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STUDY OF NORMAL DEVELOPMENT AND EXTERNAL MORPHOLOGY OF TADPOLES OF *MICROHYLA ORNATA* AND *UPERODON GLOBULOSUS* OF THE FAMILY MICROHYLIDAE (AMPHIBIA: ANURA) FROM NORTH EAST INDIA

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ABSTRACT
Normal developmental table and external morphology of stage 38 tadpoles of *Uperodon globulosus* and *Microhyla ornata* has been described. The development rate of *U. globulosus* has been found to be slower in comparison to *M. ornata* in laboratory condition. Larva hatches at stage 20 in both *M. ornata* and *U. globulosus.* Clutch size in *U. globulosus* is larger (750-900 eggs) than in *M. ornata* (300-510 eggs), but hatching success of larvae are less in *U. globulosus* (66.6-80%) in comparison to *M. ornata* (91.1- 93.3%). The tadpole of *M. ornata* is transparent dorsally with oval shaped body and thin tail musculature. On the other hand tadpole of *U. globulosus* has dark pigmentation with roughly oval body and moderate tail musculature. At stage 38 Pineal ocellus is visible in *M. ornata* whereas it is absent in *U. globulosus*. Spiracle is median in both the species. Oral disc is more developed in *U. globulosus* than in *M.ornata*. Toe pads are well developed in metamorphosing stages in both the species at stage 40 whereas toe pads are absent in the adult of *U. globulosus.*

KEY WORDS: Anura, Microhylidae, breeding, tadpole, morphology, ontogeny.

INTRODUCTION

The normal development in anurans follow a similar pattern however, the time taken for completion of life cycle, seasonality etc. are determined by a variety of factors. According to Duellman and Trueb (1986) most of the amphibians are highly dependent upon aquatic habitats because of their physiology and natural history. Rainfall plays an important role in the normal development of amphibians and any change in rainfall pattern can reduce amphibian reproduction (Lips, 1996) as a part of their life cycle is spent in water. Study of breeding biology and life cycle of a species is important for making conservation effort for that species. Anurans breed in a variety of ecosystems ranging from highly torrential water bodies to permanent ponds or temporary rain pools. Frogs of the family Microhylidae are mainly found in sub- tropical regions. They are generally pond and rain pool breeders. Microhylids come overground only during breeding season. A detailed breeding biology of many species of the family Microhylidae is yet to be studied.

Nineteen species of the family Microhylidae are found in India (www.amphibiaweb.org, 2011), of which breeding ecology and life history study of only a few species are known. Dutta et al. (1990-91) studied the complete life cycle of south Indian Microhylid frog *Ramanella variegata.* Vijayalaxmi et al. (2004) described breeding ecology of *Ramanella montana* from western ghat, India. In North East India, Dey and Gupta (2002) provided some information related to *Microhyla ornata*. Bhaduri and Daniel (1956) described the tadpole morphology of *Uperodon globulosus*. Gosner (1960) suggested a generalized table of 46 stages covering the period from fertilization of the egg to completion of metamorphosis and this table has been used for comparison of developmental stages of Anurans world over. In the present study Normal developmental table of *U. globulosus* and *Microhyla ornata* has been prepared according to Gosner's 46 stages. Shimizu and Ota (2003) described the developmental table of *M. ornata* from Japan. In their study larva hatching stage was 21stage (Gosner's 18 stage) whereas in our study *M. ornata* larva hatches in Gosner's stage 20.

The present study gives the time taken for attainmentt of landmark characters of various stages in the present ecological condition of Assam during monsoon. Completion of life history of both the species has been studied in the same habitat therefore a comparative study of the two species has been possible.

MATERIAL AND METHODS

Study site was Ramfalbil area of Kokrajhar district (26°32′N-26°35′N and 90°09′E-90°15′E), altitude 64.5m MSL), western Assam, India. Breeding activity of *Uperodon globulosus* and *Microhyla ornata* were monitored during the breeding season (March to June, 2007). Amplecting pairs of both the species were collected from the habitat and kept in an aquarium for collection of fertilized eggs. Number of eggs in different clutches was counted. Eggs were transferred to rectangular trays keeping 100 eggs in each tray. Tadpoles were fed with plankton collected from the habitat. They were reared in the laboratory at 25- 27ºC. Tadpoles were staged as per Gosner (1960). The eggs and tadpoles were preserved in 10% formaldehyde solution and measured with a dial

vernier caliper. Morphological terminology was followed after Altig and McDiarmid (1999).).

The abbreviations used in description of tadpoles are as follows:

BH, maximum body height; BL, body length; BW, maximum body width; ED, maximum diameter of eye; HL, head length; HLL, hindlimb length; IOD, inter ocular distance; UF, maximum height of upper tail fin; LF, maximum height of lower tail fin; NN, inter narial distance; NP, nare pupilar distance; ODW, oral disc width; SE, snout eye distance; RN, snout nare distance; SN, spiracle-nare distance; SpE, spiracle eye distance; SS, distance from tip of snout to spiracle; TAL, tail length; TL, total length; TMH, maximum tail muscle height; MTH, maximum tail height; UF, maximum height of upper tail fin.

RESULTS

Microhyla ornata (Dumeril and Bibron, 1841)

Breeding activity of *M. ornata* is March to June. Calling males were found at night. Amplecting pairs are observed in early evening. Females lay eggs in early morning (3.30- 4.00A.M.) or just before sunrise. Clutch size is from 300- 510 eggs (three clutches contained eggs numbering 300, 340 and 510 respectively), of which hatching success of larvae were from 280, 310, 470 eggs respectively. Egg masses were circular in shape and their diameter was 7-10cm. The color of the egg was brown in animal pole and white in vegetal pole. The total time taken for completion of life cycle in laboratory condition is 1176 hrs (49 days).

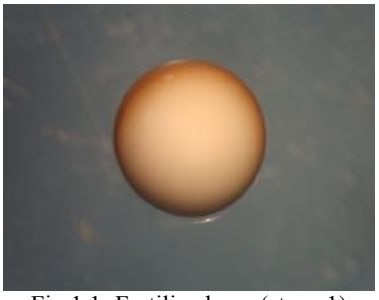

Fig.1.1: Fertilized egg (stage 1)

Fig.1.2: 16-cell (stage 6)

Fig.1.3: Mid-cleavage (stage 8)

Fig.1.4: Dorsal lip (stage 10)

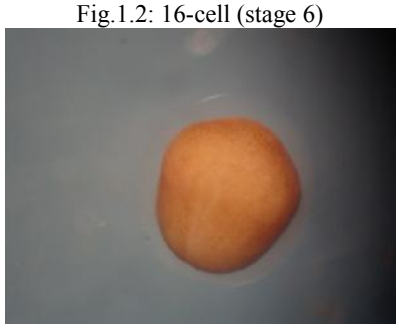

Fig.1.5: Neural plate (stage13)

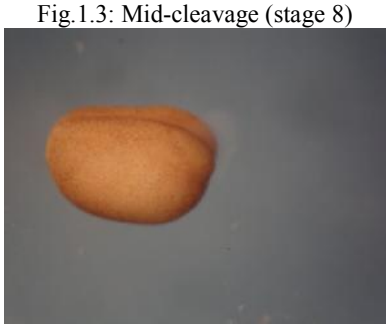

Fig.1.6: Closure of neural fold (stage 15)

PLATE1: photographs showing development stages (Gosner's stage1,6,8,10,13 and 15) of *Microhyla ornata*

Normal development of *Microhyla ornata*

Stage.1: Fertilization (zero hours)

Diameter of egg is 0.8mm. The egg is spherical in shape with brown animal pole and white vegetal pole.

Stage.2: One cell stage (30 min.)

Diameter of egg is 0.9mm. The grey crescent appears between the animal and vegetal pole towards the pigmented hemisphere.

Stage.3: Two cell stage (one hour)

Diameter of egg is 0.9mm. The first cleavage plane is meridional. It cuts the egg through its median animal-vegetal polar axis and results in two equal sized blastomeres.

Stage.4: Four cell stage (1.30 hours)

Size of egg is 0.9mm. The second cleavage is meridional and is at right angles to the first plane and four equal sized blastomeres result.

Stage.5: Eight cell stage (2 hours)

Diameter of egg is 0.9mm. The third cleavage is latitudinal, but unequal. Therefore, eight-cell stage consists of four small-sized micromeres and four large sized macromeres.

Stage.6: Sixteen cell stage (2.30 hours)

Diameter of egg is 0.9 mm. The fourth cleavage is vertical to previous one, resulting in 8 pigmented micromeres and followed by division of 4 un-pigmented macromeres, resulting in a total of 16 cells.

Stage.7: Thirty two cell stage (3 hours)

Diameter of egg is 0.9mm. The fifth cleavage is latitudinal, as a result 16 micromeres and 16 macromeres are formed.

Stage.8: Mid blastula stage (3.30 hours)

Diameter of the egg is 0.9mm. The number of cells increased to more than 64 and takes the shape of a ball and the surface of animal pole looks like a cluster of beads.

Stage.9: Late blastula stage (4.30 hours)

Diameter of egg is 0.9 mm. The surface of the animal pole has a granular appearance. The pigmented region of the animal pole extends over the vegetal pole.

Stage.10: Crescent dorsal lip (6 hours)

Size of the embryo is 0.9 mm. Crescent shaped dorsal lip of blastopore appears. Micromere cells migrate towards vegetal pole as a result size of un-pigmented region is reduced.

Stage.11: Mid gastrula (7 hours)

Size of the embryo is 0.9 mm. Horse shoe shaped dorsal lip of blastopore is surrounded by un-pigmented macromeres.

Stage.12: Late gastrula (9 hours)

Size of the embryos ranges from 0.9-0.91mm. The ventral lip of blastopore shifts to posterior end and small yolk plug is visible.

Stage.13: Neural plate (10 hours)

Size of the embryos ranges from 1.3-1.32mm. The yolk plug has disappeared and the neural plate is visible clearly on the dorsal surface.

Stage.14: Neural fold (11 hours)

The embryo becomes elongated (size range 1.3-1.33mm) and the neural fold becomes distinct, separated by neural groove.

Stage.15: Closure of neural fold (12 hours)

Lengths of embryos are 1.32-1.34mm. The neural fold comes close to each other forming a shallow neural groove.

Stage.16: Neural tube (13 hours)

Lengths of embryos are 1.32-1.34mm. The neural folds are closed thereby forming a neural tube. Head region can be distinguished.

Stage.17: Tail bud stage (14 hours)

The body sizes of the larvae are 1.7-1.72mm and tail buds are 0.2-0.21mm.

Stage.18: Muscular response stage (18 hours)

The body sizes of the larvae are 2.0-2.01mm and tail buds are 0.5-0.52mm. Optic bulges , bulges of gill plates and pigmented oral suckers are present in the head.

Stage.19: Diameter of tail bud equal to its length (24 hours)

The body sizes of the larvae are 2.0-2.02mm and tail buds are 0.7-0.71mm. Oral suckers become bogged, two black ridges distinct. Stomodeal depression is distinct. External gill buds are prominent.

Fig.1.7: Larva hatch (stage 20)

Fig.1.8: Cornea transparent (stage 21)

PLATE.2: Development stages (Gosner's stage 20 and 21) of *Microhyla ornata*

Stage.20: Larva hatching (26 hours)

The body sizes of the larvae are 2.0-2.02mm and tail buds are 2.5-2.53mm. The larva emerges from the mass of jelly. Tails are straight, tail fins are prominent. Olfactory pit deepens. External gill cannot be distinguished.

Stage.21: Cornea transparent (34 hours)

The body sizes of the tadpoles are 1.7-1.73mm and tails are 2.8-2.83mm. Free swimming tadpole. Tail fins well developed. The cornea becomes transparent. External gill branched. Oral sucker become darker and ridges become larger at this stage. Mouth still not developed.

Stage.22: Tail fin circulation stage (48 hours)

The body sizes of the tadpoles are 1.8-1.82mm and tails are 3.0-3.04mm. Dorsal and ventral fins are transparent. Well developed mouth and branched external gill. Cornea transparent, eyes can be distinguished externally.

Stage.23: Opercular fold stage. (72 hours)

The body sizes of tadpoles are 1.56-1.58mm and tail lengths are 3.1-3.12mm.

Stage.24: Opercular fold closed on right side. (73 hours)

The body sizes of tadpoles are 1.56-1.58mm and tail lengths are 3.1-3.13mm.

Stage.25: Operculum closed on both sides (84 hours)

The body sizes of tadpoles are 1.64-1.65 mm and tail lengths are 4.42-4.45mm. Operculum covers the external gills. The mouth becomes wider. External eyes well developed. Spiracle develops ventrally near vent.


Fig.1.9: Operculum closed (stage 25)


Fig.1.10: Foot paddle stage (stage 31)

PLATE.3: Development stages (Gosner's stage 25 and 31) of *Microhyla ornata*

Stage.26: First hind limb bud appears (120 hours)
The body sizes of the tadpoles are 2.1-2.12mm and tail lengths are 4.45-4.52mm.
Stage.27: Length of limb bud equal to half of its diameter (132 hours).
The body sizes of the tadpoles are 2.3-2.31mm and tail lengths are 5.1-5.14mm. Length of hind limb bud at this stage equals to half of its diameter.
Stage.28: Length of limb bud equal to its diameter (240 hours)
The body sizes of the tadpoles are 3.0-3.02mm and 5.3-5.32mm. Length of hind limb bud is equal to its diameter.
Stage.29: Length of limb bud is equal to one and half times its diameter (360 hours)
The body sizes of the tadpoles are 3.4-3.41mm and tail lengths are 5.4-5.41mm. At this stage, length of limb bud is equal to one and half times of its diameter.
Stage.30: Length of limb bud is equal to twice its diameter (456 hours).
The body sizes of the tadpoles are 4.12-4.14mm and tail lengths are 7.8-7.83mm. Length of limb bud becomes twice of its diameter. Distal half of conical limb bud is slightly bent ventrally.
Stage.31: Foot paddle shaped stage (528 hours)
The body sizes of the tadpoles are 5.6-5.62mm and tail lengths are 8.9-8.92mm. Paddle shaped hind limb appear at this stage.
Stage.32: First indentation (600 hours)
The body sizes of the tadpoles are 5.7-5.71mm and tail lengths are 9.2-9.24mm. The margin of foot paddle becomes slightly indented which marks the prominence of future 4th and 5th toes.
Stage.33: Second indentation (672 hours)
The body sizes of the tadpoles are 6.2-6.21mm and tail lengths are 9.6-9.62mm. Second indentation occurs on the ventral side behind the prominence of 4^{th} toes, which marks the 3^{rd} toes.
Stage.34: Third indentation (744 hours)
The body sizes of the tadpoles are 6.2-6.22mm and tail lengths are 10.1-10.2mm. The margin of foot paddle becomes indented on ventral side, behind the prominence of 3^{rd} toe which marks the prominence of 2^{nd} toe.
Stage.35: Fourth indentation (816 hours)
The body sizes of the tadpoles are 6.36-6.38mm and tails are 10.63-10.64mm. The first toe of the hind limb is prominent. All the five toes are separated from each other. 4^{th} toe is very long.
Stage.36: Maximum incurvation between 4^{th} and 5^{th} toes directed towards the tip of 2^{nd} toe (864 hours)
The body sizes of the tadpoles are 6.36-6.38mm and tails are 10.64-10.67mm. The maximum incurvation between 4^{th} and 5^{th} toes directed towards the tip of 2^{nd} toe.


Fig.1.11: 4^{th} indentation (stag35)


Fig.1.12:Appearance of IMT (stage38)


Fig.1.13:Fore limbs emerge (stage42)

PLATE.4: Development stages (Gosner's stage35, 38 and 42) of *Microhyla ornata*

Stage.37: Maximum incurvation between 4^{th} and 5^{th} toes directed towards the tip of 1^{st} toe (912 hours)
The body sizes of the tadpoles are 6.38-6.39mm and tails are 11.2-11.24mm. The maximum incurvations between 4th and 5th toes are directed towards the tip of 1^{st} toe. Hind limbs directed towards the ventral fin.
Stage.38: Appearance of inner metatarsal tubercle (984 hours).
The body sizes of the tadpoles are 6.48-6.49mm and tails are 12.6-12.62mm. Inner metatarsal tubercle appears, limbs are creamy white. Tip of tail is long and pointed. The sizes of tadpoles become larger at this stage than other stages.
Stage.39: Appearance of subarticular tubercles (1032 hours).

The body sizes of the tadpoles are 6.49-7.01mm and tails are 12.8-12.83mm. Subarticular tubercles are prominent, shovel shaped inner metatarsal tubercles appear.

Stage.40: Appearance of toe pads (1056 hours)

The body sizes of the tadpoles are 6.5-6.52mm and tail lengths are 11.2-11.24mm. Toe pads completed. Subarticular tubercles are clearly elevated.

Stage.41: Cloacal tail piece reduced (1080 hours).

The body sizes of the tadpoles are 6.5-6.52mm and tail lengths are 10.9-10.93mm. Cloacal tail piece reduced. Tail darkening starts. The forelimbs are visible through the skin.

Stage.42: Both fore limbs emerge (1104 hours)

The body sizes ranges from 6.8-6.82mm and tail lengths are 10.9-10.95mm. Both the forelimbs emerge. Right limb emerges first by forming a pore or window and left limb appears later.

Fig.1.14:Tail reduced (stage43)

Fig.1.15:Tail stub (stage 45)

Fig.1.16:Froglet (stage 46)

PLATE.5: Metamorphosis stages (Gosner's stage43, 45 and 46) of *Microhyla ornata*

Stage.43: Angle of mouth between the eye and nostril (1116 hours)

The body sizes of the animals are 7.21-7.23mm and tail lengths are 8.3-8.36mm. The angle of mouth is between the nostrils to the eyes. Both dorsal and ventral fins shrink and the length of the tail is reduced but still longer than the hind limbs.

Stage.44: Angle of mouth reaches the middle of the eye (1128 hours)

The body sizes of the animals are 7.68-7.7mm and tail lengths are 4.4-4.43mm. The angle of mouth reaches middle of the eyes.

Stage.45: Angle of mouth reached posterior margin of the eye (1152 hours)

The body sizes of the animals are 7.4-7.46mm and tail lengths are 2.1-2.17mm. The angle of mouth reaches the posterior margin of eyes.

Stage.46: Metamorphosed into froglet (1176 hours)

The tailed froglet metamorphoses into a tailless frog. The sizes of the juveniles are 9.0-9.2mm.

General description of the tadpole

The external morphology is based on a specimen in stage 38. Morphometric data are provided for 10 tadpoles of stage 38 (**TABLE.1**).

Fig.1.17:Dorsal & ventral view of stage38 Fig.1.18: Oral disc of stage 38

PLATE.6: Tadpoles of stage 38 and oral disc of stage 38 of *Microhyla ornata*

External morphology

In dorsal view (Fig.1.17), body oval in shaped, snout rounded. In lateral view, body depressed, BW 121% of BH. Eyes moderately large in size, ED 12.42% of BL, bulging, placed laterally and directed laterally. Pineal ocellus present in stage 38. Nares rounded, distinct, positioned dorsally and directed anterolaterally. Inter ocular distance (IOD), (NN 21.42% of IOD). The spiracle is medial, situated ventral body, posterior part of the abdomen. SS 90.06% of BL. Tail musculature comparatively small, TMH 22.5% of BH and 26% of MTH. Tail fin moderate in size and upper tail fin slightly smaller than the lower one, UF 31.17% of MTH and LF 38.23% of MTH. Anal tube moderately large, tubular, medial and directed posteriorly, entirely attached to ventral fin, opening large, posteriorly directed. Mouth is antero dorsal. Upper lip and lower lip is completed as a simple curved thin layer and lower lip as a thin layer, contains a U- shaped notch. Karatodont is absent.

Live coloration

Dorsum transparent creamy white with dark diamond marking in between inter ocular space. Nares and abdominal region is dark brown. Ventrally glassy transparent, heart, blood circulation and intestinal coils are

clearly visible. Tail musculature is creamy white and a dark line is present on the dorsal side.

***Uperodon globulosus* (Gunther,1864)**

Breeding season of *U. globulosus* is April to June. Calling males were found when incessant rain occurs and amplecting pairs may occur at any time during day or night. Two females laid 750, 900 eggs respectively. The hatching successes of larvae were from 500, 720 eggs respectively. The color of the egg was dark brown in animal pole and white in vegetal pole. The total time taken to complete its life cycle in laboratory condition is 1560 hrs (72 days).

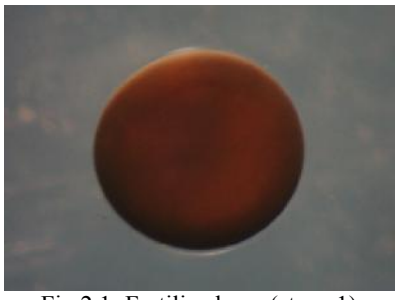

Fig.2.1: Fertilized egg (stage 1)

Fig.2.2: Two cell stage (stage 3)

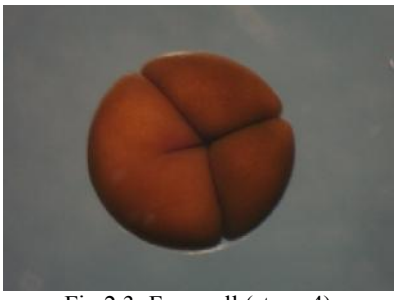

Fig.2.3: Four cell (stage 4)

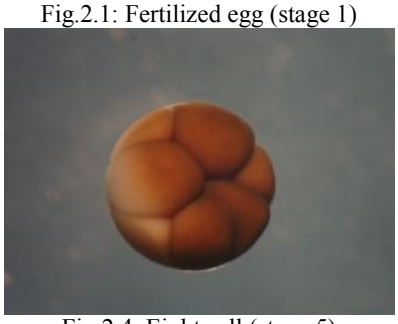

Fig.2.4: Eight cell (stage 5)

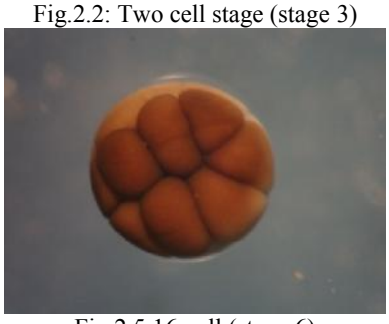

Fig.2.5:16-cell (stage 6)

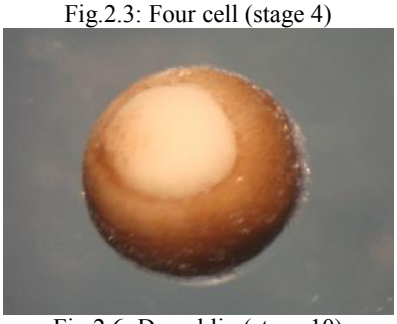

Fig.2.6: Dorsal lip (stage 10)

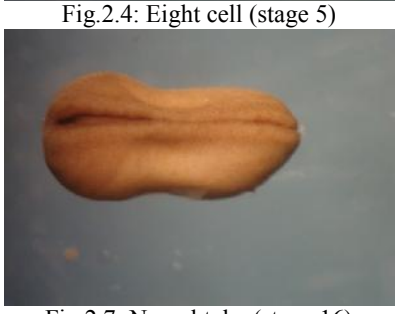

Fig.2.7: Neural tube (stage 16)

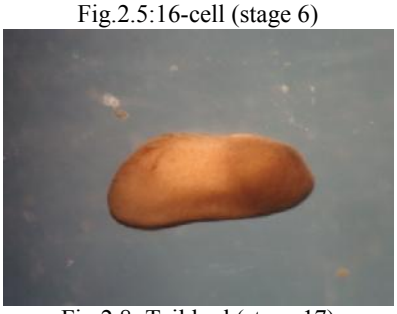

Fig.2.8: Tail bud (stage 17)

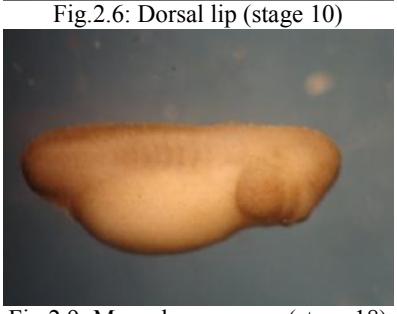

Fig.2.9: Muscular response (stage 18)

PLATE .7: Developmental stages (Gosner's stages 1,3,4,5,6,10,16,17 and 18) of *Uperodon globulosus.*

Normal development of *Uperodon globulosus*

Stage.1: Fertilization (zero hours)

Diameter of egg is 1.3 mm at this stage. The egg is spherical in shape with black animal pole and creamy white vegetal pole.

Stage.2: One cell stage (one hour)

Diameter of egg is 1.3mm. The grey crescent appears between the animal and vegetal pole towards the pigmented hemisphere.

Stage.3: Two cell stage (2 hours)

Diameter of egg is 1.3mm. The first cleavage plane is meridional. It cuts the egg through its median animal-vegetal polar axis and results in two equal sized blastomeres.

Stage.4: Four cell stage (3 hours)

Size of egg is enlarged and diameter becomes 1.4mm. The second cleavage is also meridional and is at right angle to the first plane and four equal sized blastomeres.

Stage.5: Eight cell stage (4 hours)

Diameter of egg is still 1.4mm. The third set of cleavage is latitudinal and unequal. Therefore, eight-cell stage consists of four small-sized micromeres and four large sized macromeres.

Stage.6: Sixteen cell stage (5 hours)

Diameter of egg is 1.45mm. The fourth cleavage is vertical to previous one, resulting in 8 pigmented cells of micromeres and followed by division of 4 un-pigmented macromere's resulting in a total of 16 cells.

Stage.7: Thirty two cell stage (6 hours)

Diameter of egg is 1.45mm. The fifth cleavage is latitudinal, resulting in 16 micromeres and 16 macromeres.

Stage.8: Mid blastula (7 hours)

Diameter of the egg is 1.45mm. The number of cells increased to more than 64 and takes the shape of a ball and the surface of animal pole looks like a cluster of beads.

Stage.9: Late blastula (8 hours)

Diameter of egg is 1.45mm. The surface of the animal pole has a granular appearance. The pigmented region of the animal pole extends over the vegetal pole.

Stage.10: Crescent dorsal lip (9 hours)

The size of the embryo is 1.45mm. The crescent shaped dorsal lip of blastopore has formed. Due to continuous

migration of micromeres towards the vegetal pole, un-pigmented region is reduced in size.

Stage.11: Mid gastrula (11 hours)

The size of the embryo is 1.45mm. Horseshoe shaped dorsal lip is surrounded by un-pigmented macromeres.

Stage.12: Late gastrula (12 hours)

The sizes of the embryos are 1.55-1.56mm. The ventral lip of blastopore shifts to posterior end and small yolk plug is seen.

Stage.13: Neural plate (12.30 hours)

The sizes of the embryos are 1.7-1.71mm. The yolk plugs disappears and the neural plate develops on the dorsal surface.

Stage.14: Neural fold (13 hours)

The embryo becomes elongated and ranges from 1.9-1.92 mm and the neural fold becomes distinct, separated by neural groove.

Stage.15: Closure of neural fold (13.30 hours)

Sizes of embryos are 2.0-2.02mm. The neural fold comes close to each other forming a shallow neural groove.

Stage.16: Neural tube (14 hours)

Sizes of embryos are 2.5-2.51mm. The neural folds close forming a neural tube. Head can be distinguished at this stage.

Stage.17: Tail bud stage (14.30 hours)

The body sizes of the larvae are 2.5-2.52 mm and tail buds are 0.45-0.47mm.

Stage.18: Muscular response stage (15 hours)

The body sizes of the larvae are 2.7-2.73 mm and tail buds are 0.65-0.67mm. Optic bulges and bulges of gill plates are prominent in head. Pigmented oral suckers are crescent shaped.

Stage.19: Diameter of tail bud equal to its length (18 hours)

The body sizes of the larvae are 3.0-3.02 mm and tail buds are 0.7-0.73mm. Oral suckers become prominent, two black ridges distinct. Stomodeal depression is distinct. External gill buds are prominent.

Fig.2.10: Cornea transparent (stage 21) Fig.2.11: Operculum closed (stage 25)

PLATE .8: Developmental stages (Gosner's stage 21 and 25) of *Uperodon globulosus*

Stage.20: Larva hatch (20.30 hours)

The body sizes of the larvae are 3.05-3.07mm and tail buds are 0.85-0.87mm. The larva emerges from the mass of jelly. Tails are straight, tail fins are prominent. Olfactory pit deepens. External gills not yet distinguished.

Stage.21: Cornea transparent (24.30 hours)

The body sizes of the tadpoles are 3.4-3.42mm and tails are 1.0-1.05mm. Free swimming tadpole. Tail fins well developed. The cornea becomes transparent. External gill branched. Oral sucker becomes darker and ridges become larger at this stage. Mouth still not developed.

Stage.22: Tail fin circulation stage (29 hours)

The body sizes of the tadpoles range from 3.85-3.86mm and tails are 1.55-1.58mm. Dorsal and ventral fins are transparent. Mouth develops and external gills are branched. Cornea is transparent, but eyes cannot be distinguished externally.

Stage.23: Opercular fold stage (36 hours)

The body sizes of the tadpoles are 2.4-2.43 mm and tail length varies from 4.0- 4.04 mm. operculum covers bases of external gills. Eyes can be distinguished externally.

Stage.24: Opercular fold closes on the right side (48 hours).

The body sizes of the tadpoles are 2.4-2.45mm and tails are 4.05-4.1mm. Operculum covers the external gills on right side. Bulging eyes appear.

Stage.25: Operculum closed on both sides (56.30 hours).

The body sizes of tadpoles are 2.54-2.56mm and tail lengths are 4.28-4.3mm. Operculum covers the external gills. The mouth becomes wider. External eyes well developed. Spiracle developed ventrally near vent.

Stage.26: First hind limb bud appears near cloacal tail piece (72 hours)

The body sizes of the tadpoles are 3.0-3.02mm and tails lengths are 4.94-4.97mm.

Stage.27: Length of limb bud equals half of its diameter (96 hours).

The body sizes of the tadpoles are 3.7-3.71mm and tail length 5.7-5.73mm. Length of hind limb bud at this stage is equal to half of its diameter.

Stage.28: Length of limb bud equal to its diameter (216 hours)

The body sizes of the tadpoles are 5.0-5.03mm and tail length 7.0-7.08mm. Length of hind limb bud equals its diameter.

Stage.29: Length of limb bud is equal to one and half times its diameter (408 hours)

The body sizes of the tadpoles are 5.86-5.87mm and tail lengths are 9.27-9.3mm. Length of limb bud equals to one and half times its diameter.

Stage.30: Length of limb bud is equal to twice its diameter (600 hours)

The body sizes of the tadpoles are 6.14-6.18mm and tail lengths are 9.75-9.79mm. Length of limb bud at this stage

becomes twice its diameter. Distal half of conical limb bud slightly bends ventrally.

Stage.31: Foot paddle shape stage (792 hours).

The body sizes of the tadpoles are 6.7-6.75mm and tail lengths are 10.6-10.65mm. Transparent or colorless paddle shaped hind limb first appear at this stage.

Stage.32: First indentation (864 hours).

The body sizes of the tadpoles are 7.0-7.06mm and tail lengths are 11.18-11.2mm. The margin of foot paddle becomes slightly indented which marks the prominence of future 4th and 5th toes.

Stage.33: Second indentation (936 hours).

The body sizes of the tadpoles are 7.92-7.95mm and tail lengths are 12.1-12.2mm. Second indentation occurs on the ventral side behind the prominence of 4^{th} toes, which marks the 3^{rd} toes.

Stage.34: Third indentation (1008 hours)

The body sizes of the tadpoles are 8.36-8.38mm and tail lengths are 12.76-12.8mm. The margin of foot paddle becomes indented on ventral side, behind the prominence of 3^{rd} toe which marks the prominence of 2^{nd} toe.

Stage.35: Fourth indentation (1056 hours)

The body sizes of the tadpoles are 9.66-9.69mm and tail lengths are 16.0-16.05mm. The first toe of the hind limb is prominent. All the five toes appear. 4^{th} toe is very long.

Stage.36: Maximum incurvations between 4^{th} and 5^{th} toes directed towards the tip of 2^{nd} toe (1128 hours)

The body sizes of the tadpoles are 9.68-9.7mm and tail lengths are 16.84-16.87mm. The maximum incurvations between 4th and 5th toes are directed towards the tip of 2^{nd} toe.

Stage.37: Maximum incurvations between 4^{th} and 5^{th} toes are directed towards the tip of 1^{st} toe (1152 hours).

The body sizes of the tadpoles are 9.86-9.89 mm and tails are 17.24-17.3mm. The maximum incurvations between 4^{th} and 5^{th} toes are directed towards the tip of the 1^{st} toe. Hind limbs are directed posteriorly.

Fig.2.12:4^{th} indentation (stage35)

Fig.2.13: Appearance of IMT (stage 38)

PLATE .9: Gosner's stage 35 and 38 of *Uperodon globulosus*

Stage.38: Appearance of inner metatarsal tubercle (1176 hours).

The body sizes of the tadpoles are 10.16-10.2mm and tails are 18.1-18.2mm. Inner metatarsal tubercle appeares, limbs are pigmented. Tip of tail is pointed. Tadpoles attain largest size at this stage. Pineal ocellus is not visible.

Stage.39: Appearance of subarticular tubercles (1224 hours).

The body sizes of the tadpoles are 10.2-10.25mm and tails are 16.26-16.5mm. Sub articular tubercles are prominent. Shovel shaped inner and outer metatarsal tubercles appear.

Stage.40: Appearance of toe pads (1272 hours).

The body sizes of the tadpoles are 10.02-10.1mm and tail lengths are 14.76-14.8mm. Toe pads are appeared. Subarticular tubercles are clearly elevated. Toes of hind limbs become stronger.

Stage.41: Cloacal tail piece reduced (1296 hours).

The body sizes of the tadpoles are 9.4-9.47mm and tail lengths are 14.5-14.58mm. Cloacal tail piece reduced. Tail is pigmented. The forelimbs are visible through the skin.

Stage.42: Both fore limbs emerge (1412 hours).

The body sizes of the juveniles are 9.24-9.25mm and tail lengths are 13.4-13.49mm. Both the forelimbs emerge. Right fore limb emerges first by forming a pore or window and left forelimb emerges through spiracle. The tail becomes completely pigmented and dark brown in color. Position of the spiracle is changed, present on the middle of the belly.

Fig. 2.14: Fore limbs emerge (stage42)

Fig.2.15: Tail reduced (stage 43)

Fig.2.16: Froglet(stage 46).

PLATE .10: Gosner's stages 42,43 and 46 of *Uperodon globulosus*

Stage.43: Angle of mouth between the eye and nostril (1436 hours).

The body sizes of the animals are 10.2-10.23mm and tail lengths are 11.6-11.64mm. The angle of mouth is between the nostrils and the eyes. Both dorsal and ventral fins shrink and the length of the tail is reduced but still longer than the hind limbs.

Stage.44: Angle of mouth reaches the level of middle of the eye (1460 hours).

The body sizes of the animals are 10.2-10.21mm and tail lengths are 6.4-6.48mm. The angle of mouth reaches the level of middle of the eyes. Spiracle remains as a median pore.

Stage.45: Angle of mouth reaches posterior margin of the eye (1484 hours).

The body sizes of the animals are 9.2-9.25mm and tail lengths are 1.0-1.1mm. The angle of mouth reaches the posterior margin of the eyes. Spiracle completely closed.

Stage.46: Metamorphosed into froglet (1560 hours).

The tailed frog metamorphoses into a tailless frog. The sizes of the froglets vary from 9.0-9.2mm.

General description of the stage 38 tadpole.

The external morphology is based on a specimen in stage 38 (TL 25.82mm and BL 10.2mm). Morphometric data are provided for 10 tadpoles of stage 38 (**TABLE.1**).

Fig.2.17: Dorsal & ventral view of stage 38 tadpole Fig.2.18: Oral disc of stage 38 tadpole.

PLATE.11: Tadpoles of stage 38 and oral disc of stage 38 tadpole of *Uperodon globulosus*.

TABLE.1: Morphometric data of a sample of 10 tadpoles in Gosner's stage 38 of *Uperodon globulosus* and *Microhyla ornata.* Values shown are mean (mm), ± standard deviation and range in parentheses.

Measurement	*Uperodon globulosus*	*Microhyla ornata*
TL	*24.51±0.980 (23.34-25.82)*	17.94±0.511 (17.46-18.48)
BL	9.37±0.627 (8.44-10.2)	6.68±0.360 (6.44-7.1)
BW	6.41±0.450 (5.86-7.14)	4.84±0.011 (4.84-4.86)
BH	4.60±0.653 (4.1-5.74)	4.11±0.120 (4.0-4.24)
IOD	6.07±0.218 (5.77-6.4)	2.83±0.063 (2.8-2.91)
NN	1.72±0.140 (1.48-1.84)	0.63±0.057 (0.6-0.7)
ED	*1.02±0.1 (0.92-1.2)*	0.82±0.034 (0.8-0.86)
NP	2.52±0.386 (2.1-3.1)	1.4±0.087 (1.3-1.46)
ODW	2.17±0.163 (2.0-2.44)	1.12±0.087 (1.02-1.18)
SS	8.87±0.538 (8.0-9.3)	5.86±0.057 (5.8-5.9)
RN	2.16±0185 (1.87-2.4)	1.7±0.1 (1.6-1.8)
SE	3.74±0.509 (3.1-4.5)	2.75±0.05 (2.7-2.8)
SpE	6.02±0.426 (5.4-6.4)	4.0±0.1 (3.9-4.1)
SN	6.93±0.529 (6.2-7.4)	4.78±0.076 (4.7-4.85)
TAL	15.13±0.420 (14.66-15.8)	11.0±0.632 (10.46-11.7)
UF	2.29±0.254 (2.08-2.73)	1.13±0.07 (1.06-1.2)
LF	2.44±0.399 (2.1-3.1)	1.35±0.05 (1.3-1.4)
MTH	6.49±0.491 (6.14-7.34)	3.51±0.120 (3.4-3.64)
TMH	2.33±0.338 (2.0-2.9)	1.0±0.1 (0.9-1.1)
HLL	2.45±0.531 (1.98-3.32)	2.98±0.102 (2.9-3.1)

External morphology

In dorsal view, body roughly oval in shape, snout rounded. In lateral view, body depressed, BW 143.51% of BH. Eyes moderate, ED 9.96% of BL, bulging, positioned dorso laterally and directed laterally. Pineal ocellus is not found. Nares rounded, positioned dorsally and anterolaterally, inter naral distance comparatively small than inter ocular distance, NN 30% of IOD. The spiracle is medial above the anal aperture, SS 95.53% of BL. Tail musculature moderate, TMH of BH and 36.18% of MTH. Tail fin moderate in size and upper tail fin slightly larger than lower one, UF 35.09% of MTH and LF 33.85% of MTH. Anal tube moderately large, tubular, medial and directed posteriorly, entirely attached to ventral fin, opening large, postero ventrally directed. Mouth is antero dorsal, not visible from ventral side. Upper lip is completed as a simple curved, thick flesh without jaw sheath and continuous with a folded flap on each side of mouth. Lower lip is a thin layer, with a U- shaped notch.

Live coloration

Dorsally body is with brown pigments. Ventrally light brown with slight pinkish coloration. Tail musculature is dark brown with light stripes in stage 38. Both the fins are light brown and transparent with dark pigments. Tip of tail is black.

TABLE.2: Developmental rate of embryonic phases of *Microhyla ornata* and *Uperodon globulosus* under laboratory conditions. Time is measured from time of egg laying and the number of specimens measured is five for each stage (n=5), BL, body length, TAL, tail length.

Gosner's Stages	*Microhyla ornata*			*Uperodon globulosus*		
	Age in hours	BL in mm	TAL in mm	Age in hours	BL in mm	TAL in mm
1	0.00	0.8	-	0.00	1.3	-
2	0.30	0.9	-	1.00	1.3	-
3	1.00	0.9	-	2.00	1.3	-
4	1.30	0.9	-	3.00	1.4	-
5	2.00	0.9	-	4.00	1.4	-
6	*2.30*	0.9	-	5.00	1.45	-
7	3.00	0.9	-	6.00	1.45	-
8	3.30	0.9	-	7.00	1.45	-
9	4.30	0.9	-	8.00	1.45	-
10	6.00	0.9	-	9.00	1.45	-
11	7.00	0.9	-	11.00	1.45	-
12	9.00	0.9-0.91	-	12.00	1.55-1.56	-
13	10.00	1.3-1.32	-	12.30	1.7-1.71	-
14	11.00	1.3-1.33	-	13.00	1.9-1.92	-
15	12.00	1.32-1.34	-	13.30	2.0-2.02	-
16	13.00	1.32-1.34	-	14.00	2.5-2.51	-
17	14.00	1.7-1.72	0.2-0.21	14.30	2.5-2.52	0.45-0.47
18	18.00	2.0-2.01	0.5-0.52	15.30	2.7-2.73	0.65-0.67
19	24.00	2.0-2.02	0.7-0.71	18.00	3.0-3.02	0.7-0.73
20	26.00	2.0-2.02	2.5-2.53	20.30	3.05-3.07	0.85-0.87
21	34.00	1.7-1.73	2.8-2.83	24.30	3.4-3.42	1.0-1.05
22	48.00	1.8-1.82	3.0-3.04	29.00	3.85-3.86	1.55-1.58
23	72.00	1.56-1.58	3.1-3.12	36.00	2.4-2.43	4.0-4.04
24	73.00	1.56-1.58	3.1-3.13	48.00	2.4-2.45	4.05-4.1
25	84.00	1.64-1.65	4.42-4.45	56.00	2.54-2.56	4.28-4.3
26	120.00	2.1-2.12	4.45-4.52	72.00	3.0-3.02	4.94-4.97
27	132.00	2.3-2.31	5.1-5.14	96.00	3.7-3.71	5.7-5.73
28	240.00	3.0-3.02	5.3-5.32	216.00	5.0-5.03	7.0-7.08
29	360.00	3.4-3.41	5.4-5.41	408.00	5.86-5.87	9.27-9.3
30	456.00	4.12-4.14	7.8-7.83	600.00	6.14-6.18	9.75-9.79
31	528.00	5.6-5.62	8.9-8.92	792.00	6.7-6.75	10.6-10.65
32	600.00	5.7-5.71	9.2-9.24	864.00	7.0-7.06	11.18-11.2
33	672.00	6.2-6.21	9.6-9.62	936.00	7.92-7.95	12.1-12.2
34	744.00	6.2-6.22	10.1-10.2	1008.00	8.36-8.38	12.76-12.8
35	816.00	6.36-6.38	10.63-10.64	1056.00	9.66-9.69	16.0-16.05
36	864.00	6.36-6.38	10.64-10.67	1128.00	9.68-9.7	16.84-16.87
37	912.00	6.38-6.39	11.2-11.24	1152.00	9.86-9.89	17.24-17.3
38	984.00	6.48-6.49	12.6-12.62	1176.00	10.16-10.2	18.1-18.2
39	1032.00	6.49-7.01	12.8-12.83	1224.00	10.2-10.25	16.26-16.5
40	1056.00	6.5-6.52	11.2-11.24	1272.00	10.02-10.1	14.76-14.8
41	1080.00	6.5-6.52	10.9-10.93	1296.00	9.4-9.47	14.5-14.58
42	1104.00	6.8-6.82	10.9-10.95	1412.00	9.24-9.25	13.4-13.49
43	1116.00	7.21-7.23	8.3-8.36	1436.00	10.2-10.23	11.6-11.64
44	1128.00	7.68-7.7	4.4-4.43	1460.00	10.2-10.21	6.4-6.48
45	1152.00	7.4-7.46	2.1-2.17	1484.00	9.2-9.25	1.0-1.1
46	1176.00	7.3-7.36	0.0	1560.00	9.0-9.2	0.0

Ontogeny of the tadpoles

M. ornata completed its life cycle in 1176 hrs (49 days) and *U. globulosus* in 1560 hrs (72 days), (**TABLE.2**). The size of eggs, larvae and tadpoles of *U. globulosus* are larger than *M. ornata*. Development of *M. ornata* is faster during cleavage to first tail bud stage (Gosner's stage 17) than in *U. globulosus* (14hours in *M. ornata* and 14.30 hours in *U. globulosus*). From stage 18, *U. globulosus* development is faster and it reaches feeding larval stage (Gosner's stage 25) in 56 hrs, whereas *M. ornata* takes 84 hrs. Hatching occurs in both the species at stage 20 (Gosner's) and the time taken for hatching in *M. ornata* is 26 hrs. Which is longer than the time taken for hatching in *U. globulosus* (20.30 hrs). In post embryonic stages, *U.*

globulosus, take 1176 hrs to reach morphological climax stage (Gosner's stage 38) whereas *M. ornata* takes only 984 hrs. Metamorphosis stages (stage 42 to stage 46) are also faster in *M. ornata* than in *U. globulosus* (*M. ornata*, 1104- 1176 hrs, total time taken is 72 hrs and *U. globulosus*, 1412- 1560 hrs, total time taken is 148 hrs). Hind limb is comparatively longer and well developed in *M. ornata* but is shorter in *U. globulosus*. Forelimbs are visible through transparent skin at stage 38 in *M. ornata* but are not prominent in *U. globulosus*. Rounded inner metatarsal tubercle becomes shovel shaped at stage 40 in *U. globulosus* but it remains rounded in froglet of *M. ornata*. Toe pads are developed in both the species at stage 40.

DISCUSSION

Most anuran larvae have considerable flexibility in their rates of growth and development (Wilbur and Collins, 1973). In the present study development of two species belonging to same genus was monitored in uniform ecological conditions and variations in time taken for attaining different landmark characteristics were noted. A host of factors has been suggested as potential influences on the rates of growth, development and survival of tadpoles in nature (Alford and Jackson, 1993). A model of influences on development, growth and survival of tadpoles proposed by Wilber and Collins(1973) stimulated a great deal of work. They noted that the developmental rates and metamorphic sizes of individuals of the same species often vary widely within a given physical environment. They suggested that this might be caused by an evoloutionary advantage conferred on species that are able to adjust their developmental rate in response to their growth rate.

TABLE .3: Comparision of external morphological characters between *U. globulosus* and *M. ornata* (Gosner's stage 38)

Characters	*M. ornata*	*U. globulosus*
Body shape	Oval	Roughly oval
Position of mouth	Antero-dorsal	Antero-dorsal
Position of eyes	Lateral	Lateral
Interorbital distance compare to snout ocular distance	Big	Big
Position of spiracle	Posterior part of the abdomen	Above the anal aperture
Position of vent	At the end of the body	At the end of the body
Pineal ocellus	Visible	Not visible
Shape of tail tip	Taper	Taper
Tail musculature	Unicolored	White striped
Tail fins	Not uniform	Uniform
Origin of dorsal fin	Tail body junction	Tail body junction
Life color	Creamy white (transparent)	Brown (not transparent)
LTRF	Absent	Absent
Papillae in middle of upper labium	Absent	Absent
Papillae in middle of lower labium	Absent	Absent
Submarginal papillae	Absent	Absent
Jaw sheath	Absent	Absent

Clutch size is positively correlated with female size (Salthe and Duellman, 1973). Clutch size *U. globulosus* is larger (750-900 eggs) than *M. ornata* (300-510 eggs), but hatching success of larvae are comparatively less than *M. ornata* (66.6-80% in *U. globulosus* & 91.1- 93.3% in *M. ornata*). Therefore, population size of *M. ornata* is larger than *U. globulosus*.

The size of the egg is large in *U. globulosus* than in *M. ornata* (1.7mm and 1.3mm). Larger eggs can be the result of a greater genomic size, which gives rise to larger cells that must be less numerous to maintain scaling ratio so their division rate must be reduced (Chipman, 2002). Growth of *U. globulosus* is slower in cleavage stages than in *M. ornata*. After tail bud formation, from stage 18 the picture changes and the larva develops faster but again slow down from stage 25.

In microhylid frogs, the normal development rate is fast. But development rate of complete life cycle of *U. globulosus* is slower in comparison to *M. ornata* (72 days or 1560 hrs in *U. globulosus* and 49 days or 1176 hrs in *M. ornata*) and south Indian microhylid, *Ramanella variegata* (32 days, Dutta et al., 1990-91), but longest in *R. Montana* (160 days, Krishna et al., 2004). *U. globulosus* has slower development rate like other ranid frogs studied in North East India namely 1680 hrs in *Rana Khare* (Ao et al., 2006), 1350 hrs in *Hylarana humeralis* and 1313 hrs in *Hylarana leptoglossa* (Bortamuli et al., 2010).

Both the species hatch at stage 20 (Gosner's stage) and the embryo of *U. globulosus* hatch into larva in 20.30 hrs, 5.30 hrs earlier than in *M. ornata* which hatches at 26 hrs. Later this development rate slows down after becoming free swimming tadpole and takes longer period to reach morphological climax i.e. stage 38 in1176 hrs whereas *M. ornata* takes only 984 hrs. In comparision to *M. ornata,* oral disc is well developed in *U. globulosus*. Well developed oral disc probably helps in better utilization of food material available in the habitat.

External morphology is similar to other Microhylid tadpoles, which have a single mid ventral spiracle and non-keratinized mouthparts (Orton,1953). In *M. ornata*

and *U. globulosus* tadpoles vary only in a few morphological structures (TABLE 3). The tadpole of *M. ornata* has a dorsally transparent, oval shaped body and tail musculature of the tadpole is very thin. On the other hand tadpole of *U. globulosus* has dark pigmented, roughly oval body and tail musculature is moderate in size. Pineal ocellus is visible at stage 38 in *M. ornata* but it is not visible in *U. globulosus*. Oral disc is more developed in *U. globulosus*. In *U. globulosus* dorsal and ventral tail fins are not uniform in *M .ornata*. Hind limbs and toe pads are well developed in metamorphosis stages in *M. ornata*, whereas hind limb is very short and very poor development of toe pads in the tadpole of *U. globulosus*.

Living organisms have distinguishing morphological adaptation at different stages in their life as surrounding environment. Metamorphosis in amphibian is not new in this regards. There is an observation that toe pads develop at stage 40 of metamorphic tadpoles of both the species and remain present at juvenile and adult in *M. ornata*. But it disappeares in juvenile and completely absent in adult frog of *U. globulosus*. Presence of toe pad in metamorphosis stages is an adaptation to help landing of animal from aquatic to terrestrial life and proved that it is common at this stage for all species.

In the present study it has been found that breeding and development of the microhylid frogs are influenced by season, habitat, water quantity and food availability in the habitat. The study will enrich the database of frog development for taking future conservation measures.

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