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### A Complete Embryonic and Larval Development of Sphaerotheca pashchima (Amphibia: Anura: Dicroglossidae) from Western India

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#### **ABSTRACT**

The normal developmental staging table for embryonic development and tadpole metamorphosis of Sphaerotheca pashchima (Dicroglossidae) was studied in Jath Tehsil in Sangli District, Maharashtra State, India, 16.5907° N and 75.1078° E, an elevation 726m asl. Sphaerotheca pashchima is a Western burrowing frog from Western parts of peninsular India. Breeding activities started from the month of June and continued till the month of October. It breeds in shallow temporary rain water pools. A clutch contains near about 1000 to 1500 eggs. The eggs measured about 1.5 mm in diameter covered by double jelly layers. Completion of development and metamorphosis took about 36 ± 2 days at temperature between 22°C to 30°C and pH 6.7 to 7.2 in the natural environment. After about 26 hours of egg laying the larva hatched out at stage 20 and started feeding at stage 25. Keratodont formula 1:1+1/3 was observed in stage 31-40. Complete absorption of the tail was observed after 36 days and metamorphosed froglet measured about 12.5 mm in length. Tadpoles are bottom dwelling.

Key words: Embryonic development, metamorphosis, tadpole, pond breeding, Western India

#### INTRODUCTION

The genus Sphaerotheca (Günther, 1859) is monophyletic group, endemic to South Asia. It includes nine species viz., S. breviceps (Schneider, 1799), S. dobsonii (Boulenger, 1882), S. leucorhynchus (Rao, 1937), S. maskeyi (Schleich & Anders, 1998), S. pluvialis (Jerdon, 1853), S. rolandae (Dubois, 1983), S. strachani (Murray, 1884), S. swani (Leviton et al., 1956) and a newly described species S. pashchima from Western parts of peninsular India (Padhye et al., 2017). S. pashchima is the Common, nocturnal and burrowing frog remains deep down into the soil for months or even years. After a period

of heavy rain in monsoon they emerge from the ground to feed and mate. It is widely distributed in the states Gujarat, Maharashtra and Karnataka (Padhye *et al.*, 2017) and found near temporary rain water pools, on the bank of river, in agricultural field and in semi arid grasslands.

The Normal staging tables of development have been described for many species viz., Bufo melanostictus (Khan, 1965), Indian bull frog Rana tigerina (Khan, 1969; Agarwal and Niazi, 1977), Rana limnocharis (Roy and Khan, 1978). In Ramanella variegata a microhylid; twenty five developmental stages were described by Dutta et al., (1994a). The breeding biology and life history of Rana crassa was described by Dutta et al., (1994b). The breeding biology and life history of Uperodon systoma, Rana cyanophlyctis and Microhyla ornata was studied by Mohanty-Hejmadi et al., (1979, 1977 and 1980), Mohanty-Hejmadi and Dutta (1988) studied on the life history of the common Indian tree frog, Polypedates maculates. Genus Sphaerotheca include nine species, of which breeding ecology and life history studies of only few species are known. Mohanty-Hejmadi et al., (1979) studied the complete Life history of the Indian burrowing frog, Rana breviceps. The detailed life history of S. pashchima is unknown. Therefore, this work was undertaken to figure out the complete embryonic and larval development.

#### **Species description:**

Sphaerotheca pashchima shows following morphological characters (Fig-2 & 3): Medium sized frog (SVL- 50.0 mm); body globular, stout; head small, broder than long; snout rounded; the lips are barred; pupil diamondshaped; nostrils nearer to snout than to the eye; tympanum about two- third the diameter of eye; supratympanic fold distinct; Inter narial width greater than the distance between eyes; vomerine teeth in two rows at the anterior border of choanae; tongue bifid, without papilla. Dorsal skin granulated, slightly warty; ventral skin granular. Dorsum dark brown to yellowish-brown coloured with dark brown spots or patches, with or without reddish granulation, dorsolateral yellowish patches behind the shoulders, inverted V-shaped mark on the back, mid dorsal line may be present, tympanum mottled, white spot behind tympanum at the angle of jaws, ventral side creamy white, yellowish marbling in the groins. Throat of male frogs are blackish with two subgular vocal sacs (Fig-2). Fingers free, femoral gland present. Finger tips not dilated. Fore limbs short, relative finger lengths from shortest to longest - F2 < F4 < F1 < F3; palmer tubercles present. Hind limbs long; Thighs barred, relative toe lengths from shortest to longest are - T1 < T2 < T3 < T5 < T4; inner metatarsal tubercle large, very prominent, compressed, shovel shaped and present obliquely at the base of the first toe. Outer metatarsal tubercle absent; webbing formula was  $10-1111-21112-3\frac{1}{2}1V3\frac{1}{2}-2V$ .

#### **MATERIAL METHODS**

Study site was a small temporary rain water pool (Fig-1) in Jath Tehsil at Sangli District, Maharashtra State, India, 16.5907° N and 75.1078° E; an elevation 726 m asl. Breeding activity of Sphaerotheca pashchima was monitored during breeding season (June to October 2017). Amplecting pairs of the species were sited early in the morning at 5.00 AM (Fig-4). The study begins when egg masses were deposited in the pond. Number of eggs in different clutches was counted. The eggs and tadpoles of different stages were measured with a digital vernier calliper (nearest 0.01mm). Morphological terminologies were followed after Altig and McDiarmid (1999).The normal developmental table of S. pashchima has been prepared according to Gosner's 46 stages and Duellman and Trueb, (1994). Photographs of live tadpoles were taken by a digital camera with 90 mm macro lens.

During the study period from June to October the physico-chemical parameters of breeding pond was recorded. The Ecological parameters are rain fall, relative humidity and air temperature. Physico-chemical parameters are water temperature and pH. Air and water temperature was measured by using a Mercury thermometer. Water pH was measured by a digital pH meter. Rainfall data was collected from the Regional Meteorological Center, Jath tehsil.

#### **RESULTS**

# Staging Table of Embryonic Development and Metamorphosis of Sphaerotheca pashchima:

A total of 46 developmental stages (Gosner's, 1960) of *Sphaerotheca pashchima* were studied from the time of egg laying till the embryo hatched into a tadpole (stage-1 to 20) and metamorphosis of tadpole into a froglet (stage-21 to 46) under natural environmental conditions. A brief account of various stages of embryonic development and larval metamorphosis are given bellow.

#### Gosner's Stage 1- Fertilised eggs (zero hours): (Fig-6)

The fertilised eggs are spherical in shape. The egg show pigmented gray coloured animal hemisphere and white vegetal hemisphere. The fertilized egg measured 1.5 mm in diameter. Eggs are surrounded by two jelly coats.

### Gosner's Stage 2- Beginning of cleavage furrow (30 min.): (Fig-7)

After fertilization the eggs oriented in such a way that the clear distinguishable gray pigmented animal hemisphere facing upwards and white coloured heavier vegetal hemisphere facing downwards. The gray crescent appears between the animal and vegetal hemispheres. The jelly around the egg absorbs water and swells rapidly; this stage was marked with the beginning of cleavage furrow at the animal hemisphere. The diameter of egg is 1.7 mm. Initiation of cleavage in the animal hemisphere was observed after 45 min.

#### Gosner's Stage 3- Two cell stage (55 min.): (Fig-8)

The first cleavage was holoblastic, equal and in meridional plane, originated at the median of animal hemisphere and slowly moved down to vegetal hemisphere with bisecting the gray crescent, results into two equal blastomeres. The diameter of egg is 1.7 mm.

### Gosner's Stage 4 - Four cell stage (1.15 hours): (Fig-9)

The first cleavage furrow was still cleaving the yolky cytoplasm of the vegetal hemisphere; the second cleavage has already started near the animal hemisphere. The second cleavage was holoblastic, equal and in meridional plane at right angle to the first cleavage and results into four equal blastomeres. The diameter of egg is 1.7 mm.

# Gosner's Stage 5- Eight cell stage (1.30 hours): (Fig-10)

The third cleavage was latitudinal but unequal (horizontal and slightly above the equator and at right angle to the both first and second cleavages) resulting into eight celled stage with four small sized animal blastomeres (micromeres) and four large sized vegetal blastomeres (macromeres). The diameter of egg is 1.7 mm. This unequal holoblastic cleavage establishes two major embryonic regions: a rapidly dividing region of micromeres near the animal hemisphere and a more slowly dividing region of macromere in the vegetal hemisphere.

### Gosner's Stage 6- Sixteen cell stage (2.00 hours): (Fig-11)

The fourth cleavage was vertical and double results in eight pigmented micromeres and eight un-pigmented macromeres, in a total of sixteen cells. The diameter of egg is 1.7 mm.

### Gosner's Stage 7- thirty two cell stage (2.40 hours): Early blastula stage (Fig-12)

The fifth cleavage was latitudinal and double slightly above and below the third cleavage resulting into four tiers of blastomeres, sixteen smaller micromeres at animal hemisphere and sixteen larger macromeres at vegetal hemisphere in a total of thirty two cells.

## Gosner's Stage 8- Mid blastula stage (3.30 hours): (Fig-13)

The animal hemisphere blastomeres divide rapidly and becomes more in number and smaller in size, while vegetal hemisphere blastomeres divide slower rate with comparatively larger cells. The egg looks like a small Mulberry fruit called 'morula'. Egg contains more than 64 blastomeres. The diameter of egg is 1.7 mm. (The 16 to 64 celled stage is called morula.)

## Gosner's Stage 9- Late blastula stage (4.30 hours): Beginning of gastrulation (Fig-14)

The surface of animal hemisphere appeared granular due to repeated division of blastomeres into minute sized and large numbered micromeres. This stage is considered as blastula. The diameter of egg is 1.7 mm.

### Gosner's Stage 10- Dorsal lip stage (6 hours): (Fig-15)

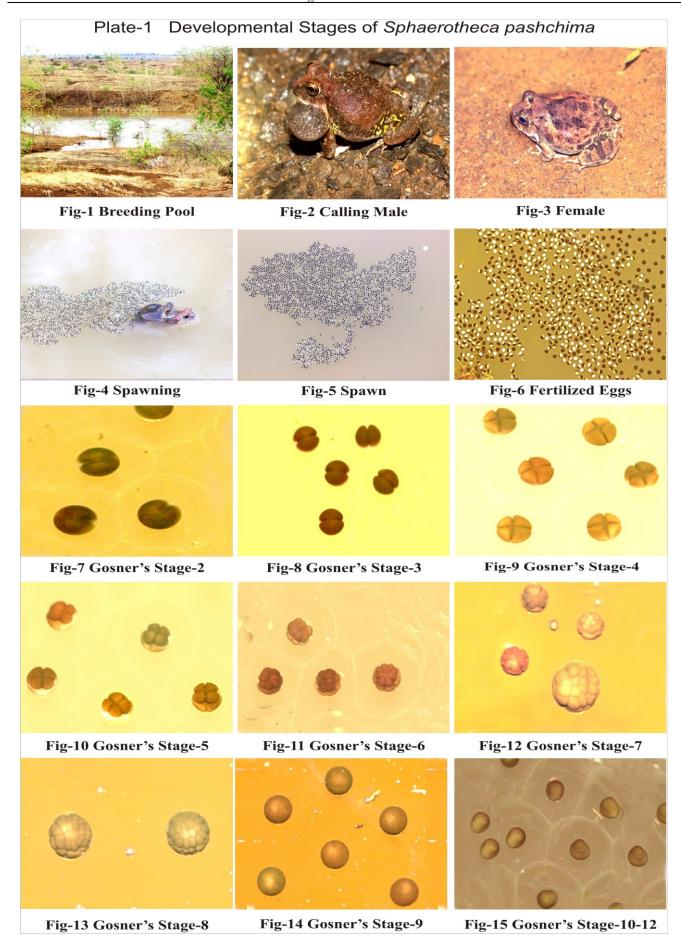
It involves the migration (involution) of micromeres towards vegetal hemisphere resulting in formation of crescent shaped dorsal lip. This is beginning of gastrulation. The diameter of egg is 1.7 mm.

### Gosner's Stage 11- Mid gastrula (7 hours): (Fig-15)

Epiboly of the animal hemisphere progressively advanced and started flattening, creating a circular blastopore. It consist yolk plug.

### **Gosner's Stage 12- Late gastrula (9 hours):** Yolk plug stage (Fig-15)

Yolk plug becomes small in size and disappeared with the epiboly going on. The diameter of egg is 1.8 mm.



#### Gosner's Stage 13- Neural plate (10 hours): (Fig-16)

The embryo becomes slightly elongated along longitudinal axis, yolk plug was disappeared and neural plate developed on dorsal surface with slightly elevated neural folds. The embryo becomes horseshoe shaped, 2 mm in length.

### Gosner's Stage 14- Neural fold (11.45 hours): (Fig-17)

The embryo becomes elongated with two prominent lateral neural folds started to develop and separated by the distinct neural groove. Embryo measures about 2.2 mm in length. Elongation proceeds in the embryo and dark pigmentation appears on dorsal surface of head.

# Gosner's Stage 15- Closure of neural fold (12.30 hours): (Fig-18)

The neural folds come closer to each other and starts closing neural fold and merged into whole trunk. Tail bud small but visible and slight uplifted. Embryo becomes 2.2 mm in length.

### **Gosner's Stage 16- Neural tube (13.15 hours):** (Fig-19)

Lengths of embryos are 2.2 mm the neural folds fused completely thereby forming a neural tube. Head region can be distinguished. The embryo was broader at the anterior region and narrow at the posterior region.

#### Gosner's Stage 17- Tail bud stage (14 hours): (Fig-20)

Tail bud protrudes at the posterior end of embryo and become slightly curved to the left side. Gill plates are visible in the cephalic region. Embryo becomes 2.6 mm in length. Tail bud is 0.3 mm.

## **Gosner's Stage 18- Muscular response stage (18 hours):** Fin formation (Fig-21)

The body elongates and larvae are 3mm in length and tail buds are 0.6 mm. The embryo shows somites and muscular response to external stimuli. Anterior region was well developed with optic bulges, gill buds bulges and pigmented oral sucker in cephalic region. Olfactory pits are visible.

### **Gosner's Stage 19- Heart beat stage (23 hours):** (Fig-22)

The heart pulsation has been seen behind gill bud. Somites become very prominent. Gill buds become prominent. Tail further elongates. Oral suckers become prominent and bogged. The body sizes of larvae are 3 mm and tail buds are 0.9 mm. Total length 3.9 mm.

### Gosner's Stage 20- Larva hatching (26 hours): (Fig-23)

The larva measures about 5mm in length during emerges from mass of jelly. Tail is straight with prominent tail fin. An olfactory pit deepens. External gill cannot be distinguished. Gill circulation started.

## Gosner's Stage 21-Cornea transparent (34 hours): (Fig-24)

Free swimming tadpole. Tail fins well developed. The cornea becomes transparent. External gills branched. Oral sucker becomes darker and ridges become prominent. Nasal pits become prominent.

# Gosner's Stage 22- Tail fin circulation stage (48 hours): 2 days (Fig-24)

Mouth widens both dorsal and ventral tail fin becomes transparent. Circulation started in tail fin. External gills branched, iris and pupils distinguishable.

## Gosner's Stage 23- Opercular fold stage (73 hours): (Fig-25)

Operculum develops and covers gill bases. Labia and teeth differentiate, jaw sheaths distinguishable, development of lower tooth rows and papilla. External gill length shortened. pigmentation develops on the body surface. Fin circulation completed. Tail increases in height. The tadpole body length was 3.5 mm and tail length was 3.2 mm.

# Gosner's Stage 24- Opercular fold closed on right side (74 hours): (Fig-26)

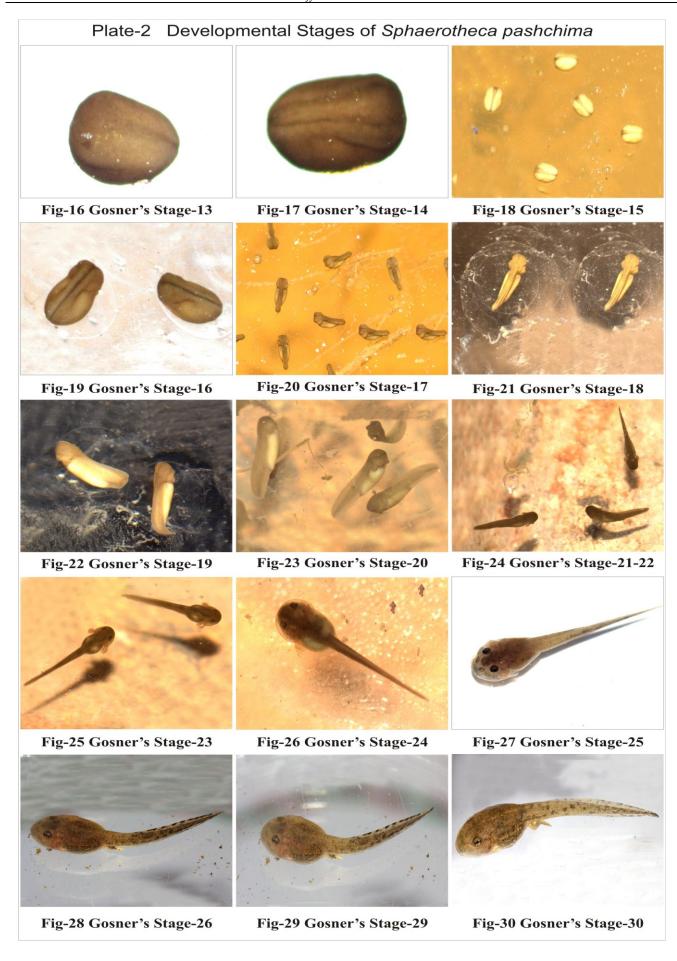
Right operculum fold closed and left external gill shortened (atrophy).

## Gosner's Stage 25- Operculum closed on both sides (84 hours): 3.5 days (Fig-27)

Mouth parts apparent. Left operculum fold closed. Operculum formation was completed and spiracle was formed on the left side *i.e.* sinistral. External gills disappeared. Larval feeding begins in this stage. Intestine visible, iris pigmented. The tadpole body length was 3.6 mm and tail length was 4 mm. Total body length 7.6 mm.

## Gosner's Stage 26- Hind limb bud development stage (168 hours): 7 days (Fig-28)

Hind limb buds were visible at the junction of the trunk and tail on either side of the vent tube. Vent tube was dextral, opening lies at the right of the plane of the



ventral fin. Coiled intestine is visible. The length of the limb bud was smaller than its width. Pigmentation observed on dorsal and ventral surface. Rows of teeth were observed in both the upper and lower labium. The tadpole body length was 3.8 mm and tail length was 6 mm

## Gosner's Stage 27- Length of limb buds equalled half of its diameter (216 hours): 9 days

The length of the limb bud increases in size and its length equalled to half of its diameter. The deposition of pigment melanophores was found to be comparatively denser than in the earlier stage. Oral structure well developed as in the previous stage. The tadpole body length was 6.5 mm and tail length was 14.2 mm.

## Gosner's Stage 28- Length of limb buds equal to its diameter (288 hours): 12 days

Length of limb bud equalled to its diameter. The pigmentations appear denser in distribution and the oral structure was found to be the same as in the stages 26 and 27.

# Gosner's Stage 29- Length of limb buds equal to one and half its diameter (360 hours): (Fig-29)

Length of hind limb bud increased in size and was equal to one and half time its diameter after 13 days. No differences in pigmentation and oral structure.

## Gosner's Stage 30- Length of limb buds equal to twice its diameter (336 hours): 14 day (Fig-30)

Length of hind limb bud further increased and was slightly curved. Deposition of dark pigmentation was found to be denser and widely distributed on the hind limbs. No change in the oral structure. The tadpole body length was 7.5 mm and tail length was 16.5 mm.

## Gosner's Stage 31- Toe differentiation and development (408 hours): 17 day (Fig-31)

The distal end of limb bud curved slightly and was spatula in shape to form the foot paddle after 17 days. The tadpole body length was 8 mm and tail length was 18 mm. At this stage, metamorphosis was also found to be present on the base of the limb bud which had taken the shape of a spatula. The numbers of blood vessels are visible on the hind limbs. Oral structure more developed than the previous stages. In the upper and lower labium teeth rows are well developed and distinct.

## Gosner's Stage 32- First indentation (456 hours): 19 day

The margin of the foot paddle becomes slightly indented on the dorsal side, which marks the fourth and fifth toe. At this stage pigmentation at the limb bud were found to be denser in distribution than in the previous stages. Oral structure was found to be the same as in the earlier stages.

## Gosner's Stage 33- Second indentation (480 hours): 20 days

The margin of foot paddle becomes indented on the ventral side behind the prominence of the fourth toe, which makes the third, fourth and fifth toes.

# Gosner's Stage 34- Third indentation (528 hours): 22 days

The margin of foot paddle becomes indented on the ventral side behind the third toe, which makes the prominence of second, third, fourth and fifth toes. Pigmentation occurs in toes. There was no difference in the oral structure.

### Gosner's Stage 35- Fourth indentation (552 hours): 23 days

The margin of foot paddle becomes indented behind the second toe demarcating the prominence of first toe, and all five toes were demarcated from each other. Blood vessels were now also visible in the toes, the knee joint becomes observable. Pigmentation occurs denser at the base of the limb, and dispersed towards the toe formation region.

## Gosner's Stage 36- First and second toes joints (600 hours): 25 days

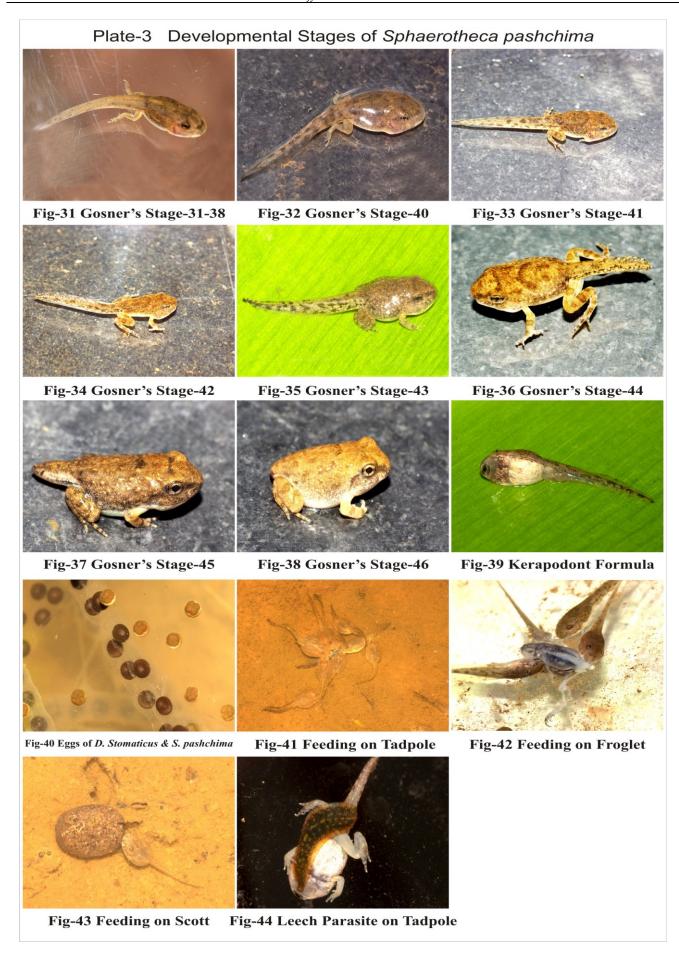
The first and second toes were still jointed, while the third, fourth and fifth toes were separated. Individual toe length increased the femur becomes more elongated. The tadpole body length was 9 mm and the tail length was 19 mm.

## Gosner's Stage 37- Toes separated (624 hours): - 26 days

All the five toes were completely separated and webbed after 26 days. The hind limb grew thinner. The tadpole body length was 10 mm and the tail length was 19 mm.

# Gosner's Stage 38- Metatarsal tubercles appeared (696 hours): 29 days (Fig-31)

There was appearance of metatarsal tubercles. The tadpole began to hold their hind limbs in a bent



configuration. Pigmentation increased on hind limbs and dark cross bands appeared on the hind limbs. The tadpole body length was 10 mm and the tail length was 19.2 mm.

#### **Gosner's Stage 39- Subarticular tubercles appeared:**

Subarticular tubercles appeared after 29 days. The tadpole body length was 11 mm and the tail length was 19.5 mm.

### Gosner's Stage 40- Toes pad complete (720 hours): 30 day (Fig-32)

Toes were fully webbed. Cross banded pattern had extended over the whole hind limbs. The iris was fully pigmented. Pigmentation increased towards the tail region. The tadpole body length was 11.5 mm and the tail length was 19.7 mm. Teeth started to shed from this stage onwards. Vent tube present.

## Gosner's Stage 41- Cloacal tail piece reduced (768 hours): 32 days (Fig-33)

The more drastic changes of metamorphosis begin after stage 40. A tadpole cloacal tail piece was reduced in size. Forelimbs started to develop laterally. The tadpole body length was 12 mm and the tail length was 20 mm. Further shedding of teeth was observed. Only few rows were remaining. Vent tube reduced.

# Gosner's Stage 42- Both limbs developed (816 hours): 34 days (Fig-34)

Tadpole developed both fore limbs and hind limbs. Mouth started widening reach the corner of nostrils, and the SVL was 12.5 mm and the tail length was 20 mm. Dark coloured cross bands were observed at the fore and hind limbs. Metamorphosis was found to be more on the tail region. Shedding of teeth almost completed, and only few teeth were observed. Vent tube lost.

# Gosner's Stage 43- Resorption of tail begins (828 hours): 34½ days (Fig-35)

Tadpole tail started to regress after 828 hrs. Body length was 12 mm and the tail length was 15 mm. At this stage longitudinal stripes were observed on the body region of the tadpole. Pigmentation was found to be present only in the tail region. Shedding of teeth was completed. The corners of the mouth were now situated below the nostrils and eyes. The pigmentation of the skin changes to a reddish brown colour. The tadpole begun to came on land and shows the typical posture of an adult frog.

### Gosner's Stage 44- Tail shortened (840 hours): 35 days (Fig-36)

There was further gradual loss of the tadpole tail and the tail was now shorter and the tadpole measures 12.5 mm in length and tail length was 10 mm. Formation of tongue took place. Mouth corner beneath the eye.

## Gosner's Stage 45- Tail stub (852 hours): 35½ days (Fig-37)

Resorption of the tail was completed and only a stub was left and the tadpole had nearly the same physical appearance as an adult frog. Mouth corner reaches beneath eye. Tadpole measures 12.5 mm in length and tail length was 5 mm. There was appearance of adult characters on the dorsum. Tongue fully developed. Typical granular surface of the skin was visible.

### Gosner's Stage 46- Metamorphosis completed (864 hours): 36 days (Fig-38)

Tail was completely disappeared and froglet looks like an adult and measures SVL 12.5 mm with fore limb length was 6.4 mm and hind limb length was 14.4 mm. Dorsum was yellowish-brown coloured with dark brown patches, with reddish granulation, inverted V-shaped mark on the back, tympanum mottled, ventral side creamy white. Dark cross bands on the fore and hind limbs. Froglet adapted to the terrestrial life.

#### **DISCUSSION:**

Completion of development and metamorphosis took about 36 ± 2 days in Sphaerotheca pashchima at temperature between 22°C to 30°C and pH 6.7 to 7.2 in the natural environment. In S. pashchima, breeding activities started immediately after the heavy rain from the month of June and continued till the month of October. Adults gather in large numbers at potential breeding habitats. Calling takes place from open spots sitting on the edge of water bodies, mainly during night from 2030 PM to 0400 AM of the following day. Males possess a paired external subgular vocal sac (Fig-2). As it is a pond breeder (lentic water), lay large number of single eggs into shallow, often temporary pools (Fig-4) at relative humidity 70% and air temperature 28°C. Amplexus was axillary. Eggs were deposited in a thin film floating above the water surface (Fig-5). The male dismounted the female after completion of egg-laying, and both male and female moved away from the egg mass, no parental attendance was noticed. Fertilization is external. A single clutch contains about 1000 to 1500

eggs. The eggs measured about 1.5 mm in diameter, spherical in shape, covered by double jelly layers, without shell and embryonic membranes. Stages 1-7 are continuous cleavage, and in stage 8 and 9 the blastocoels forms. Gastrulation occurs during stages 10-12 and neurulation during stages 13-16. Stages 17-21 involve the elongation of the body and development of the tail bud, adhesive organs, and gills. After about 26 hours of spawning and egg fertilization the larva hatched out from jelly layers at stage 20. Stages 21-25 mark the transition form a relatively immobile embryo sustained by yolk to a feeding and free-swimming tadpole. Mouthparts begin to develop in stage 23 and are essentially complete by stage 25. Feeding started at stage 25. The initial formation of patterns of pigmentation and the development of the operculum generally occur in stages 23-25. Tadpoles are bottom dwelling. It was observed that the tadpoles feed by scrapping the food particles which were attached to vegetation present in the pool. Tadpoles cannibalistic; ravens on its own species (Fig-41&42). Further development of the hind limbs and mouth was observed with complete teeth rows, keratodont formula 1:1+1/3 were observed in stage 26-40 (Fig-39). Metamorphosis begins in stage 41 and is completed in stage 46 (Fig-38). With the onset of metamorphosis, teeth rows had begin to shed and there was resorption of the tail. Complete absorption of the tail was observed after 36±2 days. Metamorphosed froglet measured about 12.5 mm in length and lead a terrestrial life. During development and metamorphosis, comprehensive and striking changes takes place where a free swim-ming aquatic tadpole was transformed into a terrestrial form. S. pashchima shares its habitat with other pond breeding species of anurans Duttaphrynus melanostictus, Duttaphrynus stomaticu, Euphlyctis cyanophlyctis, Fejervarya spp. Hoplobatrachus tigerinus, Microhyla ornate and Uperodon systoma (Fig-40).

Most of the amphibians are highly dependent upon aquatic habitats because of their physiology and life history (Duellman and Trueb, 1994), it necessary to study the ecological and physico chemical parameters. In study area rainfall ranged between 33 mm to 156 mm, relative humidity ranged between 50% to 90% and air temperature ranged between 25°C to 35°C during the period of development and metamorphosis (June to October). The water temperature of breeding pond was recorded between 22°C to 31°C and pH ranges between 6.7 to 7.2 during study period. The water temperature,

pH and rainfall are the important factors which directly influence the embryonic development. This study has shown that in arid, warm environment the development and metamorphosis of embryo and tadpole is quick and completed in short duration before the pond water dry out. The values for ecological and physico-chemical parameters of pond water are ideal, in which the tadpoles develop and metamorphose completed successfully in the natural habitat.

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