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Survey on the Morphology of Rana Leptoglosssa Cope's Severly affected Frog

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ABSTRACT

Rana leptoglossa is classified broadly as a near-threatened amphibian species. But no attempt has been made so far to study Rana leptoglossa's growth and metamorphosis in its natural environment or under captivity conditions. Keeping in mind the lack of evidence as well as R's endemic and rare status. Studies on frog growth and metamorphosis have been done on leptoglossa. Within 90 minutes, the first division of the fertilized egg of Rana leptoglossa is completed, and after 9 hours of fertilization, Morula (Gosner stage 8) is achieved. The gastrulating phase (Gosner stage 10) began after 11 hours, the forming of the neural plate (Gosner stage 13) took place after 15.30 hours, and the forming of the neural fold (Gosner stage 14) began after 18 hours. The embryo's hatching happened after 4 days of fertilization. After 7 days, the gill buds (Gosner stage 19) emerged. After 26-27 days, the hind limbs (Gosner stage 26) emerged and were fully formed (Gosner stage 40) after 55-58 days of fertilization. After 61-63 days, when the tadpole was found to have the highest length (46-47 mm), the forelimb buds (Gosner stage 42) emerged. The degeneration of the tail of the tadpole then began and the metamorphosis was finished in 68-72 days when the tadpole was turned into a frog let (stage 46 of Gosner). R. Long comparative period of metamorphosis. The predominant state of temperature and day length as well as genetic programming may be due to leptoglossa. The present research appears to be the first of its kind in which different stages of growth and metamorphosis have been identified for the endangered frog, Rana leptoglossa.

INTRODUCTION

There are 303 types of creatures of land and water in India which were recorded yet investigations of formative stages and transformation of frogs were on scarcely any species: Bufo melanostictus (Khan, 1965); Rhacophorus malabaricus (Sekar, 1989); Rana limnocharis (Roy, 1990); Polypedates maculates (Misra and Das, 1984; Kanamadi and Jirrakali, 1992); Hyla annectans (Ao and Bordoloi, 2001); Chrixalus simus (Deuti, 2001); Philautus glandulous (Biju, 2003); Philautus leucorhinus (Gururaja et al., 2005); Polypedates lecomystax (Iangrai, 2007); Rhacophorus bipunctatus (Iangrai, 2007) and Rhacophorus lateralis (Biju, 2009). Studies were additionally directed on outer morphology, bucco-pharyngeal life systems and improvement pace of the fledglings of two Asian Ranidae (Amphibia: Anura), Hylarana humeral is (Boulenger, 1887) and Hylarana leptoglossa (Adapt, 1868) (Bortamuli et al., 2010). The development and advancement rates in anurans are impacted by various ecological factors, for example, temperature (Kaplan, 1980; Saidapur and Hoque, 1995), precipitation (Lynch and Wilczynski, 2005), photoperiod (Saidapur, 1989), pool drying up (Lind et al., 2008), food gracefully and diet quality (Berven and Chandra, 1988; Nicieza et al., 2006), natural iodine levels (Dodd and Dodd, 1976), lake hydrology (Ryan and Winne, 2001), and reproducing environment (Kaplan, 1980; Hayes, 1997). The development and improvement of the anurans are additionally affected by natural factors, for example, fledgling size or egg size and yolk repositories (Duellman and Trueb, 1994). The blend of these elements at last impacts the time taken to program from eggs to a froglet (Morrison and Saint, 2003). The anuran transformation is constrained by the hypothalamo-hypophyseal-thyroid hub including activities of a few hormones (Huang and Earthy colored, 2000; Furlow and Neff, 2006; Page et al., 2008).

Ecological variables instigate the delivery by the nerve center of thyrotrophic delivering hormone (TRH), which actuates the emission of pituitary thyroid animating hormone (TSH). TSH animates the discharge from the thyroid organ of thyroid hormones (TH), separately 3, 5, 3- - triiodothyronine (T3) and 3 5 3 5- - tetraiodothyronine (T4). So as to encourage the transformation of anuran fledglings, an expanded centralization of T4 was accounted for (Page et al., 2008). Prolactin likewise assumes a fundamental part in controlling anuran larval development and transformation, notwithstanding thyroid hormones (Dodd and Dodd, 1976; Takada and Kasai, 2003). The adolescent hormone of fledglings is by and large accepted to be prolactin (PRL) and balances the stimulatory impacts of thyroid hormones on transformation (Takada and Kasai, 2003). The development of the Anuran larval is part into three separate stages , for

example pre-transformation, favorable to transformation and peak of transformation. Pre-transformation alludes to a period where, without thyroid hormones or many less thyroid hormones, embryogenesis and early fledgling development and improvement occur. Rear appendages go through morphogenesis during prometamorphosis, as exemplified by toe division and fast broad advancement of rear appendages. This time is portrayed by an ascent in endogenous thyroid hormone fixation (Rojas et al., 2003). The transformative peak is the hour of sensational changes that finish in the loss of most fledgling larval characters with quick partition described by the commencement of tail relapse, full tail resorption, and the formation of again structures and capacities that because of high thyroid hormones are critical to the grown-up (Lobby and Larson, 1998; Mc Diarmid and Altig, 2000).

METHODS

So as to consider the turn of events and transformation of Rana leptoglossa, perceptions were made on the turn of events and transformation during three back to back rearing periods in the years 2014, 2015 and 2016. New generates were gathered from the reproducing destinations, brought to the research facility and kept up in rectangular plastic plate to permit further turn of events and transformation. The water of the plastic plate containing the creating fledglings was changed consistently with the lake water. The fledglings were feed with phytoplankton, zooplankton and minced night crawlers not obligatory. Distinctive formative phases of the frog (i.e., from prepared eggs to transformed froglets) were protected in 4% formaldehyde arrangement. Outside morphology and estimations (in mm) were recorded from very much protected examples with the assistance of a Vernier caliper. During the investigation time frame, the formative phases of Rana leptoglossa were concentrated from the hour of bringing forth and preparation (0 Gosner stage, 0 h), till transformation of the fledglings into froglets under both normal and hostage conditions. Early advancements stages were gathered at a timespan minutes as long as 48 hours to discover the embryogenesis of creating eggs, which were seen under the binocular magnifying lens connected with photographic offices (Zeiss Stemi 2000C Binocular Magnifying lens with KL 1500 LCD camera). Organizing of the fledglings was proceeded according to the framework proposed by Gosner (1960). The photos of the early formative stages were taken from the safeguarded examples and introduced in Plates: 1, 2, 3, 4 and 5, while the photos of the live examples are introduced in Plate 6.

RESULTS

The first stage is fertilization of eggs the figure below will clarify:

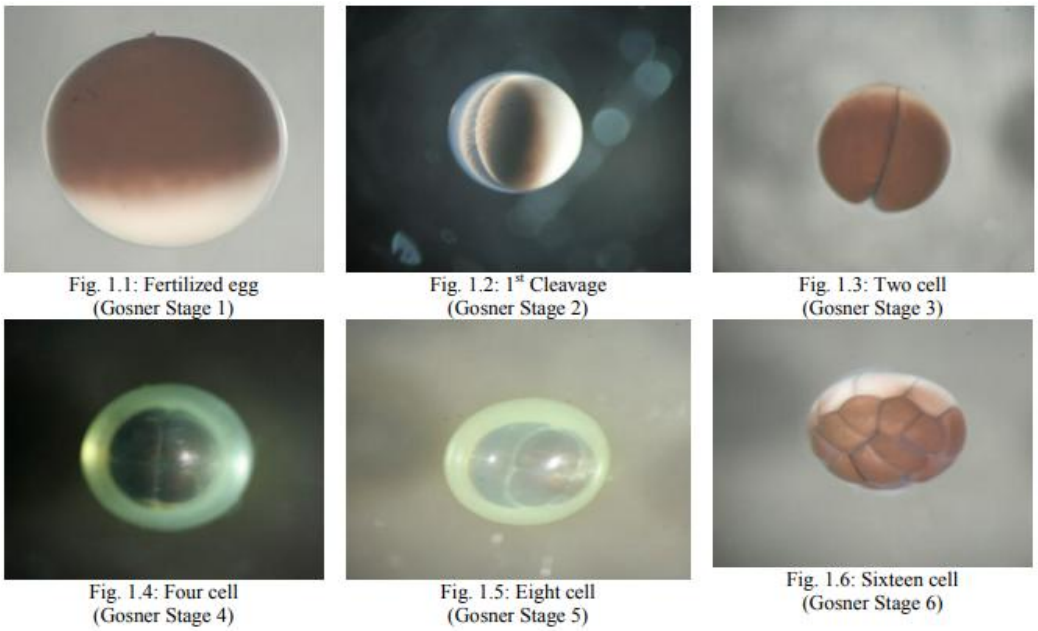

Fig. 1.1: Fertilized egg (Gosner Stage 1)
Fig. 1.2: 1st Cleavage (Gosner Stage 2)
Fig. 1.3: Two cell (Gosner Stage 3)
Fig. 1.4: Four cell (Gosner Stage 4)
Fig. 1.5: Eight cell (Gosner Stage 5)
Fig. 1.6: Sixteen cell (Gosner Stage 6)

PLATE 1: Developmental stages (Gosner stages 1 – 6) of *Rana leptoglossa*

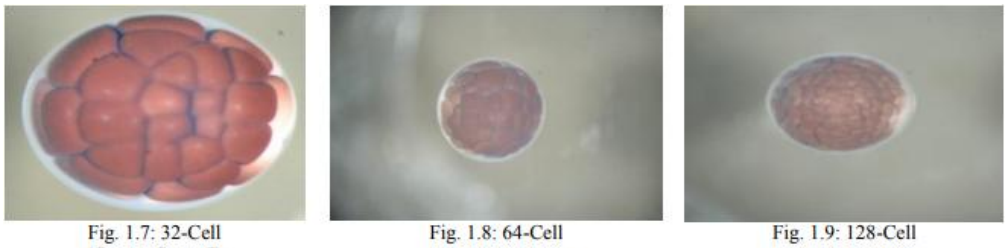

Fig. 1.7: 32-Cell (Gosner Stage 7)
Fig. 1.8: 64-Cell (Gosner Stage 8)
Fig. 1.9: 128-Cell (Gosner Stage 9)

PLATE 1: Developmental stages (Gosner stages 6 – 9) of *Rana leptoglossa*

I. Fertilized eggs

Step 1 of Gosner: Fertilized eggs (Age 0 hr, Diameter 0.5 mm)-The eggs were purple, circular in shape and about 0.5 mm in diameter. The upper pole of the animal was dark brown pigmented, and the lower pole of the carrot was white, easy to discern under the binocular microscope (Zeiss Stemi 2000C) (Fig. 1.1).

Gosner Stage 2: A lightly pigmented region (grey crescent) emerged between the animal pole and the vegetal pole towards the pigmented hemisphere (Fig. 1.2).One cell process (Age 1.00 hr, Diameter 0.6 mm)

II. Phases in cleavage:

Step 3 of Gosner: Two cell process (Age 1.30 hours, Diameter 0.8 mm)-The southern cleavage furrow starting from the animal pole continued to the vegetal pole and split the fertilized egg into two identical blastomeres (Fig. 1.3) gradually.

Step 4 of Gosner: Four cell process (Age 2.00 hrs, Diameter 1.0 mm)-The second southern furrow, which began at the animal pole, spread at the right angle to the first furrow to the vegetal pole. Four blastomeric were found in total (Fig. 1.4).

Step 5 of Gosner: Eight cell process (Age 2.30 hrs, Diameter 1.1 mm)-The third cleavage was latitudinal, slightly above the equator and eight blastomeric were produced. The four smaller micromeres of the animal pole were dark brown pigmented, where they were unpigmented as the four larger macromeres of the vegetal pole (Fig. 1.5).

Gosner Stage 6: Sixteen cell process (Age 3.00 hrs, Diameter 1.2 mm)-There were longitudinal cleavage furrows, one going through pigmented micromeres and the other through unpigmented macromeres, resulting in a total of 16 cells (Fig. 1.6).

Gosner Stage 7: 32 cell stage (Age 3.30 hrs, Breadth 1.3 mm) - The latitudinal cleavage wrinkles of the micromeres and macromeres brought about arrangement of 16 micromeres and 16 macromeres bringing about 32 cells altogether (Fig. 1.7).

Gosner Stage 8: Mid-cleavage/Morula (Age 9.00 hrs, Measurement 1.4 mm) - because of additional cleavage/cell division, the creating incipient organism accomplished the phase of morula (an assortment of 64 to 128 cells) (Figs.1.8 and 1.9).

Gosner Stage 9: Late cleavage/blastula (Age 10.30 hrs, Measurement 1.5 mm) - Because of rehashed cell divisions, the treated eggs achieved late blastula stage. The pigmented area stretched out over the vegetal shaft, which denoted the start of the epibolic development of the micromeres onto the macromeres (Fig. 1.10).

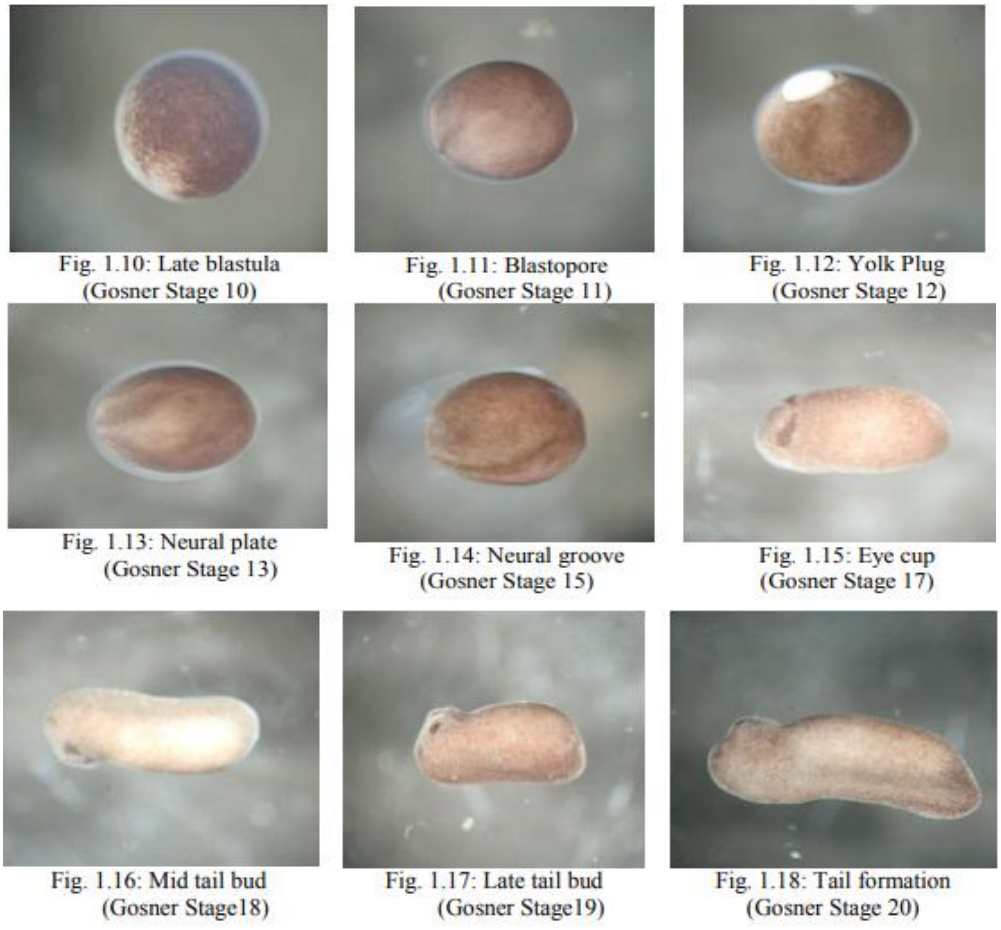

Fig. 1.10: Late blastula (Gosner Stage 10)
Fig. 1.11: Blastopore (Gosner Stage 11)
Fig. 1.12: Yolk Plug (Gosner Stage 12)
Fig. 1.13: Neural plate (Gosner Stage 13)
Fig. 1.14: Neural groove (Gosner Stage 15)
Fig. 1.15: Eye cup (Gosner Stage 17)
Fig. 1.16: Mid tail bud (Gosner Stage18)
Fig. 1.17: Late tail bud (Gosner Stage19)
Fig. 1.18: Tail formation (Gosner Stage 20)

PLATE 2: Developmental stages (Gosner stages 10 – 20) of *Rana leptoglossa*.

III. Gastrulation Level

Gosner Stage 10: Sickle shape dorsal lip (11.00 hrs, Measurement 1.7 mm) - The creating blastula went through gastrulation. It stretched and turned and estimated about 1.5 to 2 mm long. Appearance of bow molded dorsal lip because of involution of the micromeres demonstrated the start of gastrulation. The unpigmented zone of the vegetal side of the equator was decreased because of proceeded with relocation of the pigmented micromeres towards the vegetal post (Fig. 1.11).

Gosner Stage 11: Pony shoe formed dorsal lip (11.30 hrs, Measurement 1.9 mm) - The epibolic relocation of micromeres over the vegetal post diminished the uncovered territory of unpigmented macromere which was encircled by the horizontal lips of the crescent or pony shoe molded blastopore (Fig. 1.11).

Gosner Stage 12: Improvement of yolk plug (12.30 hrs, Measurement 2.1 mm) - A very much created yolk plug showed up. The ventral lip of blastopore moved to the back end. The uninvaginated macromeres, encompassed by the blastoporal lips, distended a little and comprised the yolk plug (Fig. 1.12).

IV. Developmental Stages

Gosner Phase 13: Neural plate (15.30 h, 2.3 mm long)-Mild elongation of the developing embryo. To shape the neural plate, the dorsal surface was flattened, which was distinguished along its boundaries by the concentration of pigments (Fig. 1.13).

Gosner Stage 14: Neural folds (18.00 hours, 2.6 mm long)-Broad cerebral and narrow spinal cord areas of the neural plate have been separated from the neural fold. The neural folds from the blastopore to the anterior region were increasingly approaching each other (Fig. 1.13).

Gosner Stadium 15: Elongation and rotation (Neural groove) (20.00 hours, 2.8 mm long)-Elongation of the embryo's posterior end. In both the cerebral and spinal cord areas, the neural folds got closer and reached each other, creating a shallow neural groove that was wider in the cerebral area (Fig. 1.14).

Step 16 of Gosner: Neural tube (Age 3 days, Length 3.0 mm)-The neural folds were completely fused to form a neural tube that was elevated on the mid-dorsal ridge and demarcated by a darkly pigmented strand (Fig. 1.14).

V. Periods of early tail buds:

Gosner Stage 17: Stage of the tail bud (Age 4 days, Length 3.5 mm)-The developing embryos have hatched into hatchlings / tadpoles on the fourth day. In weight, it measures 2 to 3.5 mm. At the back end of the embryo, tail buds emerged. It was broader than long, dorso-posteriorly directed, with a ventral notch marked off from the body (Fig. 1.15).

Gosner Stage 18: Stage of muscle response / olfactory pits (age 5 days, length 3.5-4.5 mm)-The area of the head was well formed with optical bulges and gill plate bulges. Two highly pigmented elongated areas joined medially by a thin lightly pigmented band below the stomodeum is indicated by oral suckers. Among oral suckers, stomodeal depression was detected. The tail begins to bend laterally to the right or left, within the contour of the vitelline membrane due to the incremental elongation of the embryo. There was also incremental elongation of the embryo and the tail began curving laterally to the left (Fig. 1.16).

VI. Stage of mid tail buds:

Gosner Stage 19: Stage of Gill buds (Age 7 days, Length 4.5- 5.0 mm)-Entirely divided into head, abdomen and tail, the developing tadpole. External gill buds (Fig. 1.17) were conspicuous.

VII. Stage of late tail buds:

Gosner Period 20: Period of gill circulation and tail elongation (age 9 days, length 5.0-6.5 mm)-elongated tail, emergence of gill buds, opening of mouth. Separate, rudimentary branching gills at the distal end and nipple-shaped oral suckers (Fig. 1.18).

PLATE 3. Developmental stages (Gosner stages 21 - 26) of *Rana leptoglossa*.

PLATE4 DEVELOPMENTAL STAGE (GOSNER STAGES 31-42) of RANA LEPTOGLOSSA

PLATE 5. Limbs (Gosner stages 26 - 42) of *Rana leptoglossa*

PLATE 6. Developmental stages (Gosner stages 44 - 46) of *Rana leptoglossa*

CONCLUSION

The results of the present study show that the first division of the Rana leptoglossa fertilised egg is completed within 90 minutes and the Morula (Gosner stage 8) is completed within 9 hours. The gastrulation process (Gosner stage 10) began after 11 hours, the forming of the neural plate (Gosner stage 13) took place after 15.30 hours, and the forming of the neural fold (Gosner stage 14) took place after 18 hours. The embryo's hatching happened after 4 days of fertilisation. After 7 days, the gill buds (Gosner stage 19) emerged. After 26-27 days, the hind limbs (Gosner stage 26) emerged and were fully formed (Gosner stage 40) after 55-58 days of fertilisation. After 61-63 days, when the tadpole was found to have the highest length (46-47 mm), the forelimb buds (Gosner stage 42) emerged. After that, after 63 days, the degeneration of the tail of the tadpole began, and the metamorphosis was finished in 68-72 days when a tadpole was turned into a froglet (stage 46 of Gosner). During Rana leptoglossa metamorphosis, the prevailing climatic conditions were as follows: optimum temperature 25.520C ± 0.430C to 29.830C ± 0.230C, day period 12.71 h ± 0.03 h to 13.16 h ± 0.04 h, relative humidity 75.83 percent ± 1.19 percent to 80.67 percent ± 0.97 percent. R. Long comparative period of metamorphosis. Leptoglossa can be due to the prevailing temperature conditions and the duration of the day. In India, it was observed that the length of growth and metamorphosis of anurans differed from species to species. For eg, in Rana cyanophlyctis, 68 days in Rhacophorus malabaricus, 64 days in Hyla annectans, 60-61 days in Polypedates leucomystax, 59-60 days in Rhacophorus bipunctatus, 55 days in Polypedates maculates, 35-50 days in Bufo melanostictus, 28 days in Philautus glandulosus, and 19 days in Philautus leucorhinus, metamorphosis is reported to have been completed in 94 days. It can be inferred, on the basis of the present findings, that the growth and metamorphosis of Rana leptoglossa was completed within 68-72 days during the time from April to August, when climate conditions were favorable. The present research appears to be the first of its kind in which different stages of development and metamorphosis have been identified for the endangered frog, Rana leptoglossa, and its life cycle (Plate 7). These observations should be used in the preparation of the frog's survival in its natural environments and its captive breeding conditions.

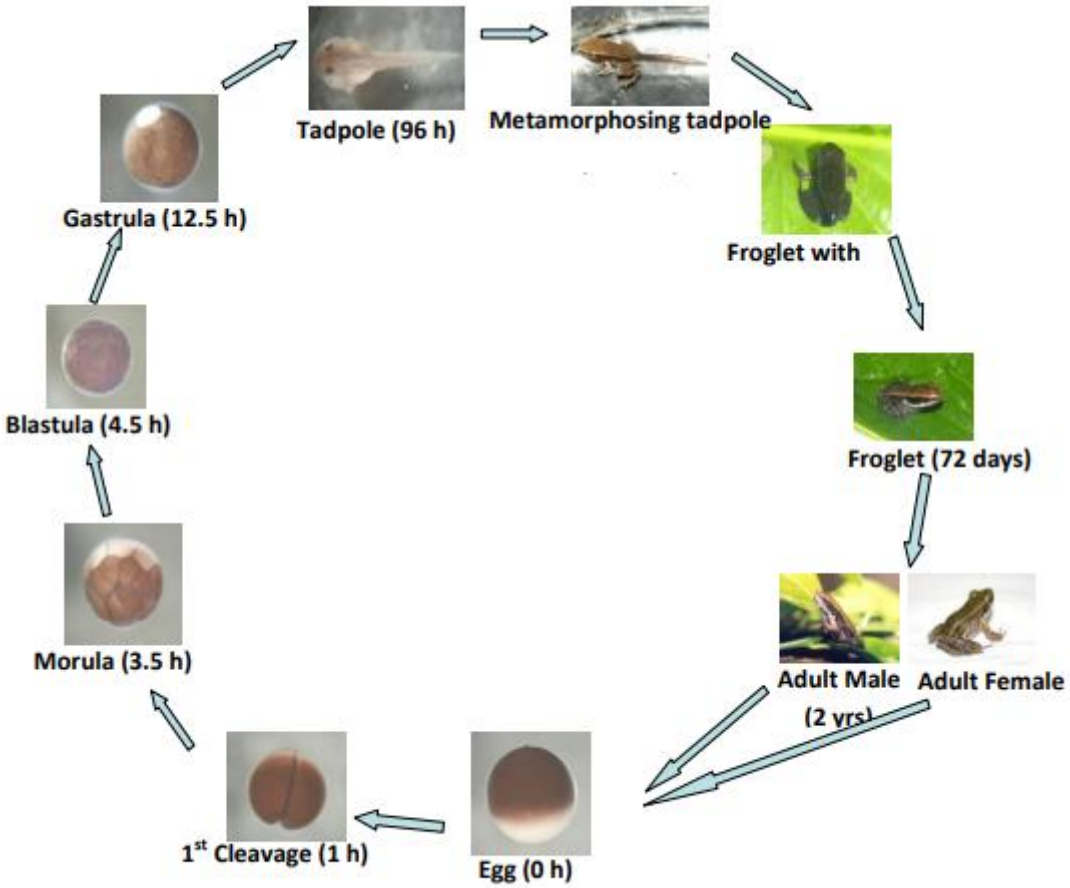

PLATE 7. Life cycle of *Rana leptoglossa*

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