membranes as it passes along sperm oviduct. Each fertilized egg contain usually a single zygote about $57 \pm 20 \mu m$ length. The eggs are isolecithal with moderate amount of yolk. The zygote is eccentrically located within the egg and remained in the same position upto the trochophore stage.

**Day I:** Freshly deposited eggs just after laying were found to contain a relatively yolk-free zone at one end, called the animal pole and a yolk rich zone at the other end called as vegetal pole. No polar bodies were present in eggs examined immediately after they had been laid. The first polar lobe and polar body were extruded out from the zygote within 50 to 90 minutes. The polar body and polar lobe were both resorbed after 15 to 25 minutes after their formation. The second polar lobe was formed within next 40 minutes. First cleavage initiates about 2 hr after deposition of egg mass and completed within next one hour. The type of cleavage is holoblastic, spiral and meridional. The cleavage furrow first appeared as a very deep vertical groove on the top of the zygote and gradually proceeded downwards dividing it into two blastomeres (Plate 1, Fig. E and F). The first division was meridional as it passed through animal vegetal pole. It might be inclined somewhat due to absorption of first and second polar lobes and first polar body which gave rise to a bit larger blastomere labelled as CD and other smaller blastomere labelled as AB (Plate 1, Fig. E and F). Second cleavage was also meridional and holoblastic. It began near about 40 minutes after first cleavage and about 4 hr after the egg mass has been deposited. The second cleavage furrow was longitudinal to the first cleavage

furrow and produced four blastomeres labelled as A, B, C and D (Plate 1, Fig. G and H). This cleavage, like the first cleavage, was also of spiral type and equal. After each division, daughter cells were almost spherical and later flattened against each other. The third cleavage occurred between 8 to 9 hr after the egg mass has been deposited. The eight-celled stage was formed after completion of four cell stage. The third cleavage was horizontal or equatorial. It was unequal and produced eight blastomeres. These eight cells were comprised of two different kinds of cells: the four cells were smaller in size and called as micromeres and remaining four cells were larger in size called macromeres. Micromeres were located towards the animal pole of the embryo. The remaining four cells of embryo were larger in size as compared to the micromeres and were located towards the vegetal pole (Plate 1, Fig. I). These cells are called as macromeres. At the time of third cleavage, the second polar body appeared and was subsequently resorbed. The sixteen cell stage was observed 10 hr after eight cell stage, that is, between 19 to 21 hr after the deposition of egg mass. Thus, on the first day embryo reached upto 16 cell stage.

**Day II:** The fifth cleavage occurred on second day or about 26 to 28 hr after the egg mass has been deposited. Further stages of cleavage could not be followed with precise timing but these divisions resulted into the formation of the blastoderm after about 40 hr of incubation period. As the cleavage progresses, the size of blastomeres decreases. This phenomenon was also observed by Cumming (1993) in opisthobranch, *Turbonella*. The blastula was a spherical mass with an outer layer of larger cells and a core of smaller inner cells (Plate 1, Fig. K and L). We could not observe the blastocoel in the blastula stage. This was the important difference between the blastula of higher animals and that of these snails. We could not record exact process of gastrulation. Blastulation was then followed by gastrulation. In the present experiment, gastrulation started at an early phase of development after about 47 hr of incubation and the process continued until next 3 to 6 hr. At the time of gastrulation the embryo was more than 64 cells in its development. The micromeres and their descendants were situated at vegetal half and underwent change in shape. At this stage, the egg flattened at vegetal pole and gastrula was formed. At the time of gastrulation, the size of developing embryo was about $97 \pm 27 \mu m$ in length (Plate 2, Fig. M).

**Day III:** The gastrulation continued even upto some hours after beginning of the third day. The day third was characterized by the appearance of the rudiments of the foot as a short ventral protuberance behind the mouth and rudiments in the form of slight elevation in the preoral or the velar area. In later half of the third day, that is, after about 66 hr of incubation, the developing embryo showed developing mouth, velum, head vesicle and jerking movements that hinted the beginning of trochophore stage.

**Day IV, V and VI:** On the day four, the trochophore stage continued its progress and took a bit elongated shape. The embryo showed the revolving movements that was indication of complete development of trochophore stage. The next 24 hr of incubation period were passed for the attainment of adequate development as the late trochophore (Plate 2, Fig.
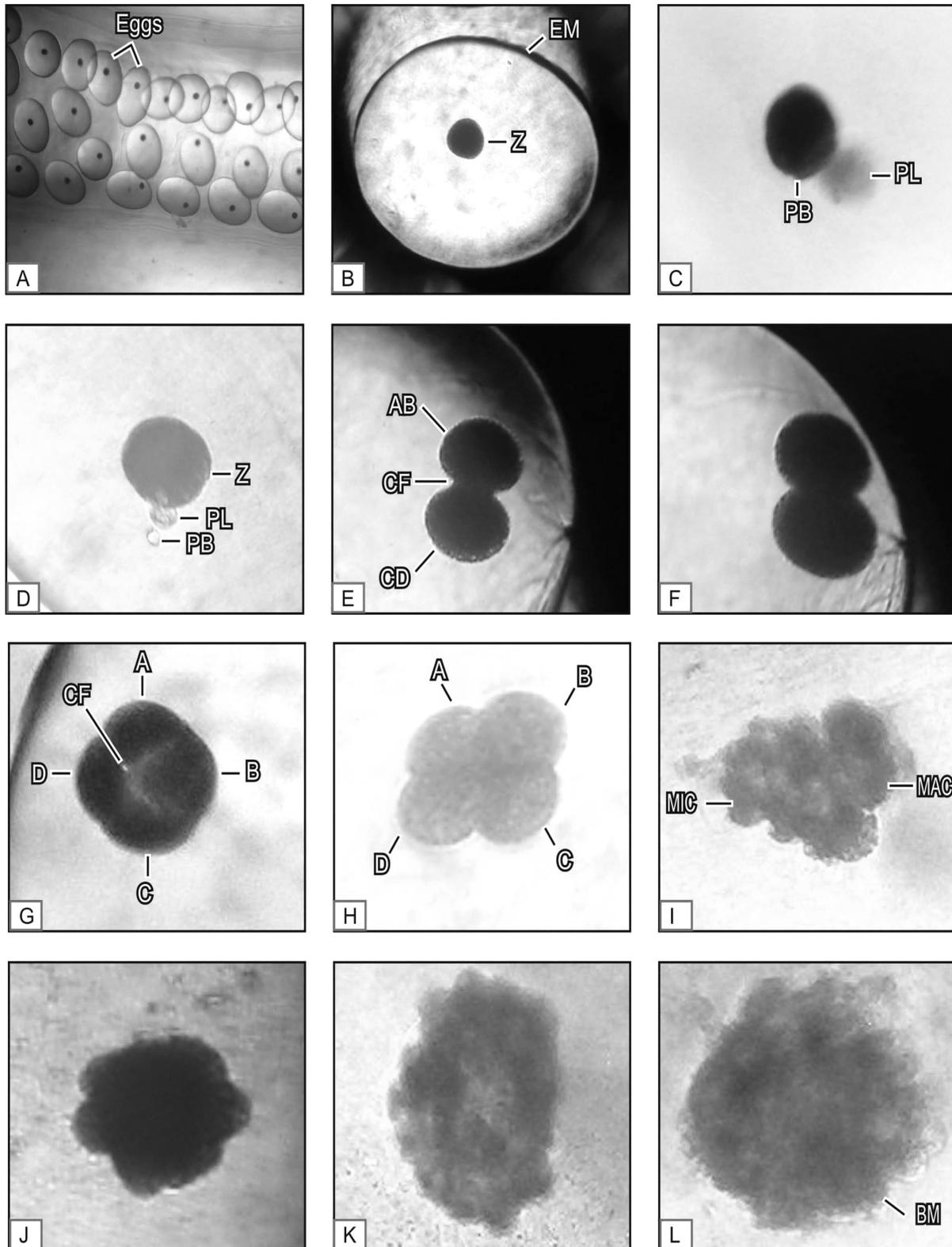
**Plate 1: Photographs of successive embryonic stages of *Lymnaea acuminata* Fig. A: An egg mass, Fig. B: An egg with zygote, Fig. C: Zygote with polar lobe, Fig. D: Zygote with polar lobe and polar body, Fig. E: First cleavage, Fig. F: Two cell stage, Fig. G: Second cleavage, Fig. H: Four cell stage, Fig. I: Eight cell stage, Fig. J: Sixteen cell stage, Fig. K: Thirty two cell stage, and Fig. L: Blastula stage. Abbreviations: (A, B, C and D) = First four blastomeres; AB and CD = First two blastomeres; MAC = Macromeres; MIC = Micromeres; P = Polar body; PL = Polar lobe) Magnification: 100X for all photographs**
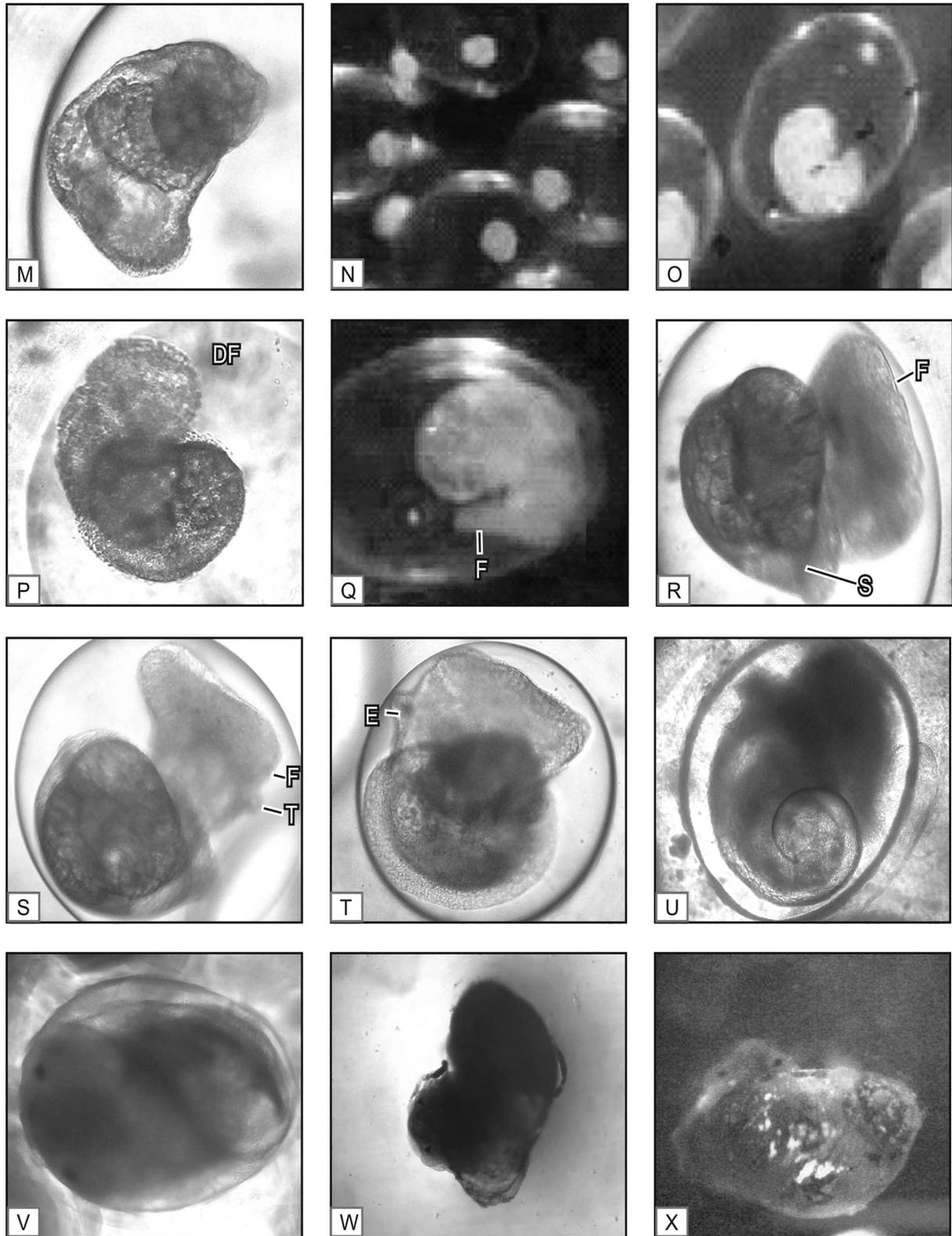
**Plate 2: Photographs of successive embryonic stages of *Lymnaea acuminata* Fig. M: Gastrula stage, Fig. N: Trochophore stage, Fig. O: Early veliger stage, Fig. P: Late veliger stage, Fig. Q: Hippo stage, Fig. R: Eight-day old embryo, Fig. S: Nine-day old embryo, Fig. U: Ten-day old embryo, Fig. V: Eleven-day old embryo before hatching, Fig.W: Hatched out young snal, Fig. X: Young snail two days after hatching. Abbreviations: (DF= Developing foot; E= Eye; S= Shell; T = Tentacle). Magnifications: 100X for all photographs except 10X for Fig. 'W' and 'X'**

N). There was no metamorphosis but rather a gradual assumption of the adult features. At the end of fourth day, the embryos started to obtain the elongated shape, had curved foot muscle and embryo turned into early veliger (Plate 2, Fig., O and P). Both trochophore and veliger stages are passed inside the egg in *L. acuminata*, however, in many snails these stages occur outside the egg as free swimming larvae. The heart beat was clearly observed during sixth day and the embryo exhibited considerable coordination of movements. The visceral mass showed periodic contraction movements towards the head-foot region. The foot has increased in size and with some glandular masses which may be the adhesive gland, secreted the sticky substances for attachments. The whole embryo became transparent and increased in length and width. The tentacle was a little more differentiated from the velar region and the invagination was deeper than before. The veliger stage extended upto 7 day.

**Day VII and VIII:** The veliger stage passed gradually and then was followed by hippo stage. The hippo stage was characterized by complete metamorphosis to young snail (Plate 2, Fig. Q). By this time the embryo was fully developed, eyes and the heartbeats could be seen; shell and foot were clearly separated. A pair of eyes, a left eye and the right eye, appeared each as a black dot at the base of left and right tentacle respectively. This is an important feature of order Basommatophora. The heart beat now could be easily observed. During this period the mantle fold could be identified. There were slight ciliary movements at the anterior opening. The length of the capsule was also increased as the embryo made movements within the capsule. After this, the foot increases in the size and becomes as long as to extend longer than the body of the embryo. Shell striations also appear during the same period. The heartbeat in the rotatory condition was much faster as compared to the resting condition. The mantle fold then became clearly distinguished.

**Day IX and X:** The shell increased in size which was too transparent and margins of the spires could be distinguished. The foot became stout. The embryo has increased in size and assumed all adult features (Plate 2, Fig. S, T and U). All parts of the body undergo further development. Tentacles increased in length and eyes were permanently observed. The coiling of the shell was clearly observed. During this period, the mantle fold was formed and began to extend forward. The pulmonary sac was clearly seen and it was light blue in colour. The foot became much stouter and broader and developed a sole. The radular movements were increased. The mantle fold was extended towards the anterior side. The mantle collar was broader and thicker. The pneumostome or opening in to mantle cavity was differentiated. The foot was slightly tapering towards the anterior end and the muscular activity increased. The dimensions of the shell were 568 $\mu m$ × 232 $\mu m$. Capsule also increased in dimensions as the embryo increased in its dimensions. This was the period of nearly complete development in which mantle obtained its original position and shell increased in size. The mantle now started the secretions of the shell because it was co-associated with the shell. Due to transparency of the shell, the internal organs could be easily observed. The radular movements became too fast. The shell with a single gyre was clearly seen. Now the embryo occupied major portion of the capsule (Plate 2, Fig. S, T and U).

**Hatching Process:** The eleventh day is period of hatching. The fully developed young snail was by that time ready to emerge out of the egg capsule and to breathe its first in free freshwater. The dimension of the young snail was 709 $\mu m$ × 369 $\mu m$ at the time just before hatching contain the longest lengths of all developmental stages. The shell which had, only a single gyre showed little resemblance to the high spires of the adult. The head and foot were well developed. The latter being very heavily ciliated towards its anterior tip. A single egg membrane surrounded the young one. The young animal glided around over the inner surface of egg membrane, the shape of which remained unaltered during these activities. Eventually, however, the membrane became more flexible and the foot could be seen to have suction effect upon it as its gliding activity continued. Thereafter the membrane became more and more collapsed in folds around the young animal, so that its diameter decreased greatly. Ultimately, the repeated tearing movements resulted into the rupture of the eggs membrane; in the course of events the foot and proboscis protruded outsider to enlarge the hole. When the size of tear was increased, the animal rather quickly emerged out. The process of hatching began on eleventh day and was completed at the end of it. After hatching, the empty egg membrane collapsed. Outside the egg, the snail moved freely in the jelly of the egg capsule and escaped out through a hole made by it or through an opening made by another snail previously.

**Juveniles:** At the beginning of the eleventh day, the miniature snail possessed all the structures found in adult individuals. The hatched out juvenile snails had a mantle covered by thin transparent shell. In addition, the foot and tentacles are also well developed in juveniles (Plate 2, Fig. W and X).

## DISCUSSION

The egg type determined was isolecithal as found in many pulmonate snails (Morill, 1982; Sarker and Jahan, 1998; Sarker, 2002) and it was found to contain moderate amount of yolk. Formation of polar body and so-called polar lobe is noted in the present study. The polar body formation usually begins about an hour after the egg mass has been deposited. However, Sarker *et al.* (2007) noticed that the first polar body appears usually after first cleavage. In *Dentalium* also the polar lobe is formed before first cleavage (Wilson, 1904). The polar lobes seems to be important structures as Clement (1952) noted that the removal of polar lobe results in the changes in symmetry and lack of development of some adult features. The timing of appearance of polar bodies seems to vary with the species of snails. For example, in turban shell, *Marmarostoma stenogyris*, the first and second polar bodies were discharged within 30 minutes after fertilization (Naoaki and Yamakawa, 1999). The polar bodies are formed in almost all molluscs except cephalopods (Gilbert, 1991). The type of cleavage is typical of all freshwater pulmonates in being holoblastic, spiral and meridional as has been supported by Verdonk and Biggelaar (1983). The first division was meridional as it passed through animal vegetal pole (Sarker *et al.*, 2007). Various stages characteristic of molluscan development such as veliger, trochophore was also observed

during embryonic development in this snail.

It was observed that the development of all adult features, except maturation of gonads, occurs by the day IX or X after the egg has been deposited. Borale (2002) and Sarker *et al.* (2007), also noted the similar observation in their respective studies on *L. acuminata*. In this study we observed the incubation period of egg of *L. acuminata* to be about 11 days at temperature between 23-28ºC. However, incubation period varies with temperature and this has been reported in number of molluscs by many workers (Calabrese and Davis, 1966; Raut, 1981; Aziz and Raut, 1996; Caveriviere *et al.,* 1999; Przeslawski, 2004 and Sarker *et al.,* 2007). Sarker *et al.* (2007) reported that the incubation period of *L. acuminata* was maximum ($17.50 \pm 0.86$ days) in January when the water temperature was $17.50 \pm 0.38$ºC. It was minimum ($6.90 \pm 0.12$ days) in July when the water temperature was $30.92 \pm 0.24$ºC.

## ACKNOWLEDGEMENT

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