

**STUDIES ON ECOLOGY, BREEDING BEHAVIOUR AND METAMORPHOSIS
OF TREE FROGS *Polypedates leucomystax* AND *Rhacophorus bipunctatus*
(ANURA: RHACOPHORIDAE) IN MEGHALAYA (NORTH-EAST INDIA)**

(ABSTRACT)

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Head
Department of Zoology
North Eastern Hill University
Shillong - 793022

BY

**ASORPHI JANAI IANGRAI
DEPARTMENT OF ZOOLOGY**

**SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT OF THE
DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY**

OF

**NORTH EASTERN HILL UNIVERSITY
SHILLONG - 793022**

ABSTRACT

The thesis incorporates studies on ecology, breeding behaviour and metamorphosis of tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* (Anura: Rhacophoridae) in Meghalaya, Northeast India. The family Rhacophoridae consists of small to large sized frogs. Rhacophorids are arboreal frogs, and exhibit specialized breeding habits. From the review of literature, it revealed that earlier work in the state of Meghalaya had been mainly focused on the survey of the amphibian fauna. Although *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 have been reported from Meghalaya, there is practically no information on ecology of habitat, breeding behaviour and metamorphosis of these two tree frogs found in Meghalaya. Therefore, the present study was undertaken to investigate distribution, breeding behaviour, habit and habitat, development and metamorphosis and the ecological factors and water chemistry (e.g., temperature and pH) of the breeding sites, food and feeding of the tadpoles and adult frogs, and the morphological changes of the oral structure of the tadpoles, and ultrastructure of the skin of these two species of tree frog in Meghalaya.

Objectives: The objectives of the proposed investigation were to study the following:

1. Distribution of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Meghalaya.
2. Breeding behaviour, habit and habitat of *Polypedates leucomystax* and *Rhacophorus bipunctatus*.

3. Development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the ecological factors and water chemistry (e.g., temperature and pH) of the breeding sites.
4. Food and feeding of the tadpoles and adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the morphological changes of the oral structure of the tadpoles.
5. Ultrastructure of the skin of *Polypedates leucomystax* and *Rhacophorus bipunctatus*.

The findings of the present study have been presented in five chapters given below:

Chapter I - Distribution of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Meghalaya:

Extensive survey and collection were conducted during the years 2002, 2003 and 2004 throughout the state of Meghalaya. The morphometric measurements of the collected frogs were recorded following the criteria given by Chanda (1994). *Polypedates leucomystax* was collected from nineteen surveyed sites, namely Cherrapunjee, Shillong, Mawsynram, Mawphlang, Pynursla, Balat, Mawkyrwat, Nongstoin, Mairang, Umsning, Nongpoh, Jowai, Dawki, Baghmara, Williamnagar, Rongjeng, Tura, Garobadha and Dadenggiri. These collection sites ranged from higher mid-altitude between 1300 m – 1700 m asl to lower mid-altitude between 120 m – 770 m asl, and lower altitude regions ranging between 15 m – 40 m asl. The morphometric measurements of the males collected from different parts of the state ranged from 42 to 66 mm with a mean of

45.0±5.23 mm, and than that of the females ranged from 45 to 78 mm with a mean of 62.0±7.60 mm.

While *Rhacophorus bipunctatus* was collected from only ten survey sites, namely Cherrapunjee, Shillong, Mawsynram, Pynursla, Nongstoin, Mawkyrwat, Jowai, Thadlaskein, Nongpoh and Umsning. These collection sites of *Rhacophorus bipunctatus* ranged from higher mid-altitude regions between 1300 m – 1560 m asl, and also from lower mid-altitude regions ranging from 560 m to 770 m asl. The morphometric measurements of the males collected from different parts of the state ranged from 39 mm to 45 mm with a mean of 43.5±0.42 mm, and the females ranged from 63 mm to 65 mm with a mean of 64.0±0.21mm.

Thus, this investigation provides an information about the present status of distribution of *Polypedates leucomystax* collected from nineteen sites and *Rhacophorus bipunctatus* collected from ten sites located in different parts of Meghalaya. The present study revealed that *Polypedates leucomystax* was widely distributed and was collected from all the seven districts of Meghalaya, namely East Khasi Hills, West Khasi Hills, Jaintia Hills, Ri Bhoi, West Garo Hills, East Garo Hills and South Garo Hills districts, while *Rhacophorus bipunctatus* was collected from only four districts, namely East Khasi Hills, West Khasi Hills, Jaintia Hills and Ri Bhoi districts. *Polypedates leucomystax* inhabits the forests of Meghalaya irrespective of the altitudes ranging from 15 m to 1700 m asl indicating that it has diverse adaptations to survive at low and high altitudes. While *Rhacophorus bipunctatus* preferred higher and lower mid-altitude regions ranging

from 560 m to 1560 m asl with cooler climate. However, both tree frogs preferred ephemeral pools with some vegetation surrounding it as their habitat during breeding season. These temporary pools and puddles, which are formed during the monsoon, provide excellent habitat for breeding, development and metamorphosis of these tree frogs.

Chapter II - Breeding behaviour, habit and habitat of *Polypedates leucomystax* and *Rhacophorus bipunctatus*:

This chapter deals with studies on breeding behaviour, habit and habitat of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in relation to some ecological factors (temperature, relative humidity and rainfall) at Cherrapunjee, Meghalaya. Cherrapunjee (locally known as Sohra) located on the regular course of the south-west monsoons is renowned for receiving maximum rainfall in the world. Breeding behaviour of the tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* was studied with the help of photographic documentation and observations were made on the courtship, mating calls, foam nest construction, size of amplexing frogs, clutch size, and sex ratio at the study sites I, II, III and IV at Cherrapunjee. Collection and preservation of plant specimens were done following the method described by Jain and Rao (1976). Identification of the collected specimens was done by comparing with herbarium specimens in the Department of Botany, North-Eastern Hill University, Shillong and Botanical Survey of India, Eastern Circle, Shillong.

The four study sites I, II, III and IV were temporary pools surrounded by a grassy field. Some of the plants found near these study sites were identified as *Imperata cylindrical*, *Hydrilla sp.*, *Gleichenia sp.*, *Rhododendron arboretum*, *Alnus nepalensis* etc.

The breeding phase of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* at Cherrapunjee was similar, and coincided with the rainy season (April to August), when air temperature and relative humidity were comparatively high. During the breeding phase (April to August), air temperature ranged between 21.3°C – 25.7°C, relative humidity between 68 % - 89 %, and rainfall between 501.5 mm – 5008.2 mm.

Polypedates leucomystax emerged from its hibernation in the month of March, and spawning took place from the month of April to August, when there was accumulation of water in the ephemeral pools so that the frogs could carry on their breeding activity without fear of desiccation. After emergence, males moved towards the breeding sites, and started making advertisement calls only with the arrival of the rain, which oriented the female to come to the breeding site for spawning. Each call was composed of three long notes. The female responded by moving to the breeding sites and approaching towards the male leading to courtship. In *Polypedates leucomystax*, the males were seen to choose the oviposition sites. This tree frog was observed to breed in shallow ephemeral ponds. Foam nest were constructed on margins of the pools about 5-40 cm above the water surface, in between stones or rocks and cemented tank, while some nests were also found about 1 m away from the water body attached to grassy edges, which served as substratum to the foam nest. The diameter of the foam nests

ranged between 6-12 cm, and clutch size between 252-530 in different foam nests. The eggs measured 1.5 mm in diameter.

Similarly, *Rhacophorus bipunctatus* also emerged in the month of March and its breeding activity started from the month of April and continued till August. Advertisement calls were heard only after a few showers of rain, and each call was composed of six long notes, which differed from that of *Polypedates leucomystax* indicating that advertisement calls were species specific. It was observed that in *Rhacophorus bipunctatus*, the amplexing pair was involved in choosing the oviposition site. The amplexing pair moved away from the water body and constructed foam nests on the grassy edges, which served as a substratum to the foam nests closer to a water body. This species also constructed foam nest on branches of trees hanging over a water body. The diameter of the foam nests ranged between 6-8 cm and the clutch size between 45-76 in different foam nests and were comparatively smaller than that of *Polypedates leucomystax*. The eggs measured 3.0 mm in diameter.

In both the species, it was found that the males usually outnumbered the females at the breeding sites during the breeding period. The breeding cycle of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was similar, and it was divided into four phases - hibernating phase, emerging and pre-breeding phase, breeding and spawning phase, and postbreeding phase. Both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were found to hibernate during the months of November to February (air temperature: 13.5°C - 20.5°C, relative humidity: 42 % - 69 %, and rainfall: 0.0 mm –

298.6 mm). During hibernating phase the frogs were found to be hidden under the forest litters in case of *Polypedates leucomystax*, where as *Rhacophorus bipunctatus* were found hiding in banana stem. Both the frogs emerged from hibernation in the month of March (air temperature: 20.6°C – 23.8°C, relative humidity: 52 % - 88 %, and rainfall: 163.2 mm – 1119.6 mm). Further, breeding and spawning took place from the month of April to August (air temperature: 21.3°C – 25.7°C, relative humidity: 65 % - 89 %, and rainfall: 501.5 mm - 5008.2 mm). During the months of September and October (air temperature: 19.4°C – 22.6°C, relative humidity: 54 % - 79 %, and rainfall: 66.3 mm - 1917.3 mm) no more foam nests were observed at the breeding sites and the adult frogs of both the species moved away from the breeding site to hibernation site and thereby exhibited the post-breeding phase.

Histological study of the testis of *Polypedates leucomystax* and *Rhacophorus bipunctatus* showed the presence of different spermatogenic cells during the different periods of the breeding cycle. In *Polypedates leucomystax* during the hibernation phase spermatogonia and spermatocytes were present in large number, and during the emergence phase seminiferous tubules were distinct and spermatids were appeared in cluster. Spermatozoa were found during the breeding phase and were observed to be slender and filamentous. During the post-breeding phase spermatogonia and only a few spermatids were observed. In *Rhacophorus bipunctatus*, during the hibernation phase a large number of spermatogonia and spermatocytes were present. Spermatids were also found to be present but were less in number. During the emergence phase spermatids and a few spermatozoa were observed, and during the breeding phase the seminiferous

tubules were filled with spermatozoa. During the post-breeding phase a large number of spermatogonia were present. In both *Polypedates leucomystax* and *Rhacophorus bipunctatus*, during the hibernation phase the ovary appeared as a membranous structure and during the emergence phase only a few number of oogonia were present. Young and mature oocytes filled up the ovary during the breeding period and during the post breeding period no oocytes were observed.

On the basis of the present findings, it may be concluded that the breeding behaviour as well breeding cycle of both the species of the tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* are similar. The ecological factors, air temperature and rainfall seem to play a major role in regulation of breeding activity and breeding cycle of both the species. The differences observed in advertisement calls, foam nest size, clutch size and egg size in these species seem to be species-specific adaptations to ensure successful breeding in variable niches specific for these two species. The foam nest and clutch size of *Polypedates leucomystax* was larger than that of *Rhacophorus bipunctatus*, while the eggs of *Polypedates leucomystax* were found to be smaller in size than that of *Rhacophorus bipunctatus*. It may be suggested that in *Rhacophorus bipunctatus* the small clutch size provides optimal conditions for large size eggs in smaller foam nest due to its arboreal nesting behaviour.

Chapter III - Development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the ecological factors and water chemistry (e.g., temperature and pH) of the breeding sites:

This chapter deals with the studies on development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in relation to some ecological factors (rainfall, relative humidity, air and water temperature and water pH) at the study sites I, II, III and IV at Cherrapunjee, Meghalaya. Some of the foam nests collected from the study sites were maintained in a plastic tray containing pond water under laboratory conditions to allow further development and metamorphosis, which were observed under a stereoscopic dissecting binocular microscope. Staging of the different developmental stages of both the species were carried out as per the criteria described by Gosner (1960), and photographs of the different developmental and metamorphic stages were also taken.

Polypedates leucomystax completed its development and metamorphosis in about 60-61 days in the natural environment as well as under laboratory conditions. In *Rhacophorus bipunctatus*, complete development and metamorphosis took about 59-60 days in the natural environment as well as under laboratory conditions. Thus, the duration of development and metamorphosis in both the tree frog species was similar. The mortality percentage of *Polypedates leucomystax* recorded under laboratory condition ranged between 34.48 % - 84.32 % with a mean of 62.13 ± 0.20 , while in *Rhacophorus bipunctatus* it ranged between 41.13 % - 92.13 % with a mean of 68.45 ± 1.18 .

Each developmental stage has been based on the new external morphological changes. The size of the eggs of *Polypedates leucomystax* measured about 1.5 mm in diameter and were found to be comparatively smaller than those of *Rhacophorus bipunctatus*, which measured about 3 mm in diameter. Comparatively larger size of the egg and the presence of large amount of yolk in *Rhacophorus bipunctatus* might be an adaptation to its arboreal mode of life. It is important to mention that association of fertilized eggs of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* with the foam nests was found to be essential up to hatching for further normal development. It, thus, seems that the foam nest is a pre-requisite and may provide optimal conditions for the early embryonic development up to hatching in tree frogs. In both *Polypedates leucomystax* and *Rhacophorus bipunctatus*, hatching was observed after four days of fertilization indicating rapid developmental rate.

Water temperature of the study sites I and II, where development and metamorphosis of *Polypedates leucomystax* took place, ranged between 18.4°C – 24.0°C, while that of study sites III and IV of *Rhacophorus bipunctatus* ranged between 18.2°C – 23.4°C. The water pH ranged between 6.8 - 7.2 in the four study sites (I, II, III and IV) during the development of the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus*. The water temperature and water pH of the four study sites were similar and provided a suitable environmental condition for the development and metamorphosis of the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* during their life cycle. At Cherrapunjee, during the period of development and

metamorphosis (April to October) atmospheric temperature ranged between 19.4°C – 25.7°C, rainfall between 66.3 – 5008.2 mm, and relative humidity between 54 % - 89 %.

On the basis of the present findings, it may be concluded that there was a similarity in the development and metamorphosis of both *Polypedates leucomystax* and *Rhacophorus bipunctatus*. These tree frogs developed and metamorphosed successfully in the water temperature range of 18° C to 24° C and in the water pH range of 6.8 – 7.2 in the natural environment. The foam nests seem to provide optimal conditions for the early development and ensure successful normal hatching of the larva. Comparatively the eggs of *Rhacophorus bipunctatus* were larger in size than that of *Polypedates leucomystax*, and this might be an adaptation of *Rhacophorus bipunctatus* to its arboreal mode of life and higher altitude habitat. In *Rhacophorus bipunctatus*, the small clutch size, larger size eggs and large amount of yolk may serve as adaptations to ensure higher rate of survival of the embryos away from water bodies.

Chapter IV - Food and feeding of the tadpoles and adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the morphological changes of the oral structure of the tadpoles:

This chapter deals with food and feeding habits of the tadpoles and adults of *Polypedates leucomystax* and *Rhacophorus bipunctatus* and the oral structures of the tadpoles of both species. Adult frogs and different developmental stages of the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were collected from the study

sites I, II, III and IV at Cherrapunjee. The tadpoles were staged according to Gosner (1960) and autopsy was done for analysis of gut content. Identification of the food items present in the gut of the tadpoles was made following the methods described by Edmonson (1959). The oral structure of the developing tadpoles during metamorphosis comprising the premetamorphic, prometamorphic and metamorphic climax were studied using stereoscopic binocular microscope and also with the help of Scanning Electron Microscopy.

It was observed that the tadpoles of both the species started feeding from stage 25 onwards. During the premetamorphic stages the tadpoles fed mostly on the detritus, plant materials and phytoplankton, and during the prometamorphic stages they fed both on phytoplankton and Zooplankton. The tadpoles were also observed to exhibit cannibalism in the natural environment as well as under laboratory conditions. From stage 40 onwards the tadpoles stopped feeding. The food items of the tadpoles of both the species were similar suggesting that it might be due to the availability and composition of the same food items in similar habitat occupied by the tadpoles of both the tree frog species, indicating that the tadpoles utilized a wide variety of food resources available in the ephemeral pools. The food items of the adults of both the tree frog species were similar and included mostly insects.

Under scanning electron microscopic studies it was found that development of the teeth of the tadpoles of both the species began from stage 25. The number of teeth rows of the tadpoles of the two tree frog species was different and species specific, and

changed with the progress of development and metamorphosis. It may be suggested that increase in the number of teeth rows might be helpful in the feeding of a wide variety of food items consumed by the tadpoles during metamorphosis. Complete dental formula was observed at stages between 31-39. The dental formula of *Polypedates leucomystax* was 1:3+3/3, while that of *Rhacophorus bipunctatus* was 2:3+3/3. Thus, the dental formula of the tadpoles of the two species was different and species specific. The oral disc of the tadpoles of both the tree frog species was ventral, which might be correlated to the feeding habits for scrapping food and capturing of prey. The oral disc was surrounded by papillae only on the lateral and ventral sides and the arrangement of these papillae may act as a diagnostic feature. With the onset of metamorphosis teeth began to shed off and the metamorphosed froglets had a developed tongue.

Chapter V - Ultrastructure of the skin of *Polypedates leucomystax* and *Rhacophorus bipunctatus*:

Ultrastructure of the skin of the adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was also studied during the breeding phase. Adult males and females of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were collected from the study sites I, II, III and IV at Cherrapunjee, anesthetized and the sections of the skin from the dorsal and ventral parts of male and females of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were processed for transmission electron microscopic studies.

Transmission electron microscopic studies of the skin of *Polypedates leucomystax* and *Rhacophorus bipunctatus* revealed that the skin was similar in its ultrastructure. The epidermis of both the frog species was composed of four layers namely, stratum corneum, stratum granulosum, stratum spinosum, and stratum germinativum. The outer most layer the stratum corneum was thickened and keratinized and consisted of a single layer of flattened cells. In the stratum granulosum some cells were observed to be devoid of a nucleus as these cells had prepared themselves to move to the stratum corneum, as stratum corneum sloughed or shed periodically and has to be replaced. Desmosomes, ribosomes, tonofilaments, golgi bodies, vacuoles and collagen fibres were also observed in the epidermal cells.

The epidermis was separated from the inner dermis by a basement membrane composed of collagenous fibres. The dermis was composed of two layers, the stratum spongiosum and the underlying stratum compactum. During the present study, pigment cells like pterinosomes, melanophores and iridophores were observed in both the species. These pigments cells may work together to determine the varied skin colouration and patterns displayed in *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and may have important roles in adapting these frog species to their environment.

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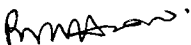
DECLARATION

I, Miss Asorphi Janai langrai, hereby declare that the subject matter of this thesis is the record of the work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University /Institute.

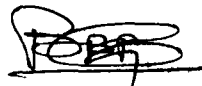
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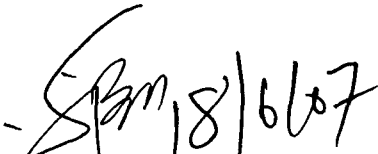
Candidate


(Prof. (Mrs.) R. N. K. Hooroo)

Supervisor


(Prof. B. B. P. Gupta)

Joint Supervisor


(Prof. S. B. Prasad)

Head

Head
Department of Zoology
North Eastern Hill University
Shillong - 793022

Dedicated to my mother

CONTENTS

	Page Number
➤ Contents	i - ii
➤ Acknowledgement	iii - iv
➤ General Introduction	1 - 5
➤ Chapter I: Distribution of <i>Polypedates leucomystax</i> (Gravenhorst, 1829) and <i>Rhacophorus bipunctatus</i> Ahl, 1927 in Meghalaya	6 - 29
❖ Introduction	6 - 12
❖ Materials and Methods	12 - 15
❖ Results	16 - 25
❖ Discussion	26 - 29
➤ Chapter II: Breeding behaviour, habit and habitat of <i>Polypedates leucomystax</i> and <i>Rhacophorus bipunctatus</i> .	30 - 63
❖ Introduction	30 - 39
❖ Materials and Methods	39 - 41
❖ Results	41 - 54
❖ Discussion	55 - 63
➤ Chapter III: Development and metamorphosis of <i>Polypedates leucomystax</i> and <i>Rhacophorus bipunctatus</i> , and the ecological factors and water chemistry (e.g., temperature and pH) of the breeding sites.	64 - 104
❖ Introduction	64 - 73
❖ Materials and Methods	73 - 75
❖ Results	75 - 98

❖ Discussion	99 - 104
➤ Chapter IV: Food and feeding of the tadpoles and adult frogs of <i>Polypedates leucomystax</i> and <i>Rhacophorus bipunctatus</i> , and the morphological changes of the oral structure of the tadpoles.	105 – 126
❖ Introduction	105 - 114
❖ Materials and Methods	114 - 115
❖ Results	115 - 121
❖ Discussion	122 – 126
➤ Chapter V: Ultrastructure of the skin of <i>Polypedates leucomystax</i> and <i>Rhacophorus bipunctatus</i> .	127 - 141
❖ Introduction	127 - 133
❖ Materials and Methods	133 - 134
❖ Results	134 - 137
❖ Discussion	138 - 141
➤ Conclusion	142 - 153
➤ References	154 - 218

Acknowledgement

First and foremost I thank God the Almighty for His countless blessings showered on me through out my research work.

My sincere gratitude goes to my supervisor Prof. (Mrs.) R. N. K. Hooroo for her guidance, encouragement, suggestions and patience. Her inspiration has helped me in completing my thesis.

I also take this opportunity to thank my Joint supervisor Prof. B. B. P. Gupta, for his guidance and valuable suggestions during the course of the investigation.

I am thankful to Prof. S. B. Prasad, Head, Department of Zoology, North Eastern Hill University, Shillong, for providing necessary laboratory facilities during the course of the investigation. I am also thankful to Prof. (Mrs.) V. Tandon, Dean, School of Life Sciences and Prof K. Chatterjee for their suggestions.

I express my gratefulness to Dr. S. Dey, SAIF, NEHU, for helping me with the scanning and transmission electron microscopic work. I express my sincere thanks to Dr. P. B. Gurung, Department of Botany, NEHU, Shillong for identification of the plant specimens. I am also thankful to Miss N. Sen, Zoological Survey of India, Shillong for her help in identification of the frogs.

My sincere thanks also go to Mr. K. Sohlang for his help in photography, Mr. E. Nongrum and Mr. S. Pradhan during my field work and Mrs. B. Dkhar, Mr. J. Nongkynrih and Mr. A. Shadap for their help rendered in many ways during my research work.

I am thankful to the office staffs and to all the non teaching staffs of the Department of Zoology, NEHU for their help rendered to me during my research work.

I remain grateful to my laboratory members Dr. Shanwell Khongwir, Miss Saipari Sailo, Mr. H. T. Lalremsanga, Mr. Duwaki Rangad, Mr. Ronald K. Lyngdoh and Miss Amesha Lyndem for their help rendered to me in many ways and without them I would not be able to execute my work.

My special thanks go to all my friends Dr. (Mrs.) C. Kikon, Miss K. Kharshiing, Mr. P. Suchiang, Dr. Y. Premabati, Dr. B. Nicol, Mr. P. Nongrum, Mr. D. Khongwir, Mr. and Mrs. D. Suiam, Mr. K. Lyngdoh, Mr. F. Kharrubon and many others for their support and encouragement.

Financial assistance in the form of a JRF awarded to me by the Department of Science and Technology, New Delhi is gratefully acknowledged.

Last but not the least I am indebted to my mother, brothers and sisters who have inspired and encouraged me, also with their dedication, perseverance and encouragement I am able to execute my work.


(Ms. Asorphi J. Iangrai)

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Amphibians are the first vertebrates that are called tetrapods. Amphibians live in a wide range of environment like grasslands, rain-forests, conifer forests, alpine areas, and even deserts. Most of amphibian species require freshwater habitats such as ponds, swamps, streams, or other wet environments for breeding. The term amphibian can be interpreted in two ways - either as an animal spending part of its life in water and then changing to an aquatic adult, or as an animal that alternates life in and out of water. They are an important ecological resource and are sensitive to a number of environmental factors, both natural and anthropogenic. Due to their sensitive skin and aquatic habit, amphibians are extremely useful as indicators of the state of our environment. Amphibians are very important components of many ecosystems and they may constitute the highest fraction of vertebrate biomass in some of these ecosystems (Blaustein *et al.*, 1994). Amphibians are both important prey and predators in many foodwebs (Duellman and Trueb, 1994) and contribute a major portion to the biomass in animal communities (Burton and Likens, 1975), any factor affecting amphibians can have far ranging effects on communities or ecosystems. Amphibians are usually least harmful in nature and never destroy or devour agricultural crops, fruits, vegetation, etc.

Annual breeding activity of amphibians in tropical and temperate regions is influenced by climatic factors. Although temperate anurans respond to rainfall, temperature seems to be a major factor initiating breeding activity, as evidenced by geographic variation in the time of breeding. In anurans, vocalizations by the male and tactile cues are important in courtship and mating. Sound production is primarily a reproductive function of male anurans. Advertisement calls attract females to breeding

areas, and announce to other males that a given territory is occupied. Advertisement calls are species specific, and any one species has a very limited repertoire of calls. Although most anurans are normally solitary, they often come together in large breeding aggregations during the spring or rainy season.

The typical pattern of reproduction in anurans begins when a male clasps a female in a process called amplexus and sheds sperm over the eggs as they are being extruded from the female into the water. Fertilization is almost always external and eggs and larvae are typically aquatic. Oviposition and fertilization are accomplished by synchronized movements by both partners. In anurans in which the pair is in amplexus prior to oviposition, the female usually chooses the oviposition site. In arboreal oviposition, the male maintains a close contact with the dorsum of the female and their vents are continuously juxtaposed. Female frogs of several species select oviposition sites to avoid predators of larvae (Resetarits and Wilbur, 1989, 1991; Petranka *et al.*, 1994) or conspecific eggs or larvae (Resetarits and Wilbur, 1989, 1991; Crump, 1991; Spieler and Linsenmair, 1997). Some amphibians lay their eggs and let them develop by themselves while others lay eggs and guard them.

Many amphibians metamorphose, or change from gill breathing aquatic larvae to air-breathing terrestrial adult. Metamorphosis is a post embryonic period of profound morphological changes by which the animal alters its mode of living. The morphological changes associated with metamorphosis in amphibians represent the change from an aquatic to a terrestrial mode of life. This variability of amphibians makes them one of the most interesting group to study, and amphibians have indeed contributed a great deal to science. They are most commonly used for studies on anatomy, reproduction and

hormones. The external fertilization and development of their eggs make them a prime candidate for investigations involving biological and mechanical manipulations.

Amphibians are grouped into three orders: Caudata (Urodela), Gymnophiona or Apoda (Caecilia) and Anura (Salientia), which are represented by a total of 5150 species all over the world, and by 209 species in India (Chanda, 1998). Among amphibians, the order Anura forms the largest living order, which is represented by a total of 4120 species under 17 families and 248 genera are known from the world, of which only 180 species under 6 families and 24 genera have been recorded from Indian region. Out of the 180 species, as many as 54 species under 6 families and 18 genera have been found to occur in Northeast India (Chanda, 1994). However, recently Sen (2004) recorded and reported 83 species of amphibian comprising 78 anurans, 4 gymnophiona and 1 caudata. The reported 78 species of anurans belong to 25 genera, 6 families and 1 order reported from the Northeast India and the state of Meghalaya is represented by 49 species of amphibians belonging to 8 families and 3 orders (Sen, 2004).

Meghalaya is part of northeast India, a "Hot-spot of Biodiversity," which houses a large number of flora and fauna. Its wide range of variations in topography and climatic conditions seems to be favourable for the distribution of anuran fauna. Meghalaya, which is known as the abode of clouds, is situated in North-eastern part of India between 25°47' and 26°10' N latitude and 89°47' and 92°47' E longitude. The state covers an area of 22,429 sq km, and consists of seven districts - East Khasi Hills, West Khasi Hills, Jaintia Hills, Ri Bhoi, West Garo Hills, East Garo Hills and South Garo Hills (Fig. 1). It is bounded on the north and east by Assam, on the west partly by Assam and partly by Bangladesh and on the south entirely by Bangladesh. It is renowned for heavy rainfall,

particularly in two of its towns, Cherrapunjee (locally known as Sohra) and Mawsynram located on the regular course of the south-west monsoons, which receive the maximum rainfall in the world. Rainfall seems to be the primary factor initiating breeding activity in amphibians. Several species of anurans belonging to the family Pelobatidae, Bufonidae, Hylidae, Microhylidae, Ranidae and Rhacophoridae have been reported from Meghalaya.

It is evident from the literature that earlier work in the state of Meghalaya has been mainly concentrated on the survey of the amphibian fauna. Although *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 have been reported from Meghalaya, there is practically no information on the distribution, ecology of habitat, breeding, development and metamorphosis of these two tree frogs in Meghalaya. Therefore, it was decided to study the distribution, breeding behaviour, development and metamorphosis, food and feeding and ultrastructure of the skin of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Meghalaya.

The objectives of the proposed investigation were to study the following:

1. Distribution of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Meghalaya.
2. Breeding behaviour, habit and habitat of *Polypedates leucomystax* and *Rhacophorus bipunctatus*.
3. Development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the ecological factors and water chemistry (e.g., temperature and pH) of the breeding sites.

4. Food and feeding of the tadpoles and adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the morphological changes of the oral structure of the tadpoles.
5. Ultrastructure of the skin of *Polypedates leucomystax* and *Rhacophorus bipunctatus*.

The present work has been divided into five chapters which deal with the distribution of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in the state of Meghalaya, the breeding behaviour, habit and habitat, development and metamorphosis in relation to some ecological factors and water chemistry (e.g., temperature and pH) of the breeding sites, food and feeding of the tadpoles and adult frogs and the morphological changes of oral structure of the tadpoles, and ultrastructure of the skin of *Polypedates leucomystax* and *Rhacophorus bipunctatus*.

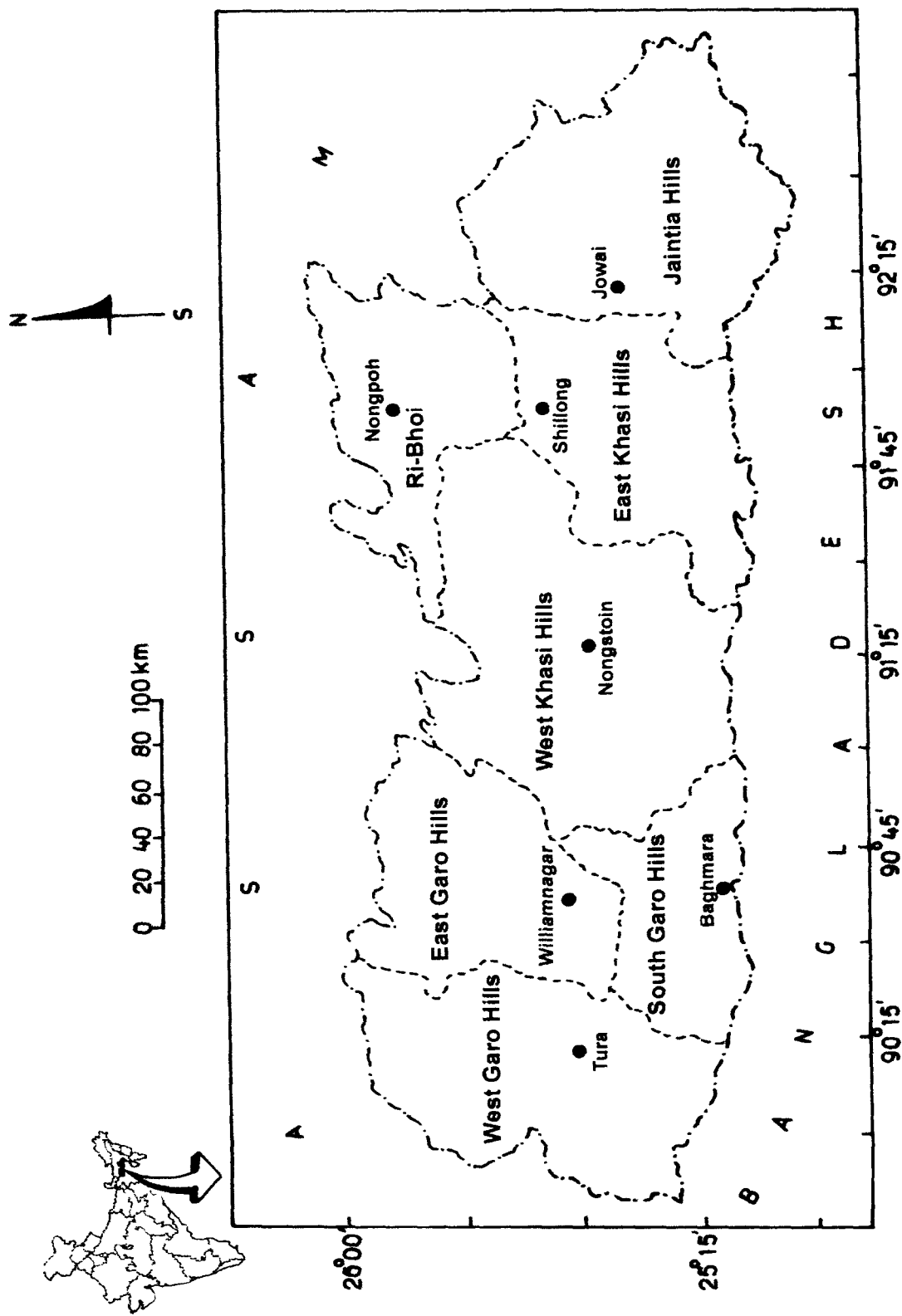


Fig. 1 - Map of Meghalaya showing the seven districts with Head quarters.

CHAPTER I

Distribution of *Polypedates leucomystax*
(Gravenhorst, 1829) and *Rhacophorus*
bipunctatus Ahl, 1927 in Meghalaya

CHAPTER I

Distribution of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Meghalaya

Introduction:

The family Rhacophoridae consists of small to large sized frogs. In India Rhacophorids inhabit the dense forest of the Western Ghats and Eastern Himalayas. Rhacophorids are arboreal frogs with long limbs and enlarged toe pads for holding on to leaves and branches of trees, and some genera have extensive webbing on the hands and feet. Frogs of this family have horizontal pupil, free and deeply notched tongue. They exhibit specialized breeding habits and construct foam nests on rocks or vegetation overhanging water bodies. At the time of hatching, the tadpoles drop into the water and undergo further development. In India, the family Rhacophoridae comprises of two sub-families (Rhacophorinae and Philautinae) and five genera (*Rhacophorus*, *Polypedates*, *Philautus*, *Chirixalus* and *Theloderma*). Sen (2004) reported two species of the genus *Polypedates*, five species of the genus *Rhacophorus* and six species of the genus *Philautus* from Northeast India.

The genus *Rhacophorus* Kuhl and van Hasselt, 1822 (Anura: Rhacophoridae), as defined by Brown and Alcalá (1994), is known to contain over 60 nominal species of which at least 41 occur in southeast Asia alone (Frost, 1985; Harvey *et al.*, 2002; Inger *et al.*, 1999; Iskander and Colijn, 2000; Ohler *et al.*, 2000; Vasudevan and Dutta, 2000). The members of the genus are distributed from southern China, Sri Lanka and southwestern and northeastern India south to Indo-China and Indo-Malaya (Frost, 1985).

Polypedates leucomystax (Gravenhorst, 1829) is an arboreal nocturnal tree frog commonly available during its breeding period of the family Rhacophoridae. It varies in colour from yellowish to brown. Similarly, *Rhacophorus bipunctatus* Ahl, 1927 is also an arboreal nocturnal tree frog of the family Rhacophoridae. It is commonly found hiding on leaves of trees during the summer. The colour of this frog is leafy green and the webs are red in colour.

Review of literature reveals that a number of reports, monograph and books have been published on the taxonomy and distribution of amphibians. The fauna of British India including Ceylon and Burma by Boulenger (1890), the amphibian fauna of Thailand by Taylor (1962), a monograph of the South Asian, Papuan, Melanesian and Australian frogs of the genus *Rana* by Boulenger (1920), Living Amphibians of the World by Cochran (1967) are some examples. Brown and Alcala (1970) studied the zoogeography of the herpetofauna of the Philippine Islands, a fringing Archipelago. Kuramoto (1973) reported the amphibians of Iriomote of the Ryu Kyu Islands with ecological and zoogeographical notes.

Hecnar and M'Closkey (1996) studied the amphibian species richness and distribution in relation to pond water chemistry in south-western Ontario, Canada. Neckel-Oliveira *et al.*, (2000) reported the diversity and distribution of frogs in an Amazonian savanna in Brazil. Measuring and monitoring amphibian diversity in tropical forest, an evaluation of methods with recommendations for standardization was studied by Rodel and Ernst, (2004). The anuran amphibians from a seasonally dry forest in Southeastern Peru and the amphibians of La Escalera region of South eastern Venezuela has been investigated (Duellman and Thomas, 1996; Duellman, 1997). An atlas of the

amphibians of China was given by Fei (1999) and further the South Asian amphibian and its adjacent islands have been studied (Dubois, 1999; Inger, 1999; Sukumaran, 2002; Stuart and Heatwole, 2004; Grismer *et al.*, 2004, Bain and Troung, 2004a). The amphibian fauna and a checklist and key to the amphibia of Bangladesh have been documented (Husain and Rahman, 1978; Khan, 2004) and that of Pakistan (Khan, 2002). Jungfer and Schiesari (1995) described a central Amazonian and Guianan tree frog, of the genus *Osteocephalus* (Anura: Hylidae) with oophagous tadpoles. Garcia *et al.*, (2003) studied on the taxonomic status of tree frog *Hyla pulchella joaquinii* (Anura: Hylidae) with description of its tadpole and vocalization.

New species of frogs have been reported from South East Asia, South American countries and other parts of the world by several workers: Dubois and Matsui (1983) reported a new species of frog (Genus: *Rana*, subgenus *Paa*) from Western Nepal (Amphibia: Anura). A new genus for an aquatic ranid (Amphibia, Anura) from Sri Lanka was reported (Dubois and Ohler, 2001). Three new species of narrow-mouth frogs (Genus: *Microhyla*) from Indochina, with comments on *Microhyla annamensis* and *Microhyla palmipes* have been described (Bain and Troung, 2004b). Recently, new species of frogs belonging to the family Rhacophoridae have been recorded e.g., *Philautus* from Northern Laos (Stuart and Heatwole, 2004), *Rhacophorus* from Myanmar (Burma) and Gunung, Gading, Sarawak (Wilkinson *et al.*, 2005; Das and Haas, 2005). Further, new species of frogs of the family Ranidae has been reported: three new species of spinule-bearing frogs allied to *Rana megatympanum* from Laos and Vietnam (Stuart and Bain, 2005), a new species of cascade frog with new data on *Rana banaorum* and

Rana morafkai from Thailand (Bain and Stuart, 2005) and three new Indochinese species of cascade frogs allied to *Rana archotaphus* (Bain *et al.*, 2006).

New species of tree frogs of the family Hylidae has been reported: *Osteocephalus* from Guyana (Smith and Noonan, 2001), *Hyla* from the Highlands of Venezuela, Guayana (Senaris and Ayarzagüena, 2002), *Scinax* from the Andes of Venezuela and Misiones, Argentina (Barrio-Amoros *et al.*, 2004; Faivovich, 2005) and *Hypsiboas benitezi* group from the Western Amazon Basin (Faivovich *et al.*, 2006). Other frogs of the family Leptodactylidae, Bufonidae and Microhylidae have also been reported. A new genus and species of frog (Anura: Leptodactylidae) have been recorded from Bahia, Brazil (Heyer, 1999) and a new species of *Eleutherodactylus* (Anura: Leptodactylidae) from the northwestern lowlands of Ecuador (Guayasamim, 2004). Maneyro *et al.*, (2004) described a new toad (Anura: Bufonidae) from Uruguay. Vences and Glaw (2003) reported a new microhylid frog (*Plethodontohyla*) with a supraocular crest from Madagascar.

The impact of human activities on the status and distribution of amphibians in Pakistan have been studied (Khan, 1990). Investigation on the decline of amphibian populations due to habitat degradation have been investigated (Blaustein *et al.*, 1994; Delis *et al.*, 1996). Vos and Stumpel (1996) compared habitat isolation parameters in relation to fragmented distribution patterns in the tree frog (*Hyla arborea*). The influence of forest fragmentation on amphibian diversity in the nature reserve of Ambohitantely, highland Madagascar has been investigated Vallan (2000). The effect of anthropogenic habitat modification on habitat use by *Afrana angolensis* along the Dodwe River in Tanzania has been investigated (Lynn and Lindle, 2002).

In India, during the last few decades several workers have contributed to distribution of amphibian fauna and new species of frogs have been reported by several workers from the Northeast India: *Philautus cherrapunjiae* from Assam (Roonwal and Kripalani, 1961), *Ansonia meghalayana* from Meghalaya (Yazdani and Chanda, 1971), *Bufoides* a new genus for the rock toad *Ansonia meghalayana* from Meghalaya (Pillai and Yazdani, 1973), *Philautus shillongensis* from Meghalaya (Pillai and Chanda, 1973), two new species of frogs (Ranidae) from Khasi hills (Pillai and Chanda, 1977), *Rana mawlyndipi* from Khasi hills, Meghalaya, (Chanda, 1990) and *Kaloula* (Anura: Microhylidae) from north eastern India (Das *et al.*, 2004).

New species of tree frogs of the genus *Polypedates* (Anura: Rhacophoridae) have been reported from Great Nicobar and the Western Ghats, India (Das, 1995; Das and Ravichandran, 1998). New species of frog of the genus *Philautus* from Central India the Western Ghats, have been reported (Das and Chanda, 1997; Biju and Bossuyt, 2005a, 2005b). A new genus and species of Ranidae (Amphibia, Anura) from south-western India have been recorded (Dubois *et al.*, 2001) and a new species of frog of the genus *Nyctibatrachus* (Anura: Ranidae) from Western Ghats, India have been reported (Krishnamurthy *et al.*, 2001; Das and Kunte, 2005). Vasudevan and Dutta, (2000) reported a new species of *Rhacophorus* (Anura: Rhacophoridae) from the Western Ghats, India.

The first record of frogs from Northeast India and other parts of the country have been recorded by several workers: *Hoplobatrachus crassus* from North eastern region in Assam and Arunachal Pradesh (Bordoloi and Borah, 1999), *Paa annandalii*, *Megophrys robusta* and nine new records of amphibian from Arunachal Pradesh (Bordoloi *et al.*,

2001; Bordoloi and Borah, 2001; Borah and Bordoloi, 2001), *Kaloula pulchra* from Cherrapunjee, East Khasi Hills district, Meghalaya (Hooroo *et al.*, 2002), *Chirixalus vittatus* from Mizoram (Deuti and Dutta, 2002), *Micrixalus borealis* from Meghalaya (Matthew and Sen, 2003), *Pterorana khare* Kiyasetuo and Khare 1986 from Aizawl district, Mizoram (Dey and Ramanujam, 2003) *Euphlyctis hexadactylus* from north east India (Sen and Matthew, 2004) and *Kaloula pulchra* (Gray, 1831) from Mizoram (Sailo *et al.*, 2005). Sekar (1999) reported four new records and checklist of amphibians from Maharashtra.

The amphibian fauna of Khasi hills, Meghalaya have been described (Pillai and Chanda, 1979). Further, Chanda (1994) reported the anuran (amphibian) fauna of north east India and in 1995 compiled the state fauna series 4 of anuran (amphibia) of Meghalaya. Bordoloi *et al.*, (2000) presented an overview of amphibian fauna of Arunachal Pradesh, India. Investigation on the amphibian fauna of Kamrup district, Assam with notes on their natural history and studies on the distribution of *Bufo stomaticus* Lutken, in Assam, North east India have been studied (Choudhury *et al.*, (2001a, 2001b).

An overview of the amphibian fauna of India have been given (Inger and Dutta, 1986). Daniel and Sekar (1989) described species of the sub genera *Discodeles* and *Hylorana* of the family Ranidae and tree frogs of the family Rhacophoridae in field guide to the amphibian of Western India, Part 4. Amphibians of India with their distribution records have been updated (Dutta, 1992). The distribution of amphibians in Gujarat state, India have been described (Naik and Vinod, 1993). A checklist and bibliography on amphibians of India and Sri Lanka, and only on the amphibians of India with English

common names have been given (Dutta 1997; Das and Dutta, 1998). The amphibian fauna of India in Faunal Diversity in India and a catalogue of amphibian types in the collection of the Zoological Survey of India have been compiled (Chanda, 1998; Chanda *et al.*, 2000). The structure and composition of rainforest floor amphibian communities in Kalakad-Mundanthurai Tiger Reserve have been studied (Vasudevan *et al.*, 2001). Chandra and Pandey (2004) gave a note on anuran fauna of Achanakmar Wildlife Sanctuary, of Bilaspur, Chhatisgarh. Andrews *et al.*, (2005) surveyed the amphibian fauna of Kerala and described their distribution and status. Further, notes on state wise distribution of the amphibian fauna of northeast India was compiled (Sen, 2004).

From the literature surveyed, it is evident that there is scarcity of information on the distribution of tree frogs in North east India, in particular in Meghalaya. Hence, this chapter deals with the distribution of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Meghalaya, with the descriptions and morphometric measurements of the two tree frogs collected from different parts of Meghalaya.

Materials and Methods:

The study was conducted on two species *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927.

Polypedates leucomystax (Gravenhorst, 1829) is an arboreal nocturnal tree frog commonly available during its breeding period of the family Rhacophoridae. It varies in colour from yellowish to brown on the dorsum and the ventral surface was creamy white. Three to four longitudinal stripes are present on the dorsum as well as fore limbs and hind

limbs of this frog. Skin is smooth and vomerine teeth present. Similarly, *Rhacophorus bipunctatus* Ahl, 1927 is also an arboreal nocturnal tree frog of the family Rhacophoridae. It is commonly found hiding on leaves of trees during the summer. The colour of this frog is leafy green on the dorsal side, yellowish on the ventral side and the webs are red in colour. Skin is smooth, vomerine teeth present and tympanum distinct.

Extensive survey on the distribution of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was conducted through out the years in 2002, 2003 and 2004 in all the seven districts of the state of Meghalaya. The survey areas in different districts of Meghalaya included Cherrapunjee, Shillong, Mawsynram, Mawphlang, Pynursla, Nongstoin, Mawkyrwat, Mairang, Balat, Jowai, Thadlaskein, Dawki, Nongpoh, Umsning, Tura, Garobadha, Dadenggiri, Williamnagar, Rongjeng and Baghmara (Fig. 1.1).

Field survey was conducted in the evenings from 4:00 PM and continued till late at night during the breeding season (April to August), which coincides with the rainy season and also during the daytime. The surveys were based on extensive search and physical verification. The animals were captured by hand or net and photographed in live condition. Collections were made from different habitats such as forest covers, ponds, rain fed pools, tree holes, open fields with vegetation, etc. Colour of the skin was noted down during collection and noted down in the morphometric data sheet, which incorporated the following:

Specimen No.:

Museum No.:

Scientific Name:

Place:

Habitat:

Microhabitat:

Temperature:

Relative humidity:

Rainfall:

Colour:

Stage: Juvenile/Adult

Sex:

Date:

Time:

Sky: Cloudy/Clear

Morphometric measurements of the frogs were carried out using a dial vernier caliper (Mitutoyo series No. 505-671). While majority of the frogs were released back, some were killed by anesthetization with chloroform and then fixed in 4 % formaldehyde for preservation, but before keeping in formaldehyde, a small incision was made on the abdomen for proper preservation. The preserved specimens were then identified with the help of established criteria (Chanda, 1994), as well as with the help of Zoological Survey of India, Shillong. Morphometric measurements of the following body parts, which were taken into account, as given by Chanda (1994) were as follows:

- | | |
|--------------------------|---|
| 1. Total length | : From tip of snout to vent. |
| 2. Length of head | : From tip of snout to angle of jaws. |
| 3. Width of head | : At angle of jaws. |
| 4. Length of snout | : From tip of snout to anterior corner of eye. |
| 5. Maximum length of eye | : From anterior corner to posterior corner of eyes. |
| 6. Inter orbital space | : Maximum gap between the two eyes. |

7. Length of arm : From proximal end of junction of arm with the body to tip of longest finger.
8. Length of hand : From the base of palm to tip of longest finger.
9. Length of first finger : From the base of palm to tip of first finger.
10. Length of second finger : From the base of palm to tip of second finger.
11. Length of third finger : From the base of palm to the tip of third finger.
12. Length of fourth finger : From the base of palm to the tip of fourth finger.
13. Length of leg : From mid-ventral line of attachment of leg with body to tip of longest toe.
14. Length of foot : From base of foot to tip of longest toe.
15. Length of first toe : From the base to tip of first toe.
16. Length of second toe : From the base to tip of second toe.
17. Length of third toe : From the base to the tip of third toe.
18. Length of fourth toe : From the base to the tip of fourth toe.
19. Length of fifth toe : From the base to the tip of fifth toe.



Results:

A brief account of diagnosis, description, habit and habitat, and distribution of *Polypedates leucomystax* (Gravenhorst, 1829) is given below:

Diagnosis: Colour yellowish, sometimes brown or reddish brown with black spots on the dorsum. A black streak on canthal and temporal region often present. Three to four longitudinal bands extending from snout to vent. Limbs with dark cross bands, ventral surface creamy white, belly and ventral surface of thigh granulated. During the present study, colour of the dorsum was locality-dependent: some were reddish brown, light yellow or brown in colour.

Description: A moderate size frog, snout-vent (S – V) length of males 42 – 66 mm and females 45 – 78 mm (Figs. 1.2a and 1.2b). Head broader than long, depressed, snout pointed, nostrils nearer to tip of snout than to eyes, tympanum distinct, half the diameter of eyes, interorbital width equal to the diameter of eyes (Fig. 1.2c). Vomerine teeth small, in oblique series, originating from the anterior border of choanae (Fig. 1.2d). Forelimbs moderately long, fingers with rudimentary webs and well-developed discs at the tips, length of fingers $1 < 2 < 4 < 3$, the third finger longest, subarticular tubercles small and not well-developed (Fig. 1.2e). Hindlimbs long, tibiotarsal articulation reaching anterior corner of eyes, heels strongly overlapping when hindlimbs folded at right angle to the body, toes two-third webbed and tips of toes dilated into well-developed discs, inner metatarsal tubercles small and prominent, outer metatarsal tubercles absent, length of toes

1 < 2 < 3 < 5 < 4 (Figs. 1.2f and 1.2g). Mean morphometric measurements of 50 males and 50 females were recorded (Table 1.1).

Habit and Habitat: *Polypedates leucomystax* was found to be a nocturnal arboreal frog. This species was commonly found in tall grasses near the vicinity of water bodies, while visiting temporary pools or ponds for breeding. During winter months they were found to hibernate under forest litters and in the holes in tree trunks. In the present study, this species was collected mostly during the months of March to August during the years 2002, 2003 and 2004.

Distribution: *Polypedates leucomystax* was collected from nineteen surveyed sites, namely Cherrapunjee, Shillong, Mawsynram, Mawphlang, Pynursla, Balat, Mawkyrwat, Nongstoin, Mairang, Umsning, Nongpoh, Jowai, Dawki, Baghmara, Williamnagar, Rongjeng, Tura, Garobadha and Dadenggiri (Fig. 1.3).

A brief account of diagnosis, description, habit and habitat, and distribution of *Rhacophorus bipunctatus* Ahl, 1927 is given below:

Diagnosis: Dorsal surface leafy green to slate. Ventrally yellowish. Latero-ventral sides yellowish white. Two characteristics black spots are very prominent on flanks, anterior spot larger than the posterior one; sometimes only one spot or three spots are present on one side of the body with two on the other side. Webs reddish in colour.

Description: A medium size frog with S - V length of males 39 – 45 mm and females 63 – 65 mm, respectively (Figs. 1.4a and 1.4b). Head broader than long, slightly depressed, snout obtusely pointed, projecting a little beyond lower jaw, interorbital width greater than eye diameter, tympanum distinct, two thirds of eye (Fig. 1.4c). Vomerine teeth in two oblique series (Fig. 1.4d). Forelimbs long, fingers moderately long with prominent discs, subarticular tubercles moderately large and prominent, length of fingers $1 < 2 < 4 < 3$, the third finger longest (Fig. 1.4e). Hindlimbs long, tibiotarsal articulation reaching posterior corner of eye, toes fully webbed with large discs at the tips, subarticular tubercles small and prominent, inner metatarsal tubercle present, outer metatarsal tubercles absent, length of toes $1 < 2 < 3 < 5 < 4$ (Figs. 1.4f and 1.4g). The mean morphometric measurements were recorded from 50 males and 50 females (Table 1.2).

Habit and Habitat: An arboreal nocturnal frog mainly found hiding during daytime in leaves of trees and was commonly found hiding during winter in tree holes and in banana leaves. It was more common during the breeding period i.e., in the months of April to August.

Distribution: This species was collected only from ten survey areas, namely Cherrapunjee, Shillong, Mawsynram, Pynursla, Nongstoin, Mawkyrwat, Jowai, Thadlaskein, Nongpoh and Umsning (Fig. 1.3).

A brief description of the survey areas and sites of collections of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 is furnished below:

Meghalaya have a unique array of vegetation, ranging from tropical and sub-tropical to temperate or near temperate. The differences in natural vegetation are due to variation in topography, soil and climatic conditions. In western part, the northern and southern low hills with warm and humid climate have dense tropical mixed forest while in higher parts where the climate is relatively cooler have temperate forests. The central and eastern parts with moderate climate have mixed tropical forest and temperate forests.

In order to gain a better understanding of distribution of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in Meghalaya, it is desirable to have collections from numerous sites. Hence, the sites where extensive survey and collections have been made were Cherrapunjee, Shillong, Mawsynram, Mawphlang, Pynursla, Nongstoin, Mawkyrwat, Mairang, Balat, Jowai, Thadlaskein, Dawki, Nongpoh, Umsning, Tura, Garobadha, Dadenggiri, Williamnagar, Rongjeng and Baghmara. The altitude of the survey sites were categorized as follows: higher mid-altitude regions ranging between 1300 m – 1700 m, lower mid-altitude between 120 m – 770 m and lower altitude between 15 m – 40 m above sea level (asl). The distribution of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in the seven districts of the state is shown in Fig. 1.3.

Cherrapunjee was the main study site selected to study the breeding behaviour, development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus*

bipunctatus. The frogs collected from other parts of the state was particularly to know their distribution and for morphometric measurements.

Cherrapunjee: The study site Cherrapunjee, locally known as Sohra is located (91°44'E longitude and 25°16'N latitude) at an altitude of 1300 m asl and is about 53 km south of Shillong and it is well known for receiving highest rainfall in the world. The main vegetation is mostly the sub-tropical rain forest. The over all climate and topography is favourable for the distribution of anurans. A thorough survey was conducted in this area and *Polypedates leucomystax* was collected from the grasses and vegetation, which were present at the breeding site. *Rhacophorus bipunctatus* was collected from temporary pools and ponds and also from the leaves of plants and shrubs. In 2002 a total number of sixteen males and sixteen females of *Polypedates leucomystax* and ten males and seven females of *Rhacophorus bipunctatus* were collected from the month of April to August. A total of twenty two males and twenty five females of *Polypedates leucomystax* and sixteen males and fifteen females of *Rhacophorus bipunctatus* were collected in 2003 and in 2004 twelve males and nine females of *Polypedates leucomystax* and twenty four males and twenty eight females of *Rhacophorus bipunctatus* were collected (Tables 1.3 and 1.4).

Shillong: The capital of Meghalaya state is located (91°53'E and 25°34'N) at an altitude of 1450 m asl. *Polypedates leucomystax* were collected from a forest cover in Malki and near a residential area from Mawlai locality, while *Rhacophorus bipunctatus* were collected from banana leaves near a rain fed pool from Mawlai locality. A total number

of nine males and five females of *Polypedates leucomystax* and six males and four females of *Rhacophorus bipunctatus* were collected (Tables 1.3 and 1.4).

Mawsynram: It is located (91°34'E and 25°17'N) at an altitude of 1400 m asl. Like Cherrapunjee, it is also well known for its heavy rainfall and dense forest covers. During the survey period 2002, 2003 and 2004 a total of five males and two females of *Polypedates leucomystax* and two males and one female of *Rhacophorus bipunctatus* were collected (Tables 1.3 and 1.4.).

Mawphlang: It is a small village about 22 km from Shillong and located (91°45'E and 25°27'N) at an altitude of 1700 m asl. It is well known for its sacred groves 'Ka law Lyngdoh'. These sacred groves have thick vegetation as no one is allowed to cut trees. Five males and two females of *Polypedates leucomystax* were collected from this village (Table 1.3).

Pynursla: It is located (91°53'E and 25°18'N) at an altitude of 1320 m asl in the East Khasi Hills and at a distance of about 75 km from Shillong. It is a small village with thick forest covers and a few streams running near the edges of these forests. A total of five males and two females of *Polypedates leucomystax* and four males and one female of *Rhacophorus bipunctatus* were collected during the survey period (Tables 1.3 and 1.4).

Balat: It is located (91°22'E and 25°11'N) at an altitude of 15 m and about 120 km from Shillong bordering Bangladesh. A number of rain fed pools are found during the summer

season and thick forest cover with thick bushes of shrubs and herbs are present in this area. Five males and four females of *Polypedates leucomystax* were collected from a cemented tank near a residential area as well as from a banana leaf present near a temporary pool (Table 1.3).

Nongstoin: It is the Head Quarter of West Khasi Hills District located (91°16'E and 25°31'N) at an altitude of 1340 m asl and about 99 km from Shillong. A total of four males and three females of *Polypedates leucomystax* and four males and one female of *Rhacophorus bipunctatus* were collected from this village (Tables 1.3 and 1.4).

Mawkyrwat: It is a small village surrounded with forests and it is located (91°28'E and 25°22'N) at an altitude of 1560 m asl in the West Khasi Hills. From this village five males and three females of *Polypedates leucomystax* and four males and one female of *Rhacophorus bipunctatus* were collected (Tables 1.3 and 1.4).

Mairang: It is also a small village located (91°28'E and 25°33'N) at an altitude of 1660 m asl in the West Khasi Hills. During the survey period a total of five males and two females of *Polypedates leucomystax* were collected (Table 1.3).

Jowai: It is the Head Quarter of Jaintia Hills located (92°11'E and 25°26'N) at an altitude of 1320 m asl and about 60 km from Shillong. A number of streams and thick forest covers are present which gives an ideal habitat for anurans. A total number of five males

and two females of *Polypedates leucomystax* and five males and two females of *Rhacophorus bipunctatus* were collected during the survey period (Tables 1.3 and 1.4).

Thadlaskein: It is located (92°10'E and 25°29'N) at an altitude of 1373 m asl. It is famous for its lake known as Thadlaskein Lake. Dense forests are still present and a total number of six males and three females of *Rhacophorus bipunctatus* were collected from a cemented tank near a residential area (Table 1.4.).

Dawki: It is a small village with thick forest covers located on the southern part of the state bordering with Bangladesh about 85 km from Shillong and located (92°0'E and 25°10'N) at an altitude of 120 m asl. Six males and five females of *Polypedates leucomystax* were collected from this village during the survey period (Table 1.3).

Nongpoh: It is a small town located (91°52'E and 25°54'N) at an altitude of 560 m asl and it is the Head Quarter of Ri Bhoi and a total of five males and five females of *Polypedates leucomystax* and six males and three females of *Rhacophorus bipunctatus* were collected (Tables 1.3 and 1.4).

Umsning: It is located (91°53'E and 25°45'N) at an altitude of 770 m asl and about 27 km from Shillong in the Ri Bhoi district. A total number of seven males and four females of *Polypedates leucomystax* and six males and one female of *Rhacophorus bipunctatus* were collected from a banana plantain and temporary pools (Tables 1.3 and 1.4).

Tura: It is the Head Quarter of West Garo Hills and located (90°13'E and 25°30'N) at an altitude of 370 m asl and about 323 km from Shillong. A total number of six males and three females of *Polypedates leucomystax* were collected during the survey period (Table 1.3).

Garobadha: It is a small village located (90°01'E and 25°34'N) at an altitude of 40 m asl. A number of rain fed pools are found in the forest covers and five males and two females of *Polypedates leucomystax* were collected from the rain fed water bodies surrounded with thick bushes adjacent to a forest cover (Table 1.3).

Dadenggiri: It is located (90°11'E and 25°42'N) at an altitude of 240 m asl. Thick forest covers are still present which serves as a good habitat for the anuran fauna. A total number of six males and two females of *Polypedates leucomystax* were collected from an ephemeral pool (Table 1.3).

Williamnagar: It is the Head Quarter of East Garo Hills and about 208 km from Shillong. It is located (90°37'E and 25°30'N) at an altitude of 260 m asl. Thick forests are found in this area and a total of five males and two females of *Polypedates leucomystax* were collected during the survey period (Table 1.3).

Rongjeng: Located on (90°48'E and 25°39'N) at an altitude of 360 m asl. It is at a distance of 111 km from Tura. Mixed tropical vegetation is found in the area. During the

survey period six males and five females of *Polypedates leucomystax* were collected from a thick forest near a slow flowing stream (Table 1.3).

Baghmara: It is the Head Quarter of South Garo Hills located (90°39'E and 25°11'N) at an altitude of 30 m asl and at a distance of about 122 km from Tura. This place has thick vegetation with evergreen forest and a total of six males and five females of *Polypedates leucomystax* were collected from this place (Table 1.3).

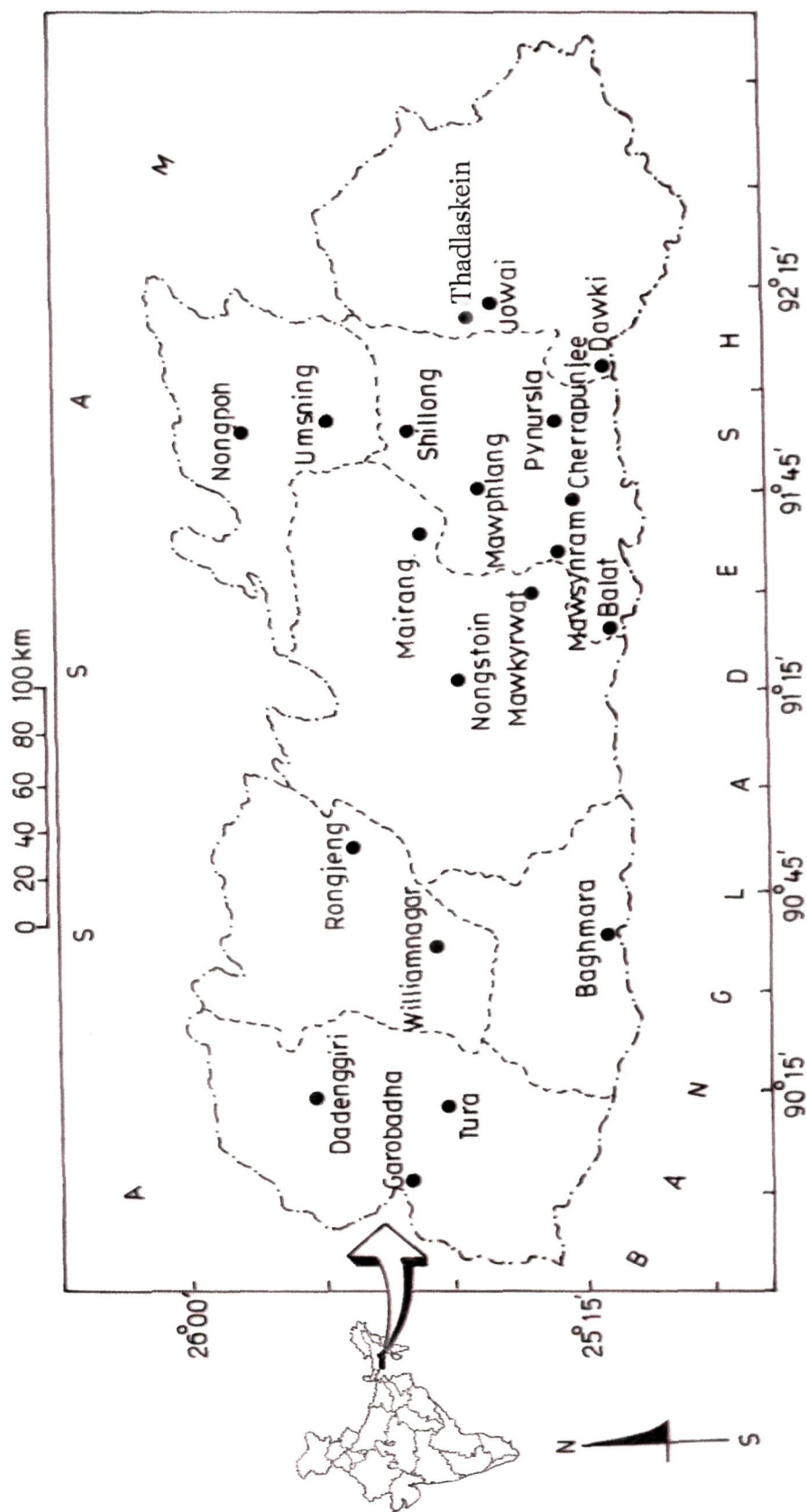


Fig. 1.1: Map of Meghalaya showing the survey areas.

● Survey areas



Fig. 1.2a – *Polypedates leucomystax* (female).



Fig. 1.2b – *Polypedates leucomystax* (male).

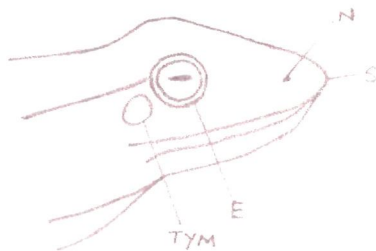


Fig. 1.2c – Lateral view of head.

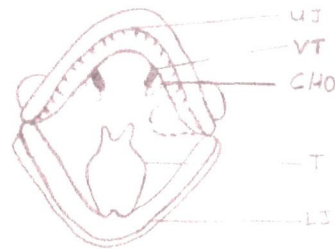


Fig. 1.2d – Showing palate, teeth and choanae.



Fig. 1.2e – Ventral view of forelimb.



Fig. 1.2f – Tibio tarsal articulation.

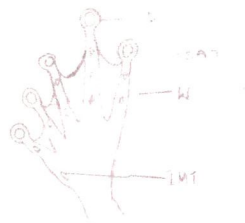


Fig. 1.2g – Ventral view of hindlimb

Abbreviations:

CHO=Choanae; **D**=Disc; **E**=Eye; **IMT**=Outer metatarsal; **LJ**=Lower jaw; **N**=Nostril; **S**=Snout; **SAT**=Subarticular tubercles; **T**=Tongue; **TYM**=Tympanum; **UJ**=Upper jaw; **VT**=Vomerine teeth; **W**=Web.

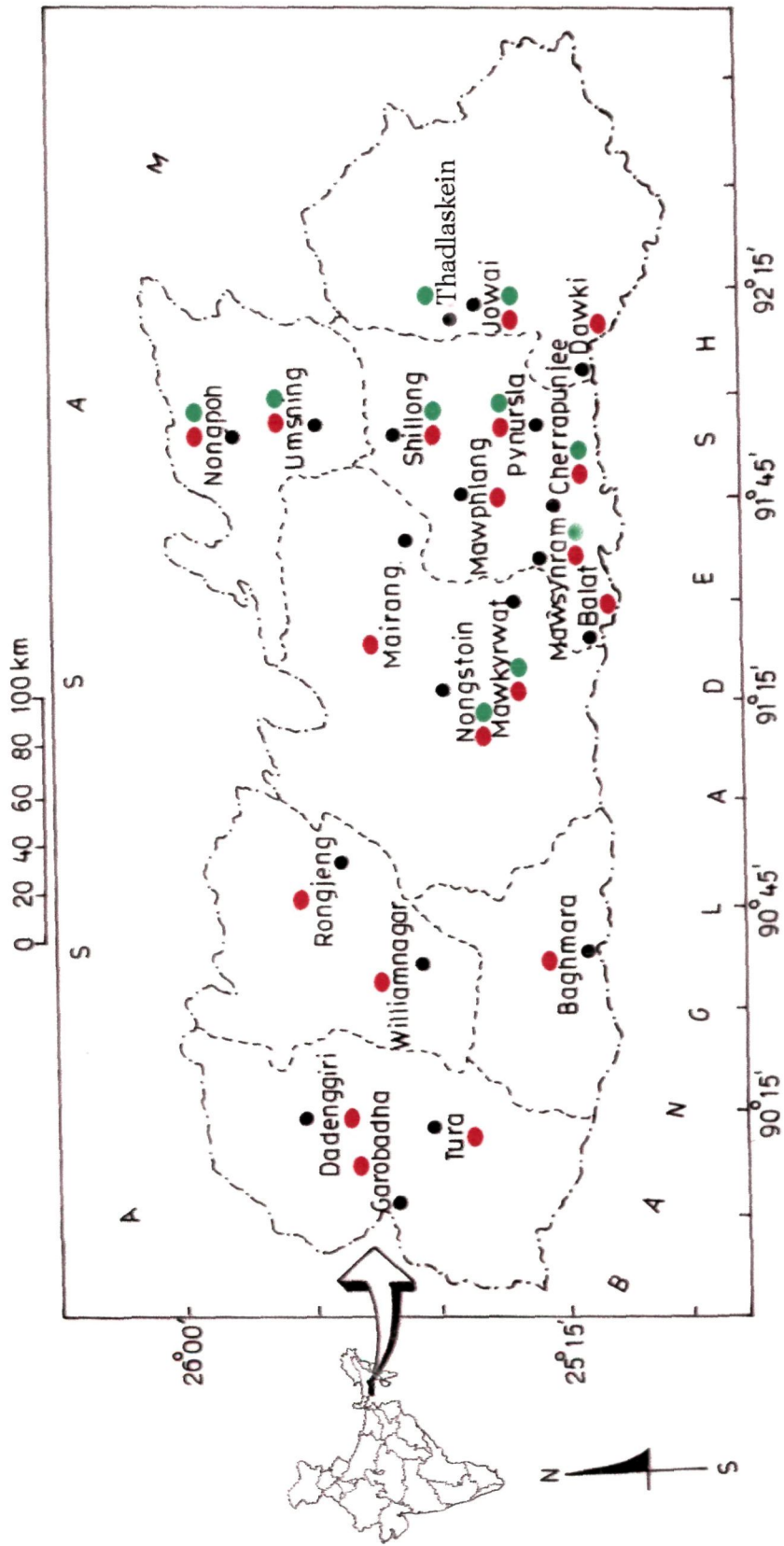


Fig. 1.3: Map of Meghalaya showing the distribution of *Polypedates leucomystax* and *Rhacophorus bipunctatus*.



Fig. 1.4a – *Rhacophorus bipunctatus* (female).



Fig. 1.4b – *Rhacophorus bipunctatus* (male).

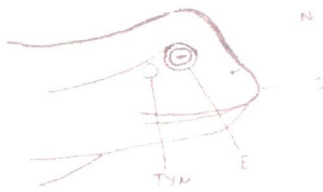


Fig. 1.4c – Lateral view of head.

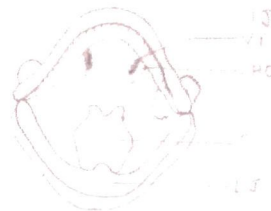


Fig. 1.4d – Showing palate, teeth and choanae.



Fig. 1.4e – V entral view of Forelimb.



Fig. 1.4f – Tibio tarsal articulation.



Fig. 1.4g – Ventral view of hindlimb.

Abbreviations:

CHO=Choanae; **D**=Disc; **E**=Eye; **IMT**=Outer metatarsal; **LJ**=Lower jaw; **N**=Nostril; **S**=Snout; **SAT**=Subarticular tubercles; **T**=Tongue; **TYM**=Tympanum; **UJ**=Upper jaw; **VT**=Vomerine teeth; **W**=Web.

Table 1.1: Morphometric measurements of *Polypedates leucomystax*

Sl No.	Characters	Males N=50		Females N=50	
		Range (mm)	Mean $\pm$ SE	Range (mm)	Mean $\pm$ SE
1.	Total length	42-66	45.0 $\pm$ 5.23	45-78	62.0 $\pm$ 7.60
2.	Head length	12.3-12.5	12.5 $\pm$ 0.15	19.0-19.1	19.0 $\pm$ 0.21
3.	Head width	15.0-15.2	15.0 $\pm$ 0.36	21.4-21.5	21.5 $\pm$ 0.63
4.	Snout length	8.9-9.1	9.0 $\pm$ 0.18	9.5-9.6	9.5 $\pm$ 0.25
5.	Eye diameter	5.4-5.5	5.5 $\pm$ 0.74	6.4-6.5	6.5 $\pm$ 0.43
6.	Inter-orbital space	5.4-5.5	5.6 $\pm$ 0.45	6.4-6.5	6.5 $\pm$ 0.24
7.	Forelimb length	27.0-27.1	27.0 $\pm$ 0.20	30.5-30.6	30.5 $\pm$ 0.32
8.	Hand length	12.9-13.0	13.0 $\pm$ 0.79	14.9-15.0	15.0 $\pm$ 0.56
9.	1 st finger length	4.9-5.1	5.0 $\pm$ 0.30	6.0-6.1	6.0 $\pm$ 0.23
10.	2 nd finger length	8.4-8.5	8.5 $\pm$ 0.36	11.0-11.1	11.0 $\pm$ 0.74
11.	3 rd finger length	13.0-13.1	13.0 $\pm$ 0.62	14.9-15.0	15.0 $\pm$ 0.40
12.	4 th finger length	11.0-11.2	11.0 $\pm$ 0.33	13.0-13.1	13.0 $\pm$ 0.63
13.	Hindlimb length	70.0-70.1	70.0 $\pm$ 0.42	83.4-83.5	83.5 $\pm$ 0.26
14.	Foot length	19.9-20.0	20.0 $\pm$ 0.16	22.4-22.5	22.5 $\pm$ 0.28
15.	1 st toe length	7.0-7.1	7.0 $\pm$ 0.53	6.9-7.0	7.0 $\pm$ 0.53
16.	2 nd toe length	8.9-9.1	9.0 $\pm$ 0.25	10.4-10.6	10.5 $\pm$ 0.45
17.	3 rd toe length	13.0-13.1	13.0 $\pm$ 0.28	14.0-14.1	14.0 $\pm$ 0.40
18.	4 th toe length	20.0-20.1	20.0 $\pm$ 0.63	22.4-22.5	22.5 $\pm$ 0.22
19.	5 th toe length	13.9-14.0	14.0 $\pm$ 0.08	16.0-16.1	16.0 $\pm$ 0.30

Table 1.2: Morphometric measurements of *Rhacophorus bipunctatus*

SI No.	Characters	Males N=50		Females N=50	
		Range (mm)	Mean±SE	Range (mm)	Mean±SE
1.	Total length	39-45	43.5±0.42	63-65	64.0±0.21
2.	Head length	11.9-12.1	12.0±0.21	15.9-16.2	16.2±0.18
3.	Head width	18.9-19.6	19.5±0.42	21.0-21.3	21.0±0.35
4.	Snout length	7.7-8.1	8.0±0.32	10.2-10.6	10.5±0.24
5.	Eye diameter	4.9-5.1	5.0±0.50	5.9-6.1	6.0±0.56
6.	Inter-orbital space	9.6-10.2	10.0±0.40	11.2-11.6	11.5±0.64
7.	Forelimb length	29.8-31.2	30.5±0.75	40.8-41.2	41.0±0.23
8.	Hand length	8.8-9.1	9.0±0.42	12.9-13.0	13.0±0.70
9.	1 st finger length	4.9-5.2	6.0±0.54	6.9-7.1	7.0±0.40
10.	2 nd finger length	5.8-6.1	5.0±0.73	7.8-8.1	8.0±0.36
11.	3 rd finger length	8.8-9.1	9.0±0.20	12.9-13.1	13.0±0.53
12.	4 th finger length	6.8-7.2	7.0±0.28	11.0-11.1	11.0±0.25
13.	Hindlimb length	80.2-81.5	81.0±0.44	84.9-85.2	85.0±0.26
14.	Foot length	19.8-20.1	20.0±0.26	21.9-22.1	22.0±0.32
15.	1 st toe length	8.8-9.0	9.0±0.60	10.9-11.0	11.0±0.42
16.	2 nd toe length	11.7-12.1	12.0±0.51	14.9-15.1	15.0±0.39
17.	3 rd toe length	14.9-15.1	15.0±0.45	17.0-17.1	17.0±0.25
18.	4 th toe length	19.9-20.0	20.0±0.36	22.0-22.2	22.0±0.48
19.	5 th toe length	16.8-17.1	17.0±0.34	18.9-19.0	19.0±0.53

Table 1.3: Survey areas and sites of collections of *Polypedates leucomystax*

Places	Location	2002			2003			2004			Total	
		Months	Males	Females	Months	Males	Females	Months	Males	Females	Males	Females
Cherrapunjee	91°44'E 25°16'N; 1300 m asl	April to August	16	16	April to August	22	25	April to August	12	9	50	50
Shillong	91°53'E 25°34'N; 1450 m ask	May	3	1	June	4	1	April	2	3	9	5
Mawsynram	91°24'E 25°17'N; 1400 m asl	May	2	1	April	1	1	May	2	0	5	2
Mawphlang	91°45'E 25°27'N; 1700 m asl	July	2	0	May	2	1	June	1	1	5	2
Pynursla	91°53'E 25°18'N; 1320 m asl	June	2	1	May	2	0	June	1	1	5	2
Balat	91°22'E 25°11'N; 15 m asl	July	3	1	May	1	2	June	1	1	5	4
Nongstoin	91°16'E 25°31'N; 1340 m asl	May	1	2	April	1	1	June	2	0	4	3
Mawkyrwat	91°28'E 25°22'N; 1560 m asl	June	2	0	April	2	1	July	1	2	5	3
Mairang	91°38'E 25°33'N; 1660 m asl	May	2	0	April	1	1	May	2	1	5	2
Jowai	92°11'E 25°26'N; 1320 m asl	July	2	1	May	1	1	April	2	0	5	2
Dawki	92°0'E 25°10'N; 120 m asl	April	2	2	June	3	1	May	1	2	6	5
Nongpoh	91°52'E 25°54'N; 560 m asl	June	2	1	July	2	2	April	1	2	5	5
Umsning	91°53'E 25°45'N; 770 m asl	May	4	1	June	2	1	April	1	2	7	4

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Table 1.3: Survey areas and sites of collections of *Polydectes leucomystax*

Places	Location	2002			2003			2004			Total	
		Months	Males	Females	Months	Males	Females	Months	Males	Females	Males	Females
Tura	90°13'E 25°30'N; 370 m asl	June	2	0	May	2	1	July	2	2	6	3
Garóbadha	90°01'E 25°34'N; 40 m asl	June	2	0	May	2	1	July	1	1	5	2
Dadenggiri	90°11'E 25°42'N; 240 m asl	June	2	1	May	3	0	July	1	1	6	2
Williamnagar	90°37'E 25°30'N; 260 m asl	July	1	1	May	2	1	April	2	0	5	2
Rongjeng	90°48'E 25°39'N; 360 m asl	July	2	2	April	3	1	July	1	2	6	5
Baghmara	90°39'E 25°11'N; 30 m asl	June	1	2	April	2	2	July	3	1	6	5

Table 1.4: Survey areas and sites of collections of *Rhacophorus bipunctatus*

Places	Location	2002			2003			2004			Total	
		Months	Males	Females	Months	Males	Females	Months	Males	Females	Males	Females
Cherrapunjee	91°44'E 25°16'N; 1300 m asl	April to August	10	7	April to August	16	15	April to August	24	28	50	50
Shillong	91°53'E 25°34'N; 1450 m asl	May	2	1	June	1	1	April	3	2	6	4
Mawsynram	91°24'E 25°17'N; 1400 m asl	May	0	0	April	1	0	May	1	1	2	1
Pynursla	91°53'E 25°18'N; 1320 m asl	June	1	1	May	1	0	June	2	0	4	1
Nongstoin	91°16'E 25°31'N; 1340 m asl	May	2	0	April	1	0	June	1	1	4	1
Mawkyrwat	91°28'E 25°22'N; 1560 m asl	June	1	0	April	2	0	July	1	1	4	1
Jowai	92°11'E 25°26'N; 1320 m asl	July	2	0	May	2	1	April	1	1	5	2
Thadlaskein	92°10'E 25°29'N; 1373 m asl	July	3	1	May	2	2	April	1	2	6	3
Nongpoh	91°52'E 25°54'N; 560 m asl	June	3	1	July	1	1	April	2	1	6	3
Umsning	91°53'E 25°45'N; 770 m asl	May	2	0	June	2	1	April	2	0	6	1

Discussion:

Amphibians are ancient creatures valued by biologists and naturalists around the world. The distribution and abundance of amphibians are known to depend on forest attributes, such as coarse woody debris, canopy characteristics, proximity to water and related environmental factors all of which vary naturally and are influenced by management activities. Anurans make up 91 % of the total number of species of Indian amphibians, which is just slightly more than their percentage on a worldwide basis (Inger and Dutta, 1986). The abundance of amphibian species is very much uneven in India, the highest concentration of genera and species being represented in the northeast region and Western Ghats. The present study revealed the current status on the distribution of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in exact localities collected from different parts of Meghalaya.

Polypedates leucomystax was collected mostly from rain-fed pools and puddles surrounded by vegetation during its breeding season and cemented tanks in residential areas. Chanda (1994) reported *Polypedates leucomystax* only from five places of Meghalaya (Shillong, Cherrapunjee, Barapani, Umtyngar and Rongjeng). However, in the present investigation it was collected from nineteen surveyed sites of Meghalaya (Cherrapunjee, Shillong, Mawsynram, Mawphlang, Pynursla, Balat Nongstoin, Mawkyrwat, Mairang, Jowai, Dawki, Nongpoh, Umsning, Tura, Garobadha, Dadenggiri, Williamnagar, Rongjeng, and Baghmara; Table 1.3; Fig. 1.3). The above collection sites ranged from higher mid-altitude between 1300 m – 1700 m asl in the East and West Khasi Hills and Jaintia Hills to lower mid-altitude between 120 m – 770 m asl in Ri Bhoi, Jaintia Hills, East and West Garo Hills districts, and lower altitude regions ranging

between 15 m – 40m asl in East Khasi Hills and South Garo Hills districts. It, thus, seems that *Polypedates leucomystax* inhabits the forests of Meghalaya irrespective of the altitudes ranging from 15 m to 1700 m asl, indicating that *Polypedates leucomystax* has diverse adaptations to live in low and high altitudes. It has been reported that anurans possess physiological, behavioural and morphological plasticity that enables them to explore and survive in many unusual habitats, such as arid environments, high elevations and arboreal ponds (Ultsch *et al.*, 1999a). Though amphibians occupy many diverse habitats across the globe, they are highly dependent upon aquatic habitats because of their physiology and natural history (Duellman and Trueb, 1994).

Chanda (1994) reported the presence of *Polypedates leucomystax* from different states other than Meghalaya such as, West Bengal, Assam, Arunachal Pradesh, Nagaland, Manipur and Sikkim from India, and Sri Lanka, China, Malaysia and Philippines from other parts of the world. Similarly, Dutta (1997) reported this species from West Bengal, Gujarat, Assam, Meghalaya, Manipur, Arunachal Pradesh, Nicobars and Sikkim and from other parts of the world such as Myanmar, Nepal, China, Taiwan, Singapore, Thailand, Philippines, Java, Sumatra and Borneo, the Malay Peninsula and Japan (introduced). Recently, Sen (2004) reported *Polypedates leucomystax* from Meghalaya, Assam, Arunachal Pradesh, Manipur, Nagaland, Mizoram and Tripura from Northeast India.

Rhacophorus bipunctatus is a small green tree frog and it was collected from rain-fed pools and puddles during its breeding period and also from the leaves of trees and cemented tanks near residential areas. During the present study, it was collected from ten surveyed sites, which includes Cherrapunjee, Shillong, Mawsynram, Pynursla,

Nongstoin, Mawkyrwat, Jowai, Thadlaskein, Nongpoh and Umsning (Table 1.4; Fig. 1.3). Earlier, Chanda (1994) reported the presence of *Rhacophorus bipunctatus* only from four places of Meghalaya including Shillong, Pynursla, Umroi and Thadlaskein. In the present investigation it was collected from higher mid-altitude regions ranging from 1300 m to 1560 m asl in East and West Khasi Hills and Jaintia Hills districts, and also from lower mid-altitude regions ranging from 560 m to 770 m asl in Ri Bhoi district. In Thailand and southern Vietnam, *Rhacophorus bipunctatus* has been recorded at altitudes of 100 m – 900 m asl but up to 1800 m asl in Pennisular Malaysia (Manthey and Grossmann 1997). In the present investigation, however, it was observed that *Rhacophorus bipunctatus* preferred higher and lower mid altitude regions ranging between 560 m – 1560 m asl with cooler climate.

On the basis of the present observations, it may be suggested that the distribution of *Rhacophorus bipunctatus* in different parts of Meghalaya is influenced by regional and local factors, such as the seasonal distribution of rainfall, moisture, temperature, vegetation and physiological tolerances of the species.

Chanda (1994) reported *Rhacophorus bipunctatus* from different states such as Assam, Arunachal Pradesh, Manipur, Nagaland and Tripura from India and only from Indonesia from other parts of the world. Dutta (1997) reported the species from Meghalaya, Assam, Arunachal Pradesh, Manipur, and Tripura from India and Thailand, Myanmar and the Malay Peninsula from other parts of the world.

The diversified topography, geographic location, high rainfall and humidity have likely supported the distribution of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in different parts of Meghalaya. This investigation provides an accurate

information about the distribution of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in different parts of Meghalaya. Such investigations need to be accomplished in the very near future because each year witnesses more destruction of the natural environment. Alfords and Richards, (1999) suggest that alterations in local weather conditions caused by global climate change will influence the ecology of amphibians in a number of ways.

Our field survey and collections revealed that *Polypedates leucomystax* and *Rhacophorus bipunctatus* preferred ephemeral pool with some vegetation surrounding it as their habitat during the breeding season. These temporary pools and puddles, which are formed during the monsoon, provide excellent habitat for breeding and development of these tree frogs. Several authors have suggested that amphibians may be particularly sensitive to habitat modification and fragmentation (Lavilla, 2001; Young *et al.*, 2001) because of their complex life history patterns (Vos and Stumpel, 1996; Delis *et al.*, 1996) and particularly owing to the fact that during their ontogenesis they are exposed to different environmental factors (Vallan, 2000). As suggested by Krebs and Davies, (1997) thus, it is important to understand the habitat use and life history of species as different life history strategies can cause spatial patterns to emerge in distributions. For amphibians, this is of particular importance, as more species are associated with the terrestrial and aquatic habitats, imbuing them with a unique role in the ecosystem. Local habitat factors related to pond structure or regional landscape factors appear to play a more important part in affecting amphibian species richness and distribution.

CHAPTER II

Breeding behaviour, habit and habitat
of *Polypedates leucomystax* and
Rhacophorus bipunctatus

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Introduction:

An essential attribute of any surviving species or population is the ability to produce a succeeding generation. In amphibians, successful propagation of an individual's genes depends on the location of potential mates, stimulation of mates, selection of breeding site, fertilization of the eggs, development of the eggs and tadpoles metamorphosis. Location of mates may be achieved by visual, olfactory, auditory, or tactile means, or a combination of these. In some vertebrates, mating occurs with little or no specialized behaviour, whereas in other groups, especially those characterized by complex, social interactions, diverse kinds of courtship are an integral part of mating and mate selection. Generally, during courtship, males are the more aggressive sex, and their courtship activities depend on female response. Much of the social behaviour of amphibians, like that of most other ectothermic vertebrates, is centered on reproductive activities.

The concept of reproductive mode in amphibians was defined by Salthe (1969) and Salthe and Duellman, (1973) as being a combination of traits that includes oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatchling, and type of parental care, if any. Although Duellman and Trueb (1994) recognized 29 modes of reproduction for anurans, new reproductive modes have been described in recent studies on ecology and natural history (Haddad and Hodl, 1997;

Haddad and Pombal Jr., 1998; Haddad and Sawaya, 2000). Anurans exhibit a wide variety of reproductive patterns ranging from the species that lay large clutches of small-sized pigmented eggs which require aquatic media for development, to species that lay small clutches of large-sized un-pigmented eggs which develop under terrestrial conditions. It is well known that the majority of frogs lay their eggs in water which subsequently develop into tadpoles that metamorphose later into adults.

Many amphibians, especially those that have terrestrial eggs, carry out courtship and reproductive activities within their normal home ranges. In these species, there is no congregation of individuals at particular breeding sites. This also may be true for anurans that live along mountain streams in cloud forests. On the other hand, most amphibians that deposit eggs in or above ponds, and some species that breed in streams congregate for breeding. In some instances, migrations to breeding sites cover distances up to several kilometers. The major factor in anuran courtship is the production of advertisement calls by males. Advertisement call in frogs is the primordial factor in breeding: it advertises the male's presence to reproductive females, and plays an important role in species recognition, mate choice and establishment of males territories (Blair, 1956, 1958, 1962, 1964; Bogert, 1960; Littlejohn, 1977; Gerhardt, 1991). There is considerable variation among anurans in terms of the diversity and complexity of their vocal repertoires (Littlejohn, 1977). While a large proportion of species have a male advertisement call, a minority have, in addition, distinct aggressive or territorial calls and encounter calls. The advertisement call has two important functions: (1) announcement of occupied territory to other males of the same or different species, and (2) attraction of conspecific females (Bogert, 1960; Littlejohn, 1977). In the context of attraction of conspecific females,

anuran calls were widely referred to as mating calls. However, according to Wells (1977) these calls have dual function, and they are now recognized by the term “advertisement call”.

In most anurans, amplexus occurs at or near the oviposition site, and the pairs remain in amplexus for only an hour or two. The most common and widespread site of oviposition is in free flowing or standing water. In some groups the eggs are terrestrial or arboreal, while in others the eggs are retained in the oviduct (Duellman and Trueb, 1994). Many arboreal species often lay their eggs on leaves above water, away from aquatic predators. The humidity keeps the eggs moist, and then as soon as they hatch the tadpoles fall into the water and develop in the aquatic environment. Some females produce a mucus secretion that is beaten into a foam by the mating pair, creating a protective nest for the developing eggs through which the tadpoles fall when hatched.

Studies on the pattern of reproduction of anurans in tropical and subtropical regions have repeatedly demonstrated that reproductive phenology is closely associated with rainfall, and this is particularly true in tropical and subtropical forests that have seasonal changes in precipitation (Blair, 1961; Bowker and Bowker, 1979; Alexander *et al.*, 1979; Aichinger, 1987; Gascon, 1991; Donnelly and Guyer, 1994). Most tropical and sub-tropical species are capable of reproduction throughout the year, however, rainfall seems to be the primary extrinsic factor controlling the timing of reproductive activity. In most temperate species, reproductive activity is cyclic and dependent on a combination of temperature and rainfall. Annual variations in environmental factors regulate reproductive cycles in many vertebrates. Thus, in temperate regions, amphibians have seasonal reproduction, usually spawning only once a year. Breeding is coincident with

the increase in photoperiod and temperature in the habitat. However, in tropical areas, where climatic conditions are constant through out the year, most amphibians show continuous reproduction (Lofts, 1984).

Review of literature revealed that there are a number of studies on the reproductive behaviour of anurans and several workers have studied the breeding behaviour of anurans in relation to courtship, mating calls, oviposition site, mode of reproduction, foam nest construction, clutch size, sexual dimorphism and breeding cycle at the breeding site. The reproductive activity of anurans living in seasonal environments is generally associated with the rainy periods, both in tropical climates (Hoogmoed and Gorzula, 1979; Aichinger, 1987) and in temperate ones (Banks and Beebee, 1986; Caldwell, 1987). Reading (2003) studied the effect of variation in climatic temperature on breeding activity and tadpole stage duration in the common toad *Bufo bufo*. Climatic factors especially rainfall and temperature exert a strong influence on the anuran reproductive activity (Telford and Dyson, 1990; Silverin and Andren, 1992). Stressful conditions can inhibit ovarian development (Silverin and Andren, 1992).

There are a few studies on the breeding habits of Rhacophorids in India (Mohanty-Hejmadi and Dutta, 1988; Girish and Saidapur, 1999; Kadadevaru and Kanamadi, 2000; Deuti, 2001; Dutta *et al.*, 2001; Biju, 2003 and Khongwir, 2004).

Courtship and Mating calls:

The term courtship is used to refer to the interaction between males and females leading to pair formation and mating. The courtship may be simple in that the male may call until a female contacts him; then he clasps the females (Wells, 1977). The major

factor in anuran courtship is the production of advertisement calls by males. Reviews dealing with various aspects of vocalization in anuran amphibians have been published (Bogert, 1960; Blair, 1963, 1968; Paillette, 1971; Straughan, 1973; Capranica, 1977; Schneider, 1977, 1990; Duellman and Trueb, 1994; Rand, 1988 and Ryan, 1988). Mating call is a determinant for attracting females and the characteristics of acoustic signal are selected through female choice (Gerhardt and Davis, 1988; Telford *et al.*, 1989; Gerhardt, 1991 and Marquez and Bosch, 1997). Males produce specific calls as an attractive courtship signal (Schneider, 1966; Crespo *et al.*, 1989; Ryan and Wilczynski, 1991; Schneider and Sinsch, 1992) and in addition, other kinds of calls were also emitted such as the territorial call (Radwan and Schneider, 1988; Schneider and Steinwarz, 1990).

Several workers from other parts of the world reported variations in the advertisement calls of anurans, and that the calls are species specific for attracting a mate (Gerhardt and Davis, 1988; McClelland *et al.*, 1996; Penna *et al.*, 1997; Heyer and De Carvalho, 2000; Grosjean and Dubois, 2001; Given, 2002; Friedl and Klump, 2002; Smith *et al.*, 2003; Murphy and Floyd, 2005 and Owen and Tucker, 2006).

However, in India vocalization of anurans have been studied by Kanamadi *et al.*, (1993, 1994), Roy *et al.*, (1998, 2004), Kadadevaru and Kanamadi (2000).

Oviposition site:

Several studies indicate that the sites where eggs are oviposited can be influenced by the physical features of breeding sites as well as by the presence of predators, competitors and communal nesters (Howard, 1978; Seale, 1982a; Resetarits and Wilbur, 1989; Petranksa, 1990). Various studies have suggested that female anurans select

oviposition sites based on factors such as water depth (Seale, 1982a; Caldwell, 1986), water temperature (Howard, 1978), or absence of predators (Howard, 1978; Resetarits and Wilbur, 1989). Crump (1991) found that *Hyla pseudopuma* females choose oviposition sites based on assessments of egg loads already present. Pairs may select a site together (e.g., *Litoria lesueuri*: Richards and Alford, (1992). But in terrestrially breeding species, more commonly males choose the nest, defend it from rival males, and call to attract females. In such circumstances, selection should favour males that choose quality nests, and females that use nest quality to discriminate between potential mates (Townsend, 1989; Resetarits and Wilbur, 1991 and Mitchell, 2001).

Oviposition-site selection by the females or males anurans or by the amplexing pairs in a suitable or in an unpredictable environment have been described in several anuran species (Kam *et al.*, 1996; Spieler and Linsenmair, 1997; Halloy and Fiano, 2000; Mitchell, 2002; Johnson and Semlitsch, 2003; Giaretta and Menin, 2004 and Shepard, 2004).

Mode of Reproduction:

The diversity of reproductive modes in amphibians is much greater than that observed in other groups of vertebrates. Such modes include breeding terrestrially and arboreally, making foam nests, parental transport of eggs and or tadpoles, and direct development (copulating on land, laying the eggs in terrestrial sites, fully metamorphosed juveniles hatching, obviating the free-living larval stage). Many lineages of frogs have reproductive modes that involve the deposition of eggs outside of aquatic habitat, yet the tadpoles complete their development in water (Duellman and Trueb, 1994; Haddad and

Sawaya, 2000). Numerous surveys have concerned evolutionary trends in amphibian reproductive modes and descriptions of life histories (Noble, 1927, 1931; Lutz, 1947, 1948; Orton, 1951; Jameson, 1955, 1957; Goin, 1960; Lynn, 1961; Harvey, 1963; Tihen, 1965; Licht, 1969; Heyer, 1969; Crump, 1974; Lescure and Letellier, 1983; Wake, 1982; Gibbons and McCarrthy, 1986 and Caldwell, 1992), but the most comprehensive review of reproductive mode in amphibians is by Salthe and Mecham (1974). Further studies on the reproductive patterns of anurans have been described by (Kam *et al.*, 1995; Solano 1987; Martins 1988; Arzabe, 1999; Prado *et al.*, 2002; Biju, 2003; Gibson and Buley, 2004; Tsuji, 2004 and Huang *et al.*, 2004).

Foam nests and Clutch size:

Most rhacophorids deposit their eggs in a foam nest attached to vegetation either above or near water. The spawning sites include leafy twigs, hanging over water, grassy bank of ponds, under stones and also on tree trunks. It has been observed in several studies that frogs deposit eggs in a wide range of habitats including underground breeding chambers (Kaminsky *et al.*, 1999), foam nests (Haddad *et al.*, 1990), and on tree roots or creek banks as well as aquatic habitats (Richard and Alford, 1992).

In India, investigation on the diversity of foam nests and variation of clutch size of anurans have been reported by Mohanty-Hejmadi and Dutta (1988); Dutta *et al.*, (1990-91); Girish and Saidapur (1999); Biswas (2000); Deuti, (2001) and Krishna *et al.*, (2004). Similar studies from other countries have been reported by Liu, (1950); Downie (1988); Martins and Moreira (1991); Downie (1993); Seymour and Loveridge (1994);

Hobel (1999); Kusano and Hayashi (2002); Burger *et al.*, (2002); Giaretta and Kokubum (2004) and Davis and Roberts (2005).

Sexual Dimorphism:

Attempts have been made to explain size dimorphism among anurans based on sexual selection (Woolbright, 1983) or differences between reproductive selection gradients (Arak, 1988). Pattern of growth is considered to be one of several factors responsible for sexual size dimorphism in adult anurans (Hayes and Licht, 1992). Few workers have described sexual dimorphism in anurans (Woolbright, 1989; Radojicic *et al.*, 2002; Monnet and Cherry, 2002; Lee and Corrales, 2002; Vasudevan, 2003 and Gramapurohit *et al.*, 2004).

Breeding cycle:

A large number of amphibian species shows annual reproductive cycles dependent on seasonal changes in environmental factors. It seems that temperature and photoperiod in temperate zones, and rainfall in tropical areas are the most important environmental factors for adjusting reproductive activity with the environment (Lofts, 1984; Whittier and Crews, 1987). The annual reproductive pattern of amphibian has been studied extensively from the temperate and subtropical regions, and the physiological basis for their cyclic reproductive behaviour has been dealt with (Oordt, 1960). Studies on the tropical species are comparatively less (McCann, 1932; Inger and Greenberg, 1956, 1963; Ramaswamy and Lakshman, 1959; Basu and Mondel, 1961; Basu, 1968). The seasonal breeders are found in areas where the variation in the length of day may be

insignificant but other environmental factors, like temperature, relative humidity and rainfall fluctuate markedly not only during different months but also from place to place. Three major environmental factors have been implicated in the regulation of the amphibian breeding cycles: rainfall, photoperiod, and temperature (Lofts, 1974). Annual reproductive cycle and seasonal activity have been studied in anurans (Dole and Durant, 1974; Gopalakrishnan and Rajasekarasetty, 1978; Huang *et al.*, 1996, 1997; Ao and Bordoloi, 2000).

In amphibia, as in other vertebrate groups, gonadal activity depends on gonadotropins secreted by the pars distalis of the adenohypophysis, which are themselves regulated by hypothalamic factors (Peter, 1986). Studies on seasonal changes in the testicular histology, and effects of photoperiod and temperature on seasonal spermatogenic cycles have been reported in amphibian (Delgado *et al.*, 1989, 1992; Paniagua *et al.*, 1990; Ko *et al.*, 1998; Amaral *et al.*, 1999; 2000; Grammapurohit *et al.*, 2000 and Chavadej *et al.*, 2000).

Another remarkable characteristics of amphibians is the change of ovarian cyclicity in correlation with the variation in environmental or seasonal conditions (Bragg and Wesse, 1950; Kanamadi and Saidapur, 1982). The classification of developing oocytes of anurans has been carried out by many researchers (Wittek, 1952; Kemp, 1953; Grant, 1953; Balinski and Devis, 1963; Dumont, 1972). Further, the mechanism regulating the ovarian cycle, effect of photoperiod and temperature and seasonal variations in ovarian cycle in anurans have also been studied (Saidapur and Prasadmurthy, 1987; Delgado *et al.*, 1990; Saidapur and Hoque, 1995; Sretarugsa *et al.*, 2001; Ogielska and Kotusz, 2004).

Keeping in view the scarcity of information on the breeding activities of tree frogs in Northeast India, a comprehensive study on the breeding behaviour of two tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* was conducted in Meghalaya, Northeast India.

Materials and methods:

To study the breeding behaviour of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 (courtship, mating calls, spawning, foam nest construction and clutch size), four temporary rain-fed pools located at Cherrapunjee (25°5'N and 91°45'E), which received the maximum rainfall in the world were selected, and studies were conducted during the years 2002, 2003 and 2004 during their breeding periods from April to August. The breeding behaviour were recorded by observations made from 4:00 PM (evening) to 10:00 AM (morning). Torchlights were used to locate and count the number of males and females of both the tree frogs at their breeding sites.

Breeding behaviour, amplexing pairs and foam nest construction were studied and documented with the help of photographic and video cameras. The amplexing pairs and their foam nests were photographed with a camera (Canon EOS 300) fitted with zoom lens (EF 75-300 mm). The duration when the breeding pairs remained in amplexus was noted down and the temperature and relative humidity of the study sites were recorded. Rainfall data were collected from the Regional Meteorological Centre, Guwahati.

Collection and preservation of plant specimens of the study sites were done following the method given by Jain and Rao (1976). Identification of the collected

specimens was done by comparing with herbarium specimens in the Department of Botany Department, North Eastern Hill University, Shillong and Botanical Survey of India, Eastern Circle, Shillong.

The numbers of foam nests at the breeding sites were also recorded and the location of the foam nests on the vegetation and their distance from the breeding sites were measured by a measuring tape. Foam nests constructed by the amplexing pairs were collected from the field and their measurements were taken and the clutch size was determined. The total number of eggs in the 30 foam nests was counted and the snout-vent length of 30 amplexing pairs was measured. Similarly, in the years 2003 and 2004 collections were made and recorded. The data were statistically analysed using Student's t-test.

Mating calls were recorded with the help of an audio tape recorder (Panasonic XBS RX-CT880) and the oscillogram was prepared with the help of a software tool "Soundprobe V 2.69".

The breeding cycle of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was studied with special reference to the breeding behaviour in relation to ecological factors temperature, rainfall and humidity. To study the histology of testes and ovaries, adult frogs were collected from the field and brought to the laboratory. On reaching the laboratory ovaries from the females and testes from the males were excised and immediately fixed in Bouin's solution (24 hours). Fixation was done immediately so as to avoid post-mortem changes. The gonads were then dehydrated through alcoholic grades (30 %, 50 %, 70 %, 90 % and 100 %), cleared in xylene and embedded in paraffin wax, serially sectioned at 7-8 μ thickness and stained with haematoxylin and counter-stained

with eosin. The sections were then observed under the microscope Leitz Ortholux 2. Photographs of the histological sections were taken with the help of Leitz Ortholux 2 microscope fitted with photographic attachments.

Results:

Description of the breeding sites of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927:

Observations on the breeding behaviour of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were conducted during the years 2002, 2003 and 2004 at the four breeding sites indicated as study sites I and II for *Polypedates leucomystax* and study sites III and IV for *Rhacophorus bipunctatus* at Cherrapunjee.

Study site I: It was a temporary rain fed pond and measured about 9 m long and 5 m wide with a depth of 50 cm (Fig. 2.1). The pond was surrounded by a grassy field, and the area was very close to a forest cover, which had a thick growth of vegetation.

Study site II: It was also a temporary rain fed pool located near the study site I. It had a dimension of 12 m long and 6 m wide and the depth was about 50 – 60 cm (Fig. 2.2). The most common vegetation in this study site comprised of *Imperata cylindrical*, *Hydrilla sp.* and *Gleichenia sp.*

Study site III: It was temporary pool and had a length, width and depth of about 10 m, 6 m and 30 – 40 cm, respectively (Fig. 2.3). The pond was located near a forest cover and was surrounded by a stretch of grasses.

Study site IV: It was a temporary pool and had a length of 1 m, width of 50 - 60 cm and a depth of 30 cm (Fig. 2.4). Besides the common grasses, *Rhododendron arboretum* and *Alnus nepalensis* are found near this study site.

Some other plants which were found near these four study sites included *Alnus nepalensis*, *Rhus cinensis*, *Rhododendron arboretum*, *Phyllanthus glaucus* Wall, *Elaeagnus conferta*, *Flacouttia jangomas*, *Myrica esculenta*, *Schima wallichii*, *Sabia purpurea*, *Rhus cinensis* Mill, *Pyrus communis*, *Polygonum sp*, *Eriocauton sp*, *Schoenoplectus sp*, *Panicum sp*, *Musa paradisiaca*, *Phyllanthus glaucus* Wall, etc., and a variety of wild orchids. The temporary pools at the four selected sites were filled up with water during the rainy season from the later part of March onwards when it started to rain till October and remained dried during the winter months from November to February. During the investigation it was found that these four study sites served as good habitat for the breeding activity, development and metamorphosis of both *Polypedates leucomystax* and *Rhacophorus bipunctatus*.

Breeding Behaviour (courtship, mating calls, spawning and foam nest construction) of *Polypedates leucomystax*:

During the present investigation it was observed that *Polypedates leucomystax* is a seasonal breeder and its breeding activity coincides with the monsoon. Its breeding

period lasted from April to August. It was observed that *Polypedates leucomystax* hibernates under forest litters and leaves of trees during the cold winter months from November to February with air temperature ranging from 13.5°C – 20.5°C, relative humidity between 42 % - 69 % and rainfall between 0.0 mm – 268.6 mm (Table 2.1; Figs. 2.5a, 2.5b and 2.5c). The males were found to emerge first from their hibernating place in the evening around 5:00 PM (dusk) in the later part of March following increase in air temperature (20.6°C - 23.8°C), and were found croaking between the tall grasses near the breeding sites (Figs. 2.6, 2.7 and 2.8).

After emergence, males were found to move towards the breeding sites. Then the male gave advertisement calls to orient the female to come to the breeding site for spawning. The female responded by moving to the breeding sites and approaching towards the male leading to courtship (Figs. 2.9, 2.10 and 2.11). Frequency of croaking was observed to be remarkably high in the evenings before and after rainfall. The advertisement call lasted for one to two days before amplexus was formed. When the advertisement calls of *Polypedates leucomystax* were analyzed, it was found that it had a number of calls produced in series at regular intervals. Each distinct call consisted of three long notes, and the duration of each call was found to be 0.454secs. Each note consisted of a group of pulses (Figs. 2.12a, 2.12b and 2.12c).

Amplexus lasted for about 2 - 3 hours or sometimes even longer, and then the female deposited its eggs in a foam nest attached to vegetation (Figs. 2.13, 2.14 and 2.15). During the present study it was observed that male *Polypedates leucomystax* changed its colour from yellowish to brownish during the breeding phase. Spawning started taking place only in the month of April when it is warmer and the air temperature

recorded between 22.7°C - 24.6°C, water temperature between 20.5°C - 22.6°C, relative humidity between 68 % - 86 % and rainfall between 501.5 mm - 1335.2 mm and continued till August where air temperature recorded was 21.3°C - 22.6°C, relative humidity between 65 % - 76 % and rainfall between 1306.6 mm - 1480.9 mm during the study period (2002, 2003 and 2004) (Table 2.1; Fig. 2.5a, 2.5b and 2.5c).

It was observed that construction of foam nest in *Polypedates leucomystax* was arboreal, about 5 – 40 cm above the water surface (Fig. 2.16). Some foam nests were also found to be attached to grassy edges on the sides of the pool about 1 m away from the water body. Some of the amplexing pairs constructed the foam nests attached to rocks and stones near the pond (Figs. 2.17 and 2.18). Further, some frogs were also seen to construct the foam nests in a cemented tank about 15 – 30 cm above the water level in a nearby residential area (Fig. 2.19). During field observations in the evening (around 7:00 PM) the males were found croaking from inside the thick cover of grass where the males sat and guarded the foam nests (Figs. 2.20 and 2.21). Construction of foam nests was usually observed to occur during the night time. However, under laboratory conditions *Polypedates leucomystax* was found to construct its foam nest in the morning hours in the month of May around 10:00 AM in the aquarium (Fig. 2.22). Both the male and female took part in nest construction. First the female released secretions followed by the release of seminal fluid by the male. Then the male was found to agitate the secretions with its hind limbs leading to formation of a foam nest. The male was present for a longer period guarding the foam nest, while the female moved away from the foam nest after depositing its eggs in the foam nest (Figs. 2.23 and 2.24).

In the year 2002 the total number of foam nests recorded during the breeding period i.e., from the months of April to August at study site I was 96 and at study site II was 89. Similarly, in 2003 the total number of foam nests recorded at study site I was 94 and at study site II was 81. In the year 2004 the total number of foam nests recorded at study sites I and II was 88 and 77 (Table 2.2).

Size of amplexing frogs and clutch size: In the year 2002 thirty amplexing pairs along with their foam nests were collected from the breeding sites and their snout-vent length and the diameter of the foam nests were recorded. Similarly, collections were also made in the years 2003 and 2004 (Table 2.3). The diameter of the foam nests recorded ranged from 6 to 12 cm. Distinct sexual size dimorphism was observed in this species where the females were larger (71.17 ± 0.09 mm) than the males (59.03 ± 0.48 mm; $t=5.80$; $p<0.001$) (Table 2.4). The frogs were released back after recording the snout-vent length.

It was found from the field observations that the foam nests, which were attached to vegetation, were slightly creamy white in colour, while those located near the pond margins and exposed the outer crust of the foam nests became dry and were slightly brownish in colour. Foam nests were more or less rounded in shape, while some of them were oval and irregular. In order to know the clutch size, the above thirty numbers of foam nests recorded in the year 2002 were collected and brought to the laboratory. The eggs were counted and kept in a tray containing pond water for further development. Similarly, collections were also made during the years 2003 and 2004 to record the clutch size (Table 2.3). The clutch size varied from 252 to 530 in different foam nests with a mean of 416 ± 5.41 (Table 2.4) and the egg measured 1.5 mm in diameter.

Sex ratio at the breeding sites: During the breeding period, it was observed that the males usually outnumbered the females at the breeding sites. In 2002, during the months of April to August the total number of males and females observed and recorded at the study site I was 96 and 71 respectively. Similarly, at the study site II the total number of males and females observed and recorded were 96 and 79 respectively. Observations were also conducted in the year 2003 and 2004. The total number of males observed and recorded during the breeding season from April to August in the year 2003 at site I and site II were 87 and 85, and females were 70 and 68 respectively. Similarly, in the year 2004 the total number of males observed and recorded at site I and site II were 92 and 89, and females were 64 and 72 respectively (Table 2.5; Fig. 2.25). The number of frogs observed and recorded at study sites I and II were more in the months of June and July (Table 2.5). By September the number of adult frogs was limited and only a few were seen near the breeding sites and a number of metamorphosed froglets were seen clinging on tree leaves.

Breeding behavior (courtship, mating calls, spawning and foam nest construction) of *Rhacophorus bipunctatus*:

Observations on the breeding behaviour of *Rhacophorus bipunctatus* was conducted at two temporary rain-fed pools, study sites III and IV at Cherrapunjee (Figs. 2.3 and 2.4). Field observations indicated that *Rhacophorus bipunctatus* is also a seasonal breeder. It emerged from hibernation during the later part of March and breeding took place from April to August. *Rhacophorus bipunctatus* hibernated in tree holes and on the

banana stems during the winter from November to February (Fig. 2.26). *Rhacophorus bipunctatus* came out of its hibernating place in the month of March and was seen perching on tree leaves near the breeding site. The atmospheric temperature ranged between 20.6°C - 23.8°C, relative humidity between 52 % - 88 % and rainfall between 163.2 mm – 1119.6 mm in the month of March during the years 2002, 2003 and 2004 (Table 2.1; Figs. 2.5a, 2.5b and 2.5c).

The males were observed to arrive first at the breeding site and were found hiding in between the tall grasses and emitted advertisement calls which induced the female to come out of their hiding place into the breeding site (Fig. 2.27). The males were heard calling in the evenings around 5.00 PM till late nights, and the calls lasted for a few days before amplexus took place. The advertisement call of *Rhacophorus bipunctatus* consisted of a number of calls produced in a series. Each call consisted of six notes, and each note was composed of a continuous group of pulses (Figs. 2.28a, 2.28b and 2.28c).

As the female approached the breeding site, the male moved towards the female and amplexus took place (Figs. 2.29, 2.30 and 2.31). The pair remained in amplexus for 3 to 4 hours or even longer. Courtship was seen to take place from the month of April when the atmospheric temperature recorded ranged between 22.7°C – 24.6°C, relative humidity between 68 % - 86 % and rainfall between 501.5 mm – 1335.2 mm and continued till August when temperature ranged between 21.3°C – 22.6°C, relative humidity between 65 % - 76 %, and rainfall between 1306.6 mm – 1480.9 mm during the study period, i.e., 2002, 2003 and 2004 (Table 2.1; Figs. 2.5a, 2.5b and 2.5c).

An unique behaviour of this frog observed was that the amplexing pair moved away from the water body to the main land for spawning and to construct their foam nests

on sloping grassy edges or vegetation which served as a substratum for the foam nest (Figs. 2.32, 2.33, 2.34, 2.35 and 2.36). During field observations some amplexing pairs were also seen to construct foam nests on branches of a plant (*Hibiscus rosasinensis*), which hanged over a water body (Fig. 2.37). Further, some foam nests were also seen to be attached to a cemented tank about 30 to 40 cm above the water surface near a residential area (Fig. 2.38).

In general, construction of the foam nests, took place at night. However, in May 2004, under laboratory conditions, *Rhacophorus bipunctatus* constructed its foam nest during the day time around 2:00 PM where the temperature recorded was 24.5°C and relative humidity was 81 % (Figs. 2.39, 2.40 and 2.41). During oviposition the male moved a little down, closer to the vent of the female. As the female laid the eggs, the male began secreting large amount of seminal fluid. Simultaneously, the male also started beating its hindlegs upwards and downwards so that it whipped up the seminal fluid resulting in construction of a foam nest. After foam nest construction, the male crawled out of the foam nest leaving the female with the foam nest (Fig. 2.42). But after sometimes, the female was seen leaving the breeding site, while the male was found to stay for a longer period of time around the foam nest.

The total number of foam nests observed and recorded in the year 2002 during the months of April to August at the study site III was 78 and at study site IV the total number of foam nests were 56 respectively. Similarly, in 2003 the total number of foam nests observed and recorded at study site III and site IV were 75 and 66 and 66 and 64 in the year 2004 (Table 2.6).

Size of amplexing frogs and clutch size: A total of twenty five amplexing pairs of *Rhacophorus bipunctatus* together with their foam nests were collected, and their snout vent length and the diameter of the foam nests were recorded in the year 2002. Similarly, collections were also made in the years 2003 and 2004 (Table 2.7). The diameter of the foam nests recorded ranged between 6 – 8 cm. *Rhacophorus bipunctatus* also exhibited a distinct sexual size dimorphism where females were larger (61.58 ± 0.60 mm) than the males (41.66 ± 0.28 mm; $t=14.89$; $p<0.001$) (Table 2.4).

The foam nests deposited on tree leaves were irregular in shape, while those deposited on grassy edges or vegetation were more or less round in shape (Fig. 2.43). The foam nests were usually observed to be light brown in colour, and after a few days the outer layer became dried and hard. In order to know the clutch size twenty five numbers of foam nests were collected from the field and brought to the laboratory in the year 2002. Similarly, in the years 2003 and 2004 collections were also made to know the clutch size (Table 2.7). The eggs were counted and recorded, and allowed to hatch under the laboratory conditions. The clutch size varied from 45 to 76 with a mean of 62 ± 0.47 in different foam nests (Table 2.4) and the egg measured 3.0 mm in diameter.

Sex ratio at the breeding site: As in case of *Polypedates leucomystax*, in *Rhacophorus bipunctatus* also the males outnumbered the females at the breeding sites. The number of males observed and recorded in the year 2002 in the months of April, May, June, July and August was 87 and that of females was 72 respectively at study site III. While the total number of males and females observed and recorded at study site IV were 87 and 59 respectively. In 2003 the total number of males and females observed and recorded were

90 and 67 in study site III and 91 and 73 in study site IV. Similarly, in the year 2004 the total number of males and females observed and recorded at site III were 84 and 62 and 84 and 60 in study site IV. The number of frogs captured increased from April to July and started decreasing in the month of August (Table 2.8). In the month of September no more adult frogs were seen and only a number of metamorphosed froglets were seen.

Breeding cycle of *Polypedates leucomystax* and *Rhacophorus bipunctatus*:

It was observed that the annual breeding cycles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were similar. The breeding behaviours of these two tree frogs were studied in relation to temperature, relative humidity and rainfall (Table 2.1; Figs. 2.5a, 2.5b and 2.5c). Our observations indicated that the annual breeding cycle of *Polypedates leucomystax* and *Rhacophorus bipunctatus* comprised four phases, namely (1). Hibernating phase (2.) Emerging and pre-breeding phase (3.) Breeding and spawning phase, and (4.) Post-breeding phase (Fig. 2.44). A brief account of different phases of their breeding cycle is given in the following sections.

1. Hibernation phase (November to February): Both the species were found to be physically inactive during the hibernation phase (Fig. 2.44). At Cherrapunjee, average ambient temperature recorded during the study period (2002, 2003 and 2004) ranged between 13.5°C and 20.5°C. Relative humidity ranged between 42 % and 69 %. Rainfall recorded was lowest during this phase and ranged between 0.0 mm and 298.6 mm (Table 2.1; Figs. 2.5a, 2.5b and 2.5c). It was very difficult to locate the frogs during the

hibernation phase. *Polypedates leucomystax* was found to hibernate under forest litters, while *Rhacophorus bipunctatus* was seen hiding in tree holes and banana stem.

2. Emergence and pre-breeding phase (March): *Polypedates leucomystax* and *Rhacophorus bipunctatus* came out of their hibernation ground during the month of March (Fig. 2.44). The temperature recorded during this month ranged between 20.6°C and 23.8°C. Relative humidity ranged between 52 % and 88 % and rainfall ranged between 163.2 mm and 1119.6 mm (Table 2.1; Figs. 2.5a, 2.5b and 2.5c). This phase of emergence was very short, and after emergence *Polypedates leucomystax* and *Rhacophorus bipunctatus* were found in the vegetation present near the breeding site. This phase was followed by the breeding phase.

3. Breeding and Spawning phase (April to August): Advertisement calls made by the males of both the species indicated the beginning of the breeding phase. This phase extended from the month of April to August (Fig. 2.44). During this phase, ambient temperature ranged between 21.3°C and 25.7°C. Average relative humidity recorded during this study period ranged between 65 % and 89 %. Rainfall recorded ranged between 501.5 mm – 5008.2 mm (Table 2.1; Figs. 2.5a, 2.5b and 2.5c). During the breeding phase, *Polypedates leucomystax* and *Rhacophorus bipunctatus* exhibited advertisement calls, amplexus, spawning, and foam nest construction.

4. Post-breeding phase (September to October): During this phase September to October, the frogs started moving away from the breeding sites towards the hibernation

sites (Fig. 2.44). Ambient temperature varied between 19.4°C and 22.6°C. Relative humidity ranged between 54 % and 79 %. Rainfall during the study period ranged between 66.3 mm and 1917.3 mm (Table 2.1; Figs. 2.5a, 2.5b and 2.5c). No more foam nests were observed at the breeding sites during this phase and the tree frogs moved to the nearby forest covers and were found mostly on leaves of trees and below the covering sheaths of banana stems. Physical inactivity of the frogs signaled the beginning of hibernation phase.

Histology of the testis and ovary of *Polypedates leucomystax*:

The testes and ovary of *Polypedates leucomystax* was studied and observed histologically during the different phases of the breeding cycle. The testis of *Polypedates leucomystax* weighed between 0.41 – 0.80gm.

During the breeding cycle the histological sections of the testis of *Polypedates leucomystax* showed the presence of different stages of spermatogenic cells and sertoli cells. During the hibernation phase, boundaries of the seminiferous tubules were visible but not distinct. Spermatogonia and spermatocytes were present in large number. Spermatogonia were spherical in shape. Spermatids were also observed (Fig. 2.45). The spermatocytes under go further development into spermatids during the emergence phase. During the emergence phase the seminiferous tubules and connective tissue were distinct. The spermatids appeared in a cluster and their number increased. Spermatozoa were not observed during this phase (Fig. 2.46).

During the breeding phase the seminiferous tubule were clearly distinct and appeared more or less rounded in shape, and large in size. Presence of spermatozoa in the

seminiferous tubule indicated the beginning of the breeding phase. Spermatozoa were observed to be slender and filamentous. Mature spermatozoa occur in a cluster with their tail extending into the lumen of the tubules (Fig. 2.47). The seminiferous tubule during the post breeding phase was not clearly distinct and appeared smaller in size. During this phase and spermatogonia were found to be present and a few spermatids in cluster were observed (Figs. 2.48).

During the hibernation phase the ovary appeared as membranous structure and during the emergence phase a few number of oogonia were present. During the breeding period the weight of the ovaries ranged between 1.82 – 2.31 gm. The sections of the ovary during the breeding period showed the presence of young as well as mature oocytes (Figs. 2.49 and 2.50). Each mature oocyte was found to be covered by a vitelline membrane. Presence of large amount of yolk was observed. The size of the oocytes ranged between 0.2 - 1.5 mm in diameter. Oocytes which varied between 0.2 - 0.7 mm in diameter can be classified as young oocytes and those which ranged between 0.8 – 1.5 mm in diameter as matured oocytes. During the post breeding phase all the eggs were released.

Histology of the testis and ovary of *Rhacophorus bipunctatus*:

The testes and ovary of *Rhacophorus bipunctatus* was also studied and observed histologically during the different phases of the breeding cycle. The weight of the testis varied between 0.18 – 0.26 gm.

During the breeding cycle the histological sections of the testis of *Rhacophorus bipunctatus* showed the presence of different stages of spermatogenic cells. During the

hibernation phase the seminiferous tubule showed the presence of a large number of spermatogonia and spermatocytes. Spermatids were also found to be present but less in number (Fig. 2.51). During the emergence phase seminiferous tubules were distinct and spermatids were found to be present and appeared in a cluster, and a few spermatozoa were also present (Fig. 2.52).

During the breeding phase the seminiferous tubule was clearly distinct and more or less oval in shape. The seminiferous tubule was filled with spermatozoa. Spermatozoa were observed to be slender and filamentous. Different stages of spermatogonial cells, primary and secondary spermatocytes were also observed during this phase (Fig. 2.53). During the post breeding phase the seminiferous tubule had decreased in size no more spermatozoa were observed as they have been released during the breeding phase. A few spermatids in cluster and a large number of spermatogonia were found to be present during this phase (Figs. 2.54).

Similarly, during the hibernation phase the ovary appeared as membranous structure in *Rhacophorus bipunctatus* and during the emergence phase a few number of oocytes were present. During the breeding phase there are several lobes on each side and the ovary size varied depending on the maturity of the ova in the ovary (Figs. 2.55 and 2.56). The weight of the ovaries ranged between 1.86 - 2.31 gm during the breeding phase. Both young and mature oocytes were found to be present during the breeding phase and the size of the oocytes ranged between 0.4 and 3 mm in diameter. Oocytes which ranged between 0.4 and 0.8 mm in diameter were classified as young oocytes and those between 0.9 and 3 mm in diameter as mature oocytes.



Fig. 2.1 – Study site I – Breeding site of *Polypedates leucomystax*.



Fig. 2.2 – Study site II – Breeding site of *Polypedates leucomystax*.



Fig. 2.3 – Study site III – Breeding site of *Rhacophorus bipunctatus*.



Fig. 2.4 – Study site IV – Breeding site of *Rhacophorus bipunctatus*.

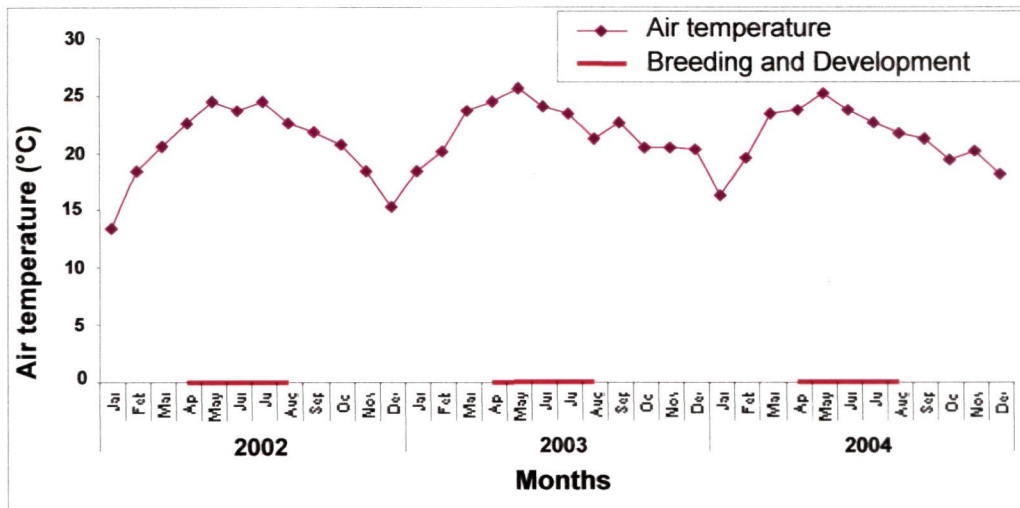


Fig. 2.5a – Monthly variation in air temperature at Cherrapunjee 2002, 2003 and 2004.

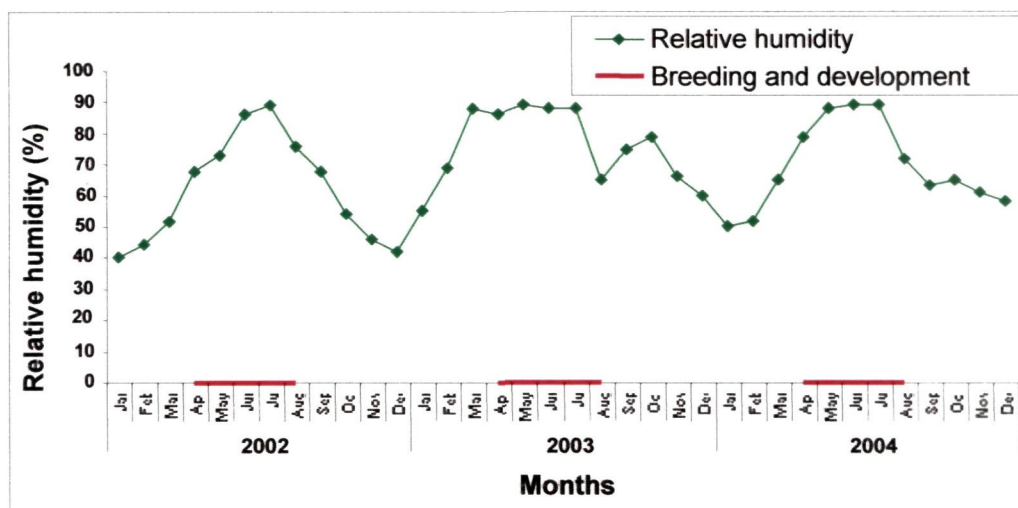


Fig. 2.5b – Monthly variation in relative humidity Cherrapunjee 2002, 2003 and 2004.

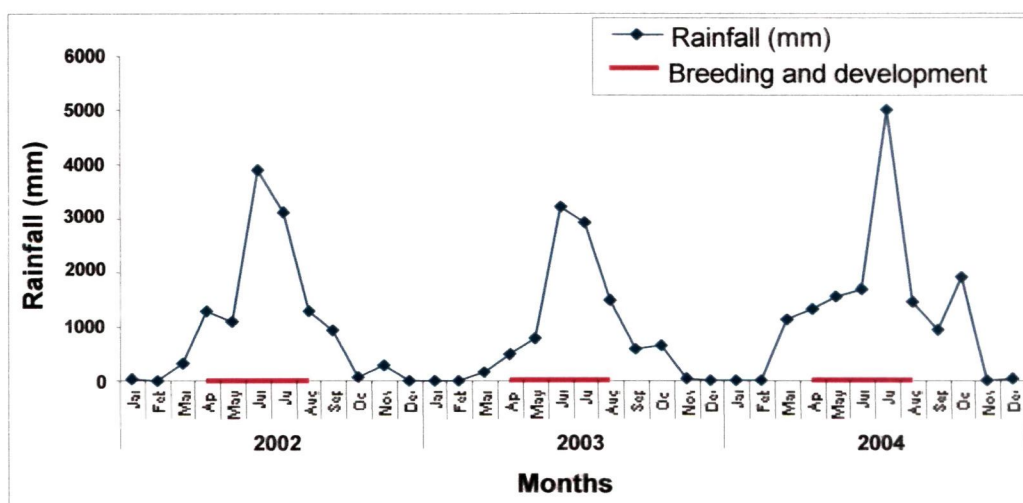


Fig. 2.5c – Monthly variation in rainfall at Cherrapunjee 2002, 2003 and 2004.



Fig. 2.6 – *Polypedates leucomystax* (Hibernating).



Fig. 2.7 – Male emerging from hibernation.



Fig. 2.8 – Male croaking at the breeding site.



Fig. 2.9 – Female emerging from its hibernation.



Fig. 2.10 – Female moving towards the breeding site.



Fig. 2.11 – Male and female at the breeding site.

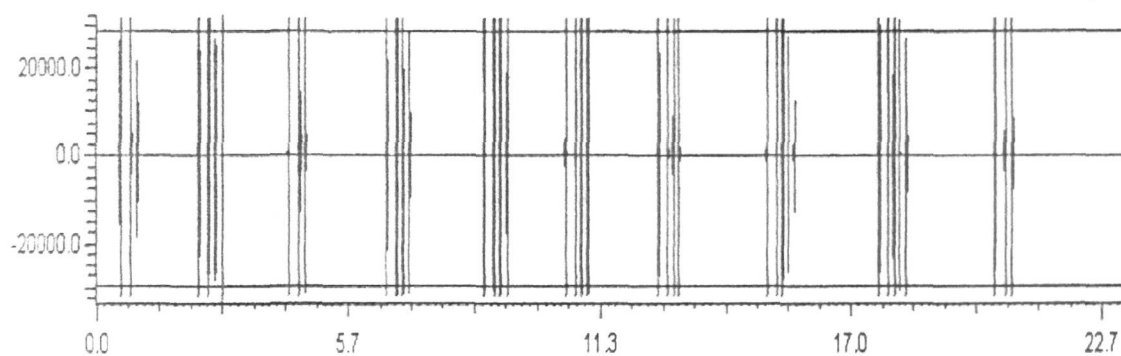


Fig. 2.12a – Oscillogram with time scale 22.7 secs

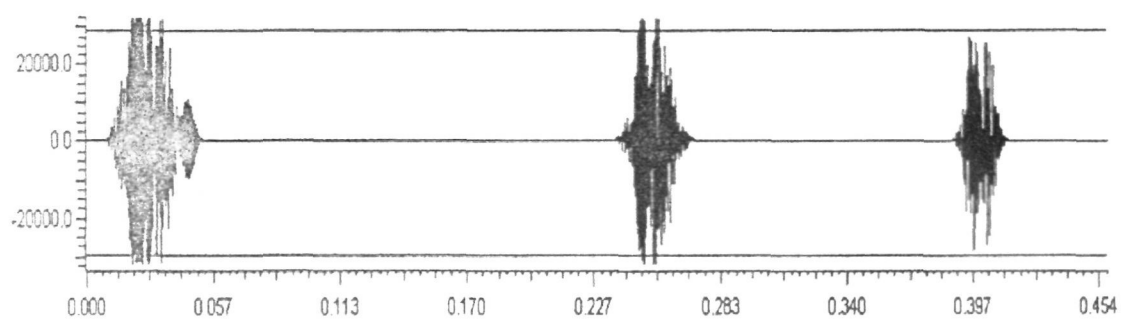


Fig. 2.12b – Selected piece of upper oscillogram showing three notes

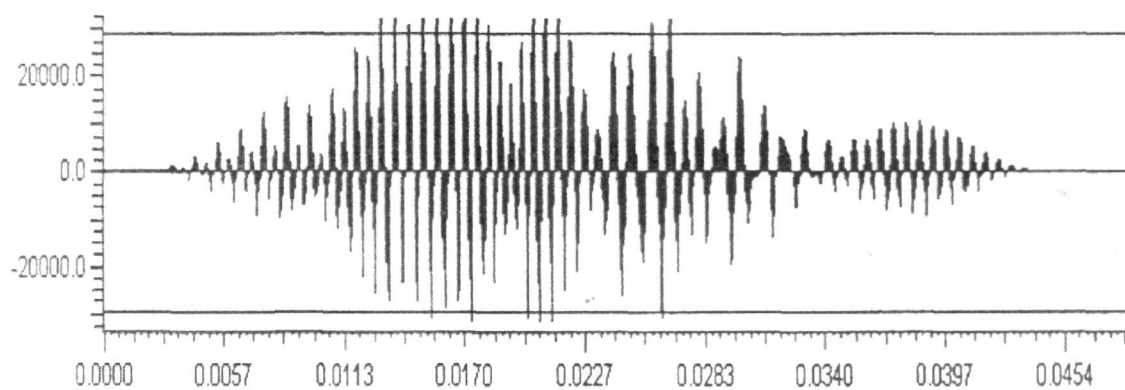


Fig. 2.12c – Detailed oscillogram of one note



Fig. 2.13 – Amplecting pair at the breeding site.



Fig. 2.14 – Amplecting pair at the breeding site.



Fig. 2.15 – Amplecting pair at the breeding site.



Fig. 2.16 – Foam nest about 30cm above the water surface.



Fig. 2.17 – Foam nest attached to rocks and stones.



Fig. 2.18 – Foam nest attached to rocks and stones.

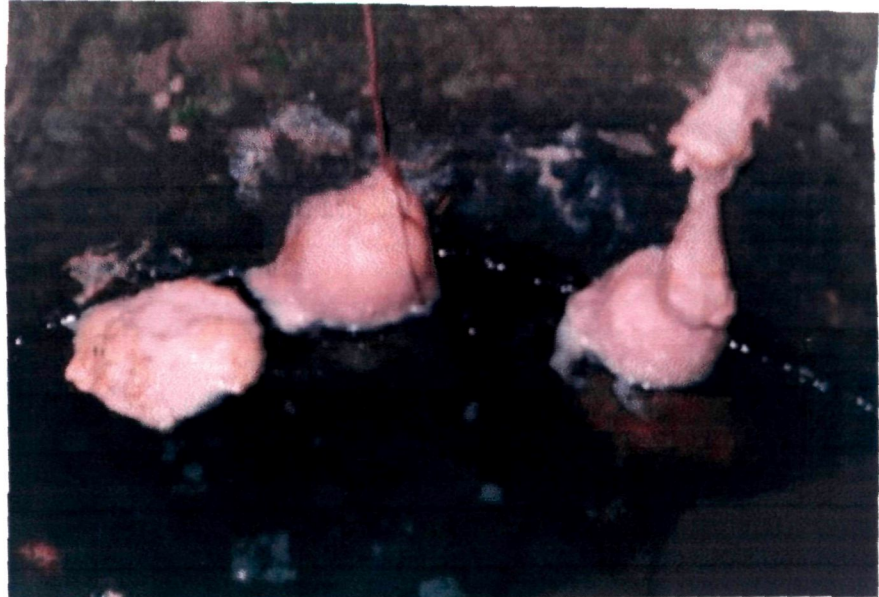


Fig. 2.19 – Foam nests in a cemented tank.



Fig. 2.20 – Males guarding the foam nests under grass cover.



Fig. 2.21 – Male guarding the foam nests under grass cover.

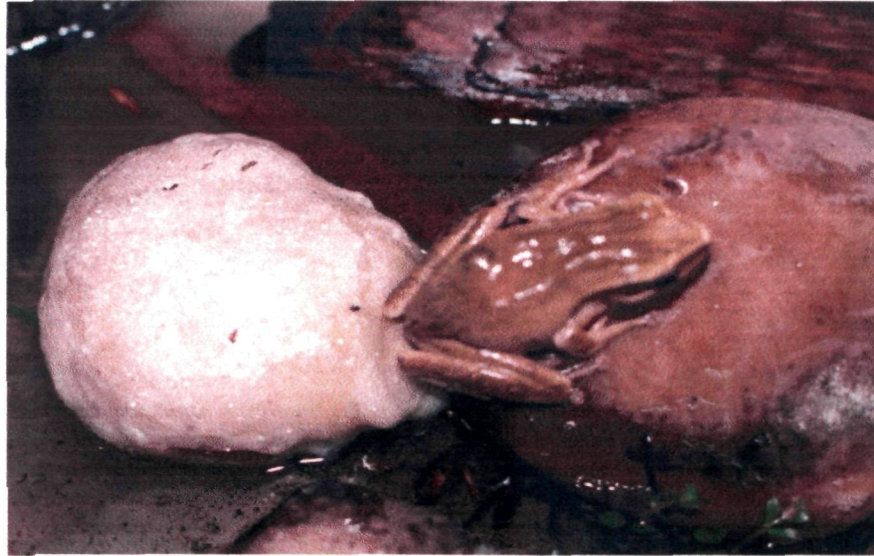


Fig. 2.22 – Construction of foam nest under laboratory condition.



Fig. 2.23 – Male guarding the foam nest.



Fig. 2.24 – Female moving away from the foam nest.



Fig. 2.25 – Males at the breeding site.



Fig. 2.26 – *Rhacophorus bipunctatus* (Hibernating).



Fig. 2.27 – Male emerging from hibernation.

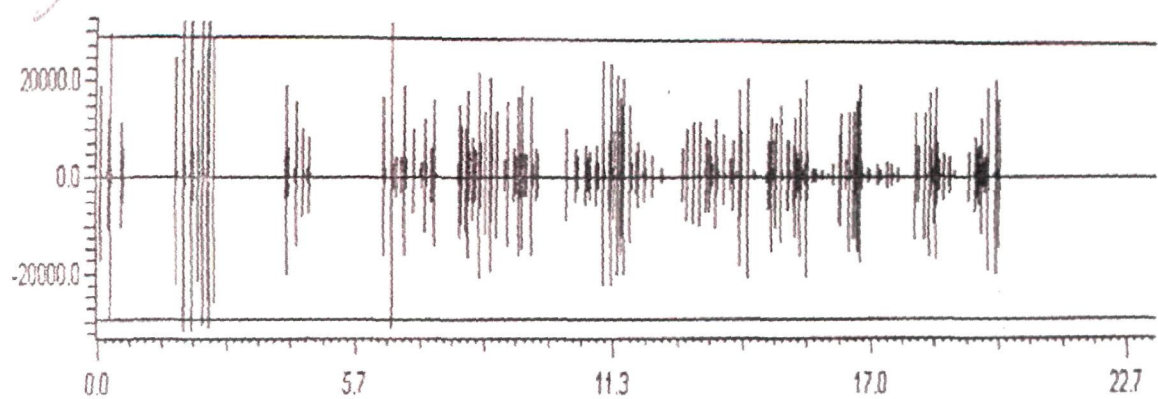


Fig. 2.28a – Oscillogram with time scale 22.7 secs.

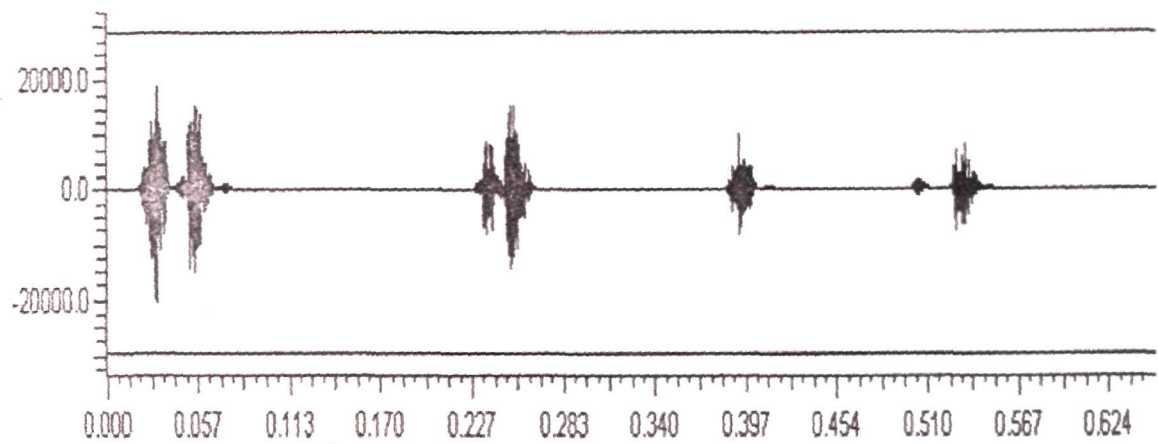


Fig. 2.28b – Selected piece of upper oscillogram showing three notes.

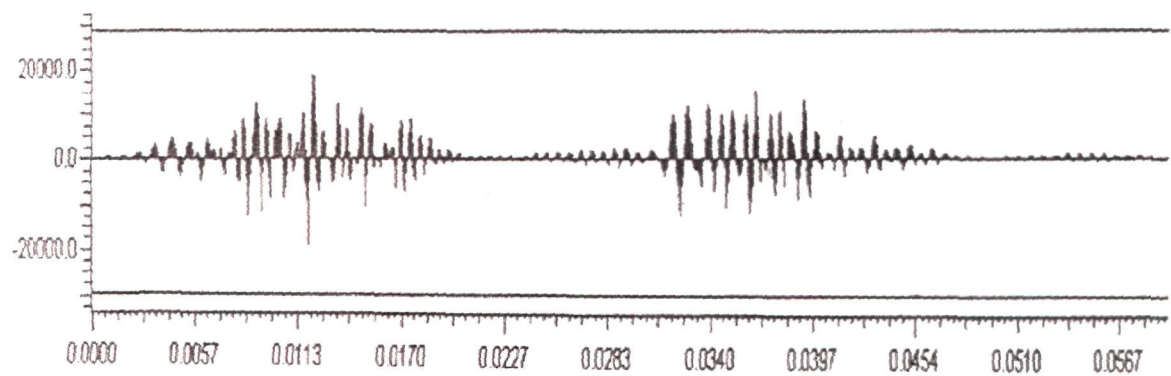


Fig. 2.28c – Detailed oscillogram of one note.



Fig. 2.29 – Female emerging at the breeding site.



Fig. 2.30 – Male and female emerging at the breeding site.



Fig. 2.31 – Amplexing pair in the water at the breeding site.



Fig. 2.32 – Amplecting pair in the water at the breeding site.



Fig. 2.33 – Amplecting pair moving towards main land.



Fig. 2.34 – Amplecting pair moving towards main land.



Fig. 2.35 – Amplecting pair in main land.



Fig. 2.36 – Amplecting pair with foam nest.



Fig. 2.37 – Foam nest attached to a leaf (*Hibiscus rosasinensis*).



Fig. 2.38 – Female with foam nest in cemented tank.



Fig 2.39 – Construction of foam nest under laboratory condition.



Fig 2.40 – Construction of foam nest under laboratory condition.



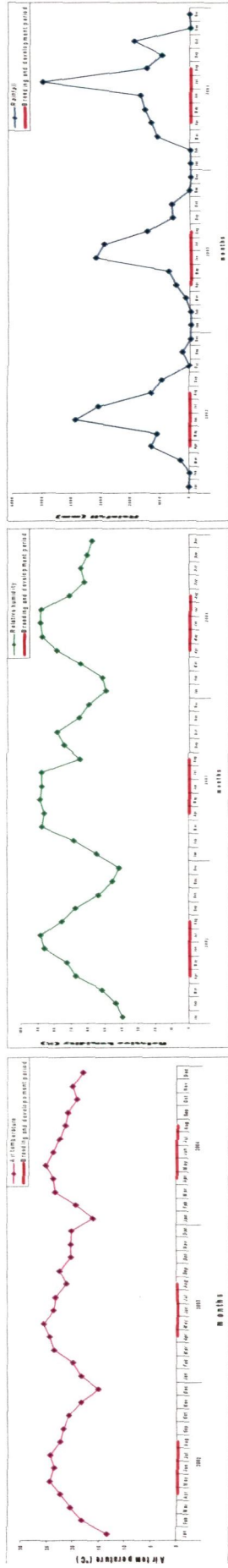
Fig 2.41 – Female with foam nest under laboratory condition.



Fig: 2.42 – Female near the foam nest.



Fig: 2.43 – Foam nest on a tree leaf.



- Hibernating phase
- Emergence phase
- Breeding phase
- Post-breeding phase

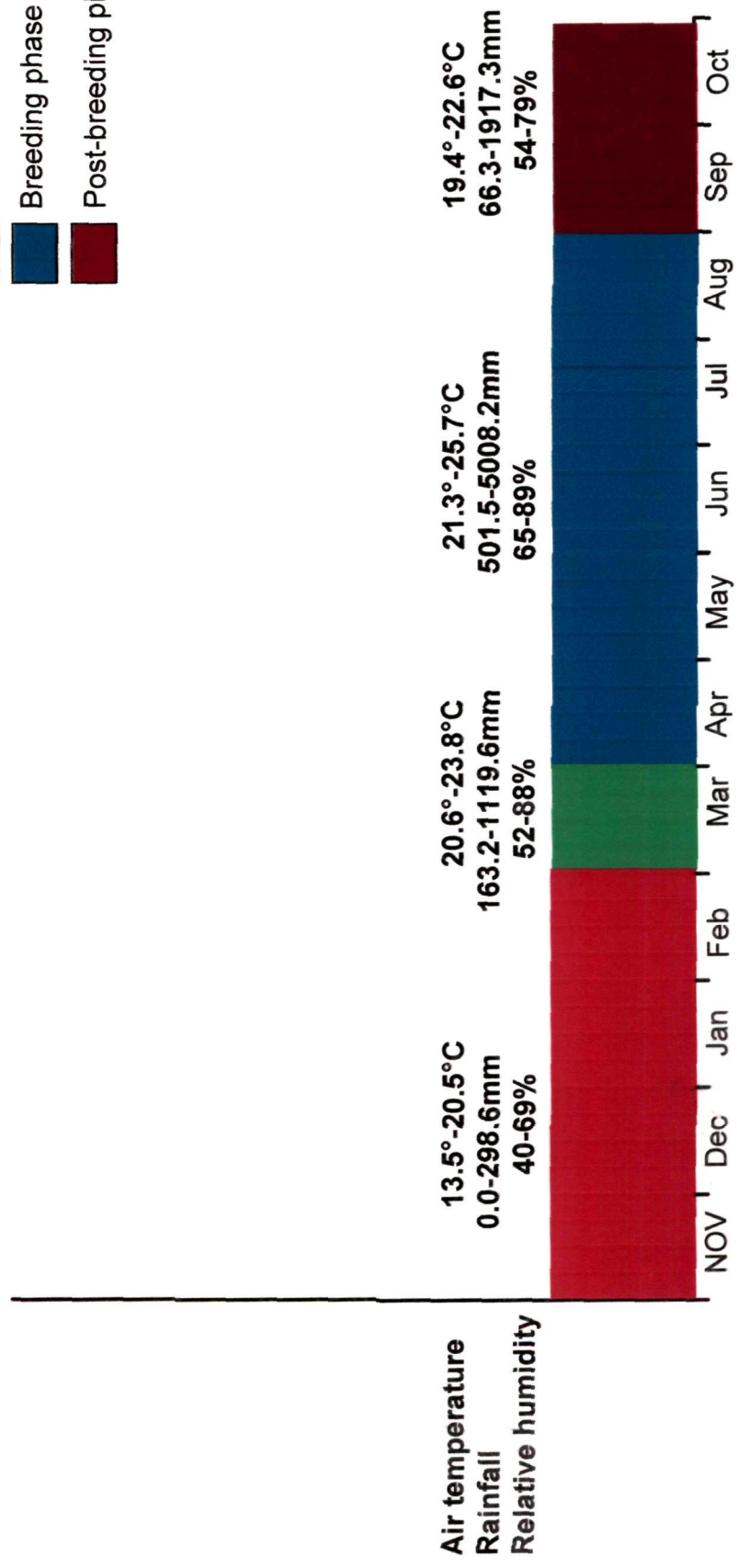


Fig. 2.44 – Breeding cycle of *Poypedates leucomystax* and *Rhacophorus bipunctatus*

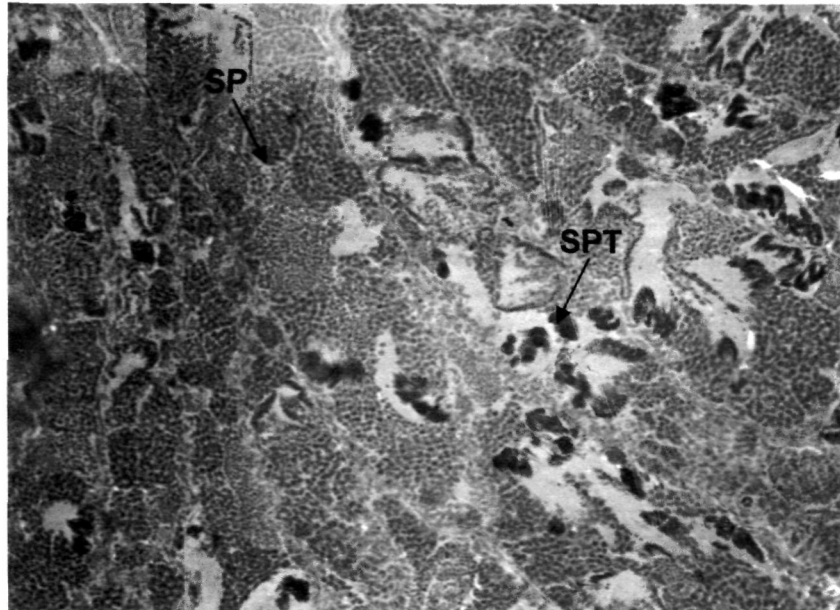


Fig. 2.45 – Histological section of testis of *Polypedates leucomystax* during hibernating phase (X160).

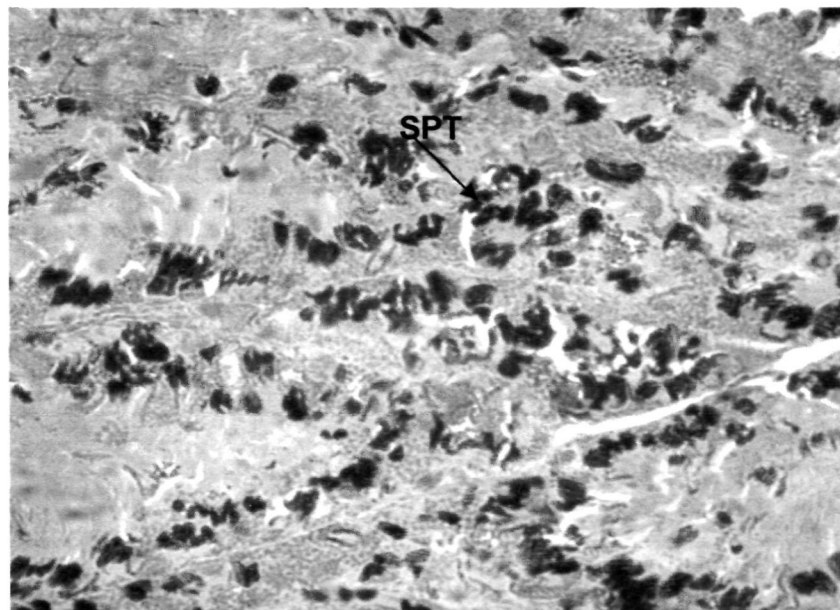


Fig. 2.46 – Histological section of testis of *Polypedates leucomystax* during emerging phase (X160).

SPT=Spermatids; SPZ=Spermatozoa.

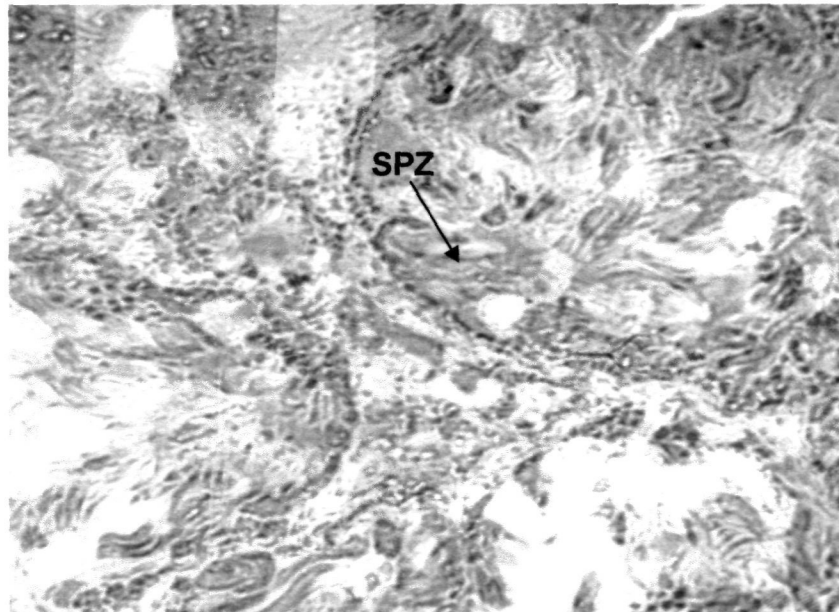


Fig. 2.47 – Histological section of testis of *Polypedates leucomystax* during breeding phase (X160).

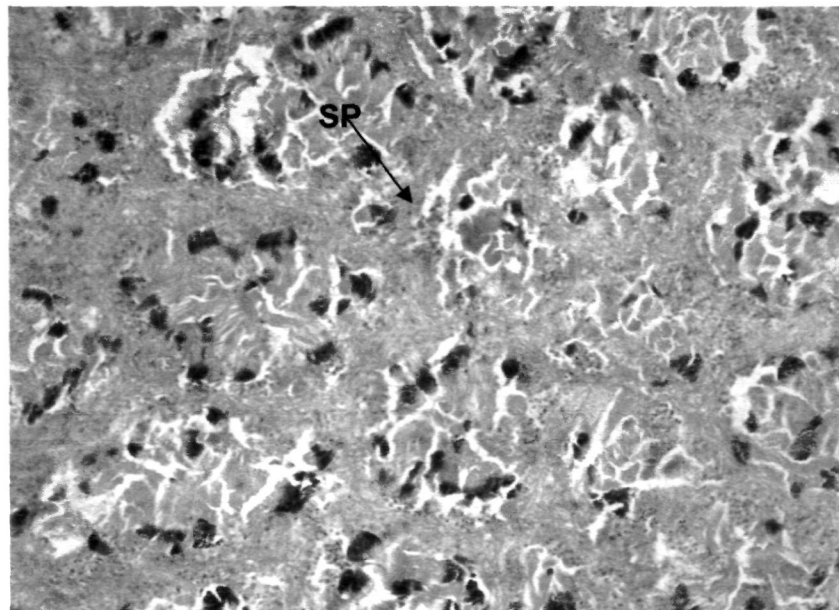


Fig. 2.48 – Histological section of testis of *Polypedates leucomystax* during post breeding phase (X160).

SP=Spermatocytes; SPZ=Spermatozoa.

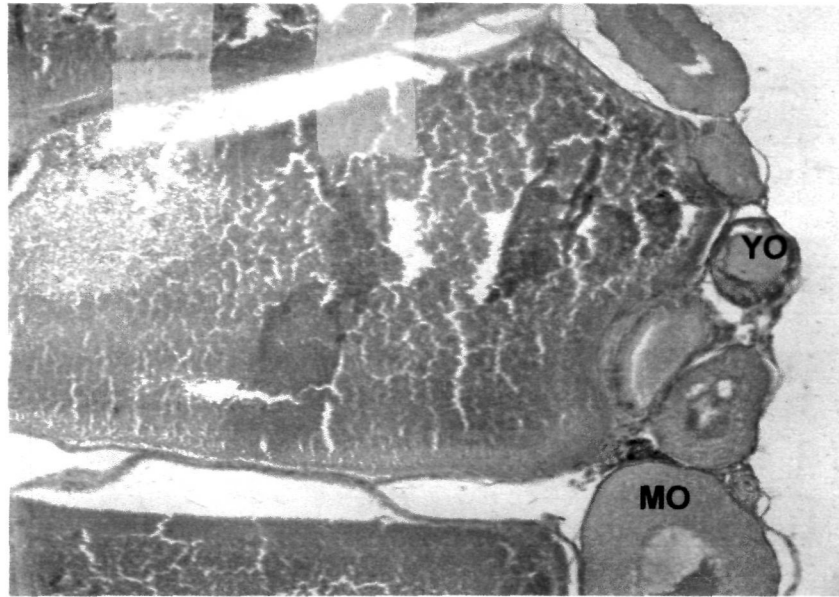


Fig. 2.49 – Histological sections of ovary of *Polypedates leucomystax* during the breeding phase showing the young and mature oocytes (X160).

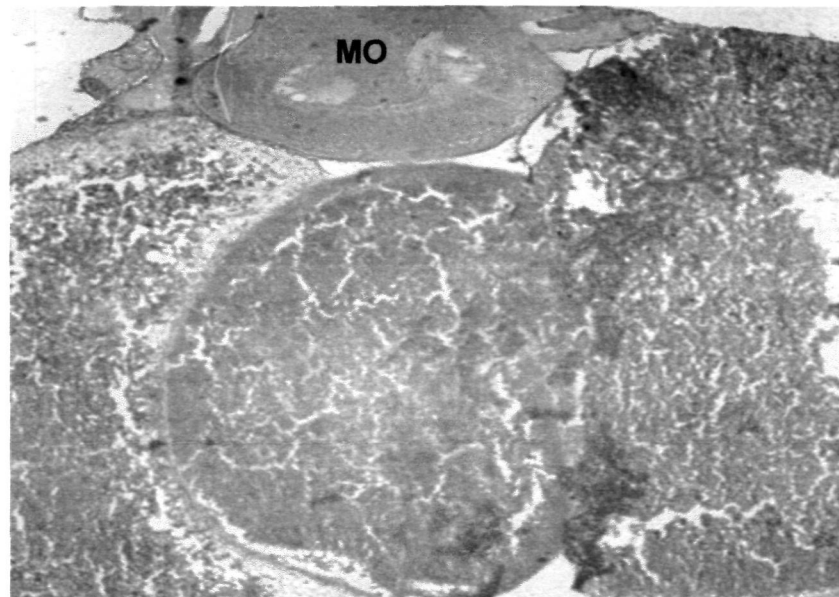


Fig. 2.50 – Histological sections of ovary of *Polypedates leucomystax* during the breeding phase showing the mature oocyte (X160).

MO=Mature oocyte; YO=Young oocyte.

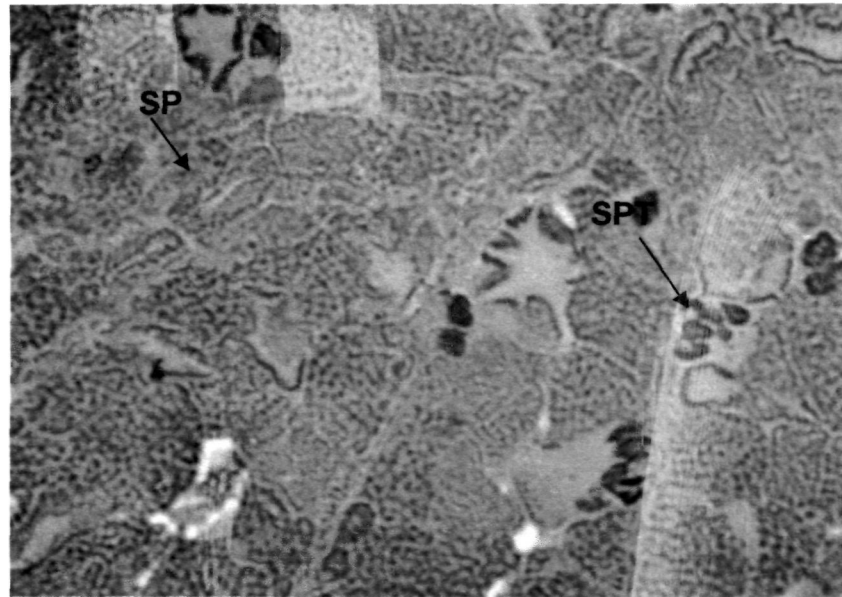


Fig. 2.51 – Histological section of testis of *Rhacophorus bipunctatus* during hibernating phase (X160).

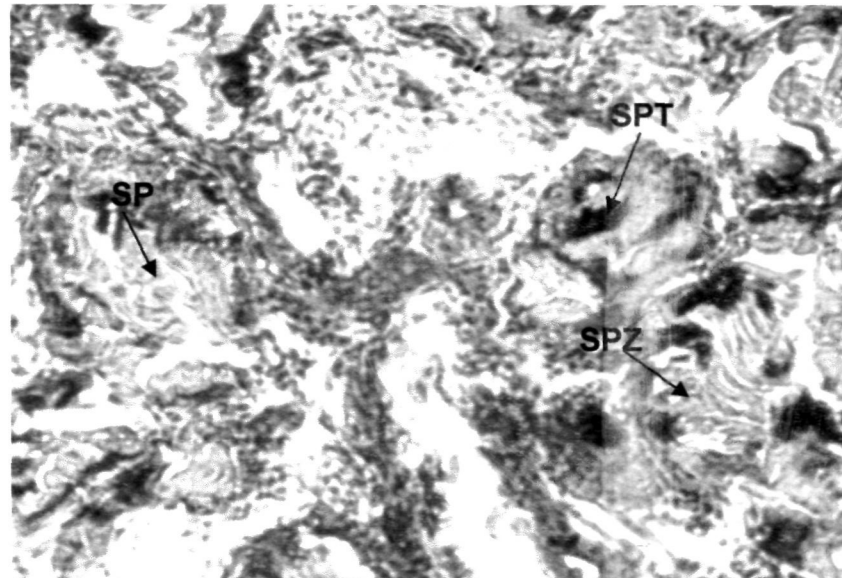


Fig. 2.52 – Histological section of testis of *Rhacophorus bipunctatus* during emerging phase (X160).

SP=Spermatocytes; SPT=Spermatids; SPZ=Spermatozoa.

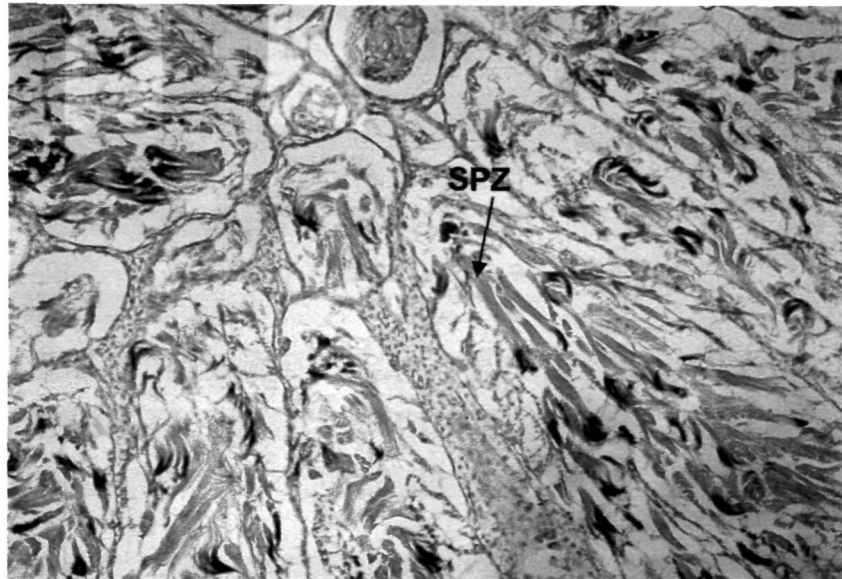


Fig. 2.53 – Histological section of testis of *Rhacophorus bipunctatus* during breeding phase (X160).

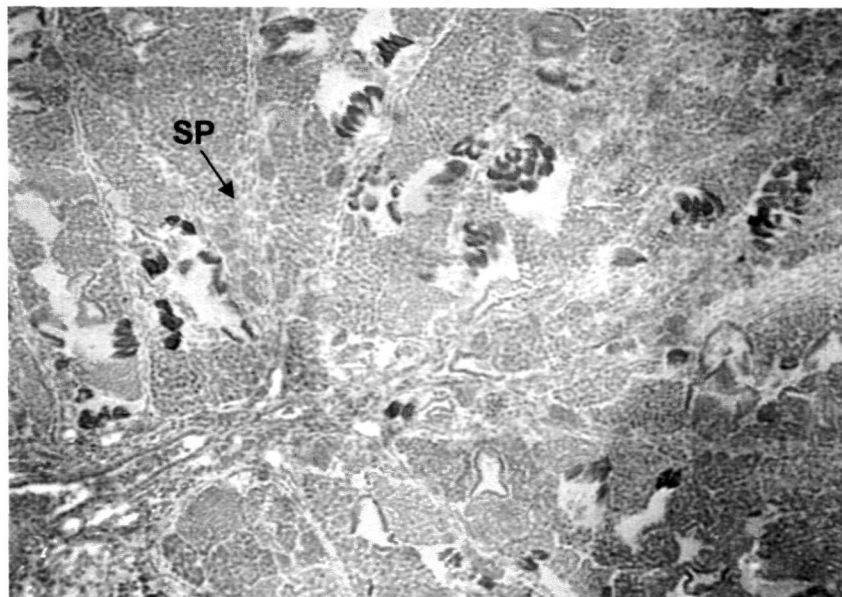


Fig. 2.54 – Histological section of testis of *Rhacophorus bipunctatus* during post breeding phase (X160).

SP=Spermatocytes; SPZ=Spermatozoa.



Fig. 2.55 – Histological sections of ovary of *Rhacophorus bipunctatus* during the breeding phase (X160).

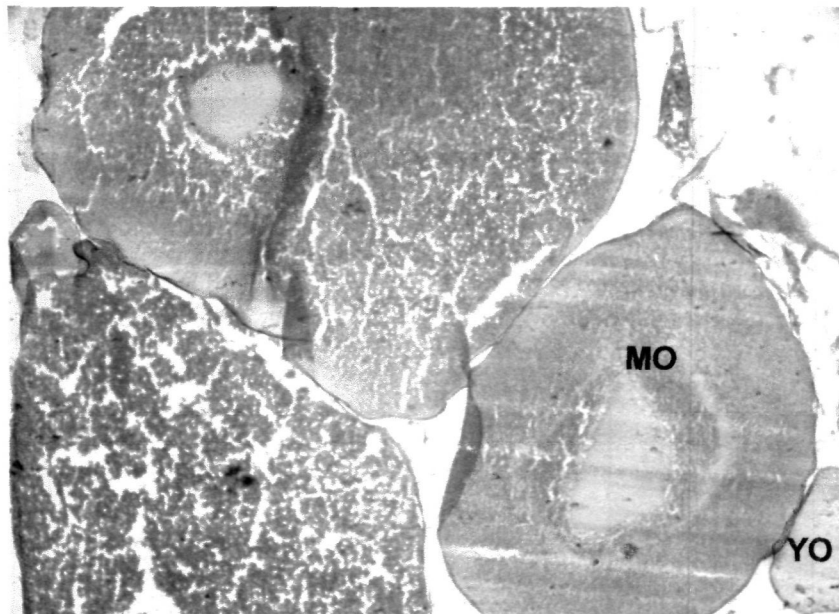


Fig. 2.56 – Histological sections of ovary of *Rhacophorus bipunctatus* during the breeding phase (X160).

MO=Mature oocyte; YO=Young oocyte.

Table 2.1: Average atmospheric temperature, relative humidity, and rainfall at the study sites I, II, III and IV (Cherrapunjee) during the study period (2002, 2003 and 2004)

Months	Temperature(°C)			Relative humidity (%)			Rainfall (mm)2004		
	2002	2003	2004	2002	2003	2004	2002	2003	2004
Jan	13.5±0.71	18.5±0.39	16.2±0.32	40±0.12	55±0.17	50±0.26	39.9	4.7	4.2
Feb	18.5±0.41	20.2±0.41	19.5±0.15	44±0.31	69±0.27	52±0.58	1.1	9.2	12.6
Mar	20.6±0.22	23.8±0.28	23.5±0.45	52±0.42	88±0.51	65±0.42	316.8	163.2	1119.6
Apr	22.7±0.20	24.6±0.13	23.8±0.72	68±0.44	86±0.43	79±0.30	1294.2	501.5	1335.2
May	24.6±0.31	25.7±0.70	25.2±0.25	73±0.51	89±0.70	88±0.12	1093.9	764.5	1548.5
Jun	23.8±0.14	24.0±0.09	23.8±0.62	86±0.12	88±0.26	89±0.70	3893.4	3221.9	1677.5
Jul	24.6±0.52	23.5±0.52	22.6±0.53	89±0.19	88±0.36	89±0.56	3100.2	2927.1	5008.2
Aug	22.6±0.48	21.3±0.41	21.7±0.42	76±0.20	65±0.41	72±0.62	1306.6	1480.9	1472.9
Sep	21.9±0.40	22.6±0.15	21.2±0.20	68±0.30	75±0.20	63±0.48	948.8	598.9	947.1
Oct	20.8±0.33	20.5±0.21	19.4±0.12	54±0.22	79±0.18	65±0.18	66.3	651.0	1917.3
Nov	18.5±0.18	20.5±0.27	20.1±0.58	46±0.26	66±0.26	61±0.38	298.6	16.4	0.0
Dec	15.3±0.12	20.3±0.34	18.2±0.62	42±0.33	60±0.31	58±0.56	1.0	11.0	28.0

Table 2.2: Total number of foam nests of *Polypedates leucomystax* recorded during the study period (2002, 2003 and 2004)

Study period	Months	<i>Polypedates leucomystax</i>	
		Study site I	Study site II
2002	April	12	10
	May	15	17
	June	21	23
	July	26	21
	August	22	18
	Total	96	89
2003	April	14	10
	May	17	15
	June	21	19
	July	24	22
	August	18	15
	Total	94	81
2004	April	10	12
	May	14	12
	June	24	19
	July	23	20
	August	17	14
	Total	88	77

Table 2.3: Size of amplexing pairs and clutch size of *Polypedates leucomystax* during the study period (2002, 2003 and 2004)

Sl No.	2002					2003					2004				
	SVL male (mm)	SVL female (mm)	Diameter of foam nest (cm)	Clutch size		SVL male (mm)	SVL female (mm)	Diameter of foam nest (cm)	Clutch size		SVL male (mm)	SVL female (mm)	Diameter of foam nest (cm)	Clutch size	
1.	56	74	7	508		61	75	10	521		58	63	9	369	
2.	45	63	6	454		66	79	6	507		61	71	10	289	
3.	61	79	10	252		56	66	8	489		55	70	10	397	
4.	58	68	8	321		52	69	12	369		60	70	6	436	
5.	43	63	10	393		64	70	9	387		66	75	11	412	
6.	65	76	10	405		63	72	9	456		63	74	8	306	
7.	63	79	7	361		51	75	6	279		62	70	12	298	
8.	57	68	8	278		55	69	10	306		59	69	12	518	
9.	49	61	10	530		56	70	10	458		58	71	6	506	
10.	51	77	10	457		58	68	9	478		57	69	10	469	
11.	55	71	9	395		61	72	8	423		58	70	8	480	
12.	58	69	12	487		58	70	10	314		56	73	11	390	
13.	63	78	9	356		64	73	10	508		61	75	9	455	
14.	66	80	12	412		66	80	8	506		65	71	10	276	
15.	64	79	10	519		63	78	9	459		63	70	10	381	
16.	59	68	8	398		62	67	11	426		60	69	6	473	
17.	69	81	10	506		60	76	7	357		57	68	12	451	
18.	61	75	9	493		59	69	10	478		60	69	12	375	
19.	56	66	8	364		56	68	9	416		59	72	10	513	
20.	59	72	12	457		55	73	12	319		58	71	9	426	
21.	53	71	9	389		60	71	8	426		53	72	9	478	
22.	66	77	10	489		61	74	7	487		56	69	8	382	
23.	55	69	11	402		57	68	9	433		63	75	8	362	
24.	56	69	10	456		54	69	9	294		61	73	10	446	
25.	61	67	8	397		54	70	10	419		65	76	10	458	
26.	60	73	10	336		56	69	10	349		60	77	9	476	
27.	53	67	9	506		62	75	11	410		59	71	11	372	
28.	58	71	10	518		61	68	9	455		57	69	7	378	
29.	59	70	10	479		63	67	10	362		61	68	9	289	
30.	66	70	8	463		59	70	10	379		64	70	10	339	
Mean	58.16±1.11	71.20±1.24	9.33±0.26	426.03±13.47		59.10±0.73	71.33±0.67	9.2±0.27	415.39±12.78		59.83±0.57	71.0±0.52	9.4±0.31	407.33±13.03	

Table 2.4: Range and mean of amplexing pairs, diameter of foam nest and clutch size of *Polypedates leucomystax* and *Rhacophorus bipunctatus* during the study period (2002, 2003 and 2004)

	<i>Polypedates leucomystax</i>		<i>Rhacophorus bipunctatus</i>	
	Range	Mean	Range	Mean
SVL male (mm)	43 – 49	59.03±0.48 *	36 – 47	41.66±0.28 **
SVL female (mm)	61 – 80	71.17±0.09	55 – 68	61.58±0.60
Diameter of foam nest (cm)	6 – 12	9.31±0.05	6 – 8	7.16±0.08
Clutch size (number of eggs)	252 – 530	416±5.41	45 – 76	62±0.47

* Differ significantly from females ($t=5.80$; $p<0.001$)

** Differ significantly from females ($t=4.89$; $p<0.001$)

Table 2.5: Number of males and females of *Polypedates leucomystax* at the study sites I and II during the study period (2002, 2003 and 2004)

Months	2002				2003				2004			
	Site I		Site II		Site I		Site II		Site I		Site II	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
April	16	10	20	12	15	12	12	8	17	8	14	10
May	22	15	18	16	21	16	17	13	16	11	20	16
June	21	15	23	18	19	14	22	16	20	17	22	17
July	28	21	23	25	21	19	26	23	25	20	23	21
August	9	10	12	8	11	9	8	8	14	8	10	8
Total	96	71	96	79	87	70	85	68	92	64	89	72

Table 2.6: Total number of foam nests of *Rhacophorus bipunctatus* recorded during the study period (2002, 2003 and 2004)

Study period	Months	<i>Rhacophorus bipunctatus</i>	
		Study site III	Study site IV
2002	April	8	6
	May	12	11
	June	18	15
	July	23	14
	August	17	10
	Total	78	56
2003	April	10	8
	May	12	11
	June	15	15
	July	23	19
	August	15	13
	Total	75	66
2004	April	9	8
	May	12	12
	June	16	18
	July	18	16
	August	11	10
	Total	66	64

Table 2.7: Size of amplexing pairs and clutch size of *Rhacophorus bipunctatus* during the study period (2002, 2003 and 2004)

SI No.	2002				2003				2004			
	SVL male (mm)	SVL female (mm)	Diameter of foam nest (cm)	Clutch size	SVL male (mm)	SVL female (mm)	Diameter of foam nest (cm)	Clutch size	SVL male (mm)	SVL female (mm)	Diameter of foam nest (cm)	Clutch size
1.	41	64	6	53	42	56	8	51	44	63	6	52
2.	39	65	8	57	39	59	8	45	41	65	7	45
3.	42	68	6	62	47	63	7	61	39	56	6	66
4.	46	64	8	51	40	66	8	47	42	67	8	63
5.	36	56	7	56	41	58	7	54	43	60	8	50
6.	43	67	7	67	40	60	6	56	42	61	8	63
7.	44	65	6	45	46	61	6	63	38	59	7	54
8.	40	68	6	63	43	65	8	70	39	56	6	58
9.	39	58	8	69	39	61	8	67	39	67	8	62
10.	45	67	8	49	44	59	7	66	44	62	7	63
11.	40	56	7	58	42	58	8	70	42	63	7	69
12.	47	66	7	74	39	63	7	71	40	60	8	70
13.	44	67	6	66	39	64	8	49	43	58	6	71
14.	38	57	6	59	46	60	6	59	40	59	8	58
15.	41	65	8	71	45	63	8	55	38	63	7	66
16.	47	64	8	76	45	61	8	56	47	63	8	70
17.	38	55	7	47	47	66	7	62	39	64	6	68
18.	42	66	6	65	41	65	6	62	46	59	7	69
19.	39	67	6	72	40	59	8	60	38	59	8	73
20.	44	65	8	54	42	58	7	67	39	60	6	58
21.	40	58	8	55	39	61	8	65	42	57	6	56
22.	41	56	7	61	38	60	6	71	42	55	7	68
23.	44	63	7	66	43	59	7	68	41	64	7	63
24.	46	64	7	75	40	60	8	69	39	60	8	66
25.	39	59	8	72	45	62	8	63	41	62	8	67
Mean	41.80±0.61	62.8±0.87	7.04±0.16	61.72±1.8	42.08±0.56	61.08±0.54	7.32±0.16	61.08±1.54	41.11±0.49	60.88±0.65	7.12±0.16	62.72±1.44

Table 2.8: Number of males and females of *Rhacophorus bipunctatus* at the study sites III and IV during the study period (2002, 2003 and 2004)

Months	2002				2003				2004			
	Site III		Site IV		Site III		Site IV		Site III		Site IV	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
April	15	12	13	9	11	9	14	11	10	7	14	11
May	16	13	16	11	17	12	16	14	17	14	14	12
June	22	19	19	16	24	17	27	21	21	17	26	15
July	22	18	25	14	24	19	18	15	21	15	18	13
August	12	10	14	9	14	10	16	12	15	9	12	9
Total	87	72	87	59	90	67	91	73	84	62	84	60

Discussion:

Breeding behaviour:

Present findings indicate that the breeding phase of both the tree frogs *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Cherrapunjee, Meghalaya, coincides with the rainy season (April to August), when air temperature and relative humidity were comparatively high. Similarly, several workers reported that the reproductive activity of anurans living in seasonal environments is generally associated with the rainy periods, both in tropical climates (Hoogmoed and Gorzula, 1979; Aichinger, 1987) and in temperate ones (Banks and Beebee, 1986; Caldwell, 1987).

The emergence of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was initiated in the later part of March when it was warmer with increasing atmospheric temperature and with the arrival of the rain so that there must be some water in the pool. Earlier studies reported that climatic factors especially rainfall and temperature exert a strong influence on the anuran reproductive activity (Roy *et al.*, 2004; Telford and Dyson, 1990; Silverin and Andren, 1992). Conditions of stress can even inhibit ovarian development (Silverin and Andren, 1992). Foam nest construction and spawning took place in the month of April when there is accumulation of water in the temporary and permanent pools and continued till August. In this context, it may be suggested that increased activity with rainfall might be due to the fact that the moist soil condition acts as cue indicating that conditions are satisfactory for initiating breeding activity and to travel overland to ambient breeding ponds. High moisture content may increase the possibility that the frogs could carry on their breeding activity longer without fear of

desiccation. Some rain was found to be essential to initiate breeding activity in the tree frog *Chirixalus simus* in West Bengal, as there must be some standing water at the base of the grass for construction of its foam nests (Deuti, 2001).

In both the species of the tree frogs the process of courtship, spawning and foam nest construction was initiated by rainfall and advertisement calls given by the males. It is important to mention that despite high ambient temperature and emergence of the frog, the advertisement of the call was not recorded in the absence of the rain. In both the species, the advertisement call was triggered by first few showers of rain. Further, the advertisement calls were recorded till the month of August but became absent during the month of September with decline in rainfall. These observations seem to suggest that rainfall is the primary environmental factor, which regulates the advertisement calls of both the species. Rainfall has also been found to regulate the advertisement calls in several other species of frogs (Kanamadi *et al.*, 1994; Roy *et al.*, 2004).

It was noted that the advertisement calls of *Polypedates leucomystax* were different from that of *Rhacophorus bipunctatus*. The ossilogram of *Polypedates leucomystax* showed that each call was composed of three long notes, while that of *Rhacophorus bipunctatus* each call was composed of six notes. The difference in the number of notes per call indicates that the call is species specific. The role of species-specific mating call pattern may be an important mechanism for reproductive isolation and speciation. Species specific advertisement calls have also been reported Penna *et al.*, (1997) and Roy *et al.*, (1998). Many aspects of male calling behaviour can influence mating success, and the calls can serve both as indicators of species identity and caller quality (Wells, 1977; Gerhardt, 1991). Mating call is a determinant for attracting females

and the characteristics of acoustic signal are selected through female choice (Gerhardt and Davis, 1988; Telford *et al.*, 1989; Gerhardt, 1991; Marquez and Bosch, 1997).

In the present study, the males of *Polypedates leucomystax* were found to select a suitable spawning site closer to water body so that the tadpoles when hatched can fall into the water body for further development. In other anurans, females have been reported to select oviposition sites based on factors such as water depth (Seale, 1982a; Caldwell, 1986), water temperature (Howard, 1978), or absence of predators (Howard, 1978; Resetarits and Wilbur, 1989; 1991; Petranksa *et al.*, 1994) or conspecific eggs or larvae (Resetarits and Wilbur, 1989, 1991; Spieler and Linsenmair 1997). In *Hyla pseudopuma* females choose oviposition sites based on their assessments of egg loads already present (Crump, 1991). Shepard, (2004) suggested that selection of the oviposition site may be the primary means by which parents can directly influence their offsprings probability of survival in some species.

It may be mentioned that in some species (e.g., *Litoria lesueri*) pairs may select a site together (Richards and Alford, 1992). However, in terrestrially breeding species only males choose the oviposition site, defend it from rival males, and call to attract females. In this investigation, the amplexing pair of *Rhacophorus bipunctatus* was found to select a suitable site for foam nest construction. It was seen that *Rhacophorus bipunctatus* constructed its foam nests closer to a water body and also on leaves of trees. Location of the foam nests closer to water bodies and on tree leaves above water might be an adaptation to avoid predators in the aquatic habitat during early development. Similarly, the female of *Rhacophorus malabaricus* has been reported to choose leaves of a tree branch overhanging the water body for spawning (Kadadevaru and Kanamadi, 2000). It

may be mentioned that non-aquatic oviposition behaviour in frogs evolved independently in many lineages, and has been interpreted as a strategy to avoid predation, competition and unfavourable abiotic conditions in the water body (Crump, 1982; Duellman and Trueb, 1994).

Like other rhacophorids, *Polypedates leucomystax* and *Rhacophorus bipunctatus* showed diversity in the selection of the oviposition sites and foam nests construction. The tree frog *Hyla boans* showed diversity in basin or nest sites, and its nests can be on leaf litters or roots (Magnusson *et al.*, 1999; Martins and Moriera, 1991), on pond margins (Martins, 1993), or on margins of swamps or on wet grounds up to 30 m away from a swamp (Hobel, 1999). The preference of these two tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* for a particular type of breeding habitat reflects the differential fitness and adaptiveness of these species in the various habitats available in that particular natural environment. Some workers interpreted that a particular habit of nest building was a method to protect the early developmental stages against the high diversity of aquatic predators including cannibalism by conspecific tadpoles in adjacent streams (Kluge, 1981; Martins, 1993; Caldwell, 1992) and as a method to raise water temperature to enhance growth and development (Lamotte and Lescure, 1977). The foam nests are thought to protect eggs and embryos from predation and desiccation (Heyer, 1969; Downie, 1988; 1993). However, a crust of dried jelly sometimes forms a seal around undisturbed egg masses and in addition to reducing egg desiccation, it may also restrict the entry of smaller predators (Mitchell, 2002).

The foam nest of *Polypedates leucomystax* was comparatively larger in diameter than that of *Rhacophorus bipunctatus*. Further, the clutch size of *Polypedates*

leucomystax was also larger than that of *Rhacophorus bipunctatus* (Table 2.4). These observations suggest that the foam nest diameter is in proportion to the clutch size – bigger the foam nest diameter larger is the clutch size and vice versa. It is important to mention that the clutch size was independent of the size of females in both the species of tree frogs (Table 2.4). Since the size of a frog depends on its age, the clutch size independent of female size seems to suggest that the clutch size is independent of the age of the female. In the present study, it thus seems that the clutch size is species specific. Earlier workers have also reported on the clutch size of tree frogs *Polypedates maculatus* was 719 with an average of 454 (Mohanty-Hejmadi and Dutta, 1988), clutch size of *Chirixalus dudhwaensis* 201 - 231 (Biswas, 2000) and of *Chirixalus simus* 153 - 234 (Deuti, 2001). Similar to *Polypedates leucomystax* and *Rhacophorus bipunctatus* no significant correlation was found between SVL and clutch size in *Heleioporus albopunctatus* (Davis and Roberts, 2005). However, a positive relationship between clutch size and body size has been reported in some anurans and tropical Australian frogs (Martins, 1988; Ryser, 1988; Praderio and Robinson, 1990 and Dziminski, 2000).

A unique feature was observed that the size of the eggs of *Rhacophorus bipunctatus* were larger than that of *Polypedates leucomystax*. On the basis of these observations, it may be suggested that in *Rhacophorus bipunctatus* the small clutch size provides optimal conditions for large size eggs in smaller foam nest due to its arboreal nesting behaviour. The egg size may be one of the important fitness components in species with no parental care, since this parameter may strongly affect growth and survival of hatchlings and clutch size in females. In these two tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* there was no correlation found between egg

size and body size of the adult frogs. However, in many species, egg size is correlated with female body size; i.e., larger females produce larger eggs (Roff, 1992), while in some amphibian species, egg size does not depend on females size (Howard 1981; Kuramota, 1978).

It was observed that the males were more in number than females at the breeding sites leading to a competition among the males to mate with the limited number of females. Further, the females were found to be larger in size than the males (Table 2.4). The observed sexual dimorphism in size may help in increasing mating success. As in the case of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, in the Indian bullfrog *Rana tigerina* Daudin also the males tend to be smaller than the females at the breeding sites (Dutta and Mohanty-Hejmadi, 1981). Size dimorphism has been reported in 90 % of anuran species where females were larger than the males (Shine, 1979). Further, larger females have been reported to produce larger eggs or lay eggs in large quantities (Crump, 1974; Crump and Kaplan, 1979). Howard (1981) reported that females in a population of bullfrogs (*Rana catesbeiana*) were larger than the males because of differential predation on larger males due to increased risks involved in territorial defense and mating. Woolbright (1989) suggested that size dimorphism is the result of complex interactions among different selective pressures to which a species is subjected in its environment. The size dimorphism in anuran is supposed to be mainly due to differences in age structure between the sexes in the breeding population (Monnet and Cherry, 2002).

Breeding Cycle: Breeding activity of most anuran is associated with the rainy season both in the temperate zones and tropics (Deuti, 2001; Huang *et al.*, 1996; 1997). In

temperate areas, the increase in air and water temperature in the spring appear important for initiating certain behaviour including emergence from hibernation and initiation of reproductive activities such as male calling (Reading, 1998). In the present study, both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were seen to emerge from hibernation in the month of March when it is warmer. First shower of rain was associated with their movement towards the breeding sites. Both the species continued breeding till the month of August, after which they were seen moving towards the hibernation sites where they hibernated during the months of November to February. Seasonal activity of several amphibian species is mainly regulated by environmental temperature and rainfall/moisture (Duellman and Trueb, 1994). The activity levels have been reported to reflect the physiological conditions of organisms (Licht, 1969; Wiest, 1982; Pough *et al.*, 1983). Though the breeding and spawning phase of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was found to be longer (April to August), the post-breeding phase was short (September to October). Both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were found to exhibit distinct pre-breeding and post-breeding migrations, and they aggregate at the breeding sites during the breeding phase. Similar observations have also been made in some amphibian species (Dole and Durant, 1974; Beshkov and Jamesen, 1980; Kam *et al.*, 1995). Our observations clearly indicate that three factors namely temperature, relative humidity and rainfall play important role in the regulation of breeding activity and breeding cycle of both the tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus*.

During the present study, it was observed that there was a variation of colour in *Polypedates leucomystax* from yellowish to brownish during the breeding period

especially in the male. Colour changes in this species may be due to seasonal variations in environmental temperature and humidity. Physiological colour change in the green treefrog *Hyla cinerea* has been reported to be influenced by environmental cues such as background colour, light, temperature, and stress (King *et al.*, 1994). Androgens have been reported to play a role in some species. Whether these factors play any role in colour change in *Polypedates leucomystax*, remains to be experimentally investigated.

The histological study of the testis and ovaries of *Polypedates leucomystax* and *Rhacophorus bipunctatus* showed different phases of spermatogenesis and oogenesis during the different phases of the breeding cycle. Presence of numerous spermatids in the testis of *Polypedates leucomystax* and few spermatozoa and a large number of spermatids in the testis of *Rhacophorus bipunctatus* during emergence and pre-breeding, seems to suggest that the males of these tree frogs remained prepared for reproduction. Our observations also suggested that while the process of spermatogenesis in *Rhacophorus bipunctatus* is independent of rainfall and dependent on increased environmental temperature, in *Polypedates leucomystax*, however, rainfall seem to be essential for spermatogenesis. It is important to mention that only rainfall trigger breeding behaviour, amplexus and release of spermatozoa. It has been reported that reproductive activities of most amphibians are highly susceptible to environmental fluctuations, and most of them exhibit markedly seasonal testicular cycles (Chavadej, *et al.*, 2000). Reproductive cyclicity seems to be correlated with the climatic condition prevailing in the habitats. Adverse annual variations in environmental conditions have been reported to disrupt breeding cycles (Sretarugsa *et al.*, 2001). In the subtropical anuran species, *Rana sauteri*,

spermatid production and testis mass were found to be inhibited by high temperature (Huang *et al.*, 2004).

Mature oocytes filled up the ovary during the pre-breeding period and breeding phase. However, no mature oocytes were seen during the post-breeding period. The absence of mature oocytes might be due to the fact that during the breeding phase all the mature oocytes were released and/or remaining mature oocytes in the ovary were lost due to follicular atresia. The ovaries regressed during summer, and ovarian weight began to increase at the end of summer and continued during autumn and winter, until the ovaries reached maximum value before the spawning period at early spring (Delgado *et al.*, 1990).

On the basis of the present findings, we conclude that there is similarity in the breeding behaviour as well as in the patterns of the breeding cycle of both the species of the tree frogs. Further, ambient temperature and rainfall seem to play a major role in regulation of gonadal activities and breeding behaviours. The differences observed in advertisement calls, nest size, clutch size, egg size and spawning sites seem to be species specific adaptations to ensure successful breeding in variable niches specific for these two species of the rare tree frogs.

CHAPTER III

Development and metamorphosis of
Polypædates leucomystax and *Rhacophorus*
bipunctatus, and the ecological factors and
water chemistry (e.g., temperature and pH) of
the breeding sites

CHAPTER III

Development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the ecological factors and water chemistry (e.g., temperature and pH) of the breeding sites

Introduction:

Most anurans have complex life cycles involving egg fertilization, embryo and larval development in aquatic habitats (Wilbur, 1980). Detailed descriptions of the ontogeny of embryonic, larval, metamorphic, and postmetamorphic frogs are important for understanding the general patterns of development of representative taxa and, by comparisons of different patterns, for resolving questions about systematic relationships. For anurans, staging tables are a condensed way of describing ontogenetic changes. A review of the literature indicated that of the few thousand known species of anurans, there are complete staging tables (fertilized eggs through the completion of metamorphosis) for fewer than 20 species. Most of the tables that have been constructed are for species contained in the genera *Bufo* and *Rana*. Two of the most detailed tables for the African clawed frog, *Xenopus laevis* (Nieuwkoop and Faber, 1956), and the northern leopard frog, *Rana pipiens* (Shumway, 1940; Taylor and Kollros, 1946) were constructed to facilitate investigations of development at the tissue and cellular levels (Fox, 1984). Amphibian development has been investigated extensively by many embryologists, who have taken advantage of the development of relatively large external eggs for both descriptive and experimental studies (Rugh, 1951; 1962; Harrison, 1969). One of the prominent life history characteristics common to most living amphibians is the

presence of an aquatic larval period, which immediately follows the initial embryonic development after fertilization and ends with the completion of metamorphosis (Duellman and Trueb, 1994; Altig and McDiarmid, 1999).

Metamorphosis is a post-embryonic period of profound morphological changes by which the animal alters its mode of living. Anuran metamorphosis is separated into three specific periods - premetamorphosis, prometamorphosis and metamorphic climax (Etkin, 1964; 1968; Dodd and Dodd 1976). Metamorphosis in anurans involves resorption of the tail, development of the front and hind limbs and large changes in most organ systems (Shi, 2000). The transformation of a tadpole into a frog is one of the most spectacular processes in nature and, consequently, one of the most thoroughly investigated. The endocrinological regulation of amphibian metamorphosis has been particularly well studied (Gilbert and Frieden, 1981). Amphibian metamorphosis, a complex developmental process controlled by thyroid hormones, results in reorganization of most tadpole tissues and organs (Weber, 1967; Dodd and Dodd, 1976; Leloup and Buscaglia, 1977; Fox, 1981; Galton, 1983; Kaltenbach, 1996).

In nature, amphibian metamorphosis is also accelerated by a number of ecological factors such as increasing temperature, decreasing food, overcrowding and pond evaporation, which play important roles in determining the metamorphic rates (Hayes, 1997). During the larval period amphibians, anurans in particular, exhibit a series of dramatic morphological changes like tail formation, perforation and closure of the spiracle, limb formation, tail reduction, etc. The changing appearance of embryos, especially during organogenesis, necessitates a method of quantifying the progress of development. After varying periods of time, larvae metamorphose and may spend

significant portions of their remaining lives in terrestrial habitats, returning seasonally to the aquatic habitat to breed. Many different methods have been developed to stage anurans during development, especially during metamorphosis (Just, *et al.*, 1981).

Appropriate staging of the larval period, is therefore, fundamental to various life history studies of amphibians. Studies on the successive ontogenic stages to record the normal developmental table are important in understanding the ecology of a species and for planning conservation measures. Duellman and Trueb (1994) suggested that complete tables of development are necessary for accurate comparison of developmental stages in different organisms. Hence, tables of normal stages of development have been worked out for a number of species by several workers. The most commonly used staging method for *Rana pipiens* and *Rana catesbeiana* is that of Taylor and Kollros (1946) whereas that for *Xenopus laevis* is that of Nieuwkoop and Faber (1956). The anuran development was described thoroughly by Rugh (1951), who also conducted experimental embryological work on amphibians in detail (Rugh, 1962). Gosner (1960) gave a simplified table for staging anuran embryos and larvae with notes on identification. Normal tables of development have been described for *Bufo melanostictus* (Khan, 1965), Indian bull frog *Rana tigerina* Daud (Khan, 1969; Agarwal and Niazi, 1977), *Rana limnocharis* (Roy and Khare, 1978) and the Japanese green frog, *Rhacophorus arboreus* (Iwasawa and Kawasaki, 1979). The most comprehensive treatment of amphibian development within a broad biological context is the work of Salthe and Mecham (1974) and Fox (1984). Twenty five developmental stages of a microhylid, *Ramanella variegata* were described by Dutta *et al.*, (1990-91).

Iwasawa and Futagami (1992) evaluated the normal stages of development of a tree frog, *Hyla japonica*. Shimzu and Ota (2003) studied the normal development of *Microhyla ornata*, and gave, for the first time a description of the complete embryonic and larval stages for the Microhylid frogs. Dziminski and Anstis (2004) determined the embryonic and larval development of the sunset frog, *Spicospina flammocaerulea* from South Western Australia, and compared with other myobatrachid genera.

Amphibian metamorphosis has been investigated thoroughly by several workers (Etkin and Gilbert, 1968; Dodd and Dodd, 1976; Gilbert and Frieden, 1981; Fox, 1981, 1984). The aquatic larval stage has received more attention from amphibian ecologists and is commonly viewed as the stage in which the advantages of rapid aquatic stage growth and large size at metamorphosis are pitted against the risks of mortality, primarily drying, in the larval stage (Pomeroy, 1981; Newman, 1989, 1992; Pfennig, 1992a). Collins (1979) evaluated the intrapopulation variation in the body size at metamorphosis and timing of metamorphosis in the bullfrog, *Rana catesbeiana*. Wassersug (1986) investigated on how does a tadpole know when to metamorphose?

Several workers have reported that the time to metamorphose and the rate of development for anuran tadpoles is influenced by the ambient temperature (Harkey and Semlitsch, 1988), size (Pearman, 1993), rate of desiccation of their habitat (Newman, 1989; Denver, 1997), density (Gromko *et al.*, 1973; Miranda and Pisano, 1993), pathogens (Beebee, 1995; Petranka, 1995), diet (Kupferberg, 1997a), photoperiods/day-lengths and feeding schedules (Wright *et al.*, 1988), hydroperiod (Ryan and Winne, 2000), maternal effects (Loman, 2002) and the effects of hatching age on development and hatchling morphology (Warkentin, 1999).

The adaptive plasticity in amphibian development and metamorphosis in desert ponds and ephemeral habitats have been worked out in the tadpoles of *Scaphiopus couchii* (Newman, 1988) and *Scaphiopus hammondi* (Denver, 1997; Denver *et al.*, 1998). Beachy *et al.*, (1999) investigated the effects of developmental and growth history on metamorphosis in the gray tree frog, *Hyla versicolor*. Shi (2000) in the book amphibian metamorphosis: from morphology to molecular biology studied in detail on amphibian metamorphosis. Girish and Saidapur (2003) reported that density-dependent growth and metamorphosis in the larval bronze frog *Rana temporalis* is influenced by genetic relatedness of the cohort. However, Patil and Kanamadi (1997) described the direct development in the rhacophorid frog, *Philautus variabilis* (Günther) in the Western Ghats.

Several workers have contributed to the breeding biology and life history of anurans in India. Studies have been conducted on the breeding behaviour and life history of the Indian bull frog, *Rana tigerina* (Daudin) (Dutta and Mohanty-Hejmadi, 1976), *Rana cyanophlyctis* (Schneider) (Mohanty-Hejmadi and Dutta, 1979), *Uperodon systoma* (Schneider) and *Microhyla ornata* (Mohanty-Hejmadi, *et al.*, 1979, 1980). Kiyasetuo and Khare (1986) reported the annual breeding cycle and development of *Rhacophorus leucomystax* (Kuhl) in Nagaland. Mohanty-Hejmadi and Dutta (1988) studied on the life history of the common Indian tree frog, *Polypedates maculatus*, and reported the average number of days to complete metamorphosis was 55 days. Sekar (1990) studied the developmental stages of tadpoles of the Malabar gliding frog, *Rhacophorus malabaricus* under laboratory conditions, where the tadpole took 68 days to complete metamorphosis. Girish and Saidapur (1999) investigated the early development in the tree frog

Polypedates maculatus and reported that the eggs complete their embryonic development in the foam nest within nine days, and complete metamorphosis in 60 ± 10 days. Ao and Bordoloi (2001) described the development of *Hyla annectans* in Nagaland, and Dutta *et al.*, (2001) studied the breeding and developmental stages of *Polypedates maculatus* in Orissa.

Recently, similar studies have also been conducted on the life history and metamorphosis of several anurans from other parts of the world. The life history of *Leptodactylus troglodytes* has been investigated in detail (Arzabe and de Almeida, 1997). Giaretta and Menin (2004) investigated on the reproduction, phenology, and mortality sources of *Physalaemus* cf *fuscomaculatus*, and reported that the frog completed its metamorphosis within 28 – 31 days. *Leptodactylus fallax* has been reported to complete metamorphosis within 42 – 57 days (Gibson and Buley, 2004).

Physico-chemical factors:

Most of the amphibians are highly dependent upon aquatic habitats because of their physiology and life history (Duellman and Trueb, 1994). Many amphibian species have highly permeable skin, and their aquatic larvae may be susceptible to the effects of chemicals present in water (Vitt *et al.*, 1990). Possible environmental cofactors that have been shown to affect amphibian survival and that might contribute to the decline in population included climate change, acid rain, and environmental contaminants. Eichler and Gray (1976) studied the influence of environmental lighting on the growth and prometamorphic development of larval *Rana pipiens*. Diaz-Paniagua (1992) investigated the variability in timing of larval season in an amphibian community in Spain. Parris and

Baud (2004) reported the interactive effects of a heavy metal and chytridiomycosis on gray treefrog larvae (*Hyla chrysoscelis*).

There is a paucity of information on the effects of low temperature on amphibians metamorphosis (Frieden *et al.*, 1965; Fry, 1972; Griswold *et al.*, 1972; Shrode, 1972; Norris and Platt, 1974; Roy and Khare, 1979 and Moriya, 1983). Studies on temperature selection in larval amphibians were carried out by Dupre and Petranka (1985), Wollmuth and Crawshaw (1988) and Blouin and Brown (2000). While Brown (1990) examined the influence of temperature and thyroxine during induced metamorphosis in tadpoles of a primitive frog, *Ascaphus truei*, and suggested that low environmental temperature can inhibit the progress of metamorphosis and showed that *Ascaphus* tadpoles in late premetamorphic and prometamorphic stages have tissues that are very responsive to this metamorphic hormone. Several workers have studied the influence of temperature on energy cost and timing of embryonic and larval development in a number of anuran species (Ultsch *et al.*, 1999b; Mitchell and Seymour, 2000; Kam *et al.*, 2001; and Gillooly *et al.*, 2002). Changes in ambient temperature have been reported to influence amphibian behaviour including those related to reproduction (Blaustein *et al.*, 2001). Potential changes in ambient temperature on a global scale could disrupt timing of breeding, periods of hibernation, and ability to find food. Mitchell and Seymour (2003) investigated the effects of nest temperature, nest substrate, and clutch size on the oxygenation of embryos and larvae of the Australian moss frog, *Bryobatrachus nimbus* and suggested that at cooler temperatures (5°C and 10°C), larvae were clustered near the nest base, and their distribution were significantly clumped. Larvae became more widely dispersed at 15°C and 20°C. At 10°C and 15°C, most larvae were oriented with their

heads at the aerated gauze walls of the nest, while at 20°C many larvae were positioned with their nostrils emerging above the jelly surface for extended periods. The effects of variation in climatic temperature were studied on breeding activity and tadpoles stage duration in the common toad, *Bufo bufo* during the years 1980-2001 and showed that the duration of the tadpole stage is significantly longer under cold, wet conditions than under warm dry conditions (Reading, 2003). Wu and Kam (2005) investigated the thermal tolerance and thermoregulation by Taiwanese rhacophorid tadpoles living in geothermal hot springs and streams.

Acidification of habitat is thought to have a major impact on amphibians and the structure of their populations. Under conditions of low pH, however, the previtelline volume fails to increase at a normal rate, leading to the growing embryo becoming tightly coiled and often failing to hatch (Gosner and Black, 1957; Salthe, 1965; Pough, 1976; Dunson and Connell, 1982). Salthe (1965) reported that during normal development and hatching of an amphibian embryo, there is a continual expansion of previtelline volume, and secretion of a hatching enzyme that weakens the embryonic membranes and allows hatching to occur. Exposure of embryos to extremely low pH has been reported to arrest development (Pough, 1976; Freda and Dunson 1985b). Generally amphibian embryos are the most sensitive to low pH, and tolerance to low pH increases with larval age (Pierce *et al.*, 1984; Freda and Dunson, 1985a; Freda, 1986). Clark (1986) studied the responses of *Ambystoma maculatum* populations to habitat acidity in Central Ontario. Field investigation on amphibian abundance and species diversity have shown a clear correlation between the acidification of breeding ponds and the decline of amphibian populations (Beebee, 1987).

Freda *et al.*, (1991) conducted long term monitoring of amphibian populations with respect to the effects of acidic deposition. Several workers have studied the effect of pH on anuran development and metamorphosis. The effects of acute and chronic exposure to pH were studied on three species of larval amphibians that breed in temporary ponds and it was indicated that habitat acidification could indeed have strong negative impacts on amphibians, which breeds in temporary ponds (Rowe *et al.*, 1992). Similarly, the genetic variation in tolerance of amphibians to low pH (Pierce and Wooten, 1992), the effects of low pH, metals, and water hardness on larval amphibians (Horne and Dunson, 1995), the effects of ultraviolet-B radiation and pH on early development of the moor frog *Rana arvalis* indicated that UV-B radiation have no negative effects on the development of moor frog embryo, but survival probability and hatchling size were reduced by low pH (Pahkala, *et al.*, 2001). However, Rasanen *et al.*, (2002) investigated the carry over effects of embryonic acidic conditions on development and growth of *Rana temporaria* tadpoles, and suggested that exposure to acidity during the embryonic stage may increase the ability of larvae to tolerate continued acid exposure. Glos *et al.*, (2003) showed a clear differences in pH tolerance of two *Rana temporaria* populations living in different pH environments.

Rainfall reportedly influences the reproductive phenology of many amphibian species, particularly in tropical and temperate forests with seasonal precipitation (Inger and Greenberg, 1963; Alexander *et al.*, 1979; Bowker and Bowker, 1979; Aichinger, 1987; Donnelly and Guyer, 1994). Annual variation in rainfall can have an effect on the number of eggs laid in a given year (Caldwell, 1987). A reduction in rainfall brought on by deforestation can have a detrimental effect on amphibian reproduction as the specific

timing and duration of rainfall act to trigger breeding (Blaustein and Wake, 1995; Lips, 1998; Alexander and Eischeid, 2001). Rainfall is an environmental factor that has been shown to affect breeding population of other amphibian species through its effect on breeding activity and past recruitment (Semlitsch *et al.*, 1996). Timing and length of the hydroperiod frequently play a significant role in metamorphosis success and body size in anurans (Wilbur and Collins, 1973; Semlitsch, 1987; Pechmann *et al.*, 1989, 1991; Rowe and Dunson, 1995). Amphibians are dependent on rainfall/moisture for survival and reproduction, and hence adverse changes in rainfall are likely to act as a stress factor (Lips, 1999).

This chapter deals with the investigation of various aspects of development and metamorphosis in life history of the tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus*. Keeping in view the paucity of information, it was decided to investigate in detail the development and metamorphosis in these two tree frog species at Cherrapunjee, Meghalaya, the wettest place on earth with special reference to ecological factors like temperature, relative humidity, rainfall and pH.

Materials and Methods:

To study the development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus* at Cherrapunjee, Meghalaya the investigation was conducted in the natural environment at the study sites I and II for *Polypedates leucomystax* and sites III and IV for *Rhacophorus bipunctatus* (Please refer to Chapter II: Figs. 2.1, 2.2, 2.3 and 2.4). These four study sites were temporary rainfed pools, which were found to be

excellent breeding grounds and served as good habitat for the development and metamorphosis of these two species of tree frogs.

During the study period from the month of March to October, when there was accumulation of water in the pools, samples of water were collected in plastic bottle from the study sites at weekly interval for the analysis of the physico-chemical factors. Water temperature was recorded in the field and pH was recorded with the help of a digital pH meter. Simultaneously, the ecological factors like air temperature and relative humidity of the study sites were recorded, and rainfall data for Cherrapunjee was collected from the Regional Meteorological Centre, Guwahati.

During the years 2002, 2003 and 2004 the foam nests were collected during the breeding phase (April to August) just after they were constructed at the study sites and brought to the laboratory for further studies. The snout-vent length of the amplexing pair was recorded at the breeding sites and after the measurement the frogs were released back at the breeding sites. The time of egg laying was taken as the time of fertilization. Development of the eggs was observed with the help of a stereoscopic dissecting binocular microscope during field observations. The four study sites were visited at a regular interval of every three days to observed and record the development and metamorphosis of *P. leucomystax* and *R. bipunctatus* in the natural environment. Some foam nests were fixed immediately in the field in 4 % formaldehyde, and on reaching the laboratory the eggs were separated from the foam nests and counted to know the clutch size.

Some of the foam nests collected from the study sites were maintained in a plastic tray containing pond water to allow further development and metamorphosis, which were

observed under a stereoscopic dissecting binocular microscope (Meopta DM 23 66223). The time of onset of each new stage was noted and some developmental stages and tadpoles were anesthetized and then were fixed in 4 % formaldehyde. Staging of the anuran embryos and larvae of both the species was carried out on the basis of a new external morphological change as per the criteria described by Gosner (1960). Photographs of the developmental stages were taken with the help of a binocular (Magnus MLX) with photographic attachments. Photographs of the different developmental and metamorphic stages were taken at different magnification.

The hatched tadpoles were reared in a plastic tray containing pond water and fed daily with boiled cabbage and algae under laboratory conditions (temperature: 20°C – 22.5°C). The percentage of mortality of the tadpoles were also recorded under laboratory conditions.

Results:

Ecological factors at the breeding sites of *Polypedates leucomystax* (sites I and II) and *Rhacophorus bipunctatus* (sites III and IV):

The ecological factors (rainfall, relative humidity and air temperature) of the four selected breeding sites were recorded during the study period, 2002, 2003 and 2004 as presented in (Please refer to Chapter II: Table 2.1; Figs. 2.5a, 2.5b and 2.5c). A brief account of annual variations in rainfall, relative humidity and temperature is given in the following sections.

Rainfall: Rainfall ranged between 0.0 – 5008.2 mm through out the study period. During the period of development and metamorphosis (April to October) rainfall ranged between 66.3 – 5008.2 mm. During the winter months (November to February) the amount of rainfall recorded was nil or very less, if any. As a result the pools remained dried during the winter season. The amount of rainfall increased from the month of March and onwards, and reached a maximum during the months of June and July. Thereafter, the rainfall slowly decreased again from the month of November during all the three years (Please refer to Chapter II: Table 2.1; Fig. 2.5c).

Relative humidity: During the study period from 2002, 2003 and 2004, relative humidity ranged between 40 % - 89 %. During the periods of development and metamorphosis (April to October), relative humidity was found to be high and ranged between 54 % - 89 %. Relative humidity increased from the month of April and onwards, and reached a maximum during the months of June and July. The relative humidity decreased during the winter months (November to February) (Please refer to Chapter II: Table 2.1; Fig. 2.5b).

Air Temperature: In Cherrapunjee atmospheric temperature ranged between 13.5°C – 25.7°C through out the study period (2002, 2003 and 2004). During the period of development and metamorphosis (April to October) atmospheric temperature ranged between 19.4°C – 25.7°C. It was observed that air temperature increased from the month of March and onwards, reached a maximum in the months of June and July, then it

gradually decreased down from the month of August and reached a minimum in the month of January (Please refer to Chapter II: Table 2.1; Fig. 2.5a).

Physico-chemical factors at the breeding sites of *Polypedates leucomystax* (sites I and II) and of *Rhacophorus bipunctatus* (sites III and IV):

Water temperature and water pH of the four breeding sites (rain fed pools, which remained dried up during the winter) were recorded and analysed during the years, 2002, 2003 and 2004 (Tables 3.1 and 3.2; Figs. 3.1a, 3.1b, 3.2a and 3.2b).

Water Temperature: During the study period (2002, 2003 and 2004), water temperature at the sites I and II ranged between 18.2°C – 24.1°C (Tables 3.1; Fig. 3.1a). From the month of April to October when development and metamorphosis of *Polypedates leucomystax* took place, water temperature ranged between 18.4°C – 24.1°C at both the study sites. It was observed that water temperature increased from the month of March and onwards, reached a maximum in the month of May and June, and then it gradually decreased again during the winter months (Table 3.1; Fig. 3.1a).

At the study sites III and IV, water temperature ranged between 18.2°C – 23.8°C. During the period of development and metamorphosis (April to October), water temperature ranged between 18.2°C – 23.8°C at both the study sites III and IV. Water temperature increased in the month of March when it is warmer, reached a maximum in the month of June and July, and then it gradually decreased again in the month of October (Table 3.2; Fig. 3.1b).

Water pH: Water pH at the study sites I ranged between 6.8 – 7.2, and at the study site II 6.8 and 7.1 (Table 3.2; Fig. 3.2a). Similarly, at study site III, the water pH ranged between 6.8 – 7.1 and at study site IV between 6.8 – 7.2 (Table 3.2; Fig 3.2b). Water pH was more or less the same during the period of development and metamorphosis (April to October) of *Polypedates leucomystax* and *Rhacophorus bipunctatus* at the four study sites.

Stages of Development and Metamorphosis of *Polypedates leucomystax*:

As mentioned earlier, *Polypedates leucomystax* deposited its eggs in a foam nest attached to vegetation near the water. During the present study the developmental stages of *Polypedates leucomystax* were recorded from the time of egg laying till the embryo hatched into a tadpole, and metamorphosis of the tadpole into a froglet at the study sites I and II under natural environment, and also under laboratory conditions. Observations were made on the external morphological changes, and altogether 46 different developmental stages were recorded. A brief account of various stages of development and metamorphosis is given in the following sections.

Stage 1 – Fertilized egg: The fertilized egg was spherical in shape and completely white in colour devoid of pigments. When the female lay eggs in foam nests the time of fertilization was taken as 0 min. The egg measured 1.5 mm in diameter (Fig. 3.3).

Stage 2 –Beginning of cleavage furrow: This stage was marked with the beginning of cleavage furrow at the animal pole. Initiation of cleavage in the animal pole was observed after 50 mins (Fig. 3.4)

Stage 3 – *Two cell stage:* It was observed that after 50 mins of fertilization the egg became flattened at the animal pole, cleavage occurred in the meridional plane on the animal pole and moved down to the vegetal pole dividing the egg into two equal halves. The first cleavage was completed in 2 hrs 45 mins after fertilization (Fig. 3.5).

Stage 4 – *Four cell stage:* The second cleavage was also found to occur in the meridional plane at right angle to the first cleavage, and progressed down the egg dividing it into four equal halves. The complete four cell stage was observed at 3 hrs 35 mins after fertilization (Fig. 3.6).

Stage 5 – *Eight cells stage:* The third cleavage was horizontal and slightly above the equator and at right angle to both the first and second cleavages resulting into four smaller micromeres and four bigger macromeres. Completion of the eight cells stage was recorded at 4 hrs 10 mins (Fig. 3.7).

Stage 6 – *Sixteen cell stage:* The fourth cleavage was found to be vertical. The furrow progressed vegetally dividing each of the four blastomeres of the animal hemisphere resulting into sixteen cells. The egg size increased and measured 1.55 mm. Completion of sixteen cells stage took place at 4 hrs 55 mins (Fig. 3.8)

Stage 7 – *Thirty two cell stage:* Horizontal cleavage furrow cut the micromeres and macromeres into tiers of cells resulting in the formation of sixteen smaller micromeres and sixteen larger macromeres. This stage was differentiated by the size of the blastomeres and took 5 hrs and 50 mins to complete (Fig. 3.9).

Stage 8 – *Mid cleavage:* The animal pole blastomeres divided rapidly and become more in number and smaller in size, while the vegetal pole blastomeres divide at slower rate due to the presence of yolk and were found to be comparatively larger. During this stage, the egg looked like a compact ball of cells commonly called morula, and measured about 1.6 mm in diameter. This stage was also differentiated by the blastomeres of different sizes. The mid cleavage stage took place after 14 hrs 5 mins (Fig. 3.10).

Stage 9 – *Late cleavage:* The blastomeres were further divided into a large number of micromeres, which were very minute in size. The surface of the egg appeared granular, and the egg was considered as blastula, and measured 1.6 mm. Completion of this stage was observed at 20 hrs 45 mins (Fig. 3.11).

Stage 10 – *Dorsal lip:* The involution of the micromeres indicated the beginning of gastrulation, when the blastopore appeared. The dorsal lip appeared and later bulged out at 27 hrs 25 mins. The egg measured 1.65 mm (Fig. 3.12).

Stage 11 – *Mid gastrula:* During this stage, the blastopore became circular. The invaginated micromeres and macromeres protruded and constituted the yolk plug at 32 hrs 30 mins and the egg measured 1.65 mm (Fig. 3.13).

Stage 12 – *Late gastrula:* The small protruding yolk plug gradually disappeared and the yolk plug became smaller in size. The egg measured 1.65 mm. This stage was observed at 37 hrs 10 mins (Fig. 3.14).

Stage 13 – *Neural plate:* The embryo was slightly elongated and the neural plate developed as a tubular area on the dorsal surface, and the lateral ridges

became slightly elevated forming neural folds. The embryo measured 1.72 mm. This stage was observed at 39 hrs 25 mins (Fig. 3.15).

Stage 14 – *Neural fold*: This stage was marked by further elongation of the embryo associated with the elongation of the two lateral ridges separated by the neural groove. The neural folds became distinct and gradually approached each other from blastopore to anterior region. The neural groove was formed at 42 hrs 45 mins and the embryo measured 1.72 mm (Fig. 3.16).

Stage 15 – *Rotation*: The neural folds came closer and met each other, and the neural groove became narrow. This stage was observed at 45 hrs 15 mins and the embryo measured 1.8 mm (Fig. 3.17).

Stage 16 – *Neural tube*: The left and right neural folds were fused completely to form the neural tube. The embryo was further elongated and measured 1.89 mm. The embryo was broader at the anterior region and narrow at the posterior region. Completion of this stage was observed at 47 hrs 50 mins (Fig. 3.18).

Stage 17 – *Tail bud*: Tail bud protruded at the posterior end of the embryo, and became slightly curved to the left side. Bulges of gill plates were distinct in the cephalic region and yolk was still present at 54 hrs 10 mins. Embryo further elongated and measured 2.0 mm (Fig. 3.19).

Stage 18 – *Muscular response*: Embryo showed muscular response to external stimuli. Anterior region was well defined with optic bulges and bulges of the gill plates. Yolk was still present. There was gradual elongation of the embryo and the tail started curving laterally to the left at 62 hrs 45 mins. The embryo measured 2.5 mm (Fig. 3.20).

Stage 19 – *Heart beat*: Pulsation of the heart was seen below and behind gill bud.

Rudimentary gill buds became prominent. Tail was further elongated. This stage was observed at 73 hrs 50 mins and the embryo measured 4.55 mm (Fig. 3.21).

Stage 20 – *Gill circulation*: At 84 hrs 25 mins gills were well developed and branched into gill filaments. Opercular fold covered the base of the gills, and oral sucker became well developed. Melanophores appeared on the dorsal surface. The embryo measured 6.5 mm (Fig. 3.22).

Stage 21 – *Cornea transparent*: After 4 days larva hatched out, and escaped from the foam nest. Tail became straight and elongated. Cornea was transparent. Oral suckers and nasal pits became prominent. The larva measured 7.65 mm (Fig. 3.23).

Stage 22 – *Tail fin circulation*: Tail fins become transparent, and mouth widened. Melanophores became dispersed towards the ventral surface and tail region after 7 days. The larva measured 8.12 mm (Fig. 3.24).

Stage 23 – *Opercular fold formation (Opercular and pigmentation)*: After 9 days the operculum developed, and gills length shortened. Small and large melanophores developed on the body surface. The larva measured 9.25 mm (Fig. 3.25).

Stage 24 – *Opercular fold closed on right side*: Right operculum fold closed after 14 days, and left external gill shortened. The larva measured 9.85 mm (Fig. 3.26).

Stage 25 – Opercular fold closed on left side: Left operculum fold closed after 20 days.

Operculum formation was completed and spiracle was formed on the left side.

Gills disappeared and the tadpole measured 10.23 mm (Fig. 3.27).

Stage 26 – Hind limb bud development: Hind limb buds appeared at the junction of the trunk and tail on either side of the cloacal tail after 22 days. Coiled intestine became visible. The length of the limb bud was smaller than its diameter/width. The tadpole head length was 7 mm and the tail length was 12 mm. Melanophores were observed to be distributed on the dorsal and ventral side. Rows of teeth were observed in both the upper and lower labium. In the upper labium, both uninterrupted and interrupted rows of teeth were observed, while in the lower labium only distinct uninterrupted rows of teeth were recorded (Fig. 3.28).

Stage 27– Length of limb bud equaled half its diameter: After 26 days, the length of the limb bud increased in size and its length equaled to half of its diameter. The tadpole head length was 7 mm and the tail length was 14 mm. Dispersion of melanophores was found to be comparatively denser than in the earlier stage. Oral structure was found to be similar as in the previous stage (Fig. 3.29).

Stage 28 – Length of limb bud equaled to its diameter: Length of limb bud equaled to its diameter on the 28 day. The tadpole head length was 7 mm and the tail length was 14 mm. Similarly, in this stage melanophores were found to be denser in distribution, and the oral structure was found to be the same as in the stages 26 and 27 (Fig. 3.30).

Stage 29 – *Length of limb bud equaled to one and half time its diameter:* Length of limb bud increased in size and was equal to one and half time its diameter after 31 days. The tadpole head length was 9 mm and the tail length was 17 mm. Distribution of melanophores was found to be similar as in the previous stage and no differences was found in the oral structure (Fig. 3.31).

Stage 30 – *Length of limb bud equaled to twice its diameter:* Length of limb bud further increased and was slightly curved, and the tadpole head length was 11 mm and the tail length was 18 mm. Dispersion of melanophores was found to be denser and widely distributed through out the head as well as tail region, and there was no changes in the oral structure (Fig. 3.32).

Stage 31 – *Foot paddle (Toe differentiation and development):* The distal end of limb bud curved slightly and was spatula in shape to form the foot paddle after 35 days. The tadpole head length was 11 mm and the tail length was 20 mm. At this stage, melanophores were also found to be present on the base of the limb bud which had taken the shape of a spatula. Oral structure at this stage was found to be more developed than the previous stages. In the upper and lower labium teeth rows are well developed and distinct. From this stage onwards till stage 39, oral structure remained the same (Fig. 3.33).

Stage 32 – *First indentation:* The margin of the foot paddle became slightly indented on the dorsal side, which marked the fourth and fifth toe. After 37 days the tadpole head length was 12 mm and the tail length was 21 mm. At this stage melanophores 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 stage (Fig. 3.34).

Stage 33 – *Second indentation:* After 38 days, the margin of the foot paddle became indented on the ventral side behind the prominence of the fourth toe, which marked the third, fourth and fifth toes. The tadpole head length was 14 mm and the tail length was 23 mm (Fig. 3.35).

Stage 34 – *Third indentation:* The margin of foot paddle became indented on the ventral side behind the third toe, which marked the prominence of second, third, fourth and fifth toes. After 39 days, the tadpole head length was 14 mm and the tail length was 25 mm. Melanophores were found to be present in the regions which marked the future third, fourth and fifth toes, and there was no difference in the oral structure (Fig. 3.36).

Stage 35 – *Fourth indentation:* The margin of foot paddle became indented behind the second toe demarcating the prominence of first toe, and all five toes were demarcated from each other on the 41 day, and the tadpole head length was 15 mm and the tail length was 26 mm. Melanophores were found to be more denser at the base of the limb, and to be dispersed towards the toe formation region (Fig. 3.37).

Stage 36 – *First and second toes joined:* After 42 days the first and second toes were still joined, while the third, fourth and fifth toes were separated. The tadpole head length was 15 mm and the tail length was 28 mm (Fig. 3.38).

Stage 37 – *Toes separated:* All five toes were completely separated and webbed after 43 days. The tadpole head length was 16 mm and the tail length was 29 mm (Fig. 3.39).

Stage 38 – *Metatarsal tubercles appeared:* There was appearance of metatarsal tubercles, and the tadpole head length was 16 mm and the tail length was 32 mm after 45 days (Fig. 3.40).

Stage 39 – *Subarticular tubercles appeared:* Subarticular tubercles appeared after 47 days, and the tadpole head length was 16 mm and the tail length was 35 mm (Fig. 3.41).

Stage 40 – *Toes pad complete:* Toes were fully webbed with developing discs after 50 days. Pigmentation increased towards the tail region, and the tadpole head length was 17 mm and the tail length was 35 mm. Teeth started to shed from this stage onwards (Fig. 3.42).

Stage 41 – *Cloacal tail piece reduced:* Following stage 40 the more drastic changes of metamorphosis began. Tadpole cloacal tail piece was reduced in size. After 52 days, the tadpole head length was 17 mm and the tail length was 38 mm. Fore limbs visible and further shedding of the teeth was observed and only a few rows were observed (Fig. 3.43).

Stage 42 – *Both limbs developed:* After 53 days, tadpole developed both fore limbs and hind limbs. Mouth started widening, and the head measured 12 mm and tail 41 mm. Length of hindlimbs measured 22 mm and fore limbs 10 mm. Presence of dark cross bands were observed at both the fore and hind limbs.

Melanophores were found to be more dense on the tail region. Shedding of teeth almost completed, and only a few teeth were observed (Fig. 3.44).

Stage 43 – *Resorption of tail begins:* Tadpole tail started to regress after 55 days. The head of tadpole measured 10 mm and the tail measured 29 mm in length. At this stage longitudinal stripes were observed on the body region of the tadpole. Melanophores were found to be present only in the tail region. Shedding of teeth was completed (Fig. 3.45).

Stage 44 – *Tail shortened:* There was further gradual loss of the tadpole tail after 58 days and the tail was now shorter and the tadpole measured 28 mm in length. Longitudinal stripes were observed to extend to the tail of the tadpole, and melanophores had disappeared. Formation of the tongue took place (Fig. 3.46).

Stage 45 – *Tail stub:* After 60 days resorption of the tail was complete and only a stub remained and the tadpole measured 22 mm in length. There was appearance of adult characteristics like three to four longitudinal stripes appeared on the dorsum. Tongue had fully developed (Fig. 3.47).

Stage 46 – *Metamorphosis completed:* After 60 – 61 days tail was completely disappeared and the frog-let looks like an adult and measured 20 mm. Dorsum was yellowish in colour with three to four stripes extending longitudinally from snout to vent with dark cross bands on the fore and hind limbs (Fig. 3.48).

Completion of development and metamorphosis took about 60 – 61 days in *Polypedates leucomystax* at temperature between 18.4° C and 24.0° C in the natural

environment. In the laboratory, the pattern and duration of development and metamorphosis was observed to be more or less the same during the peak of the breeding period, but it was slightly prolonged by few days during the later part of the breeding season. Within 4 – 5 days of spawning and egg fertilization the larva hatched into the water after reaching stage 21.

Life Cycle:

In *Polypedates leucomystax*, breeding activities started from the month of April and continued till the month of August. Eggs were deposited in a foam nest attached to vegetation a few cm above the water surface. The egg measured about 1.5 mm in diameter and developed within the soft layer of the foam. After about 4 days the larva hatched out from the foam nest at stage 21 and started feeding at stage 25. Further development of the mouth was observed and complete teeth rows with dental formula 1:3+3/3 were observed in stages 31 – 39. It was observed that the tadpoles fed mostly on phytoplankton and zooplankton. At the stage 21, pigmentation was observed on the tail of the tadpole and continued to increase as the tadpole grows. Complete absorption of the tail was observed after 60 – 61, days and the metamorphosed froglet measured about 20 mm and lead a terrestrial life (Fig. 3.49). During development and metamorphosis percentage of mortality ranged between 34.48 % and 84.32 % with a mean of 62.31 ± 2.10 (Tables 3.3 and 3.4).

During development and metamorphosis, comprehensive and dramatic changes took place where the aquatic tadpole was transformed into a terrestrial adult. Some of these changes were development of the hind limbs and fore limbs, which were observed

after 53 days. During the premetamorphic phase, the development of the limb bud was observed and the limb bud grew in length till the foot paddle stage,. Complete teeth rows with dental formula 1:3+3/3 were observed during the prometamorphic phase. With the onset of metamorphosis, teeth rows had begun to shed and there was resorption of the tail and finally complete absorption of the tail took place, which marked the end of metamorphosis (Fig. 3.50).

Stages of Development and Metamorphosis of *Rhacophorus bipunctatus*:

Similar to *Polypedates leucomystax*, *Rhacophorus bipunctatus* was also found to construct its foam nest attached to vegetation always above the water surface or the leaves of a plant (e.g., *Hibiscus rosasinensis*) hanging over a water body. Observations on the development and metamorphosis of *Rhacophorus bipunctatus* was conducted at the study sites III and IV in the natural environment and also under laboratory. Some of the foam nests were collected immediately after construction, and observed. The time of spawning and fertilization was taken as 0 hours. Various stages of the development and metamorphosis were recorded and staging was done according to Gosner's table (1960). A brief account of the stages of development and metamorphosis are given in the following sections.

Stage 1 – Fertilized egg: The fertilized egg was spherical in shape and light yellow in colour and measured 3.0 mm in diameter (Fig. 3.51).

Stage 2 – stage 6: It was observed that when the eggs of *Rhacophorus bipunctatus* were removed from the foam nest after 1 hour of spawning and fertilization, early embryonic stages of development (stage 2 to stage 6) could not be observed

due to the presence of large amount of yolk. In addition, removal of the eggs from the foam nests at stage 1 before 5 hours (stage 2 to stage 6) was found to obstruct further development. Therefore, the eggs were removed from the foam nest only after 5 hours of spawning and fertilization (after stage 6) to observe the development.

Stage – 7: *Thirty two cell stage:* Five hours after fertilization, the eggs were found to reach the cleavage stage 7. The egg was round in shape, measuring 3.20mm and composed of a number of blastomeres (Fig.3.52).

Stage 8: *Mid cleavage:* Seven hours after fertilization, the embryo developed further reaching mid cleavage stage 8 and appeared as a compact ball of cells. The number of blastomeres could not be clearly identified due to the presence of large amount of yolk (Fig. 3.53).

Stage 9 – *Late cleavage:* The late cleavage was recorded after 14 hrs 5 mins. At this stage, the blastomeres were further divided and appeared smaller in size and the egg was considered as the blastula stage (Fig. 3.54)

Stage 10 – *Dorsal lip:* During this stage, the embryo changed its shaped and became slightly oval. Involution of cells at the dorsal lip of blastopore indicated the beginning of gastrulation, which was observed after 25.15 hours (Fig. 3.55).

Stage 11 – *Mid gastrula:* The blastopore appeared semi-circular and protruded constituting the yolk plug after 30 hrs 30 mins.

Stage 12 – *Late gastrula:* The size of the blastoporal lip was reduced, and the yolk plug was observed to be smaller in size and diminished after 35 hrs 30 mins.

Stage 13 – *Neural plate*: The embryo became slightly elongated and the neural plate developed as a tubular area on the dorsal surface after 37 hrs 5 mins (Fig. 3.56).

Stage 14 – *Neural fold*: This stage was marked by further elongation of the embryo and measured 4.30 mm. Distinct elevation of the two lateral ridges separated by neural groove was observed. Neural folds became distinct after 40 hrs 25 mins (Fig. 3.57).

Stage 15 – *Rotation*: The embryo was oval in shape and measured 4.45 mm. Neural folds were distinct and approached and met each other, and the neural groove became narrow after 43 hrs 45 mins (Fig. 3.58).

Stage 16 – *Neural tube*: The embryo was further elongated and the neural folds were fused completely to form the neural tube after 45 hrs 40 mins. The embryo measured 4.56 mm (Fig. 3.59).

Stage 17 – *Tail bud*: This stage was marked by the formation of the tail bud at the posterior end. The tail bud was observed to curve to the left side after 53 hrs 15 mins. The embryo measured 4.75 mm, and the yolk was still present (Fig. 3.60)

Stage 18 – *Muscular response*: Anterior region was well defined with prominent optic bulges after 60 hrs 50 mins. Embryo showed muscular response and measured 4.78 mm (Fig. 3.61).

Stage 19 – *Heart beat*: Pulsation of the heart was seen below and behind gill bud, and yolk was still present. Rudimentary gill buds became prominent and the tail

was greatly elongated after 71 hrs 20 mins. The embryo measured 5.51 mm (Fig. 3.62).

Stage 20 – Gill circulation: After 82 hrs 45 mins gills were well developed and branched into gill filaments. Opercular fold covered the base of the gills and oral sucker became well developed. Melanophores appeared on the dorsal surface, and the embryo measured 6.64 mm (Fig. 3.63).

Stage 21 – Cornea transparent: The larva hatch out of the foam nest after 4 days and tail became straight and elongated. Gills had developed. Cornea was transparent. Oral suckers and nasal pits became prominent, and the larva measured 8.25 mm (Fig. 3.64).

Stage 22 – Tail fin circulation: Tail fins became transparent. Melanophores present on the dorsal surface dispersed towards the ventral surface and tail region. Mouth became distinct, and the tadpole measured 9.34 mm after 6 days (Fig. 3.65).

Stage 23 – Opercular fold formation (Opercular and pigmentation): The operculum developed after 8 days. Gills length shortened. Small and large melanophores developed on the body surface. The tadpole measured 10.55 mm (Fig. 3.66).

Stage 24 – Opercular fold closed on right side: After 12 days, right operculum fold closed and left external gill shortened, and the larva measured 12.25 mm (Fig. 3.67).

Stage 25 – Opercular fold closed on left side: The left operculum fold was observed to close after 19 days. Operculum formation was completed, and spiracle was formed on the left side. Gills disappeared, and the tadpole measured

13.45 mm. The tadpole started feeding during this stage, and only a few rows of teeth were present (Fig. 3.68).

Stage 26 – *Hind limb bud development:* Limb buds appeared at the junction of the trunk and tail on either side of the cloacal tail after 22 days. Coiled intestine became visible. The length of limb bud was smaller than its diameter, and the tadpole head length was 10 mm and the tail length was 18 mm. Melanophores were found to be present on the tail region. The oral structure was further developed than stage 25 with a number of teeth rows on the upper and lower labium which were not so well distinct (Fig. 3.69).

Stage 27 – *Length of limb bud equaled half its diameter:* After 25 days, the length of the limb bud increased in size and its length equaled to half its diameter. The tadpole head length was 10 mm and the tail length was 18 mm. Melanophores appeared to be more denser than in the early stages, and the oral structure was similar to that of stage 26 (Fig. 3.70).

Stage 28 – *Length of limb bud equaled to its diameter:* Length of limb bud equaled to its diameter after 27 days. The tadpole head length was 10 mm and the tail length was 19 mm. Melanophores were also found to be present at the base region of the limb bud. In the tail region, melanophores were found to be concentrated in small patches. Oral structure was found to be the same as observed in the stages 26 and 27 (Fig. 3.71).

Stage 29 – *Length of limb bud equaled to one and half time its diameter:* After 30 days length of limb bud increased in size and was equaled to one and half time its diameter. The tadpole head length was 10 mm and the tail length was 21 mm.

Melanophores were observed to be dispersed in the limb bud region, and no differences were observed in the oral structure (Fig. 3.72).

Stage 30 – *Length of limb bud equaled to twice its diameter*: The length of limb bud further increased and was slightly curved after 33 days. The tadpole head length was 11 mm and the tail length was 21 mm. Dispersion of melanophores was found to be denser at the tail region, and had dispersed towards the tip of the limb bud. There were no changes in the oral structure (Fig. 3.73).

Stage 31 – *Foot paddle (Toe differentiation and development)*: The distal end of the limb bud curved slightly and was spatula in shape to form the foot paddle after 35 days. The tadpole head length was 11 mm and the tail length was 22 mm. At this stage, melanophores were found to be present at the tip of the limb bud. There were some changes in the oral structure and the teeth rows were also found to be well developed. From this stage onwards till stage 39 maximum number of teeth rows was observed on the upper and lower labium with complete dental formula $2:3 + 3/3$ (Fig. 3.74).

Stage 32 – *First indentation*: The foot paddle became slightly indented on the dorsal side, which marked the margin of the fourth and fifth toe after 37 days. The tadpole head length was 12 mm and the tail length was 22 mm. At this stage, melanophores started to disperse toward the tip of the limb bud. Oral structure was the same as that in the earlier stage (Fig. 3.75).

Stage 33 – *Second indentation*: After 38 days, the margin of the foot paddle became indented on the ventral side behind the prominence of the fourth toe, which marked the third, fourth and fifth toes. The tadpole head length was 12 mm

and the tail length was 23 mm. Melanophores were now observed on the regions, which marked toe formation. Oral structure still remained the same as that in the earlier stage (Fig. 3.76).

Stage 34 – *Third indentation:* The margin of the foot paddle became indented on the ventral side behind the third toe, which marked the prominence of second, third, fourth and fifth toes after 39 days. The tadpole head length was 12 mm and the tail length was 24 mm. Patches of melanophores were observed at the tail region, and melanophores were found to be denser at the base of the limb bud region. There were no changes in the oral structure (Fig. 3.77).

Stage 35 – *Fourth indentation:* The margin of the foot paddle became indented behind the second toe demarcating the prominence of first toe, and all five toes were demarcated from each other after 40 days. The tadpole head length was 13 mm and the tail length was 24 mm. At the base of the limb bud, melanophores had decreased and moved towards the region of toe formation. Oral structure remained the same (Fig. 3.78).

Stage 36 – *First and second toes joined:* After 41 days, first and second toes were still joined, while the third, fourth and fifth toes were separated. The tadpole head length was 13 mm and the tail length was 26 mm. Only few melanophores were found scattered at the base of the limb bud concentrated in the tip region. Oral structure still remained the same (Fig. 3.79).

Stage 37 – *Toes separated:* All five toes were separated and webbed after 42 days. The tadpole head length was 14 mm and the tail length was 27 mm (Fig. 3.80).

Stage 38 – *Metatarsal tubercles appeared:* There was appearance of metatarsal tubercles after 44 days, and the tadpole head length was 14 mm and the tail length was 27 mm (Fig. 3.81).

Stage 39 – *Subarticular tubercles appeared:* Subarticular tubercles appeared after 46 days, and the tadpole head length was 14 mm and the tail length was 29 mm (Fig. 3.82).

Stage 40 – *Toes pad completed:* Toes were fully webbed, and distal ends with developing disc developed after 49 days. The tadpole head length was 15 mm and the tail length was 29 mm. Teeth started to shed from this stage onwards (Fig. 3.83).

Stage 41 – *Cloacal tail piece reduced:* After 51 days, cloacal tail piece of the tadpole started to reduce in size. The tadpole head length was 15 mm and the tail length was 30 mm. Head of tadpole is more or less circular. Fore limbs visible and few rows of teeth were observed at the upper and lower labium (Fig. 3.84).

Stage 42 – *Both limbs developed:* Tadpoles with well developed fore limbs and hind limbs was observed after 52 days. The head of the tadpole changed in shape and was broader at the anterior end, measured 16 mm, tapered towards the tail region, and the tail length measured 30 mm. Melanophores were clearly seen in patches at the tail region and even in the fore and hind limbs. Further shedding of teeth was observed (Fig. 3.85).

Stage 43 – *Resorption of tail begins:* The tail of the tadpole started to regress after 54 days. The tadpole head length was 16 mm and the tail length was 25 mm.

Melanophores had decreased in the tail region. Complete shedding of the teeth was found (Fig. 3.86).

Stage 44 – *Tail shortened*: There was gradual loss of the tadpole tail after 57 days and was now shorter. The tadpole head length was 17 mm and the tail length was 16 mm. Formation of tongue was observed (Fig. 3.87).

Stage 45 – *Tail stub*: After 59 days, tail was completely lost and only a stub remained which measured about 2 mm, and the tadpole measured 22 mm. The formation of tongue was completed (Fig. 3.88).

Stage 46 – *Metamorphosis completed*: Tail was completely absorbed, and adult characteristics appeared after 59 – 60 days (e.g., dorsal surface leafy green and ventral is yellowish, and webs are red in colour). Metamorphosis was completed, and the froglet measured 21 mm (Fig. 3.89).

Life cycle:

It was observed that in *Rhacophorus bipunctatus*, the frogs came out of their hibernation sites in the month of March, when the temperature became warmer (20.6°C – 23.8° C), and breeding took place in the months of April to August. This frog deposited its eggs in a foam nest attached to vegetation, which served as a substratum above the water. The eggs were spherical in shape, large in size and light yellow in colour and measured about 3 mm in diameter. The eggs developed and hatched in the soft layer of the foam. After four days, the larva came out of the foam nest at the stage 21 into the water for further development. At the stage 25, the tadpole started feeding by scrapping the food particles which were attached to vegetation present in the pool. Complete

development and metamorphosis took about 59 – 60 days, and the metamorphosed froglet measured 21 mm in length (Fig 3.90). The mortality percentage ranged between 41.13 % - 92.13 % with a mean of 68.45 ± 1.18 through out the study period (2002, 2003 and 2004) (Tables 3.4 and 3.5).

During metamorphosis, the head and body structure changed from a larval to adult type. Development of the hind limb bud took place during the premetamorphic phase. The length of the limb bud increased in size till it reached the foot paddle stage. From stage 36 onwards prometamorphic phase began, and well developed hind limbs and fore limbs were observed after 52 days. The oral structure of the tadpole was observed to developed further and complete teeth rows with dental formula $2: 3 + 3/ 3$ were observed during this phase. Teeth rows began to shed from stage 40 onwards, which marked the onset of metamorphosis, and there was resorption of the tail. Complete absorption of the tail took place after about 59 – 60 days, and marked the end of metamorphosis (Fig. 3.91).

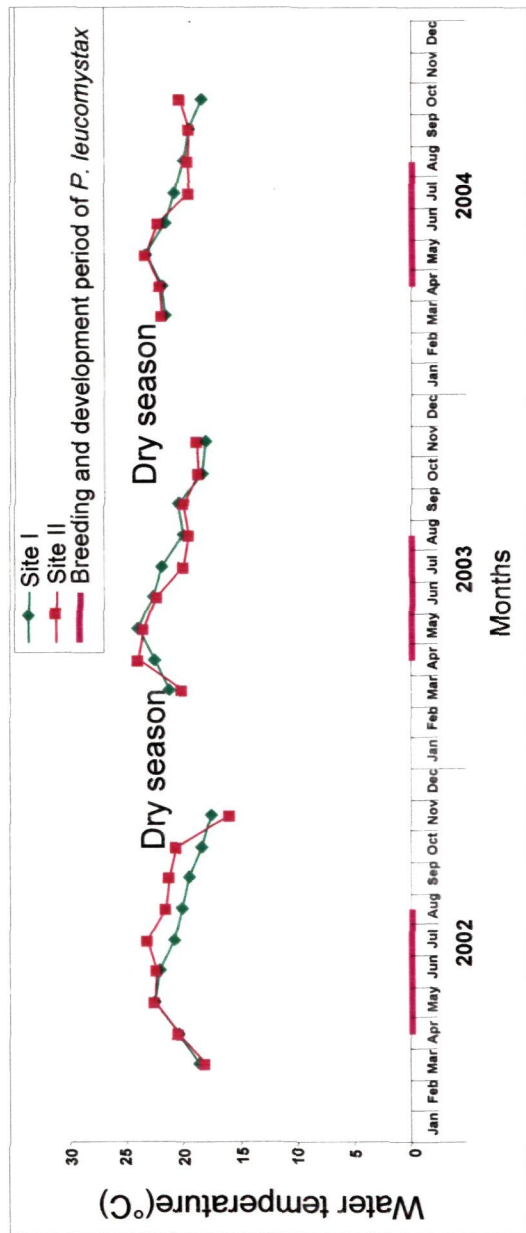


Fig. 3.1a – Monthly variation of water temperature at study sites I and II at Cherrapunjee 2002, 2003 and 2004.

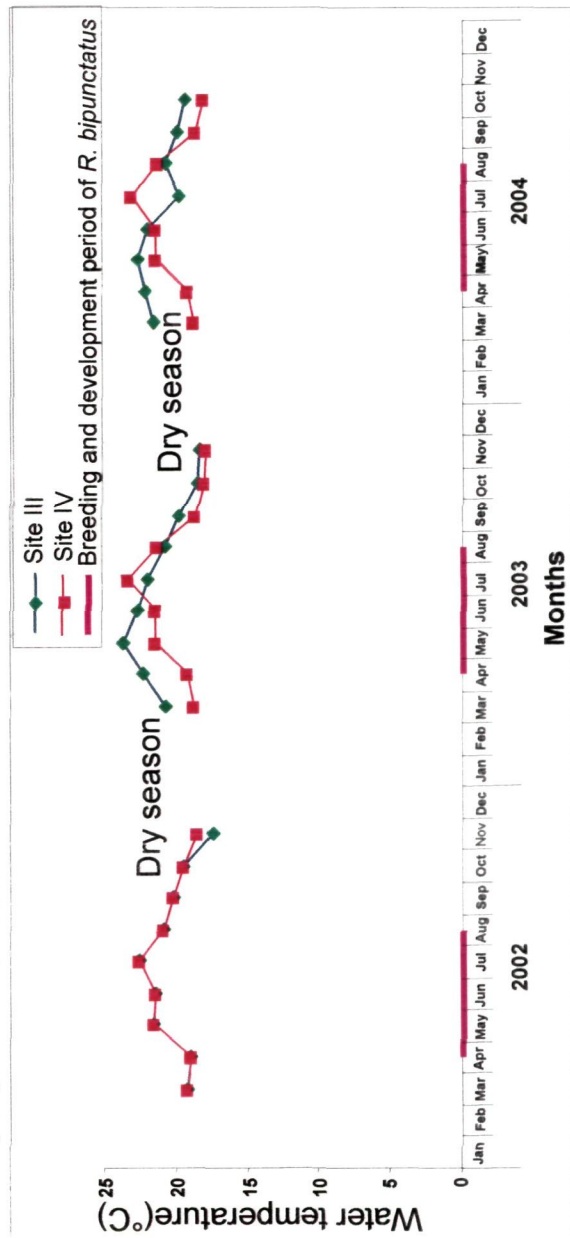


Fig. 3.1b – Monthly variation of water temperature at study sites III and IV at Cherrapunjee 2002, 2003 and 2004.

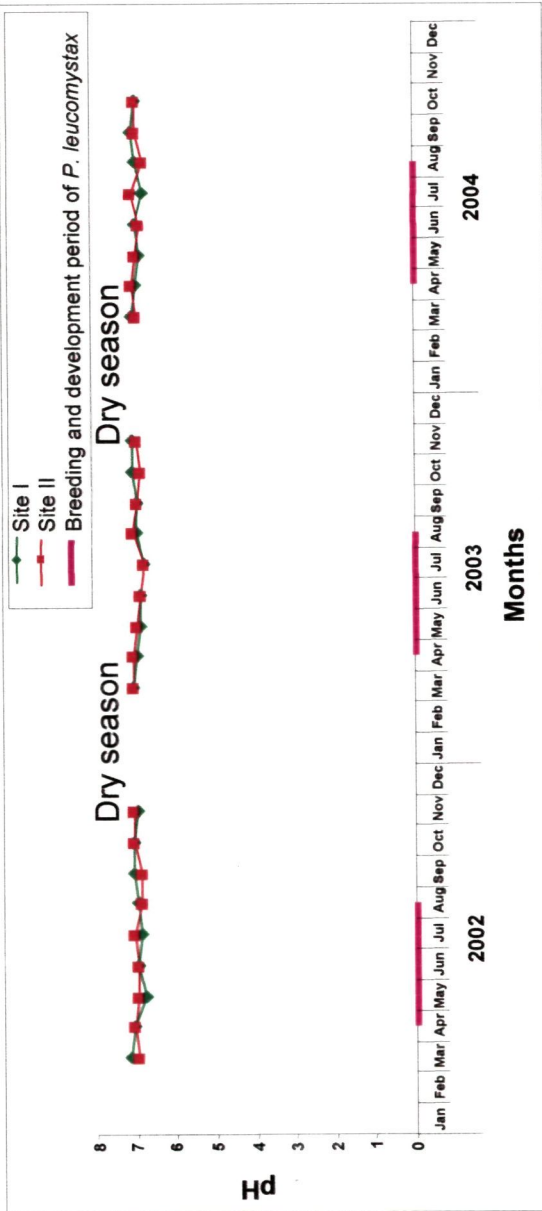


Fig. 3.2a – Monthly variation of pH at study sites I and II at Cherrapunjee 2002, 2003 and 2004.

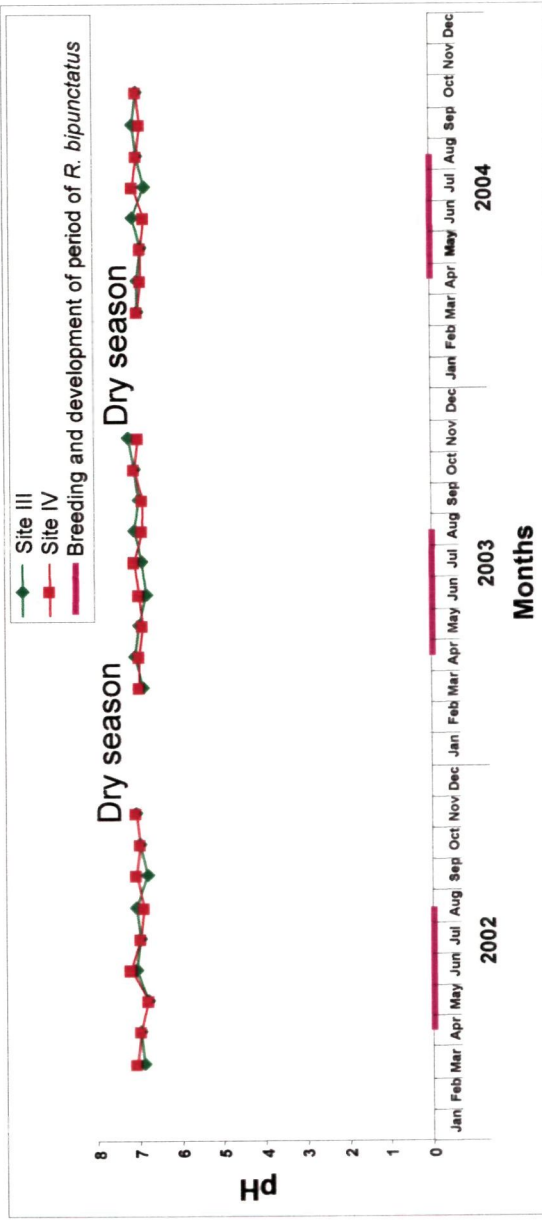


Fig. 3.2b – Monthly variation of pH at study sites III and IV at Cherrapunjee 2002, 2003 and 2004.

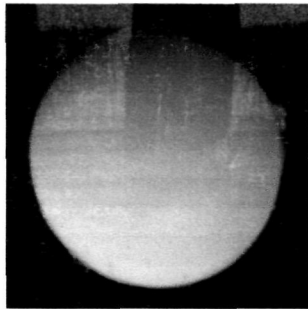


Fig. 3.3
Stage 1 - Fertilized
egg (X40).

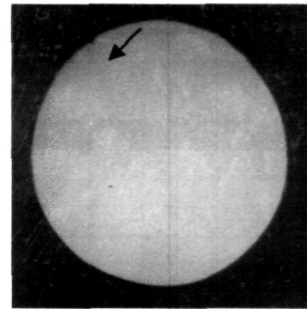


Fig. 3.4
Stage 2 - Beginning
of cleavage furrow
(X40).

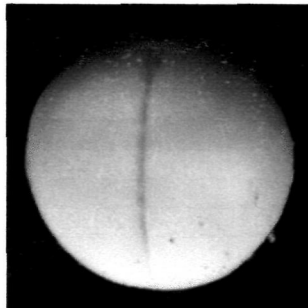


Fig. 3.5
Stage 3 - Two cell
(X40).

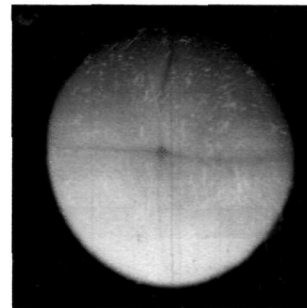


Fig. 3.6
Stage 4 - Four cell
(X40).



Fig. 3.7
Stage 5 - Eight
cell (X40).

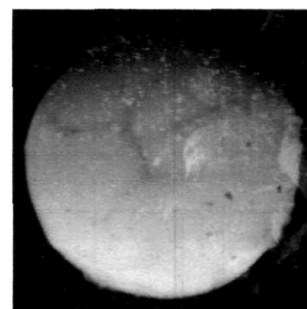


Fig. 3.8
Stage 6 - Sixteen
cell (X40).

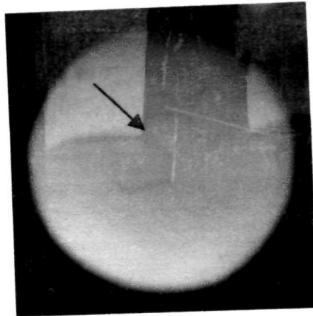


Fig. 3.9
Stage 7 - Thirty two
cell (X40).

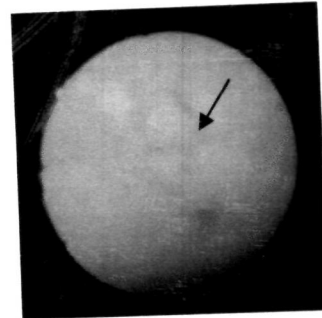


Fig. 3.10
Stage 8 - Mid
cleavage (X40).

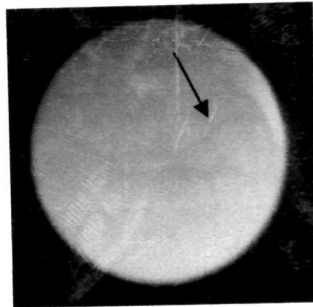


Fig. 3.11
Stage 9 - Late
cleavage (X40).

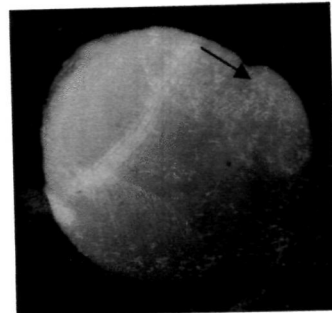


Fig. 3.12
Stage 10 - Dorsal lip
(X40).

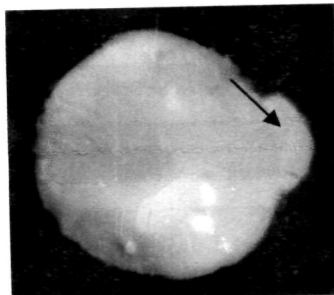


Fig. 3.13
Stage 11 - Mid
gastrula (X40).

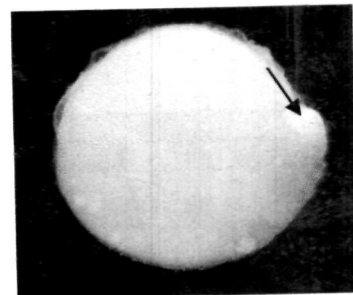


Fig. 3.14
Stage 12 - Late
gastrula (X40).

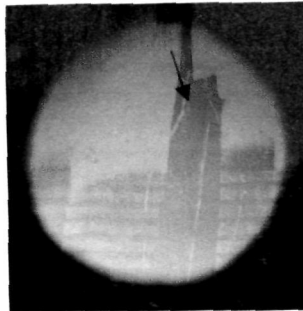


Fig. 3.15
Stage 13 - Neural
plate (X40).

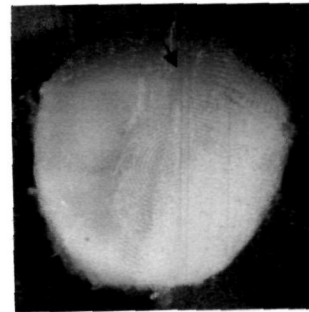


Fig. 3.16
Stage 14 - Neural
fold (X40).

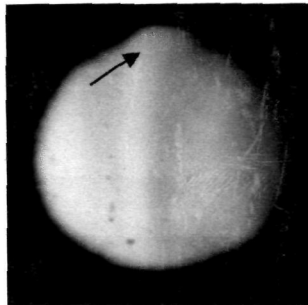


Fig. 3.17
Stage 15 - Rotation
(X40).

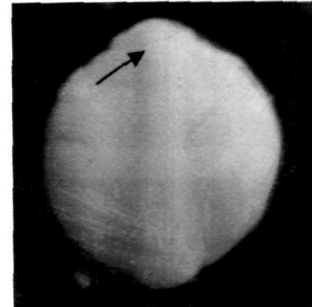


Fig. 3.18
Stage 16 - Neural
tube (X40).

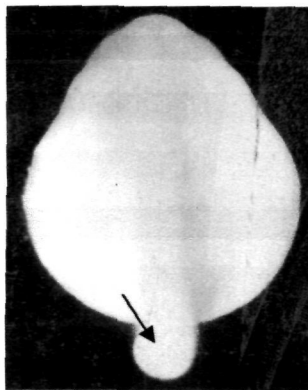


Fig. 3.19
Stage 17 - Tail bud
(X40).

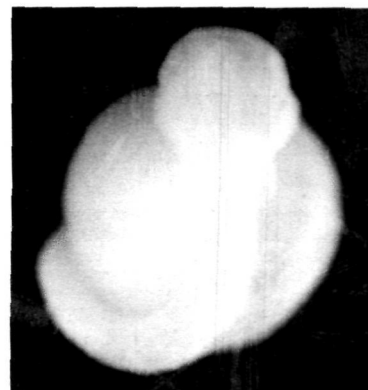


Fig. 3.20
Stage 18 - Muscular
response (X40).



Fig. 3.21
Stage 19 - Heart
beat (X40).

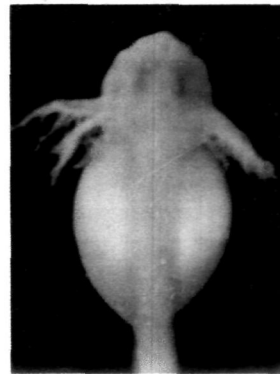


Fig. 3.22
Stage 20 - Gill
circulation (X40).

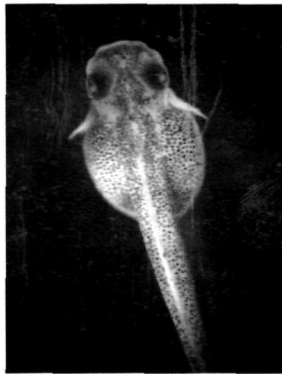


Fig. 3.23
Stage 21 - Cornea
transparent (X30).



Fig. 3.24
Stage 22 - Tail fin
circulation (X10).



Fig. 3.25
Stage 23 - Opercular fold
Formation (X30).



Fig. 3.26
Stage 24 - Opercular fold
closed on the right (X30).



Fig. 3.27
Stage 25 - Opercular fold
closed on the left.

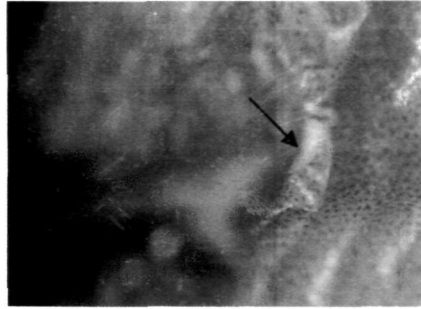


Fig. 3.28
Stage 26 - Hind limb bud.

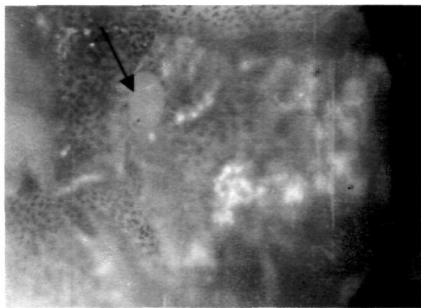


Fig. 3.29
Stage 27 - Length of limb bud
equaled to half its diameter.

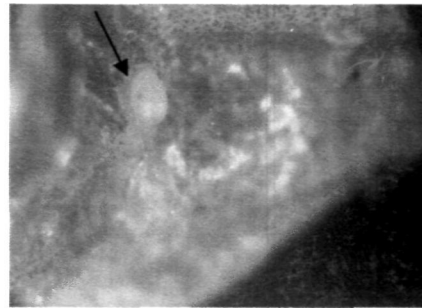


Fig. 3.30
Stage 28 - Length of limb bud
equaled to its diameter.

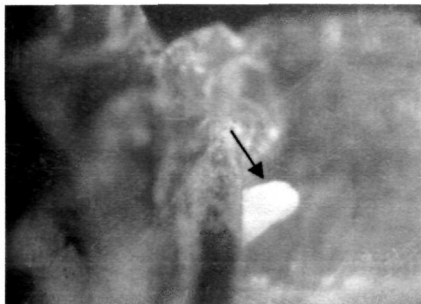


Fig. 3.31
Stage 29 - Length of limb bud
equaled to one and half its
diameter.

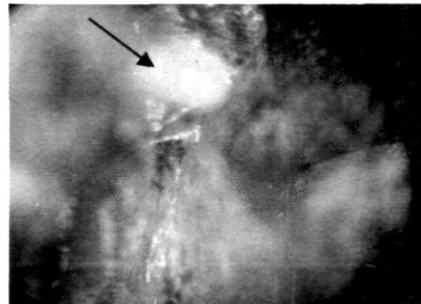


Fig. 3.32
Stage 30 - Length of limb bud
equals to twice its diameter.

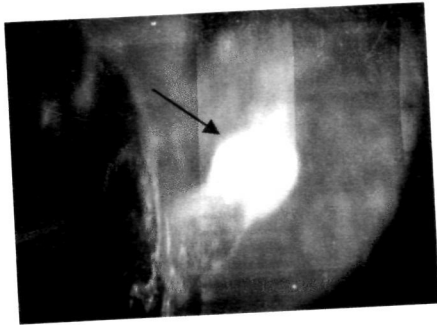


Fig. 3.33
Stage 31 - Foot paddle.

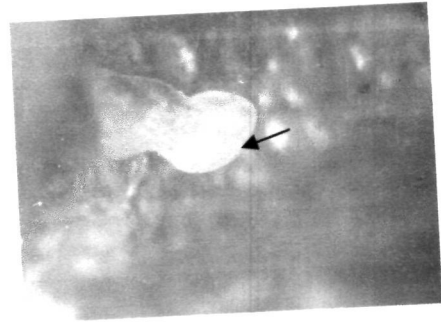


Fig. 3.34
Stage 32 - First indentation.

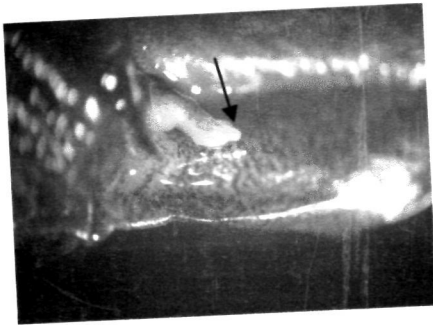


Fig. 3.35
Stage 33 - Second indentation.

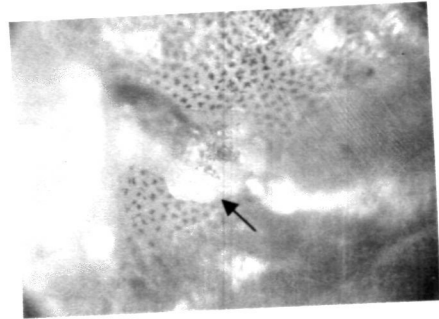


Fig. 3.36
Stage 34 - Third indentation.

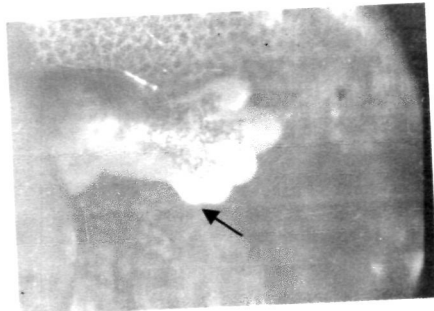


Fig. 3.37
Stage 35 - Fourth indentation.

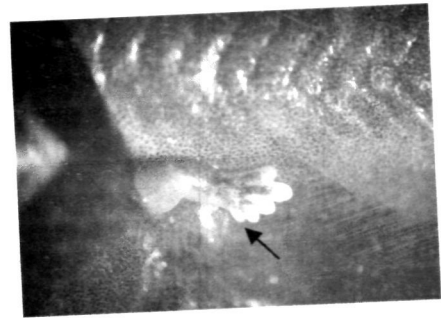


Fig. 3.38
Stage 36 - 1st and 2nd toes joined

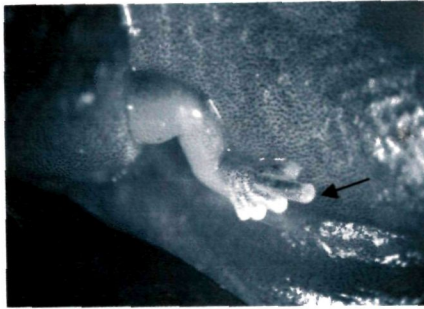


Fig. 3.39
Stage 37 - Toes separated.

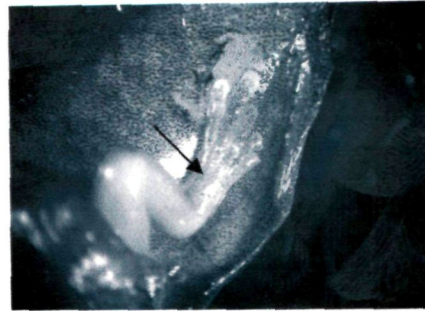


Fig. 3.40
Stage 38 - Metatarsal tubercle.



Fig. 3.41
Stage 39 - Subarticular tubercles.



Fig. 3.42
Stage 40 - Toes pad complete.

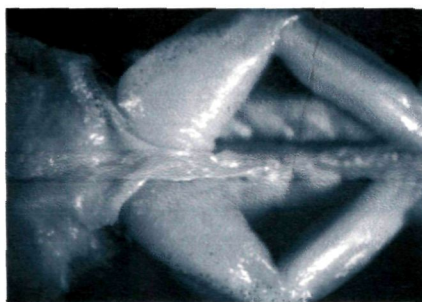


Fig. 3.43
Stage 41 - Cloacal tail reduced.



Fig. 3.44
Stage 42 - Both limbs developed.



Fig. 3.45
Stage 43 - Resorption of tail begins.



Fig. 3.46
Stage 44 - Tail shortened.



Fig. 3.47
Stage 45 - Tail stub.



Fig. 3.48
Stage 46 - Froglet.

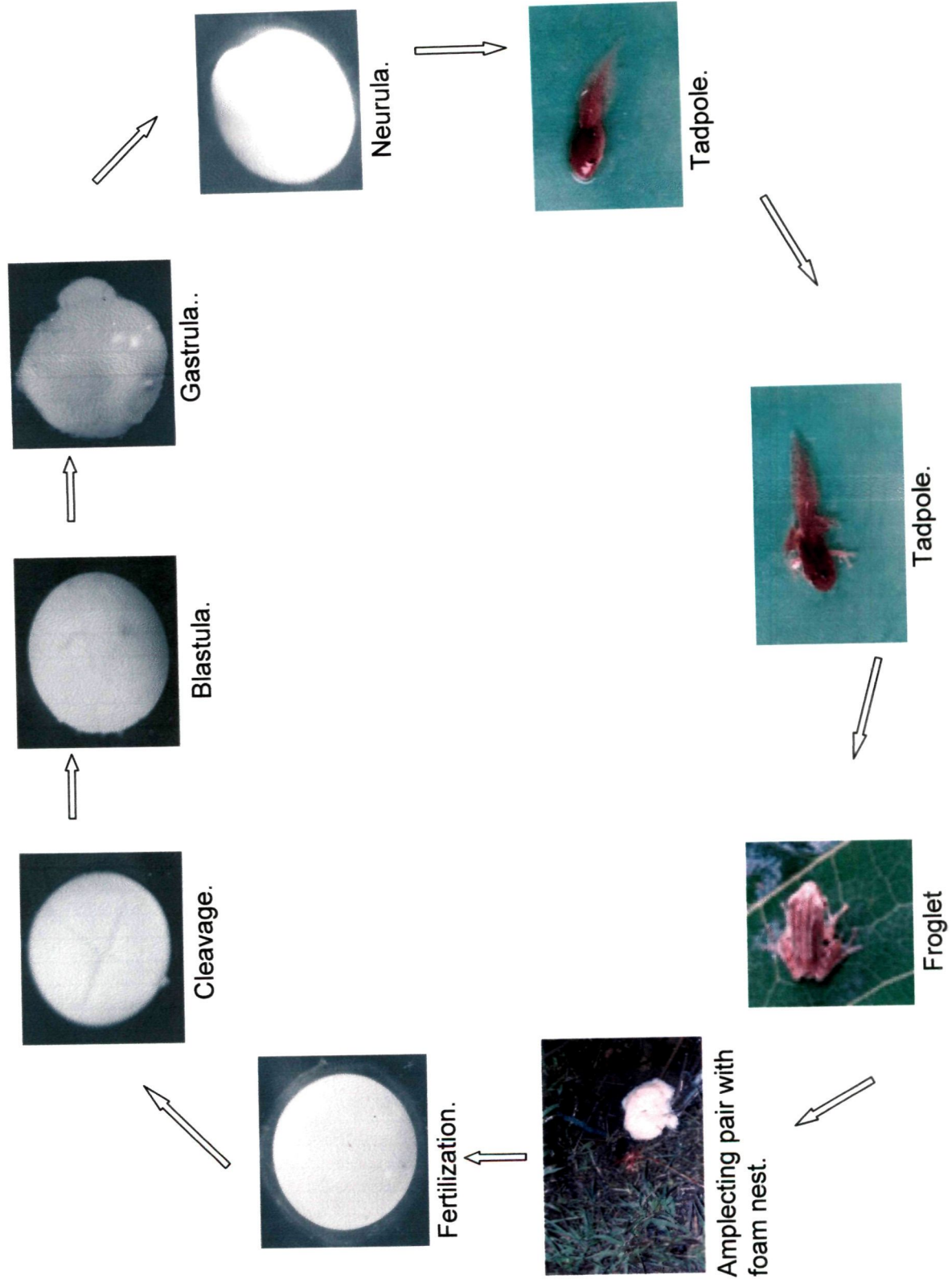


Fig. 3.49 – Life Cycle of *Polypedates leucomystax*.



Premetamorphic tadpole.



Prometamorphic tadpole.



Prometamorphic tadpole.



Metamorphic Climax stage.

Fig. 3.50 –Metamorphosis of *Polypedates leucomystax*.

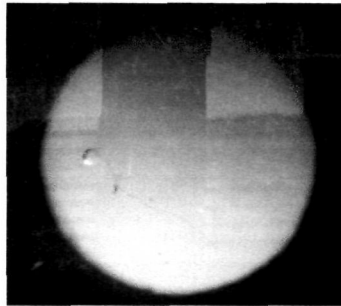


Fig. 3.51
Stage 1 - Fertilized
egg (X20).

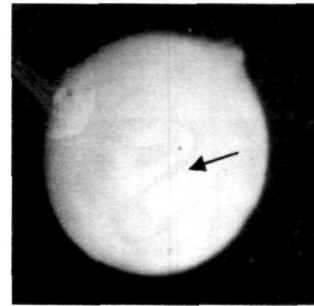


Fig. 3.52
Stage 7 - Thirty
Two cell (X20).

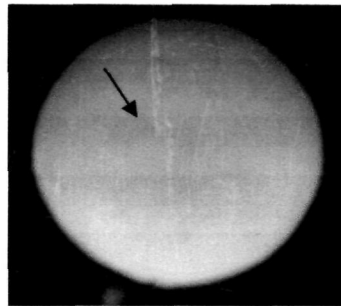


Fig. 3.53
Stage 8 - Mid
cleavage (X20).

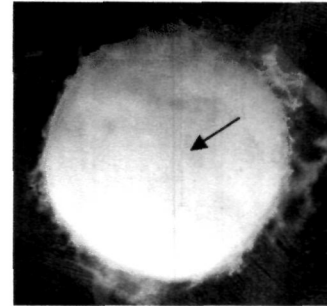


Fig. 3.54
Stage 9 - Late
cleavage (X20).

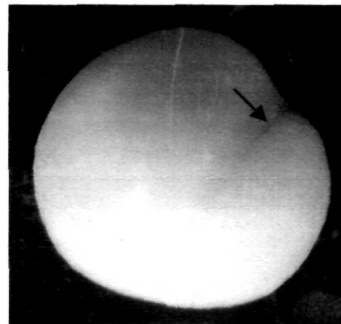


Fig. 3.55
Stage 10 - Dorsal lip
(X20).

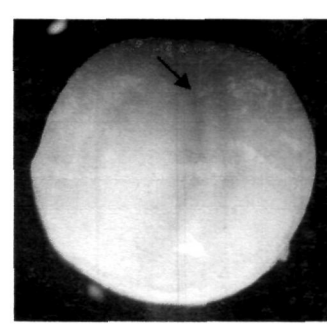


Fig. 3.56
Stage 13 - Neural
plate (X20).

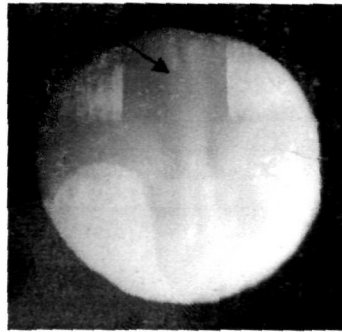


Fig. 3.57
Stage 14 - Neural fold
(X20).

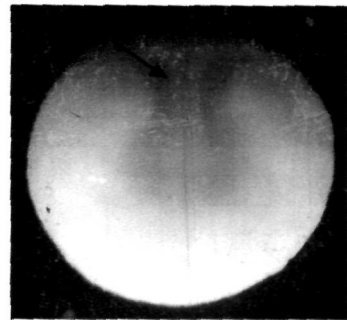


Fig. 3.58
Stage 15 - Rotation
(X20).

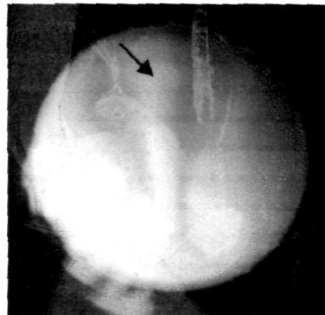


Fig. 3.59
Stage 16 - Neural
tube (X20).

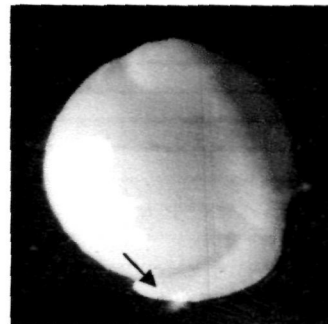


Fig. 3.60
Stage 17 - Tail bud
(X20).

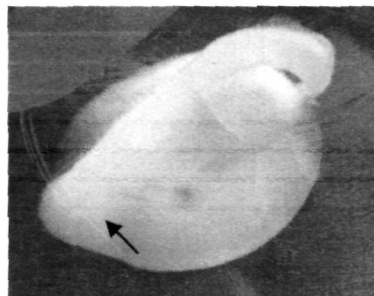


Fig. 3.61
Stage 18 - Muscular
response (X20).

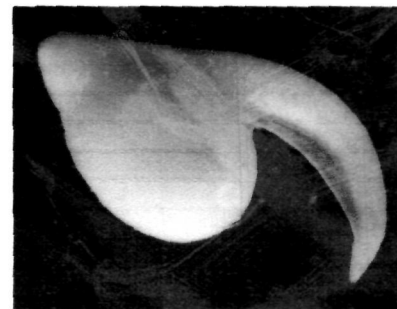


Fig. 3.62
Stage 19 - Heart beat
(X20).



Fig. 3.63
Stage 20 - Gill circulation.



Fig. 3.64
Stage 21 - Cornea transparent.



Fig. 3.65
Stage 22 - Tail fin circulation.

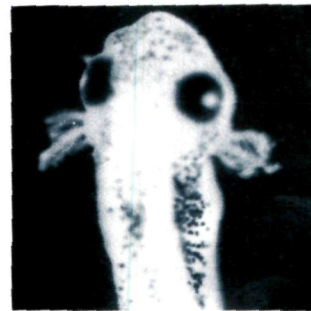


Fig. 3.66
Stage 23 - Opercular fold formation.



Fig. 3.67
Stage 24 - Opercular fold close on the right.



Fig. 3.68
Stage 25 - Opercular fold closed on the left.

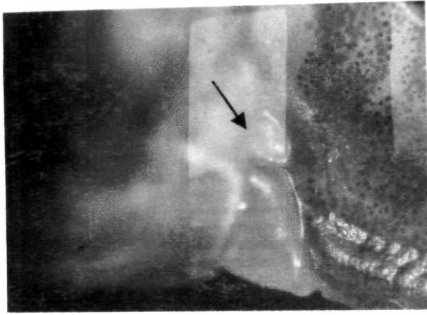


Fig. 3.69
Stage 26 - Hind limb bud.



Fig. 3.70
Stage 27 - Length of limb bud
equaled half its diameter.

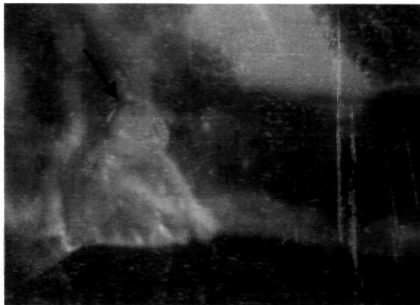


Fig. 3.71
Stage 28 - Length of limb bud
equaled to its diameter.



Fig. 3.72
Stage 29 - Length of limb bud
equaled to one and half its diameter.

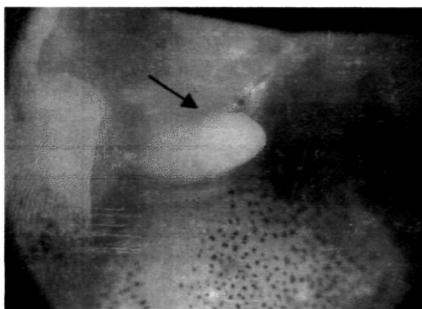


Fig. 3.73
Stage 30 - Length of limb bud
equals to twice its diameter.

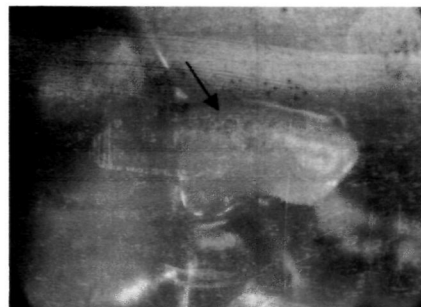


Fig. 3.74
Stage 31 - Foot paddle.

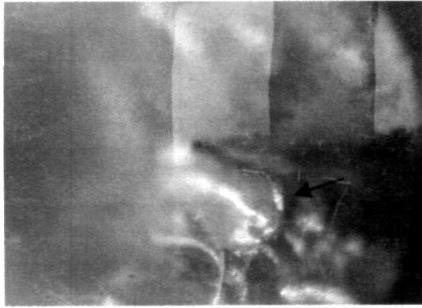


Fig. 3.75
Stage 32 - First indentation.

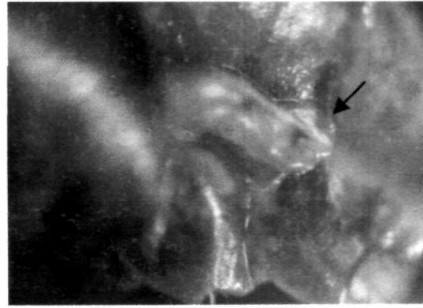


Fig. 3.76
Stage 33 - Second indentation.



Fig. 3.77
Stage 34 - Third indentation.



Fig. 3.78
Stage 35 - Fourth indentation.

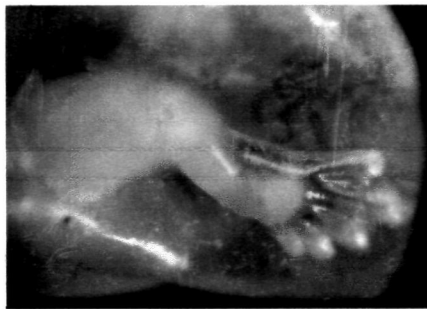


Fig. 3.79
Stage 36 - 1st and 2nd toes joined.



Fig. 3.80
Stage 37 - Toes separated.



Fig. 3.81
Stage 38 - Metatarsal tubercle.



Fig. 3.82
Stage 39 - Subarticular tubercles.

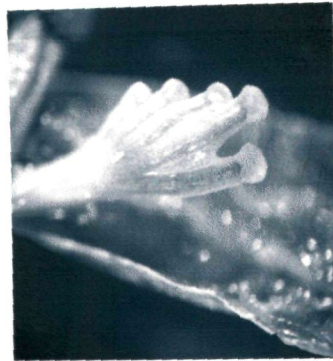


Fig. 3.83
Stage 40 - Toes pad complete.



Fig. 3.84
Stage 41 - Cloacal tail piece reduced.

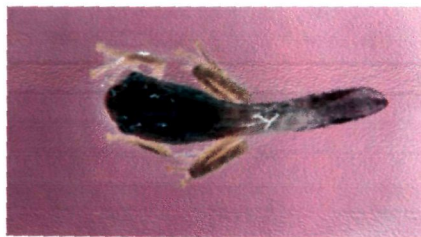


Fig. 3.85
Stage 42 - Both limbs developed.



Fig. 3.86
Stage 43 - Resorption of tail begins.

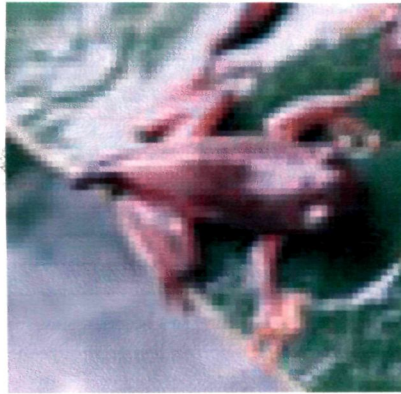


Fig. 3.87
Stage 44 - Tail shortened.



Fig. 3.88
Stage 45 - Tail stub.

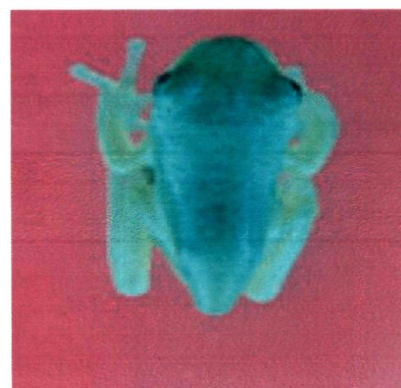


Fig. 3.89
Stage 46 - Froglet.

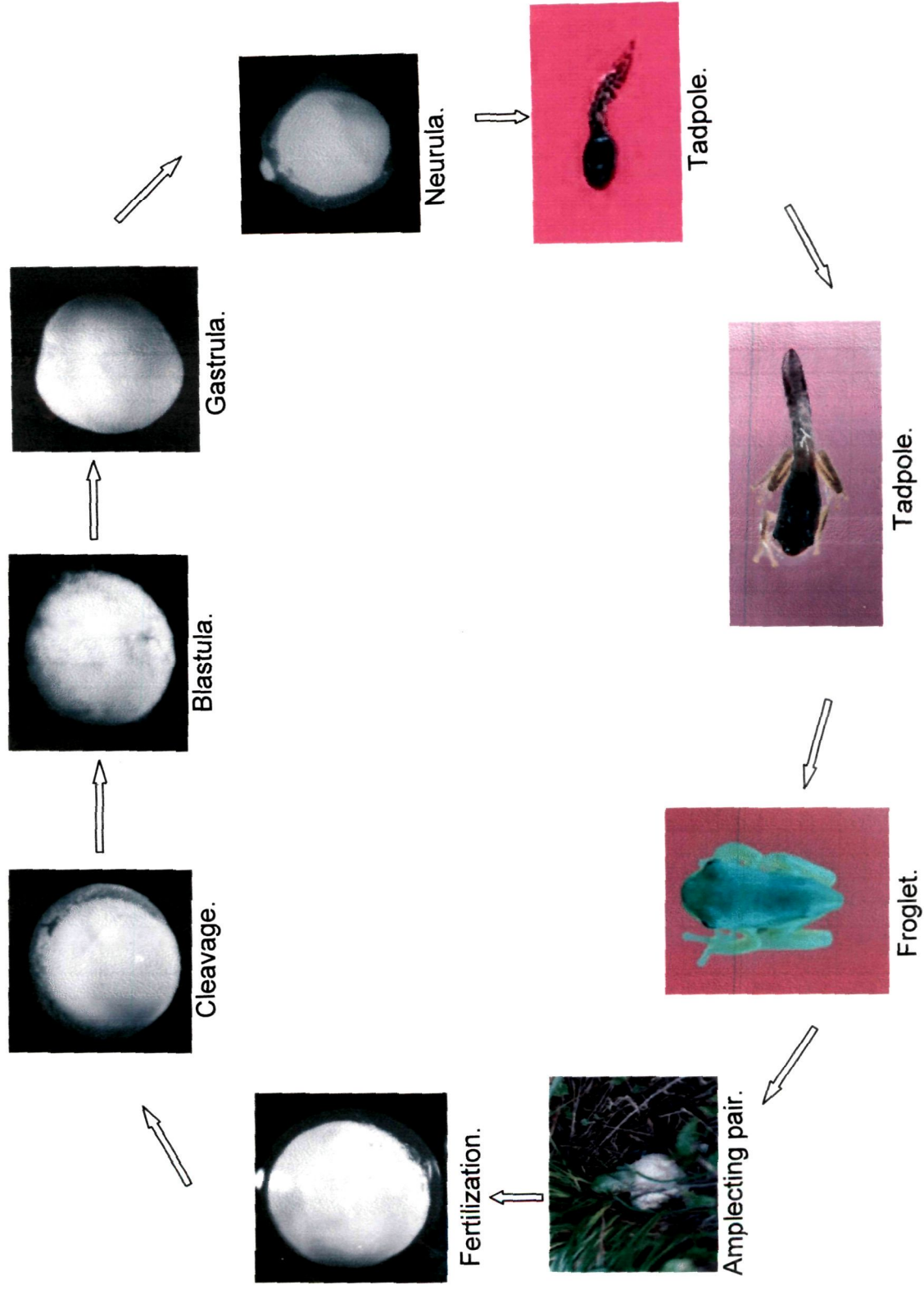
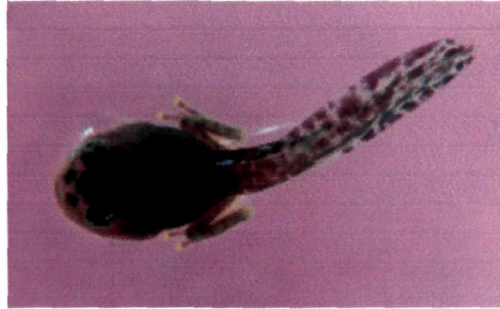


Fig. 3.90 – Life cycle of *Rhacophorus bipunctatus*.



Premetamorphic tadpole.



Prometamorphic tadpole.



Prometamorphic tadpole.



Metamorphic Climax stage.

Fig. 3.91 – Metamorphosis of *Rhacophorus bipunctatus*.

Table 3.1: Water temperature and water pH of study sites I and II at Cherrapunjee during the study period (2002, 2003 and 2004)

	Study Site I								Study Site II							
	Water temperature (°C)				pH				Water temperature (°C)				pH			
Months	2002	2003	2004	2004	2002	2003	2004	2004	2002	2003	2004	2004	2002	2003	2004	2004
Jan	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Feb	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mar	18.6±0.45	21.4±0.45	21.7±0.26	7.1±0.36	7.2±0.66	7.1±0.53	7.1±0.36	18.2±0.25	20.2±0.81	22.0±0.42	22.0±0.42	22.0±0.42	7.0±0.19	7.1±0.45	7.1±0.12	7.0±0.12
Apr	20.5±0.16	22.6±0.23	22.0±0.34	7.0±0.24	7.1±0.45	7.0±0.46	7.0±0.24	20.5±0.58	24.1±0.32	22.1±0.86	22.1±0.86	22.1±0.86	7.1±0.48	7.1±0.67	7.1±0.16	7.1±0.16
May	22.7±0.72	24.0±0.33	23.4±0.12	6.9±0.22	6.8±0.28	6.9±0.13	6.9±0.22	22.6±0.16	23.6±0.20	23.5±0.46	23.5±0.46	23.5±0.46	7.0±0.36	7.0±0.13	7.0±0.48	7.0±0.48
Jun	22.1±0.63	22.8±0.28	21.7±0.58	7.0±0.12	7.0±0.39	6.9±0.25	7.0±0.12	22.5±0.42	22.5±0.12	22.3±0.20	22.3±0.20	22.3±0.20	7.0±0.26	6.9±0.75	6.9±0.71	6.9±0.71
Jul	20.8±0.21	21.9±0.12	20.9±0.46	6.8±0.13	6.9±0.56	6.8±0.22	6.8±0.13	23.2±0.44	20.0±0.45	19.6±0.75	19.6±0.75	19.6±0.75	7.1±0.20	6.8±0.13	7.1±0.56	7.1±0.56
Aug	20.2±0.65	20.0±0.75	20.0±0.16	7.0±0.19	7.0±0.28	7.0±0.70	7.0±0.19	21.7±0.36	19.6±0.23	19.8±0.36	19.8±0.36	19.8±0.36	6.9±0.69	7.1±0.29	6.8±0.49	6.8±0.49
Sep	19.6±0.58	20.6±0.42	19.6±0.24	7.1±0.56	7.1±0.12	7.0±0.63	7.1±0.56	21.3±0.56	20.1±0.56	19.6±0.28	19.6±0.28	19.6±0.28	6.9±0.74	7.0±0.31	7.0±0.62	7.0±0.62
Oct	18.5±0.45	18.4±0.26	18.4±0.14	7.0±0.45	7.1±0.19	7.1±0.12	7.0±0.45	20.7±0.20	18.7±0.15	20.3±0.44	20.3±0.44	20.3±0.44	7.1±0.46	6.9±0.65	7.0±0.56	7.0±0.56
Nov	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Dec	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

* Dry season

Table 3.2: Water temperature and water pH of the study sites III and IV at Cherrapunjee during the study period (2002, 2003 and 2004)

	Study Site III								Study Site IV							
	Water temperature (°C)				pH				Water temperature (°C)				pH			
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004	2004
Months																
Jan	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Feb	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mar	19.3±0.26	20.8±0.42	21.6±0.36	6.9±0.12	6.9±0.50	7.0±0.46	19.3±0.30	18.9±0.33	19.2±0.33	7.1±0.16	7.0±0.23	7.0±0.13	7.1±0.16	7.0±0.23	7.0±0.13	7.0±0.13
Apr	19.0±0.45	22.3±0.12	22.2±0.12	7.0±0.36	7.1±0.26	7.0±0.23	19.0±0.16	19.3±0.56	19.9±0.56	7.0±0.37	7.0±0.52	6.9±0.46	7.0±0.37	7.0±0.52	6.9±0.46	6.9±0.46
May	21.7±0.69	23.8±0.19	22.7±0.45	6.8±0.20	7.0±0.13	6.9±0.12	21.7±0.12	21.5±0.42	20.5±0.42	6.8±0.51	6.9±0.12	6.9±0.63	6.8±0.51	6.9±0.12	6.9±0.63	6.9±0.63
Jun	21.5±0.75	22.8±0.25	22.0±0.26	7.1±0.45	6.8±0.24	7.1±0.17	21.5±0.45	21.5±0.45	21.8±0.45	7.2±0.30	7.0±0.26	6.8±0.71	7.2±0.30	7.1±0.38	7.1±0.22	7.1±0.22
Jul	22.6±0.62	22.1±0.23	19.8±0.22	7.0±0.52	6.9±0.56	6.8±0.56	22.6±0.33	23.4±0.53	21.2±0.53	7.0±0.29	7.1±0.38	7.1±0.22	7.0±0.29	7.1±0.38	7.1±0.22	7.1±0.22
Aug	21.0±0.39	20.8±0.33	20.8±0.36	7.1±0.40	7.1±0.58	7.0±0.53	21.0±0.48	21.4±0.25	20.7±0.25	6.9±0.44	6.9±0.20	7.0±0.64	6.9±0.44	6.9±0.20	7.0±0.64	7.0±0.64
Sep	20.3±0.22	19.8±0.53	20.0±0.72	6.8±0.62	7.0±0.63	7.1±0.41	20.3±0.76	18.7±0.57	18.7±0.57	7.1±0.50	6.9±0.56	6.9±0.48	7.1±0.50	6.9±0.56	6.9±0.48	6.9±0.48
Oct	19.6±0.20	18.6±0.40	19.4±0.41	7.0±0.12	7.1±0.46	7.0±0.20	19.5±0.39	18.2±0.45	18.6±0.45	7.0±0.12	7.1±0.22	7.0±0.54	7.0±0.12	7.1±0.22	7.0±0.54	7.0±0.54
Nov	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Dec	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

* Dry season

Table 3.3: Diameter of foam nest, clutch size and percentage of mortality of *Polypedates leucomystax* during the study period (2002, 2003 and 2004)

Sl No.	2002			2003			2004		
	Diameter of foam nest (cm)	Clutch size	Mortality %	Diameter of foam nest (cm)	Clutch size	Mortality %	Diameter of foam nest (cm)	Clutch size	Mortality %
1.	7	508	58.56	10	521	45.36	9	369	63.21
2.	6	454	71.23	6	507	58.63	10	289	45.16
3.	10	252	81.12	8	489	71.25	10	397	71.14
4.	8	321	42.32	12	369	65.30	6	456	66.35
5.	10	393	42.13	9	387	69.21	11	412	40.19
6.	10	405	65.22	9	456	71.26	8	306	52.06
7.	7	361	63.12	6	279	65.48	12	298	56.32
8.	8	278	56.36	10	306	46.31	12	518	72.16
9.	10	530	72.13	10	458	52.13	6	506	45.16
10.	10	457	69.16	9	478	45.16	10	469	69.34
11.	9	395	45.16	8	423	68.12	8	480	61.18
12.	12	487	54.18	10	314	72.09	11	390	42.18
13.	9	356	70.15	10	508	43.16	9	455	80.16
14.	12	412	84.32	8	506	55.47	10	276	58.36
15.	10	519	59.42	9	459	64.25	10	381	74.16
16.	8	398	71.26	11	426	66.12	6	473	81.06
17.	10	506	68.72	7	357	61.48	12	451	77.46
18.	9	493	59.12	10	478	56.57	12	375	65.13
19.	8	364	45.68	9	416	71.15	10	513	66.04
20.	12	457	80.46	12	319	68.26	9	426	56.34
21.	9	389	56.23	8	426	57.48	9	478	71.14
22.	10	489	67.48	7	487	69.22	8	382	69.21
23.	11	402	65.13	9	433	67.12	8	362	63.17
24.	10	456	34.48	9	294	54.17	10	446	59.16
25.	8	397	63.15	10	419	63.27	10	458	65.14
26.	10	336	56.48	10	349	58.47	9	476	49.58
27.	9	506	71.45	11	410	66.07	11	372	69.28
28.	10	518	72.36	9	455	73.61	7	378	67.15
29.	10	479	55.46	10	362	41.59	9	289	71.16
30.	8	463	56.38	10	379	61.14	10	339	75.14
Mean	9.33±0.26	426.03±13.47	61.95±2.20	9.2±0.27	415.39±12.78	60.96±1.72	9.4±0.31	407.33±13.03	63.44±2.02

Table 3.4: Range and mean of diameter of foam nest, clutch size and percentage of mortality of *Polypedates leucomystax* and *Rhacophorus bipunctatus* during the study period (2002, 2003 and 2004)

	<i>Polypedates leucomystax</i>		<i>Rhacophorus bipunctatus</i>	
	Range	Mean	Range	Mean
Diameter of foam nest (cm)	6-12	9.31±0.05	6-8	7.16±0.08
Clutch size (number of eggs)	252-530	416±5.41	45-76	62±0.47
Mortality %	34.48-84.32	62.31±2.10	41.13-92.13	68.45±1.18

Table 3.5: Diameter of foam nest, clutch size and mortality percentage of *Rhacophorus bipunctatus* during the study period (2002, 2003 and 2004)

Sl No.	2002			2003			2004		
	Diameter of foam nest (cm)	Clutch size	Mortality %	Diameter of foam nest (cm)	Clutch size	Mortality %	Diameter of foam nest (cm)	Clutch size	Mortality %
1.	6	53	83.13	8	51	74.16	6	52	72.27
2.	8	57	79.25	8	45	59.13	7	45	58.12
3.	6	62	61.48	7	61	69.35	6	66	65.12
4.	8	51	66.03	8	47	82.15	8	63	82.15
5.	7	56	75.42	7	54	90.16	8	50	75.46
6.	7	67	71.23	6	56	77.68	8	63	68.26
7.	6	45	42.12	6	63	66.25	7	54	57.45
8.	6	63	47.03	8	70	81.12	6	58	58.42
9.	8	69	85.14	8	67	75.16	8	62	75.26
10.	8	49	76.52	7	66	42.15	7	63	79.36
11.	7	58	56.13	8	70	56.35	7	69	66.23
12.	7	74	92.13	7	71	44.26	8	70	72.15
13.	6	66	69.18	8	49	75.26	6	71	52.17
14.	6	59	86.57	6	59	62.13	8	58	56.26
15.	8	71	62.32	8	55	66.35	7	66	69.26
16.	8	76	41.13	8	56	74.15	8	70	74.15
17.	7	47	66.54	7	62	85.26	6	68	77.25
18.	6	65	58.26	6	62	56.26	7	69	66.38
19.	6	72	78.16	8	60	44.17	8	73	69.35
20.	8	54	56.14	7	67	72.13	6	58	75.15
21.	8	55	71.16	8	65	69.16	6	56	74.16
22.	7	61	63.35	6	71	77.15	7	68	88.27
23.	7	66	62.15	7	68	69.24	7	63	74.15
24.	7	75	71.14	8	69	66.32	8	66	70.12
25.	8	72	69.56	8	63	46.28	8	67	66.35
Mean	7.04±0.16	61.72±1.8	67.65±2.64	7.32±0.16	61.08±1.54	67.27±2.64	7.12±0.16	62.72±1.44	69.73±1.71

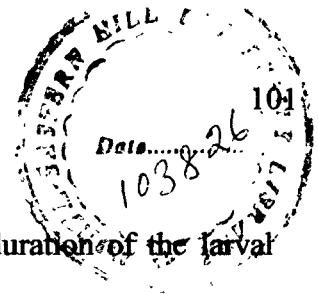
Discussion:

There are several reports on the development and metamorphosis as well as on the staging tables for several species of tree frogs *Rhacophorus arboreus* (Iwasawa and Kawasaki, 1979); *Rhacophorus malabaricus* (Sekar, 1990); *Hyla japonica* (Iwasawa and Futagami, 1992); *Polypedates maculatus* (Girish and Saidapur, 1999; Dutta *et al.*, 2001) and *Hyla annectans* (Ao and Bordoloi, 2001).

Though *Polypedates leucomystax* and *Rhacophorus bipunctatus* are endemic to Eastern and Northeast India, there is no information on the development and metamorphosis of these two tree frogs. Findings of the present study, indicate that both under the natural environment as well as in the laboratory conditions, the development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was completed in about 60 – 61 days and 59 – 60 days respectively. These two species of tree frogs were observed to be dependent on the ephemeral pools at the breeding sites to complete their life cycle. The duration of development and metamorphosis of these two tree frogs species is similar and seems to be similar to the other species of tree frogs. *Polypedates maculatus* has been reported to complete development and metamorphosis in 55 days (Mohanty-Hejmadi and Dutta, 1988), while *Hyla annectans* needs 64 days to complete its development (Ao and Bordoloi, 2001). In *Rhacophorus malabaricus*, the development and metamorphosis was completed in about 68 days (Sekar, 1990). These species seem to take advantage of transitional aquatic habitats during the monsoons. In both *Polypedates leucomystax* and *Rhacophorus bipunctatus*, hatching was observed after 4 days of fertilization indicating rapid developmental rate. The observed rapid developmental rates might be advantageous for these two tree frog species in ephemeral

habitats, which allow larvae to metamorphose quickly and escape desiccation reducing exposure to aquatic predators and diseases as reported also in other frog species (Newman, 1988; Denver *et al.*, 1998; Parris and Baud, 2004). Tadpoles from small temporary ponds have been reported to spend more time feeding and to develop faster than tadpoles from large permanent ponds, where the larvae spend more time hiding from predators, and, as a consequence develop more slowly (Peltzer and Lajmanovich, 2004).

It is important to mention that association of fertilized eggs of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* with the foam nests was found to be essential up to hatching for further normal development. When the eggs were removed from the foam nests before hatching, the embryos of both the tree frog species did not develop further. In the case of *Chirixalis simus* (Deuti, 2001) also, the fertilized eggs develop normally only in the foam nests. It, thus, seems that the foam nest is a pre-requisite for the early embryonic development up to hatching in tree frogs. The foam nests have been reported to protect the developing eggs till hatching mainly from desiccation. This confirm to the earlier reports by some workers that foam nests protect the eggs and embryos from predators and desiccation (Heyer, 1969; Downie, 1988, 1993) and thermal damage as white foam reflects heat (Gorzula, 1977). As compared to that of *Polypedates leucomystax*, the eggs of *Rhacophorus bipunctatus* were found to be soft and delicate, and separation of the eggs were difficult due to the sticky nature of the foam nests. The eggs of *Polypedates leucomystax* contained comparatively small amount of yolk, while the eggs of *Rhacophorus bipunctatus* contained a large amount of yolk. As a result, in *Rhacophorus bipunctatus* few early embryonic stages (stage 2 – stage 6) were difficult to observe and identify due to the soft and sticky nature of the foam nest.



Temperature is one of the variables known to affect the duration of the larval stage of amphibians with aquatic larvae (Beebee, 1985; Collins, 1979; Diaz-Paniagua, 1992). Some workers have reported that larvae that live in desert habitats, temporary ponds or shallow pools, in which high temperatures are common, usually have a high thermal tolerance (Brown, 1969; Cupp, 1980; Herried and Kinney, 1967; Hoppe, 1978; Miller and Packard, 1977; Ultsch *et al.*, 1999). Similarly, the thermal tolerance and thermal regulation of Taiwanese rhacophorid tadpoles (*Buergeria japonica*) collected from two different sites (Rushan and Guoshin) showed that the tadpoles collected from Rushan (27.8°C) selected higher temperatures than those collected from Guoshin (24.8°C) and suggested that the thermal tolerance of the tadpoles of *Buergeria japonica* was correlated with their geographical distribution and breeding habitats (Wu and Kam, 2005). In the present investigation, water temperature ranged between 18.4°C – 24.0°C at study sites I and II and between 18.2°C – 23.4°C at sites III and IV where *Polypedates leucomystax* and *Rhacophorus bipunctatus* completed their metamorphosis and life cycles, respectively. Hence, in this investigation the rhacophorid tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were found to thrive well and showed thermal acclimation in the water temperature range between 18°C – 24°C observed during their life cycle. Under this ranged of temperature the tadpoles showed successful development and metamorphosis in the natural habitat. Some studies have found differences in preferred temperatures among stages of development, with a tendency for later stages tadpoles to select higher temperatures (Dupre and Petranka, 1985; Wollmuth and Crawshaw, 1988). However, Brown (1990) has suggested that low environmental temperature (5°C) can inhibit the progress of metamorphosis.

In the present study, water pH ranged between 6.8 – 7.2 at the four breeding sites where development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus* took place. It may be mentioned that these four temporary rain-fed pools were located in the undisturbed areas away from agricultural run off and industrial effluents. This range of water pH between 6.8 – 7.2 seems to be optimal for the normal development and metamorphosis of the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* during their life cycle. Although some workers have suggested that some populations of certain species of frogs show a greater tolerance to low pH (Clark and LaZerte, 1987; Gosner and Black, 1957), this is not a general phenomenon (Clark, 1986). Very little is known about the potential of amphibians to adapt to low pH conditions (Andren *et al.*, 1988). Low pH reduced the survival probability as well as increased the frequency of developmental anomalies in some amphibian species (Pahkala *et al.*, 2001). During development, the embryonic stage appears to be most sensitive, low pH leads to a denaturation of the hatching enzyme (Urch and Hedrich, 1981) and subsequently to deformation of the embryo and high embryonic mortality (Glos *et al.*, 2003). In embryos, exposure to extremely low pH arrests development (Pough, 1976; Freda and Dunson 1985b). Glooschenko *et al.*, (1992) suggested that amphibians seemed to avoid high acidity and heavy metal concentrations in northern Ontario.

Mortality schedules and survivorship patterns are important attributes that influence the evolution of life history traits (Petranka, 1985). Information on the egg and larval mortality is essential to our understanding of the success of breeding and population dynamics of anuran species. In the present investigation, it was difficult to estimate the survival rates of anuran embryos and larvae under the natural condition.

Therefore, observations on the mortality percentage was made under the laboratory condition (water temperature: 20°C – 22.5°C). The mortality of embryos under the laboratory conditions ranged between 34.48 % - 84.32 % with a mean of 62.13 ± 0.20 in *Polypedates leucomystax*, while in *Rhacophorus bipunctatus* it ranged between 41.13 % - 92.13 % with a mean of 68.45 ± 1.18 . These observations indicate that mortality rate of embryos under laboratory conditions is very high. Further, there was comparatively higher mortality in the embryos of *Rhacophorus bipunctatus* as compared to that of *Polypedates leucomystax*. Warkentin (1999) reported that mortality and developmental rates in the egg and post hatching environments depend on environmental factors such as predator density, oxygen availability and temperature. Similarly, Elinson (1987) reported that few mortalities that occurred in very early embryonic stages were possibly due to developmental irregularities associated with cleavage of the eggs. It may be suggested that in the investigation, the mortality that occurred were possibly due to developmental irregularities observed during cleavage, which might have been associated with transportation/dislocation of the foam nests from the study sites to the laboratory. This may be elucidated that during early development the foam nests required a stable natural habitat free from any disturbances and environmental changes.

On the basis of the present findings, we conclude that there was a similarity in the development and metamorphosis of both *Polypedates leucomystax* and *Rhacophorus bipunctatus*. Further, these tree frogs developed and metamorphosed successfully in the water temperature range of 18°C to 24°C and in the water pH range of 6.8 to 7.2. The foam nests seem to provide optimal conditions for the early development and ensure successful normal hatching of the larva. Comparatively, larger size of the egg and the

CHAPTER IV

Food and feeding of the tadpoles and adult frogs
of *Polypedates leucomystax* and *Rhacophorus*
bipunctatus, and the morphological changes of
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Food and feeding of the tadpoles and adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the morphological changes of the oral structure of the tadpoles

Introduction:

The feeding strategies of amphibians include their choice of prey and the ways they locate, capture and ingest the prey. Amphibians are generally considered to be feeding opportunists with their diets reflecting the availability of food of appropriate size. Amphibians show major differences in ways of capturing and in taking the food, however, all terrestrial amphibians except caecilians use the tongue in capturing the prey. Many caecilians and large anurans use fang-like teeth to hold struggling prey. The tongue of amphibians have glands that produce a sticky substance, which immobilizes the prey. The limited information on amphibian diets indicates that all adult amphibians are carnivores, most of them feed principally on insects, although many species eat a wide variety of invertebrates (Duellman and Trueb, 1994).

Amphibian tadpoles exhibit structural diversities that are associated with their habitat, foraging behaviour and predator avoidance. Many morphological structures in tadpoles are associated directly with their grazing and suspension feeding way of life (Wassersug, 1980). Their specialized feeding habits require mouthparts and a digestive system that is dramatically different from those characterizing the adult frog. Anurans possess morphologically and ecologically divergent larval and post-larval stages, each

with an array of specializations. Larval specializations include keratinized teeth, a buccal pump mechanism, a long coiled intestine, and caudal locomotion.

Several studies have documented that tadpole mouthparts can exhibit variation in nature (Bresler and Bragg, 1954; Bragg and Hayes, 1963; Bragg *et al.*, 1963; Hampton and Volpe, 1963; Pfennig, 1992b). Orton (1953) defined seven adaptive types including direct development of tadpoles based on position and size of the mouth, shape of the body, and development of the caudal musculature and fins. The phenotypic plasticity of anuran larvae and environmental variables influence body shape and oral morphology in *Rana temporaria* tadpoles (Vences *et al.*, 2002). In larval amphibians, higher rearing temperatures can disrupt proper mouth development (Bresler, 1954), and competition can induce carnivorous mouthparts in a few species of tadpoles and larval salamanders (Bragg, 1956; Pedersen, 1991; Pfennig, 1992a, b; Reilly *et al.*, 1992; Walls *et al.*, 1993). In *Rana muscosa*, seasonal changes in mouthpart pigmentation has been reported in tadpoles from several populations and lab-maintained animals (Rachowicz, 2002). Further, the tadpoles of *Rana muscosa* showed variation in pigmentation of keratinized cells of the tooth rows and jaw sheaths with changes in temperature (Rachowicz, 2002). It has been reported that competitors and predators have dramatic impacts on the mouth morphology of tadpoles and that the changes likely affect the growth performance of predator- and competitor-induced individuals (Relyea and Auld, 2005). In general, competitors induced mouths that had wider oral discs, wider beaks and longer tooth rows, whereas predators induced mouths that had narrower oral discs, narrower beaks and shorter tooth rows.

The investigations to certain morphological features in the mouth of the tadpoles and comparative studies on the evolution of anuran larvae are useful in systematics of anuran amphibians. Some workers described features of the tadpole and provided the diagnostic key to their identification. Khan (1982) devised key for the identification of amphibian tadpoles (*Microhyla ornata*, *Bufo stomaticus*, *Rana tigerina*, *Rana syhadrensis*, *Rana cyanophlyctis* and *Rana breviceps*) from the plains of Pakistan. Sahu and Khare (1983) gave a field key for the identification of the tadpoles of *Rana alticola* Annandale (Anura: Ranidae) from India. Wassersug and Duellman (1984) studied the oral structures and the development in egg brooding hylid frog embryos and larvae, and revealed a wealth of structures whose taxonomic diversity could not have been predicted from an external examination of either tadpoles or embryos. These features provide much insight into both the ecology and systematics relationships of anurans. Key to the identification of the tadpoles of *Rana temporalis* have been described from South India (Hiragond and Saidapur, 1999). Hall *et al.*, (1997) studied post-embryonic ontogeny of the spadefoot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae) and indicated that mouthpart characteristics may be useful for intrageneric larval identification and the labial tooth-row formula variation is affected by temperature and geographic origin of the specimen. Grosjean (2001) investigated the rheophilous tadpoles of *Leptobrachium* (*Vibrissaphora*) *echinatum* (Amphibia, Anura, Megophryidae) including their oral internal features, and suggested that these features help in the systematic status of *Vibrissaphora* in relation to the genus *Leptobrachium* and its position among the Megophryidae. The tadpoles of *Indirana beddomei* from Karnataka, south-western India has been studied and the internal anatomy has been reported for the first time. This

investigation provided diagnostic morphological characteristics of the tadpole (Kuramoto and Joshy, 2002). The description of the morphology with an emphasis on lateral line system and mouthparts of the prometamorphic larva of the spadefoot toad, *Scaphiopus intermontanus* have been reported and the findings contributed to a comprehensive description of the ontogeny of this species (Hall *et al.*, 2002). The tadpoles of *Limnonectes tweediei* (Smith, 1935) and that of *Rana miopus* Boulenger, 1918 have been studied from Peninsular Malaysia and the salient diagnostic larval features described has helped in the identification of the tadpoles (Leong and Yaakob, 2002; Leong and Lim, 2003).

The number and arrangement of the teeth rows is species specific, and is also a useful diagnostic feature at the subfamily and family level (Martin and Watson, 1971; Watson and Martin, 1973). Dutta and Mohanty-Hejmadi (1984) studied the ontogeny of teeth row structure in *Rana tigerina* tadpoles, and reported that there was ontogenic variation in labial teeth structure. Investigation on the ontogeny of the oral apparatus of the tadpole of *Bufo americanus* showed that length of rows and keratodont number increased during anuran development (Tubbs *et al.*, 1993). It was noted that important inter-population variation in the morphology of tadpoles of the common frog *Rana temporaria*, and differences in the number of labial keratodont rows and lingual papillae have been considered as indications of taxonomic distinctness of the respective populations (Vences *et al.*, 1998). During metamorphosis, there are variations in the development of labial teeth row structure in *Rana curtipes* tadpoles, the number of teeth row changes with the stage development, and for each stage there are individual variations (James *et al.*, 2000).

The oral disc is surrounded by rows of papillae, and again the nature and arrangement of these papillae are diagnostic. The functional roles that have been proposed for oral papillae fall into two basic categories: chemosensory and tactile receptors, and structures that control water flow (Van Dijk, 1981), enhance attachment to substrates (Altig and Brodie, 1972; Gradwell, 1971, 1975), modify the shape of the oral disc during feeding and manipulate food substrate particles. Lentic species have oral discs that are least differentiated (Watson and Martin, 1973), while lotic species have elaborations of the discs that enable larvae to attach to the substrate in fast flowing waters. Such adaptations vary among species (Martin and Watson, 1971; Liem and Hosmer, 1973; Watson and Martin, 1973; Tyler *et al.*, 1979; Davies, 1989, 1991; Davies and Richards, 1990).

The habitats of amphibian larvae are as diverse as their life styles. Most amphibian larvae obtain nutrients from the environment for further development and growth. Typically, anuran tadpoles are found in streams and ponds or ephemeral situations such as puddles and road side ditches, where they glean food by scraping it off the substrates or feeding on particles suspended in the water. The majorities are aquatic and thus are subjected to different selective pressures than the adults (Duellman and Trueb, 1994). Frog larvae are usually filter feeding omnivores. They are able to feed both on the phytoplanktonic community by means of filtration and a large variety of substrates including algae, macrophytes and carrion by rasping, scraping and chopping with their jaw sheaths and labial teeth (Seale and Wassersug, 1979; Seale, 1982b). Hence, ecologists have traditionally viewed tadpoles as being herbivores that occupy lower trophic levels in aquatic communities. More precisely, trophic experts have characterized

most tadpoles as being omnivorous, microphagous suspension feeders (Wassersug, 1975; Seale, 1980; Duellman and Trueb, 1994; Stebbins and Cohen, 1995) since they possess labial teeth, keratinized jaw (beak) sheaths, buccopharyngeal gill filters and associated structures for suspension feeding and highly elongated guts for processing bulk quantities of low-quality food (Wassersug, 1980).

Many temperate *Rana* tadpoles are generalistic algae feeders (Savage, 1952; Seale, 1980). Important food categories include filamentous green algae (Dickman, 1968), epiphytic diatoms (Kupferberg, 1997b), detritus (including decomposed higher plants) and various algae (Jessen, 1967). Seale and Wassersug (1979) studied the suspension feeding dynamics of anuran larvae (*Rana sylvatica* and *Xenopus laevis*) related to their functional morphology, and reported that they are similar in several respects to those of invertebrate suspension feeders, they apparently are regulated as functions of biovolume not of particle number and they can be predicted, in part, from morphological measurements on tadpole's buccal pump. The circum-oral region of tadpoles of *Bufo stomaticus*, *Microhyla ornata*, *Rana cyanophlyctis*, *Rana syhadrensis* and *Rana tigerina* and their mode of feeding have been described. It has been found that and that every species of sympatric tadpoles differ not only in its oral disc morphology, but also in utilization of a particular part of the common food base available in pond ecosystem (Khan and Mufti, 1994). The effects of variable food types on the growth and metamorphosis in the larvae of toad *Bufo fergusonii* have been described, and it was found that the toads fed mostly on animal and plant food materials, and for large scale culture of these tadpoles, use of albumin and yolk in the laboratory is recommended as a special tadpole diet (Saxena *et al.*, 2001).

Investigations on the effects of tadpole grazing on periphytic algae in ponds suggested that grazing is potentially quantitatively important, structuring the algae community and/or affecting the feeding conditions of the tadpole population itself (Loman, 2001). Liang *et al.*, (2002) described the effects of intermittent feeding on the growth of oophagous in *Chirixalus eiffingeri* and herbivorous tadpoles of *Chirixalus idiootocus* from Taiwan, and reported that *Chirixalus idiootocus* tadpoles were unable to tolerate prolonged periods without food, in part, because their digestive tract clearance time (the time it takes food to pass through the alimentary canal) is short, and their stomach is incapable of long-term food storage. While in *Chirixalus eiffingeri* tadpoles, the trophic eggs stored in the stomach could reduce the risk of starvation between feedings. Pryor (2003) investigated the growth rates and digestive abilities of larval bullfrogs, *Rana catesbeiana* fed non-specific algal diets and reported that the filamentous blue-green alga *Anabaena* promoted better growth in *R. catesbeiana* tadpoles than the other algal species.

The qualitative composition of the diet and the range of food particle size in the tadpoles of two tree frogs, *Hyla nana* and *Scinax nasicus*, is highly variable and is linked to food availability (Candioti *et al.*, 2004). Khan (2004) evaluated the riparian tadpoles of *Hoplobatrachus tigerinus* (Daudin, 1802) with notes on breeding habits and feeding ecology, and reported that *Bufo* larvae are the staple food source of *Hoplobatrachus tigerinus* tadpoles in the diet. Larger zooplankton like copepods and *Daphnia*, which are filtered from the incoming water current, also form a considerable portion of the tadpole's diet. Other rarely ingested items include water beetles, bugs, naiads and larvae of fishes. Iwai and Kagaya (2005a) evaluated the relative suitability of different food

items (leaf litter, algae, or animal materials) for tadpoles of the Japanese toad, *Bufo japonicus formosus*, reported that all individuals successfully metamorphosed in all food treatments, and there was no difference in the ratio of metamorphosed individuals. Iwai and Kagaya (2005b) studied the difference in larval food habit of two Japanese species, *Rana japonica* and *Rana ornativentris*, and reported that their different food habits may be related to their distributional patterns.

Das and Charles (1994) observed the diet and microhabitat used by *Plytymantis dorsalis* A. Dumeril, 1853 (Anura: Ranidae) at Mount Makiling Los Banos in Philippines reported that by frequency of occurrence, spiders, insect larvae and termites were the most important prey types. Other prey type recorded include ants and beetles, while a single millipede and one centipede were found, indicating that these prey items are taken less frequently. The diet of the brown frog *Rana longicrus* in Taiwan consisted primarily of invertebrates of the classes Gastropoda, Oligochaeta, Arachnida, Crustacea, Insecta (adults and larvae) and Chilopoda (Kam *et al.*, 1995). Kam *et al.*, (1998) studied seasonal activity, reproduction, and diet of a riparian frog (*Rana swinhoana*) from a subtropical forest in Taiwan, and reported that the diet consisted primarily of invertebrates of the classes Gastropoda, Crustacea, Arachnida, Chilopoda and Insecta (adults and larvae). Insects and arachnids were the most abundant items in their diets. Sluys and Rocha (1998) studied the feeding habits and microhabitat utilization by two syntopic Brazilian Amazonian frogs *Hyla minuta* and *Pseudopaludicola* sp. (gr falcipes), and reported that the two species differed markedly in both prey type and sizes. The differences in prey types consumed by the two frog species may be primarily due to differences at the taxonomic level, whereas the observed differences in prey size can be mostly affected by

frog body size. The feeding habits of six anuran species in a rainforest fragment in Northeastern Brazil was analysed, and it was found that there was food preferences and seasonal variations in their diet (Santos *et al.*, 2004). The diet of the South American frog *Leptodactylus ocellatus* (Anura: Leptodactylidae) in Uruguay comprised mainly of Coleopterans, and there was a significant correlations between predator and prey sizes (Maneyro *et al.*, 2004).

Several studies have documented cannibalistic and interspecific predation by anuran larvae on eggs, tadpoles, and newly metamorphosed amphibians (Polis and Meyers, 1985; Petranksa and Kennedy, 1999; Dayton and Wapo, 2002). Cannibalism and interspecific predation have been documented in several species of spadefoot toads Pelobatidae (Richmond, 1947; Bragg, 1940, 1964; Woodward, 1982; Tejido, 1991). Mohanty-Hejmadi and Dutta (1981) studied the inter and intra specific predation by *Rana tigerina* tadpoles, and reported that cannibalism is concentration induced even with other food available in the surroundings. Numerous studies have demonstrated that amphibian larvae that feed on conspecifics grow faster than larvae that are not cannibalistic (Crump, 1990; Wildy *et al.*, 1998). Summers and Symula (2001) analysed cannibalism and kin discrimination in tadpoles of the Amazonian poison frog, *Dendrobates ventrimaculatus*, in the field and suggest that, even if there is some tendency to be less aggressive towards kin than towards non-kin, this tendency may usually be sufficient to prevent cannibalism of small tadpoles by larger kin in the field. All of the small tadpoles placed with larger tadpoles were cannibalized, regardless of kinship status.

The review of the literature on the food and feeding of anurans species indicates that there is scarcity of information on the food, feeding habits, and oral structures of tree

frogs in general in particular in India. Therefore, in the present study, it was decided to investigate the food and feeding habits, and oral structure of two tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* found in Meghalaya.

Materials and Methods:

To study the food and feeding habits of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, adult frogs and different developmental stages of the tadpoles were collected from the study sites I, II, III and IV described in Chapter II. After collection, the tadpoles and adult frogs were anesthetized and fixed in 4 % formaldehyde. The tadpoles were staged according to Gosner (1960) and autopsy was done for analysis of gut content. In adult frogs a small incision was made in the abdomen, and the guts were dissected and washed in 1 ml of distilled water. The gut samples were taken in a clean slide and qualitative analysis of the samples was undertaken and observed under the compound microscope. Identification of the food items present in the gut of the tadpoles was made following the works of Edmonson (1959).

Simultaneously, water samples were also collected from the four study sites where development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were studied. Fifty litres of water from the four study sites was sampled with plankton net and transferred to a plankton tube for qualitative analysis, and the organisms were fixed by adding 5 % formaldehyde. The plankton sample was taken on a slide and covered with a cover slip. Qualitative analysis of the plankton was observed and recorded.

The oral structure of the developing tadpoles during metamorphosis comprising the premetamorphic, prometamorphic and metamorphic climax were studied using stereo-scopic binocular microscope following the criteria of Van Dijk's (1966) and also with the help of Scanning Electron Microscopy.

Scanning Electron Microscopy:

For scanning electron microscopy, the samples were washed with double distilled water, fixed in 2.5 % glutaraldehyde solution (prepared in 0.1 M Na-Cacodylate buffer) for four hours and post fixed in 1 % Osmium tetroxide buffer (0.1 M Na-Cacodylate buffer) for 1 hour at 4°C. The pH of fixative and buffer was maintained at 7.4. The samples were then dehydrated through ascending acetone grades and drying was done in Tetra methyl silane (Dey *et al.*, 1989). A thin conductive coating of gold was applied to the samples using a JFC 1100 (Jeol) ion sputter and the coated samples were examined with the help of a scanning electron microscope (Jeol: JSM-35 CF, Japan) at an accelerating voltage of 20 KV.

Results:

Gut contents of *Polypedates leucomystax* and *Rhacophorus bipunctatus*:

Qualitative analysis of the gut contents of tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* revealed that the tadpoles started feeding from stage 25 onwards. The details of the gut contents of the tadpoles is shown in Table 4.1.

At the stage 25, the gut of tadpoles contained mostly detritus and plant materials, which included *Pediastrum*, *Closterium*, *Anabaena*, *Cosmarium*, and *Staurastrum*. While the tadpoles of *Rhacophorus bipunctatus* revealed that plant materials included *Pediastrum*, *Closterium* and *Anabaena* (Table 4.1).

However at stages 26 to 30, the gut of the tadpoles contained *Cosmarium*, *Zynema*, *Selenastrum*, *Scenedesmus*, *Oscillatoria*, *Pediastrum*, *Closterium*, *Anabaena*, *Navicula*, *Ulothrix*, and *Staurastrum* (Table 4.1).

Unlike earlier stages, at stage 31 to 39, besides the presence of the phytoplankton (e.g., *Pediastrum*, *Closterium*, *Cosmarium*, *Oscillatoria*, *Ulothrix*, *Navicula*, *Staurastrum*, *Cosmarium*, *Selenastrum*, *Zynema*, *Anabaena*, *Oscillatoria*, and *Scenedesmus*), the gut also contained Zooplankton (*Keratella*, *Paramecium*, *Brachionus* and *Bosmina*) (Table 4.1).

The gut of the tadpoles at stage 40 to 46 revealed absence of food items and the tadpoles had stopped feeding. It was observed that immediately after metamorphosis, the froglets did not feed, but after a few days they started feeding on a carnivorous diet.

Qualitative analysis of the gut contents of the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* revealed the presence of phytoplankton of the family Euglenophyceae, Cyanophyceae, Chlorophyceae and Bacillariophyceae, and Zooplankton (Table 4.2). The water samples of the four study sites showed the presence of the phytoplankton and Zooplankton recorded in the gut contents of the tadpoles of both to the species. It was also observed that the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* exhibit cannibalistic behaviour both in the natural environment and under laboratory conditions.

The stomach of the adult frogs was found to contain mostly insects, which included spiders, insect larvae (Arachnida), flies (Diptera) and bugs (Hemiptera) etc.

Oral structures of the tadpoles of *Polypedates leucomystax*:

When observed under the stereoscopic binocular microscope, the oral structure of the tadpole at stage 25 was characterized with serrations of one uninterrupted row of teeth and one faint and distinct interrupted row on the upper labium. Further, three distinct uninterrupted rows of teeth were observed on the lower labium along with the presence of papillae (Fig. 4.1a). Scanning electron microscopic studies of the tadpoles at stage 25 clearly indicated the V-shaped lower jaw sheath, teeth rows and papillae. The entire lower labium was lined with papillae, while in the upper labium only few were present on the lateral sides (Fig. 4.2). When magnified, the V-shaped lower jaw sheath appeared to be serrated and pointed, while the upper jaw sheath appeared serrated and blunt (Fig. 4.3). The teeth rows were arranged linearly in the lower labium, appeared club shaped and attached at the base (Fig. 4.4).

The appearance of the oral structure from stage 26 to stage 30 were the same. The upper labium consisted of one uninterrupted teeth row and three broken or interrupted teeth rows. While in the lower labium, three distinct uninterrupted rows of teeth were observed. Rostral and mental gap were distinct (Fig. 4.1b).

Scanning electron microscopic studies at stage 26 showed the presence of teeth in the upper and lower labium, papillae and a mental gap was observed to be present at the centre in the lower labium. The mental gap measured 200 μm (Fig. 4.5). Magnified view of the papillae at stage 26 showed that it appeared conical in shape and rough in surface

feature. The length of the papillae measured 41.66 μm and the width at the base measured 40 μm (Fig. 4.6). Magnified view of the serrated upper jaw sheath appeared to be columnar in shape and the tips were blunt at the centre of the opening of the mouth and measured 40 μm in length with a width of 26 μm at the base. They appeared to be pointed at the lateral sides (Fig. 4.7).

Appearance of the oral structure was the same from stages 31 to 39. In these stages, one uninterrupted tooth row and three broken or interrupted rows of teeth were observed in the upper labium, while in the lower labium three distinct uninterrupted rows of teeth were present. The entire teeth rows on the upper and lower labium were well developed. Complete teeth rows with dental formula 1:3+3/3 were observed during these stages (Fig. 4.1c).

Scanning electron microscopic studies of the entire oral structure of the tadpole of *Polypedates leucomystax* at stage 34 showed the presence of mental gap in the lower labium, which measured 200 μm (Fig. 4.8). The magnified view of the V-shaped lower jaw sheath showed the presence of pointed and serrated edges measuring 46.66 μm in length with a width of 33.33 μm (Fig. 4.9). Magnified view of upper labium showed the presence of teeth rows and upper jaw sheath. It was observed that in the upper jaw sheath the serrated edges were blunt at the centre of the opening of the mouth, while pointed at the sides (Fig. 4.10). The teeth appeared serrated and spoon-like in shape, curved and serrated at the head region measuring 72.85 μm in length and 12.85 μm in width at the base in the upper labium (Fig. 4.11). Three number of teeth rows were observed at the lower labium arranged in a linear fashion (Fig. 4.12). A magnified view of the teeth in the lower labium was observed to be similar in structure to those of the upper labium, and

measured about 57.77 μm in length and has a width of 11.11 μm at the base (Fig. 4.13). Magnified view of papillae in the lower labium appeared to be rough in surface feature and measuring 123.63 μm in length and 58.18 μm in width (Fig. 4.14).

From stage 40 onwards, the tadpoles stopped feeding. This stage was characterized by the onset of metamorphosis. Papillae were still present but the teeth had begun to shed. Further, serrations of upper and lower jaw sheath started disappearing, and only a few teeth were still remaining (Figs. 4.1d and 4.1e).

Metamorphosis was almost completed at the stage 46. Mouth started to widen and resembled to that of the adult (Figs. 4.1f and 4.1g). Magnified view of the mouth under scanning electron microscope showed complete development of the tongue, and only few papillae were present at the lateral side and complete shedding of teeth was observed at this stage (Fig. 4.15).

Oral structures of the tadpoles of *Rhacophorus bipunctatus*:

Under stereoscopic binocular microscope, the oral structure of the tadpoles at stage 25 was distinct and the tadpole started feeding. Two distinct uninterrupted as well as two interrupted teeth rows on the upper labium, and three uninterrupted teeth rows on the lower labium were observed. The teeth rows on both the upper and lower labium were not so well developed (Fig. 4.16a). At stages 26 to 30, the upper labium consisted of two uninterrupted and three interrupted teeth rows, which were not clearly visible. The lower labium consisted of three uninterrupted teeth rows, which were more developed than that of stage 25 (Fig. 4.16b). Scanning electron microscopic studies of the tadpoles of *Rhacophorus bipunctatus* showed that in the upper labium the papillae were concentrated

on the lateral sides, whereas the lower labium was entirely bordered by papillae. The teeth rows in the upper and lower labium, the upper jaw sheath and V-shaped lower jaw sheath were also observed (Fig. 4.17). A magnified view of the upper jaw sheath showed that the serrated edges were conical in shape and pointed, and measured 56.36 μm in length and 21.81 μm in width at the base (Fig. 4.18). The teeth at the upper labium appeared spoon-shaped and serrated, and narrow towards the base measuring 51.11 μm in length (Fig. 4.19). Magnified view of the papillae showed the rough surface-feature, and the papillae measured 70 μm in length and had a width of 43.33 μm at the base at the lower labium (Fig. 4.20).

However, at stages 31 to 39, the number of teeth rows were observed to reach a maximum with a dental formula of 2: 3 + 3/ 3. Upper labium consisted of two uninterrupted rows of teeth and three interrupted, which were distinct. Lower labium remained same as in the previous stages but was more developed (Fig. 4.16c). Scanning electron microscopic studies of the oral structure of the tadpoles of *Rhacophorus bipunctatus* at stage 34 showed the upper and lower labium, upper and lower jaw sheaths, and teeth rows in the upper and lower labium (Fig. 4.21). Magnified view of the upper labium showed the uninterrupted and interrupted teeth rows (Fig. 4.22). The upper jaw sheath and the V-shaped lower jaw sheath were observed to be serrated and pointed (Fig. 4.23). Teeth at the lower labium were observed to be curved or bent towards inside (Fig. 4.24). The oral structure of the tadpoles of *Rhacophorus bipunctatus* at stage 34 showed the presence of papillae on the lateral side of the upper labium while the entire lower labium was bordered by papillae. Teeth rows were present on both the upper and lower labium, the upper jaw sheath and V-shaped lower jaw sheath was also observed (Fig.

4.25). It was observed that the upper serrated jaw sheath was conical in shape and pointed measuring 62 μm in length and 30 μm in width at the base (Fig. 4.26). The teeth at the upper labium were spoon-shaped, elongated and tapered and serrated in the head region, measuring 60 μm in length (Fig. 4.27). The teeth at the lower labium were observed to be similar to that of the upper labium and measured 37.27 μm in length (Fig. 4.28).

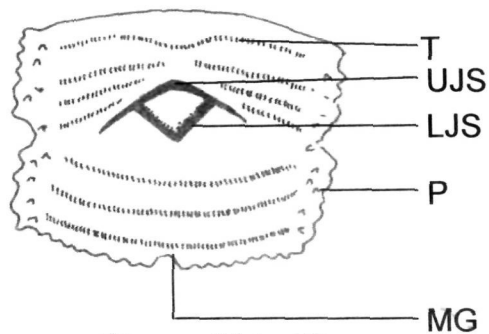
When observed under the stereoscopic binocular microscope, teeth rows had begun to shed from stage 40 onwards. Serrations were observed to disappear and only a few teeth rows were still present (Figs. 4.16d and 4.16e). Teeth had completely disappeared at the stage 46 and the tongue had developed. Mouth had widened slightly and resembled to that of the adult type (Figs. 4.16f and 4.16g). Scanning electron microscopic studies of the metamorphosed froglet revealed that all the teeth had been shed and a tongue had developed (Figs. 4.29).



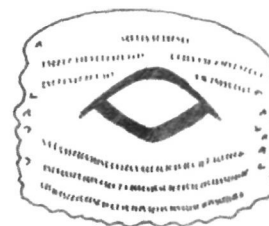
a. Stage 25



b. Stages 26 to 30



c. Stages 31 to 39



d. Stage 40



e. Stage 41



f. Stages 42 to 45



g. Stage 46

Fig. 4.1a - g – Diagrammatic representation of the oral structure of *Polypedates leucomystax*
LJS=Lower jaw sheath; Mg=Mental gap; P=Papillae; T=Teeth, To=tongue; UJS=Upper jaw sheath

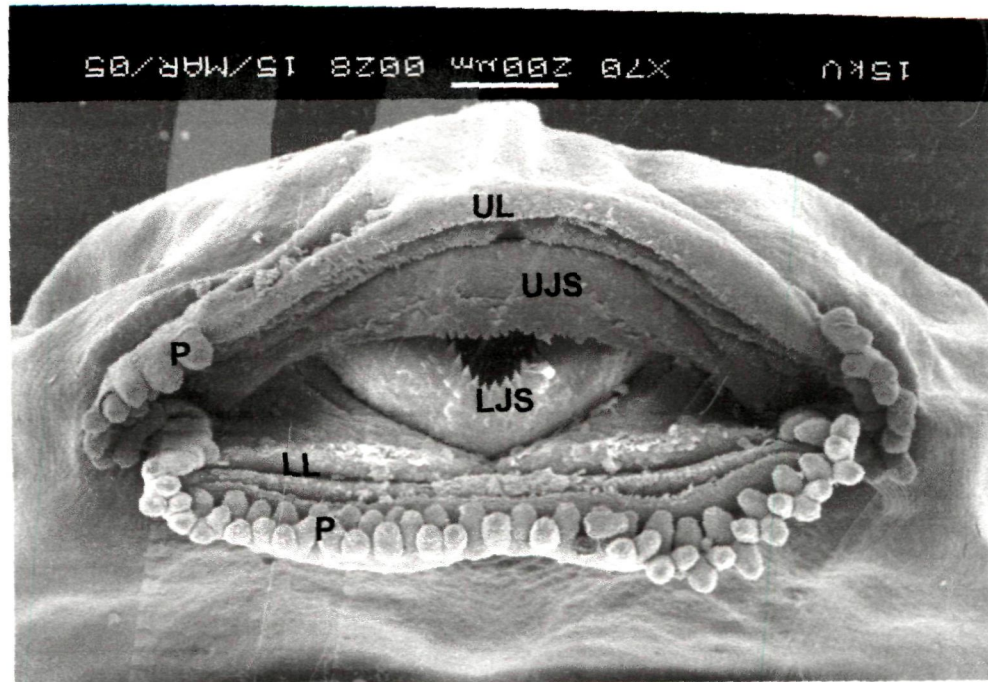


Fig. 4.2 – Scanning electron micrograph of the oral structure of *Polypedates leucomystax* (stage 25) showing upper labium, (UL), upper jaw sheath (UJS), lower jaw sheath, (LJS), papillae (PP), lower labium (LL)

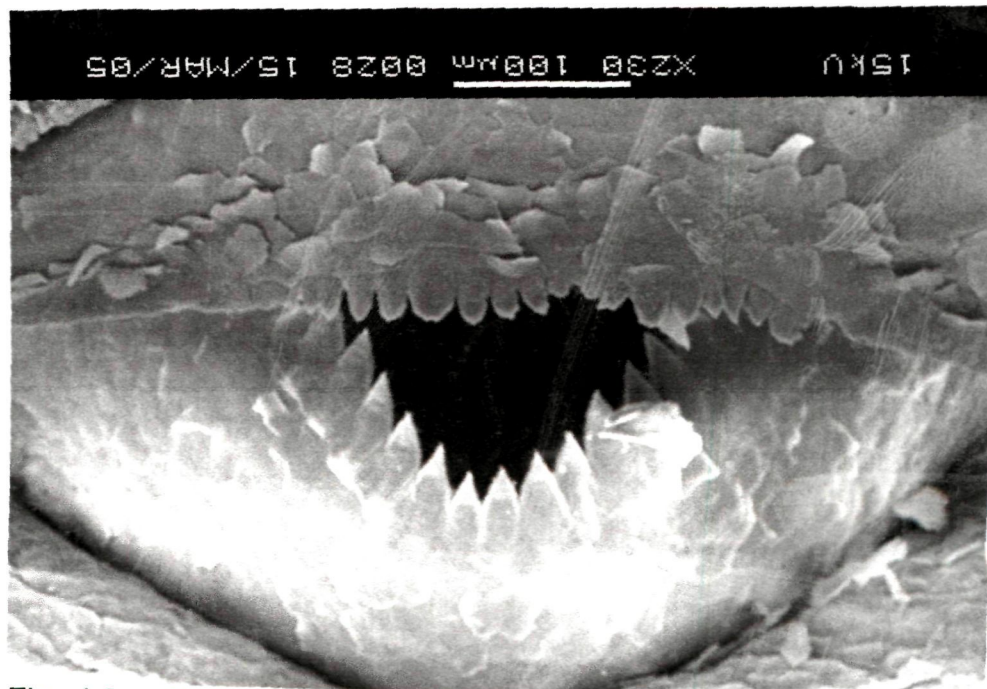


Fig. 4.3 – Scanning electron micrograph of the magnified view of the serrated upper and lower jaw sheath of *Polypedates leucomystax* (stage 25)

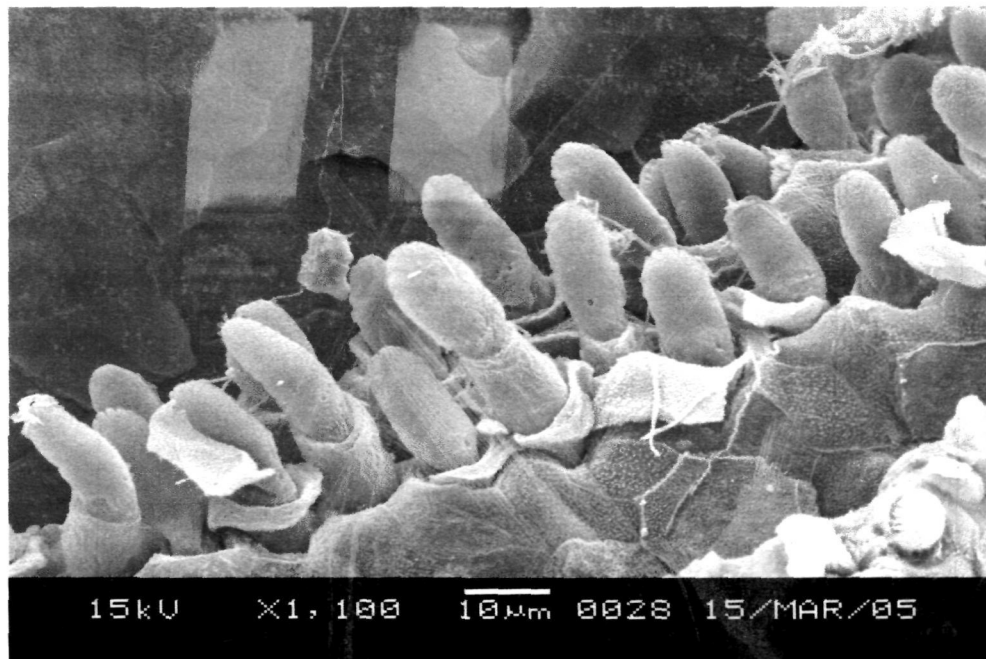


Fig. 4.4 – Scanning electron micrograph of teeth rows in the lower labium of *Polypedates leucomystax* (stage 25)

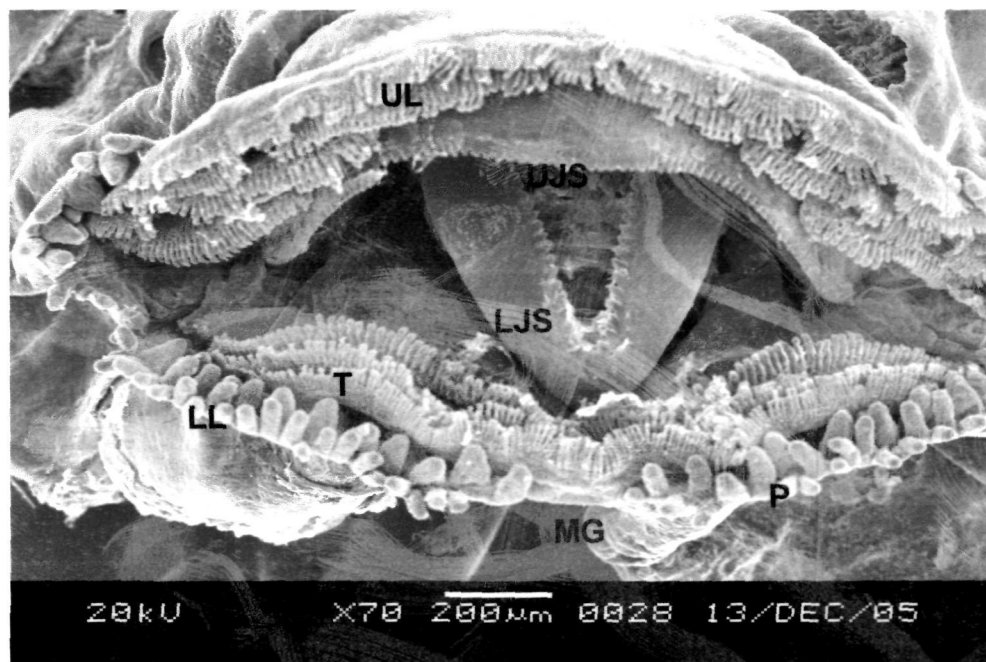


Fig. 4.5 – Scanning electron micrograph of the oral structure of *Polypedates leucomystax* (stage 26) showing upper labium (UL), Upper jaw sheath (UJS), lower jaw sheath (LJS), Lower labium (LL), teeth (T), mental gap (MG), papillae (P)

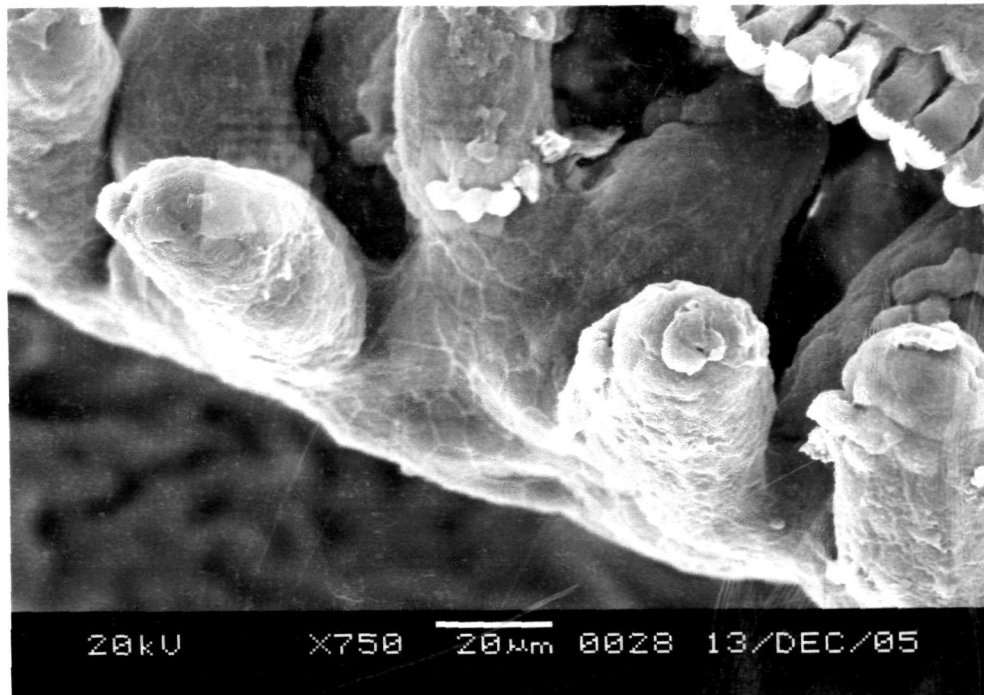


Fig. 4.6 – Magnified view of papillae of *Polypedates leucomystax* (stage 26)

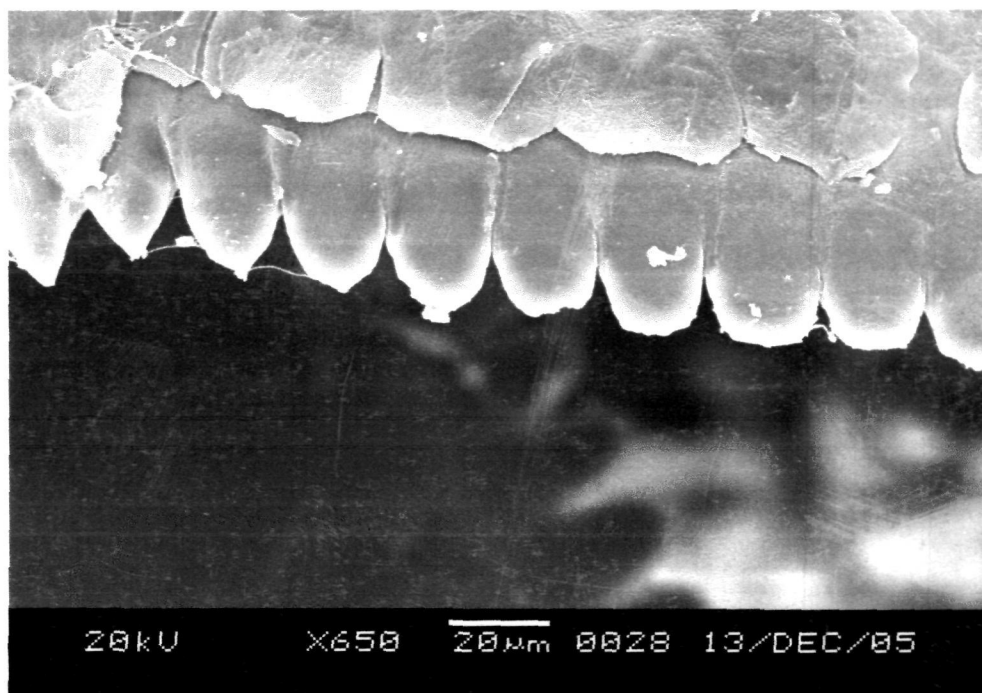


Fig. 4.7 – Magnified view of the serrated upper jaw sheath of *Polypedates leucomystax* (stage 26)

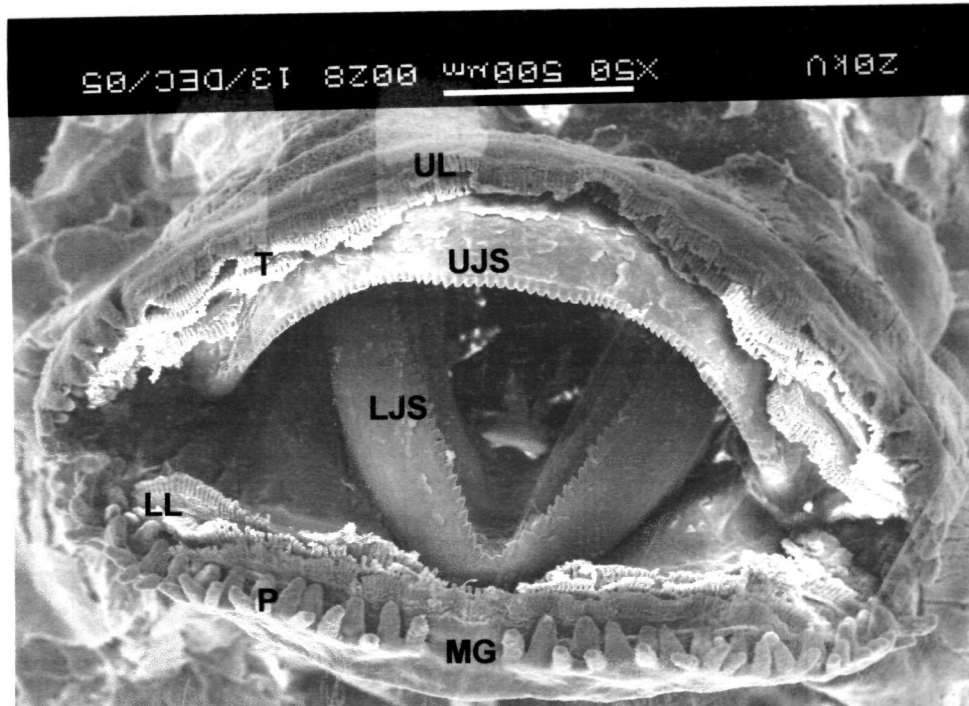


Fig. 4.8 – Scanning electron micrograph of the oral structure of *Polypedates leucomystax* (stage 34) showing upper labium (UL), upper jaw sheath (UJS), teeth (T), lower jaw sheath (LJS), lower labium (LL), papillae (P), mental gap (MG)

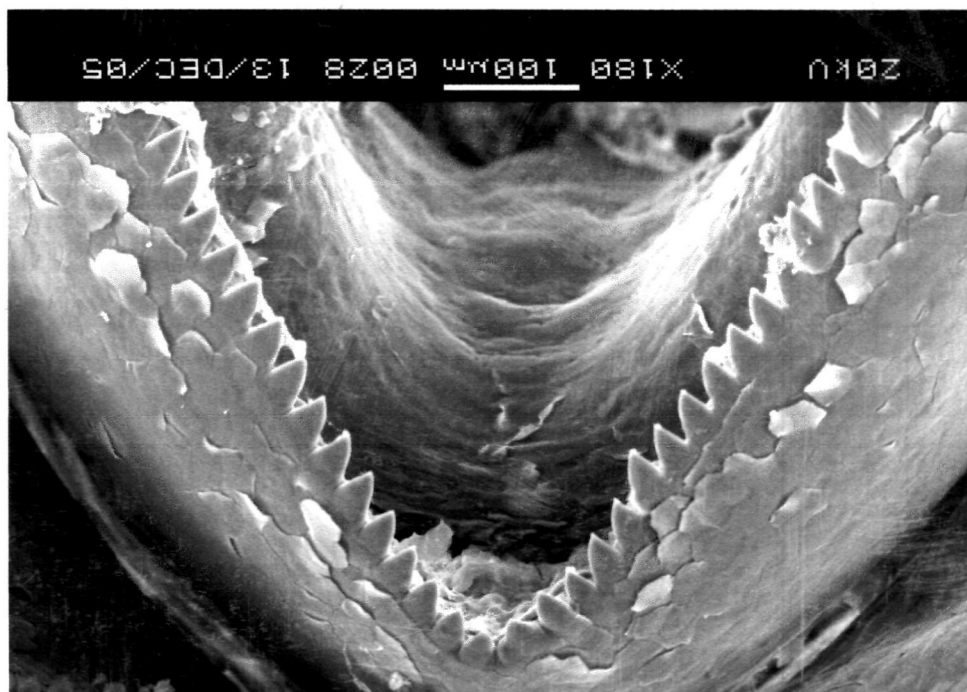


Fig. 4.9 – Scanning electron micrograph of the magnified view of the lower jaw sheath of *Polypedates leucomystax* (stage 34)

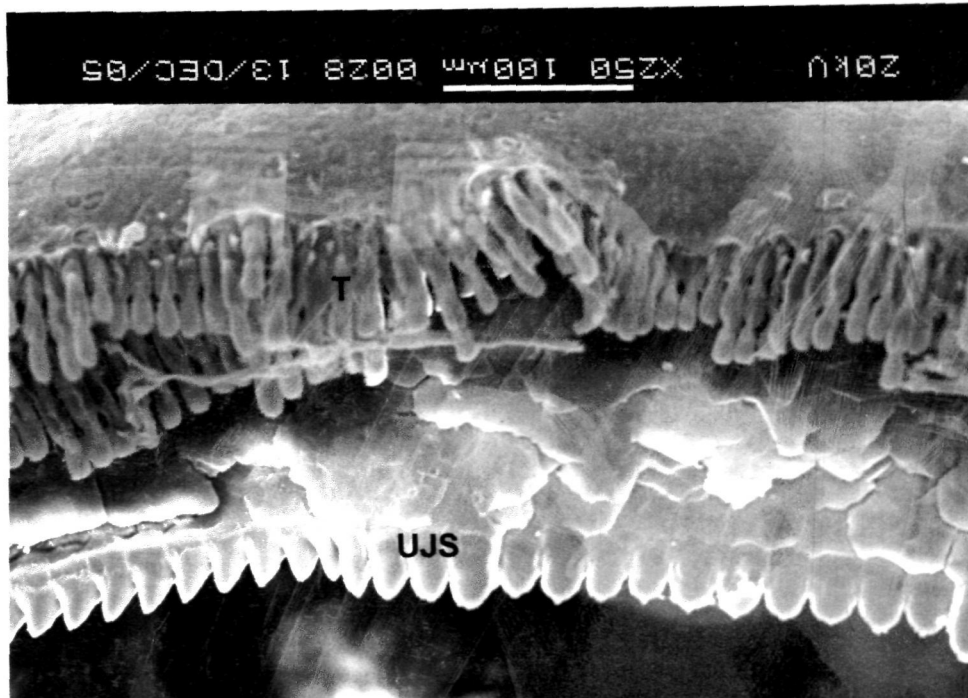


Fig. 4.10 – Scanning electron micrograph of the magnified view of upper labium of *Polypedates leucomystax* (stage 34) showing teeth (T), upper jaw sheath (UJS)



Fig. 4.11 – Scanning electron micrograph of the upper labium showing teeth row of *Polypedates leucomystax*(stage 34)



Fig. 4.12 – Scanning electron micrograph of the lower labium showing teeth (T) rows of *Polypedates leucomystax* (stage 34)

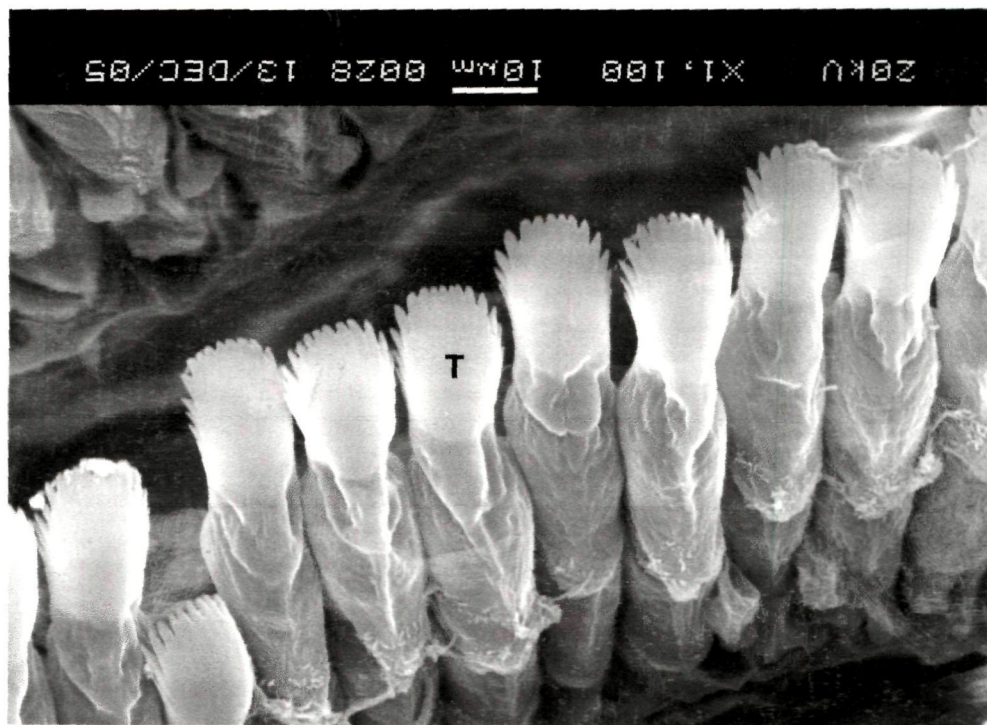


Fig. 4.13 – Scanning electron micrograph of the lower labium showing teeth (T) row *Polypedates leucomystax* (stage 34)

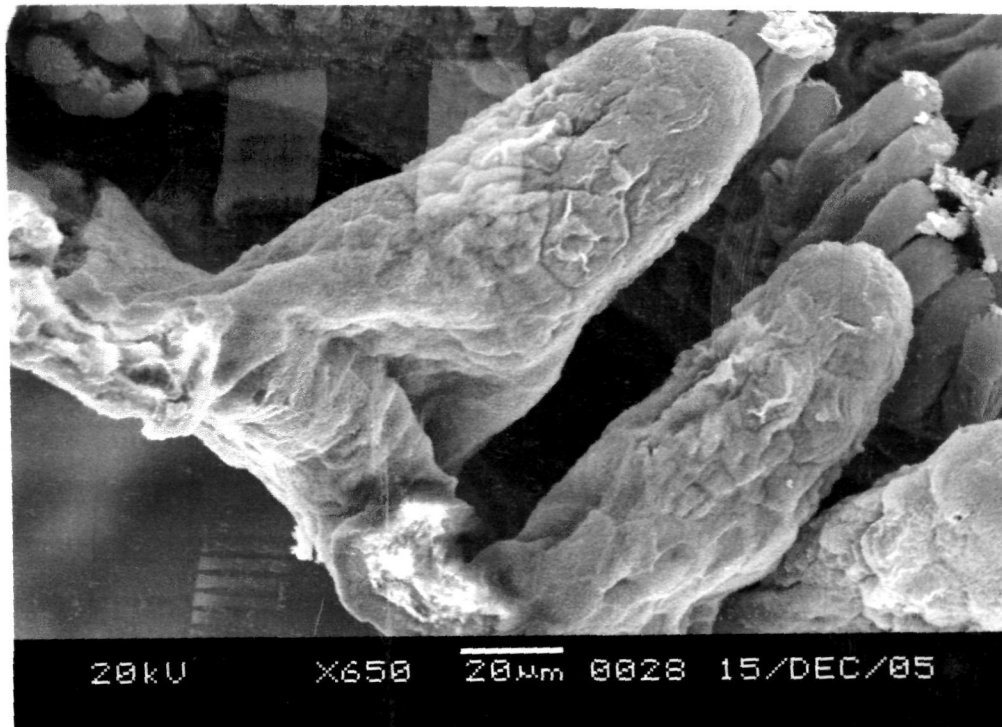
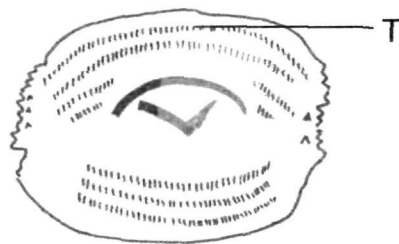


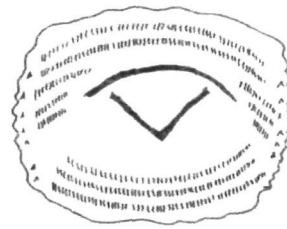
Fig. 4.14 – Scanning electron micrograph magnified view papillae of the lower labium of *Polypedates leucomystax* (stage 38)



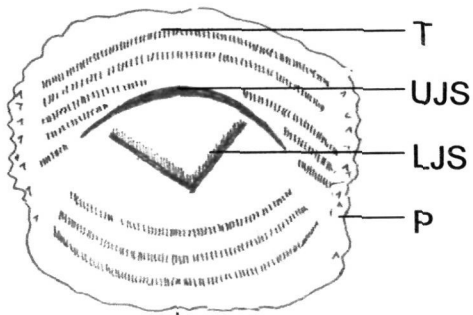
Fig. 4.15 – Scanning electron micrograph of the oral structure of *Polypedates leucomystax* (stage 46) showing tongue (To), papillae (P)



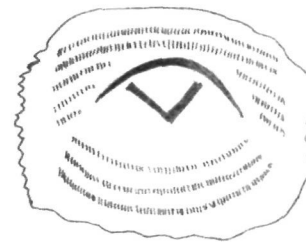
a. Stage 25



b. Stages 26 to 30



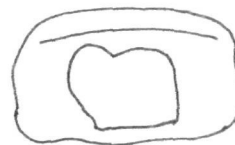
c. Stages 31 to 39



d. Stage 40



e. Stage 41



f. Stages 42 to 45



g. Stage 46

Fig. 4.16a - g – Diagrammatic representation of the oral structure of *Rhacophorus bipunctatus*
LJS=Lower jaw sheath; Mg=Mental gap; P=Papillae; T=Teeth, To=tongue; UJS=Upper jaw sheath

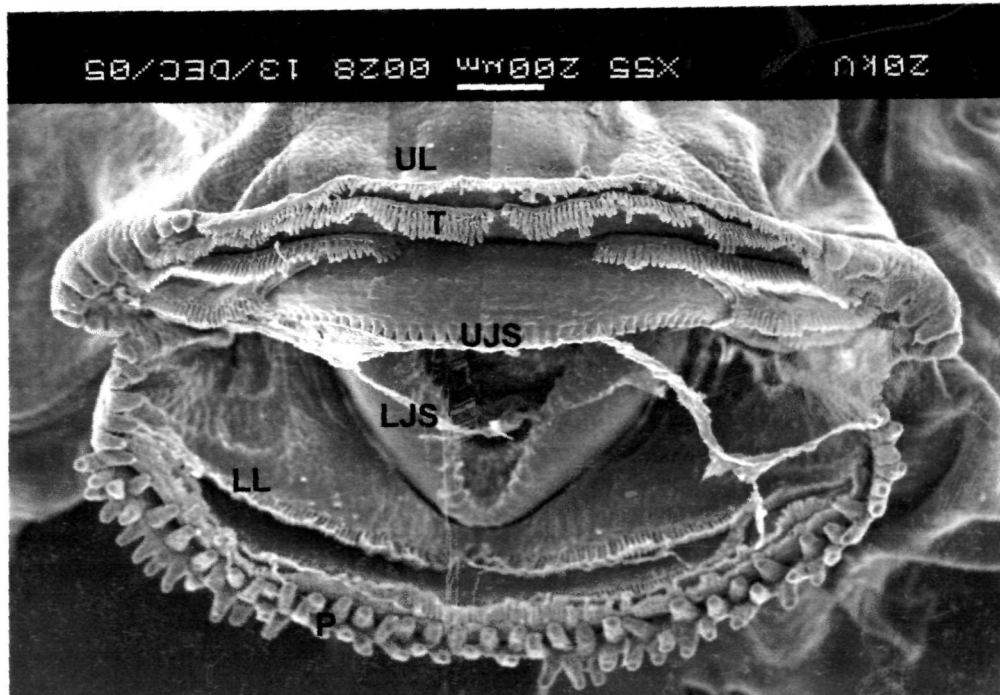


Fig. 4.17 – Scanning electron micrograph of the oral structure of *Rhacophorus bipunctatus* (stage 28) showing upper labium (UL), Teeth (T), Upper jaw sheath (UJS), lower jaw sheath (LJS), lower labium (LL), papillae (P)



Fig. 4.18 – Scanning electron micrograph of the serrated upper jaw sheath of *Rhacophorus bipunctatus* (stage 28)

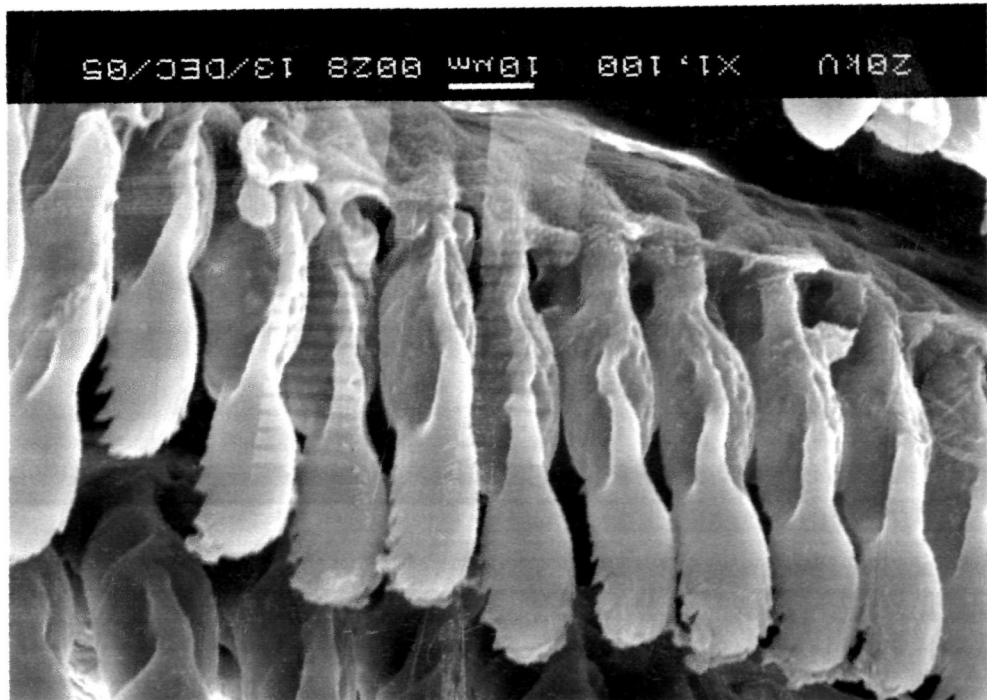


Fig. 4.19 – Scanning electron micrograph of the teeth row in the upper labium showing of *Rhacophorus bipunctatus* (stage 28).

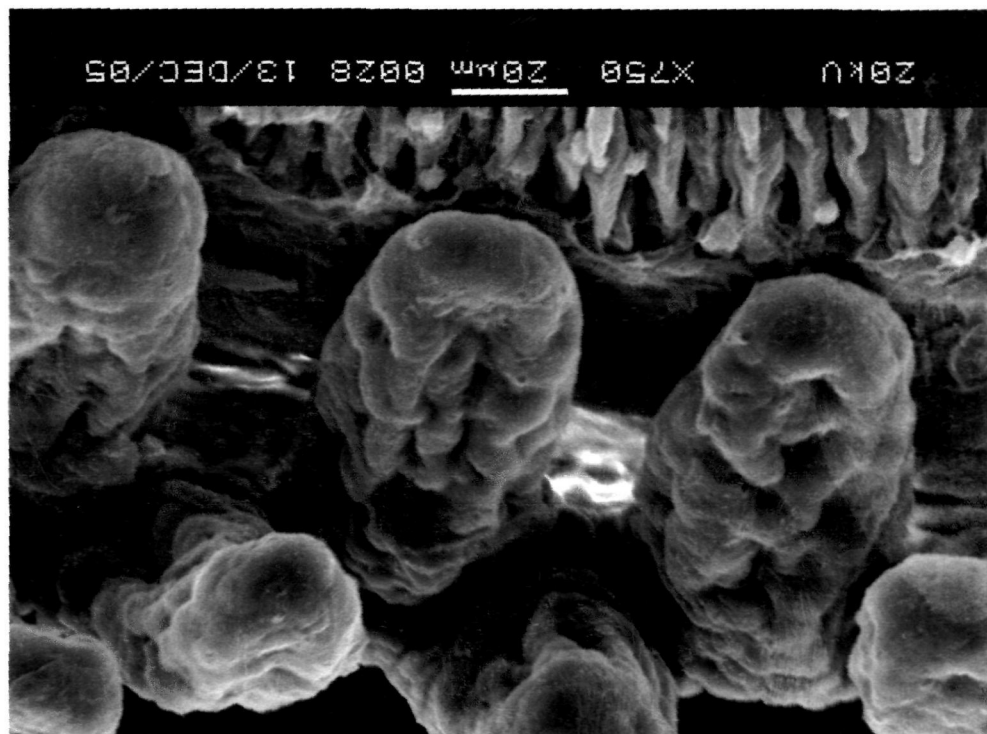


Fig. 4.20 – Scanning electron micrograph of the magnified view of papillae at the lower labium of *Rhacophorus bipunctatus* (stage 28).

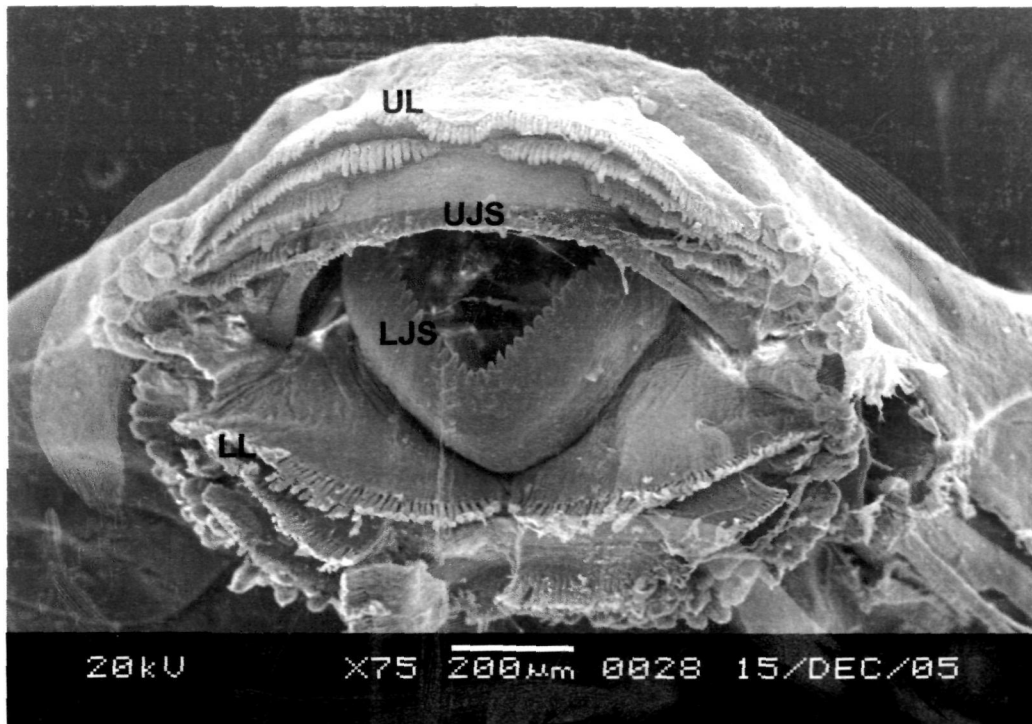


Fig. 4.21 – Scanning electron micrograph of the oral structure of *Rhacophorus bipunctatus* (stage 34) showing upper labium (UL), upper jaw sheath (UJS), lower jaw sheath (LJS), lower labium (LL).

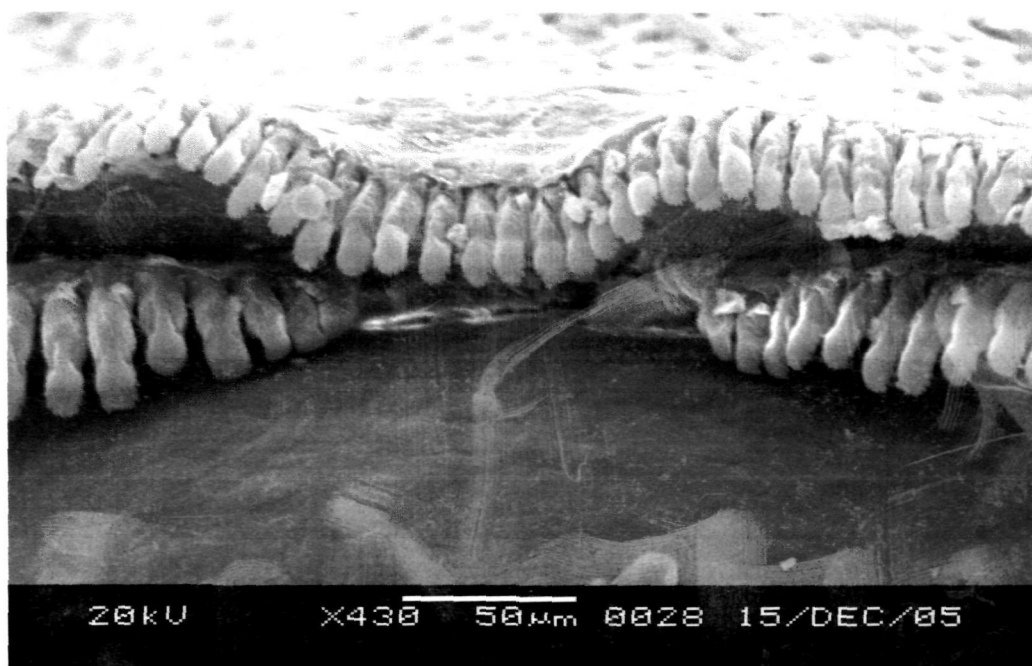


Fig. 4.22 – Scanning electron micrograph of the upper labium showing teeth rows of *Rhacophorus bipunctatus* (stage 34).

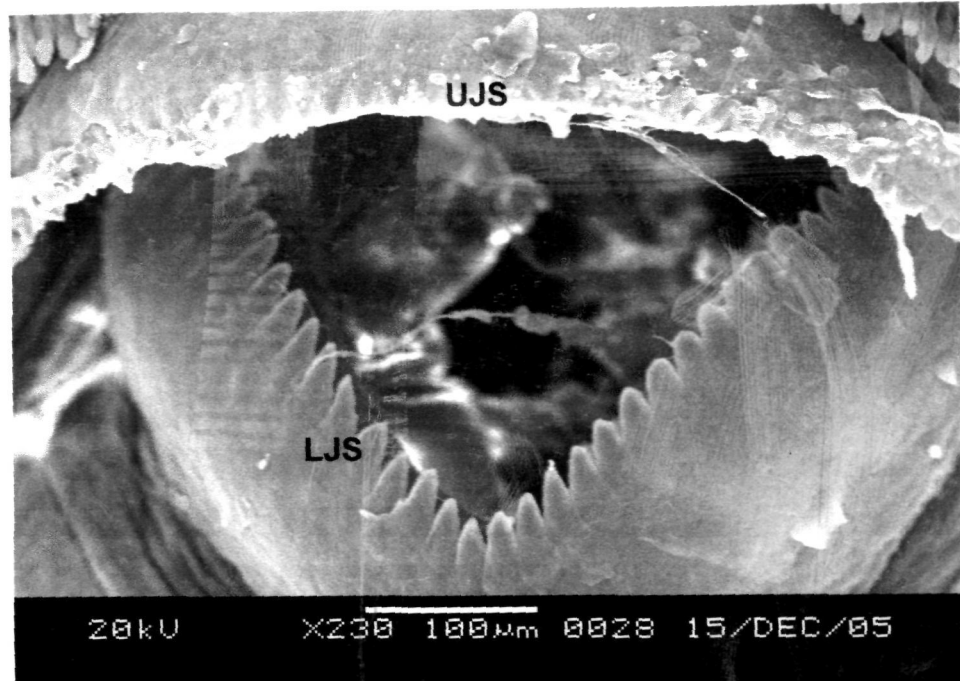


Fig. 4.23 – Scanning electron micrograph showing magnified view of the serrated upper jaw sheath (UJS) and lower jaw sheath of *Rhacophorus bipunctatus* (stage 34).

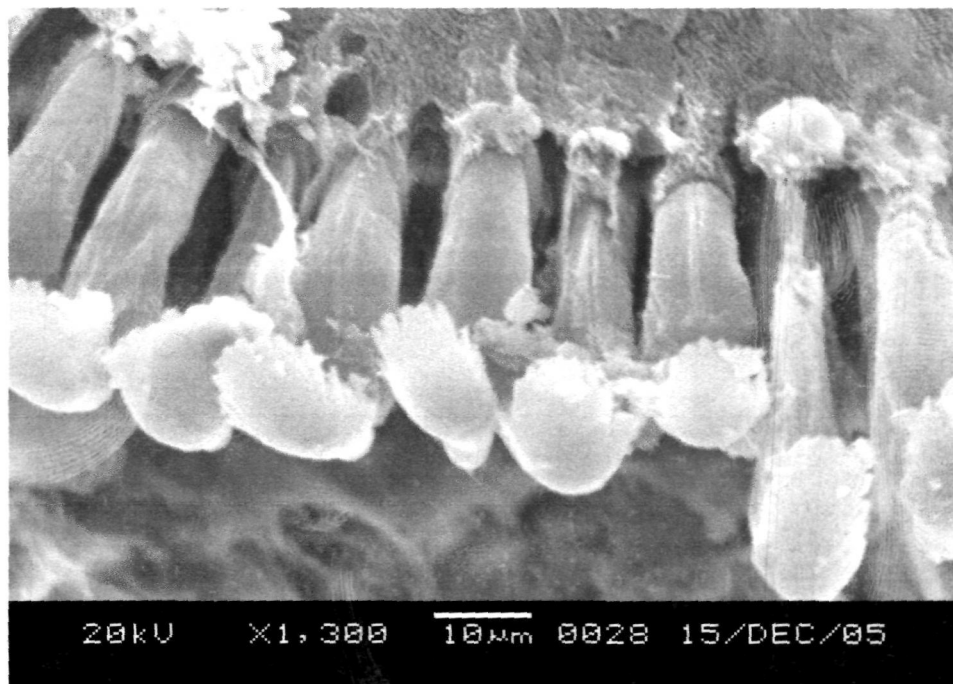


Fig. 4.24 – Scanning electron micrograph showing teeth row at the lower labium of *Rhacophorus bipunctatus* (stage 34).

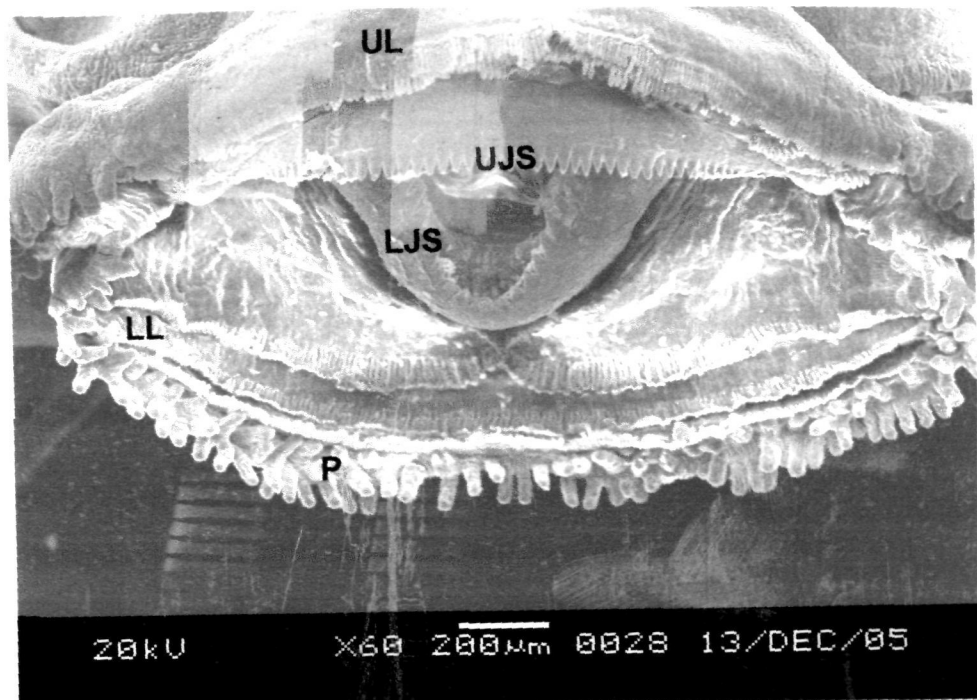


Fig. 4.25 – Scanning electron micrograph of the oral structure of *Rhacophorus bipunctatus* (stage 38) showing upper labium (UL), upper jaw sheath (UJS), lower jaw sheath (LJS), lower labium (LL), papillae (P).



Fig. 4.26 – Scanning electron micrograph showing magnified view of serrated upper jaw sheath of *Rhacophorus bipunctatus* (stage 34).

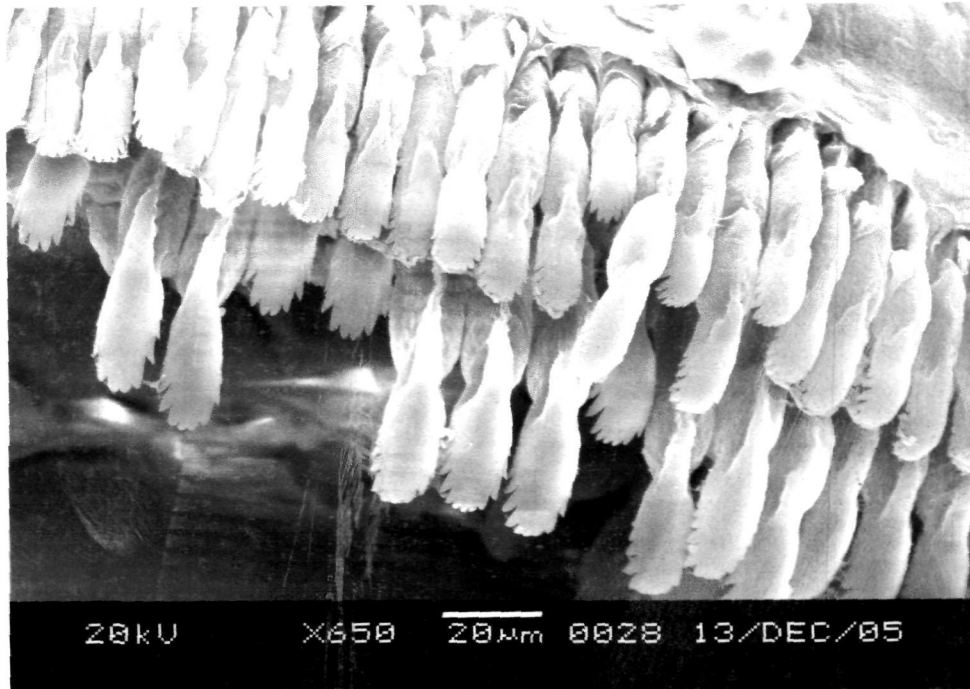


Fig. 4.27 – Scanning electron micrograph of the upper labium with teeth rows of *Rhacophorus bipunctatus* (stage 38).

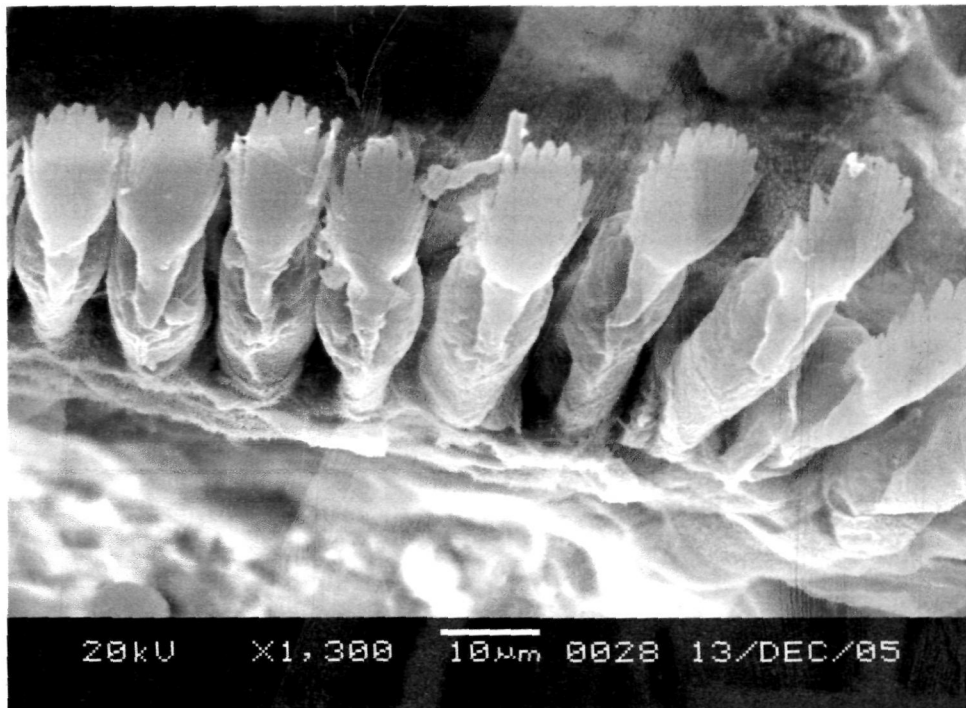


Fig. 4.28 – Scanning electron micrograph of the lower labium with teeth row of *Rhacophorus bipunctatus* (stage 38).

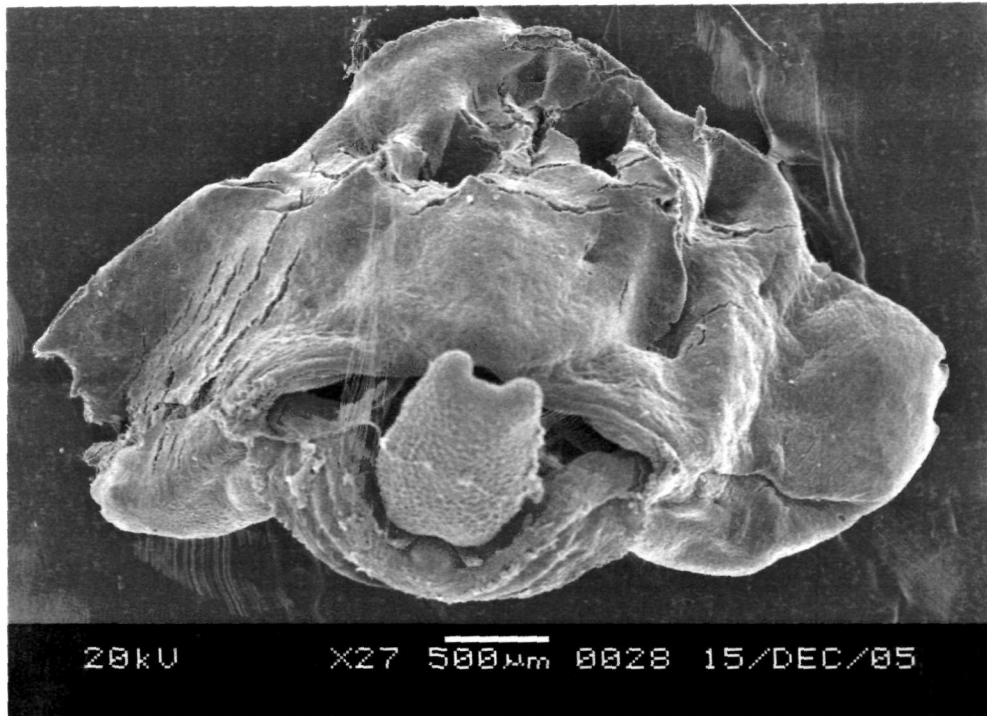


Fig. 4.29 – Scanning electron micrograph of the oral structure of *Rhacophorus bipunctatus* (stage 46) showing tongue.

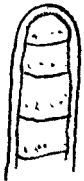
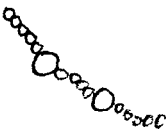
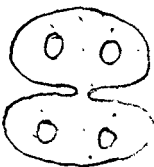
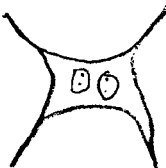
Table 4.1: Food items present in the gut of the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus*

Food items		<i>Polypedates leucomystax</i>			<i>Rhacophorus bipunctatus</i>		
		Stage 25	Stages 26 – 30	Stages 31 – 39	Stage 25	Stages 26 – 30	Stages 31 – 39
Englenophyceae	<i>Euglena</i>	-	-	+	-	+	+
Cyanophyceae	<i>Oscillatoria</i>	-	+	+	-	+	+
	<i>Anabaena</i>	+	+	+	+	+	+
Chlorophyceae	<i>Spirogyra</i>	-	-	+	-	-	+
	<i>Cosmarium</i>	+	+	+	-	+	+
	<i>Staurostrum</i>	+	+	+	-	+	+
	<i>Closterium</i>	+	+	+	+	+	+
	<i>Scenedesmus</i>	-	+	+	-	+	+
	<i>Pediastrum</i>	+	+	+	+	+	+
	<i>Ulothrix</i>	-	+	+	-	+	+
	<i>Zynema</i>	-	+	+	-	+	+
	<i>Selenastrum</i>	-	+	+	-	+	+
Bacillariophyceae	<i>Navicula</i>	-	+	+	-	+	+
Zooplankton	<i>Keratella</i>	-	-	+	-	-	+
	<i>Paramecium</i>	-	-	+	-	-	+
	<i>Brachionus</i>	-	-	+	-	-	+
	<i>Bosmina</i>	-	-	+	-	-	+
	<i>Trichocerca</i>	-	-	+	-	-	+

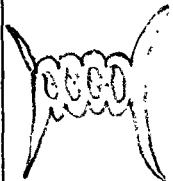
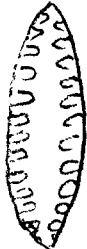
+ = Occurrence

- = Non occurrence

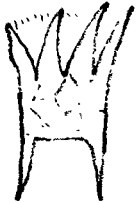
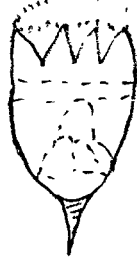
Table 4.2: Food items of the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus*

Phytoplankton	Family	Food item	Characteristics	Diagram
	Euglenophyceae	<i>Euglena</i>	Cells uniflagellate, fusiform	
	Cyanophyceae	<i>Oscillatoria</i>	Fine filament, unbranched	
		<i>Anabaena</i>	Cells form a chain like a string of beads, free floating	
	Chlophyceae	<i>Spirogyra</i>	Unbranched, spiral bands	
		<i>Cosmarium</i>	Cells oval to spherical with a deep constriction	
		<i>Staurastrum</i>	Cells bilaterally symmetrical	
		<i>Closterium</i>	Cells elongated and usually more or less crescent shape, having a narrow constriction across its centre	

contd

Phytoplankton	Family	Food item	Characteristics	Diagram
	Chlophyceae	<i>Scenedesmus</i>	Colonies of 4-8 cells stacked together. Cells arranged in a single or double series of alternating cells	
		<i>Pediastrum</i>	Cells arranged in a more or less circular, flattish plane and one layer thick	
		<i>Ulothrix</i>	Long filamentous, having its cells in a single row closely set together, being little longer than wide and very slender	
		<i>Zynema</i>	Long, cylindrical filaments or threads floating near the surface of the water unattached	
		<i>Selenastrium</i>	Colony of cells, without an outer mucilaginous envelope	
	Bacillariophyceae	<i>Navicula</i>	Central area not distinct, striae distinctly or indistinctly punctate or cross lineate	

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Phytoplankton	Family	Food item	Characteristics	Diagram
Zooplankton		<i>Keratella</i>	Dorsal plate strong with polygonal facets. Present of posterior spines	
		<i>Paramecium</i>	Free living, body slightly flattened and ciliated. Two contractile vacuoles are present	
		<i>Brachionus</i>	Head is expanded in a funnel-shaped manner and surrounded by cilia. Foot is small and short	
		<i>Bosmina</i>	Body oval, shell is normally transparent. Antennae are short and stout. Presence of beak or rostrum	
		<i>Trichocerca</i>	Body twisted in segment of helix	

Discussion:

There is paucity of information concerning the feeding habits of amphibians (Duellman and Trueb, 1994). Tadpoles in aquatic habitats feed at bottom, middle water and surface of the water column, and have characteristic morphology of the oral structure and feeding behaviour. It was found that tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were both omnivorous and microphagous feeders. During early premetamorphic stages the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* fed mostly on detritus and plant materials, while during the prometamorphic stages they consumed both phytoplankton and Zooplankton. The food items of the tadpoles of both the tree frog species were similar during their premetamorphic and prometamorphic stages, suggesting that it may be due to the availability and composition of same food items in the similar habitats of the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus*. It has been reported that the qualitative composition of the diet and the range of food particle size in the tadpoles of two tree frogs, *Hyla nana* and *Scinax nasicus*, is highly variable and are linked to food availability (Candioti *et al.*, 2004). Moreover, the diet of tadpoles in natural habitat can be influenced by food availability and presence of their competitors or predators (Bridges, 2002; Relyea, 2004; Skelly and Golon, 2003). Our observations indicated that the tadpoles of both the species were dietary generalists as they can utilize a wide variety of food resources available in the ephemeral pools. It has been reported that generalized tadpoles can function both as omnivorous microphagous suspension feeders and opportunistic macrophagous predators (Petranka and Kennedy, 1999).

Cannibalism is one of the more difficult evolutionary strategies for ecologists to understand, yet, it remains present through out the animal kingdom and occurs most frequently under conditions of high density and food limitations (Crump, 1983). The tadpoles of the tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* exhibited cannibalism both in the natural environment as well as under laboratory conditions. It has been reported that many anuran larvae under natural and experimental conditions have exhibited cannibalistic behaviour, and these interactions are increasingly recognized as relatively common (Crump, 1992; Alford, 1999). Cannibalism involving anuran tadpoles is well documented (Hoff *et al.*, 1999), but is most common in species from xeric, ephemeral habitats and tropical species that breed arboreally (Crump, 1983). Similarly, tadpoles of *Scaphiopus couchii* also exhibited cannibalism, and that anuran larvae may be important predators in aquatic communities (Dayton and Wapo, 2002). Cannibalistic tadpoles may reach metamorphosis more quickly and at larger sizes reducing the risk of desiccation as pools dry up, and ultimately increasing their fitness (Dayton and Wapo, 2002).

Adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus* fed mostly on insects and insect larvae, flies, bugs, spiders and crustaceans. Indication of insects and insect larvae as preferential prey items suggested that the two tree frog species may be important predators in the agricultural fields to help in insect control. However, other workers reported that the diet of anurans (e.g., brown frog *Rana longicrus* in Taiwan) consisted primarily of invertebrates of the classes Gastropoda, Oligochaeta, Arachnida, Crustacea, Insecta (adults and larvae) and Chilopoda (Kam *et al.*, 1995). The diet of *Rana*

swinhoana consisted primarily of invertebrates of the classes Gastropoda, Crustacea, Arachnida, Chilopoda and Insecta (adults and larvae) (Kam *et al.*, 1998).

The number of teeth rows in the tadpoles of the two tree frog species were found to be different. Further, the number of teeth rows changed with the progress of development and metamorphosis. In both the species labial teeth appeared at the external gill covered stage, and reached a full complement of rows at the foot paddle stage. Labial teeth rows maintained this full complement up to well-developed hindlimb stage. It has been suggested that the labial teeth are some of the most distinguishing features for identification of amphibian larvae and that there is ontogenic variation in labial teeth structure in *Rana tigerina* (Dutta and Mohanty-Hejmadi, 1984). Variations in the development of labial teeth row structure has also been reported in *Rana curtipes* (James *et al.*, 2000). Variation in number of teeth rows, morphology of teeth, papillae and beak differ specifically in *Bufo stomaticus*, *Rana cyanophlytis*, *Microhyla ornata*, *Rana syhadrensis* and *Rana tigerina* (Khan and Mufti, 1994). In *Polypedates leucomystax* the dental formula was found to be 1:3+3/ 3, while that of *Rhacophorus bipunctatus* was 2: 3 + 3/ 3. These observations seem to suggest that the dental formula may be species specific. This study would be usable in the description of the systematic status of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in relation to their genera, since the dental formula is one of the most important characteristics of the tadpoles for their species identification (Sahu and Khare, 1983).

We observed that the serrated upper jaw sheath and lower jaw sheath of the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* help the tadpoles for scrapping food present at the ephemeral pools. It was found that during the pre-

metamorphic stages the teeth rows of the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were distinct and few teeth rows were present. Increase in the number of teeth rows were observed with the progress of development during the pro-metamorphic stages. On the basis of this observation, it may be suggested that the increase in the number of teeth rows might be helpful in the feeding of a wide variety of food items consumed by the tadpoles during the prometamorphic stages. It has been reported that the length of rows and teeth number increased during anuran development (Tubbs *et al.*, 1993). In both the tree frog species, with the onset of metamorphosis, labial teeth began to shed and by the end of metamorphosis all the labial teeth were shed off. It has been reported that specimens in earlier developmental stages have a lower number of keratodonts and keratodonts rows, and assumed that heterochronic developmental shifts were responsible for these observations, progress of development and eventually metamorphosis, being induced before the oral structures are fully developed. This hypothesis most probably explains why growth-inhibited tadpoles in the small ponds had few keratodonts in late stages (Vences *et al.*, 2002).

In both *Polypedates leucomystax* and *Rhacophorus bipunctatus* the upper labium of the tadpoles was provided with papillae only on the lateral sides, while the entire lower labium was lined with papillae and a large mental gap was present at the centre. The closely arranged and numerous papillae presumably help in forming a tight seal between the oral disc and the irregularities of the substrate. It has been reported that in tadpoles inhabiting ponds, the mouth usually is bordered laterally and ventrally by one or two rows of papillae (Duellman and Trueb, 1994). The oral disc is surrounded by rows of papillae, and the nature and arrangement of these papillae act as diagnostic feature. It has

been reported that lentic species have oral discs that are least differentiated (Watson and Martin, 1973), while lotic species have elaborations of the discs that enable larvae to attach to the substrate in fast flowing waters. Such adaptations have been reported to vary from species to species (Martin and Watson, 1971; Liem and Hosmer, 1973; Watson and Martin, 1973; Tyler *et al.*, 1979; Davies, 1989, 1991; Davies and Richards, 1990). In the present study it was found that the oral disc of the tadpoles of both the tree frog species inhabiting ephemeral ponds was ventral, which might be correlated to the feeding habits for scrapping food and capturing of prey. There are reports suggesting that the position of oral disc on the body of tadpoles reflects its feeding ecology, food preferences and methodology employed by it for food acquisition (Altig and Johnston, 1986; 1989; Khan and Malik, 1987; Khan, 1991).

On the basis of present observations, it can be concluded that the food items of the tadpoles as well as that of the adult frogs of both the species are similar. Further, the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were omnivorous and microphagous feeders. However, the dental formula of the tadpoles of the two species was different and species specific. The number of teeth rows changed during the development and metamorphosis of the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus*.

CHAPTER V

Ultrastructure of the skin of *Polypedates*
leucomystax and *Rhacophorus bipunctatus*

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Ultrastructure of the skin of *Polypedates leucomystax* and

Rhacophorus bipunctatus

Introduction:

Amphibians live in very varied habitats. This fact probably explains the great morphofunctional diversity in their skins. Amphibian skin is a morphologically, biochemically and physiologically complex organ, which performs a wide range of functions necessary for the amphibian's survival. There are several reports on details of skin structure including its ultrastructure and function (Catton, 1976; Lindemann and Voute, 1976; Russel, 1976; Spray, 1976; Fox, 1984, 1986; Delfino, 1991 and Duellman and Trueb, 1994). The skin plays a key role in day-to-day survival of amphibians and in their ability to exploit many widely differing ecological niches. The anuran integument and its derivatives comprise an exceedingly varied and adaptable organ system, and perform several functions such as mechanical protection (Farquhar and Palade, 1965; Fox, 1986), chemical defence (Bueno *et al.*, 1981; Delfino *et al.*, 1995), sensory perception (Mearow and Diamond, 1988; Koyama *et al.*, 2001), ionic transport (Stiffler *et al.*, 1997; Sullivan *et al.*, 2000), water absorption (Hoffman and Katz, 1999; Sullivan *et al.*, 2000), and respiration (Duellman and Trueb, 1994). Further more, amphibian skin is permeable to water and as such is important in respiration, osmoregulation, and to a limited degree, thermoregulation (Duellman and Trueb, 1994). Compared with other

vertebrates, amphibian skin is quite permeable to water, ions and gases (Bentley and Baldwin, 1980), though considerable variation occurs among different species and at different times and conditions in the same animal.

Most amphibians are poorly adapted to a strictly terrestrial life. Amphibian skin interacts with biotic and abiotic factors in their environment, but it does not in general serve as a barrier to the inward or outward flow of water to and from the amphibian body (Hadley, 1980). The skin of amphibian is generally described as being naked due to the absence of the covering of scales, feathers or hair characteristics of most other classes of vertebrates.

Detailed macroscopic and histological studies of the amphibian integument by Rabl (1931) and Elias and Shapiro (1957) have shown that the epidermis varies in thickness and may have projections or indentations, and that there are elevations and thickenings of various kinds in the dermis, especially in anurans. Skin thickness and the number of epidermal skin layers vary in species specific manner. One species will also vary from another in the process of keratinization. These characteristics are related to environment - aquatic or terrestrial (Bani, 1982). Although the skin of many amphibians appears to be smooth, usually it has a texture owing to various dermal and or epidermal modifications (Duellman and Trueb, 1994). The fine structure of adult anuran skin have been described by Voute (1963), Parakkal and Matoltsy (1964), Farquhar and Palade, (1965), Lavker (1974), Fox (1974) and Whitear (1974; 1975). Increase in gap junctions in frog skin epithelium has been described, and it was demonstrated that the stratum granulosum and the more basal strata were in the route of sodium active transport

(Blankemeyer and Shahin, 1993). Keratinization in the epidermis of amphibians and the lungfish has been studied, and it was suggested that during metamorphosis a new type of keratin is produced as the epidermis stratifies (Alibardi, 2001).

Several workers have studied the skin of tree frogs, including the structure of the pigment layer (Noble, 1931; Frost-Mason *et al.*, 1994; Goniakowska-Witalenska and Kubiczek (1998), the vascularization of the respiratory surfaces (Czopek, 1965; Czopek and Szarski, 1989), and the development of the myoepithelial layer and cutaneous glands in the larval tree frog (Delfino *et al.*, 1987; Delfino, 1991; Delfino *et al.*, 1994; Lacombe *et al.*, 2000). Some workers studied the biological roles of amphibian skin secretions and described the bioactive secretions of the amphibian integument (Barthalamus, 1994; Erspamer, 1994).

Amphibians have diverse pigmentary patterns that include spots, stripes and mottles of various colours that can provide them either a cryptic or aposomatic colouration in their environment. It is well known that skin colouration is brought about by the interaction of the three kinds of chromatophores - xanthophore (erythrophore), iridophores and melanophores (Bagnara and Hadley, 1973; Oliphant, 1973; Bagnara *et al.*, 1978; 1979a, b; Frost and Robinson, 1984; Taylor, 1992). Bagnara and co-workers (1968) reported that the dermis of most amphibians contains a physiologically responsive colour producing chromatophore group termed as the dermal chromatophore unit. Further, they also studied the comparative biology of a new melanophore pigment from leaf frogs and reported that this unique pigment rhodomelanochrome is clearly different from any other melanophore pigment and is readily soluble in alkali (Bagnara *et al.*,

1976). Later, Misuraca and co-workers (1977) identified the leaf-frog melanophore pigment, rhodomelanochrome as pterorhodin on the basis of its spectral and chemical properties. Based on the studies involving dermal chromatophore unit, Nielsen (1978) showed that colour change in *Hyla arborea* is caused not only by the orientation and distribution of pigment organelles in chromatophore cytoplasm but also by the transformation and translocation of chromatophores. It has been reported that melanophores, xanthophores, and iridophores are fundamentally distinct chromatophores in their appearance, composition, and function, and all migrate from their neural crest site of origin to populate the integument (Bagnara, 1983). The development of the pale ventral pigmentation of the African clawed toad, *Xenopus laevis* has been studied and reported that typically, ventral skin of *Xenopus laevis* is white due to abundant iridophores and an apparent abundance of melanophores (Fukuzawa and Ide, 1988). It has been reported that the dermal pigmentation of the ventral skin of the two leopard frogs, *Rana pipiens* and *Rana chiricahuensis* is initially similar but becomes significantly different after metamorphosis. Only iridophores were observed in the ventrum of *Rana pipiens*, while the ventrum of the newly metamorphosed and adult of *Rana chiricahuensis* have melanophores mixed among iridophores (Fernandez and Bagnara, 1993). Morphologically the differentiation and migration of xanthophores and erythrophores and their pigmentary organelle formation during the tadpole and juvenile frog stages to determine whether or not the erythrophores originate from xanthophores in the dorsal skin of the brown frog *Rana ornativentris* was examined (Ichikawa *et al.*, 1998).

Measurement of the rates of evaporative water loss and analysis of the chemical composition of skin secretions from two Australian Hylid frogs, the arboreal *Litoria caerulea* and the terrestrial burrowing *Cyclorana australis* has been studied and reported that both species showed reduced rates of water loss compared with typical amphibians that lose water as if from a free surface. Further the chemical composition of skin secretions was similar and consisted of 5 – 10 % neutral lipids and 78 – 85 % proteinaceous materials (Christian and Parry, 1997). The natural history of amphibian skin secretions, their normal functioning and potential medical applications has been studied and reported that amphibian skin fulfils a variety of functions including respiration, water regulation and defence and also suggested that a series of mutually compatible functions take place on and in the skin (Clarke, 1997). Investigation on the visualization of the initiation and sequential expansion of the metamorphic conversion of anuran larval skin into the precursor of adult type in *Rana catesbeiana* has been studied and reported that drastic changes arise in both the epidermis and the subcutaneous connective tissue at an early developmental stage producing the precursor of adult type skin (Tamakoshi *et al.*, 1998). The effect of electrophysiological of ruthenium red and pH on the skin of the edible frog, *Rana esculenta* has been determined and showed that physiological and chemical stimuli caused dynamic changes in the transport processes in the skin (Kosik-Bogacka and Tyrakowski, 2001).

The ultrastructural localization of histidine labeled inter keratin matrix during keratinization of amphibian epidermis has been studied and it was suggested that small amounts of histidine-rich molecules which were not sufficient to form microscopically-

visible keratohyalin granules were present in ancestral amphibian epidermis (Alibardi, 2003). The morphology of *Bufo ictericus* integument was studied, using stereoscopic microscopy, low vacuum scanning electron microscopy, transmission electron microscopy and light microscopy and revealed that the dorsal integument surface is rougher than ventral and are morphologically distinct (De Brito-Gitirana and Azevedo, 2005). The structural aspects of the Eberth-Katschenko layer of *Bufo ictericus* integument: histochemical characterization and biochemical analysis of the cutaneous calcium was studied and reported that the Eberth-Katschenko layer is a discontinuous layer, being formed by hyaluronic acid and a sulfated glycoaminoglycans different from chondroitin sulfate, heparin and heparin sulfate, and keratin sulfate. In anurans integument, glycoaminoglycans play an important role in dermal hydric balance, protecting the animal against desiccation. Thus, all components of the Eberth-Katschenko layer are essential for integument integrity and functionality (Azevedo *et al.*, 2005). The inguinal macroglands of the frog *Physalaemus nattereri* (Leptodactylidae): structure, toxic secretion and relationship with deimatic behaviour was studied and it was suggested that a high degree of integument specialization, in which different glandular secretions were developed in specific regions of the body, possibly associated with deimatic behaviour (Lenzi-Mattos *et al.*, 2005).

Although the integument is the structural and functional interface between the organism and its environment, the morphological and functional complexity of amphibian skin is not fully understand (Duellman and Trueb, 1994). There is practically no information on the integument of the tree frogs *Polypedates leucomystax*

(Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 found in Cherrapunjee (East Khasi Hills, Meghalaya, India) – the wettest place on the earth. These two frog species are arboreal and come to the ephemeral pools only for breeding, and hibernate during the winter. As far as the skin colour is concerned, *Polypedates leucomystax* shows brownish to light brown-yellowish colouration. During the breeding phase, male skin pigmentation is darker as compared to the female, which shows lighter pigmentation.

In contrast, *Rhacophorus bipunctatus* shows green pigmentation of the skin on the dorsal surface, while the ventral surface is light yellowish in colour. The pigmentation however, does not change even during breeding season. Since the existing literature exhibits a number of reports on ultrastructural specialization of amphibian skin, it was felt that an answer to the remarkable difference in skin colouration of *Polypedates leucomystax* and *Rhacophorus bipunctatus* may be found in ultrastructural features of the skin. Keeping this in view, a comparative transmission electron microscopic study on the integument of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was undertaken to have a clear understanding on the role of ultrastructural specialization, if any, on the observed differences in the skin colour of the two species.

Materials and Methods:

To study the ultrastructure of the skin of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, adult males and females were collected from the study sites I, II, III and IV from Cherrapunjee, East Khasi Hills, Meghalaya (Please see Chapter II: Figs 2.1, 2.2, 2.3 and 2.4) during the breeding season. The collected adult frogs of

Polypedates leucomystax and *Rhacophorus bipunctatus* were anesthetized and the sections of the skin from the dorsal and ventral parts of males and females of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were processed for transmission electron microscopic studies.

The skin sections of the dorsal and ventral parts taken from both the male and female frogs of the two tree frog species during the breeding period were fixed in 2.5 % Gluteraldehyde (prepared from 0.1M Na-Cacodylate buffer) at 4°C for 2 hours and post fixed with 1 % buffered Osmium tetroxide (prepared in 0.1M Na-Cacodylate buffer) at 4° C for 1 hour. The samples were then dehydrated through acetone grades (30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % and 100 %) and then cleared in Propylene oxide. The samples were then infiltrated in a mixture of clearing agent propylene oxide and embedding medium. After infiltration the tissues were embedded in epoxy resins using beam capsules, and blocks were prepared. Ultrathin sections (60 – 90 nm) were cut with the help of an ultra microtome (Ultratome V, LKB), stained with uranyl acetate and lead citrate and were examined with a Transmission electron microscope (JEM 100C x II Jeol) at an accelerating voltage of 80 KV.

Results:

Transmission Electron Microscopic study of the skin of *Polypedates leucomystax*:

The electron micrograph of the skin of the male and female of *Polypedates leucomystax* revealed that the epidermis consisted of four layers of epithelial cells namely

stratum corneum, stratum granulosum, stratum spinosum, and stratum germinativum. The stratum corneum, which is the outer most layer, was thickened and keratinized and consisted of a single layer of flattened cells. Protuberances on the outer surface of the epidermis were also observed (Figs. 5.1 and 5.2). The keratinized stratum corneum was separated from the underlying stratum granulosum by intercellular spaces. In the stratum granulosum some cells were observed to be devoid of a nucleus. These cells had prepared to move to the stratum corneum, as stratum corneum sloughed or shed periodically and have to be replaced. Tonofilaments and desmosomes were seen in the epidermal layers (Figs. 5.1 and 5.2).

Following the stratum granulosum was the stratum spinosum and stratum germinativum. Cells of different shapes with prominent nucleus were observed in the stratum spinosum and stratum germinativum of *Polypedates leucomystax* (Fig. 5.3). These cells with prominent nucleus were joined together by desmosomes. Cell organelles like ribosomes, tonofilaments and mitochondria were present in the stratum germinativum.

Located beneath the epidermis was the dermis, which was separated from the epidermis by the basement membrane composed of collagenous fibres (Fig. 5.4). Transmission electron microscopy revealed that the dermis of *Polypedates leucomystax* consisted of two layers, the stratum spongiosum and the underlying stratum compactum. In the stratum spongiosum pigment cells like Pterinosomes and melanophores were present (Figs. 5.4, 5.5 and 5.6). Higher magnification of the Pterinosomes showed that it was arranged in concentric layers (Fig. 5.7). Iridophores or reflecting platelets were also

observed in the dermis. The stratum compactum includes a collagenous fibres arranged in a series of alternating layers (Fig. 5.8).

Transmission Electron Microscopic study of the skin of *Rhacophorus bipunctatus*:

Similarly, transmission electron microscopy of the skin of *Rhacophorus bipunctatus* also revealed that it exhibits a typical anuran appearance, where the epidermis consists of a number of layers. The stratum corneum was the outer most layer and was thickened and keratinized, and consisted of a single layer of flattened cells. As in the case of *Polypedates leucomystax*, protuberances were also present in the outer layer of the epidermis in *Rhacophorus bipunctatus*. The stratum corneum was separated from the underlying stratum granulosum by intercellular space (Figs. 5.9 and 5.10). In the stratum granulosum and stratum germinativum, the epidermal cells with nucleus, desmosomes and ribosomes were observed (Figs. 5.11 and 5.12).

As in the case of *Polypedates leucomystax*, in *Rhacophorus bipunctatus* also the epidermis was separated from the dermis by the basement membrane composed of collagenous fibres (Fig. 5.13). The dermis consisted of two layers, the stratum spongiosum and the stratum compactum. The outer stratum spongiosum was made up of connective tissues with interlacing fibres and various types of cells, including the pigment bearing chromatophore. The underlying stratum compactum was composed of compactly arranged collagenous fibres. In the stratum spongiosum, pigment cells like pterinosomes were observed to be present (Fig. 5.13). In higher magnification view of the pigment cells, the pterinosomes are shown in Fig. 5.14.

As observed in *Polypedates leucomystax*, the pterinosomes in *Rhacophorus bipunctatus* were also arranged in concentric layers. Another type of pigment cells called melanophores were also present just beneath the pterinosomes (Figs. 5.15 and 5.16). The melanophores were black in colour and were of different sizes, and endoplasmic reticulum was also observed (Fig. 5.16). Besides the pterinosomes and melanphores, iridophores were also seen to be present in the dermis of *Rhacophorus bipunctatus* (Figs. 5.17 and 5.18).

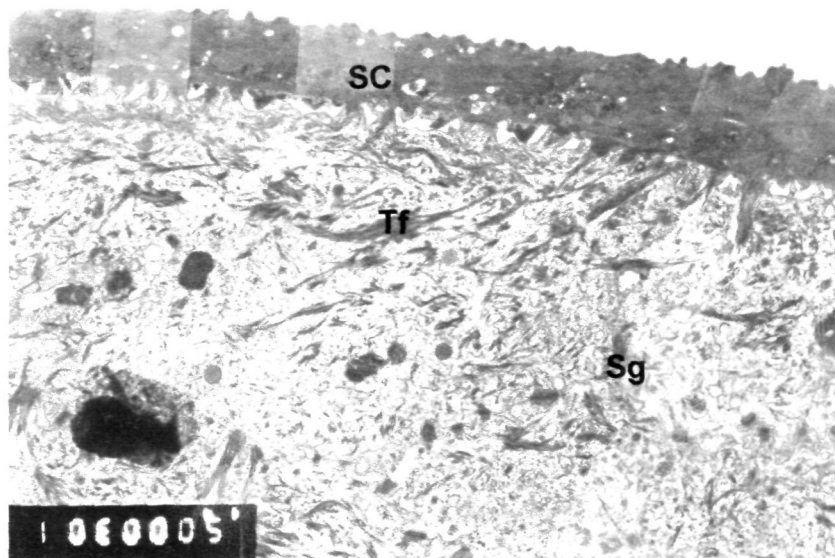


Fig. 5.1 – Transmission electron micrograph (TEM) of the female skin of *Polypedates leucomystax* showing the stratum corneum (Sc), stratum granulosum (Sg), tonifilaments (Tf) (X7250).

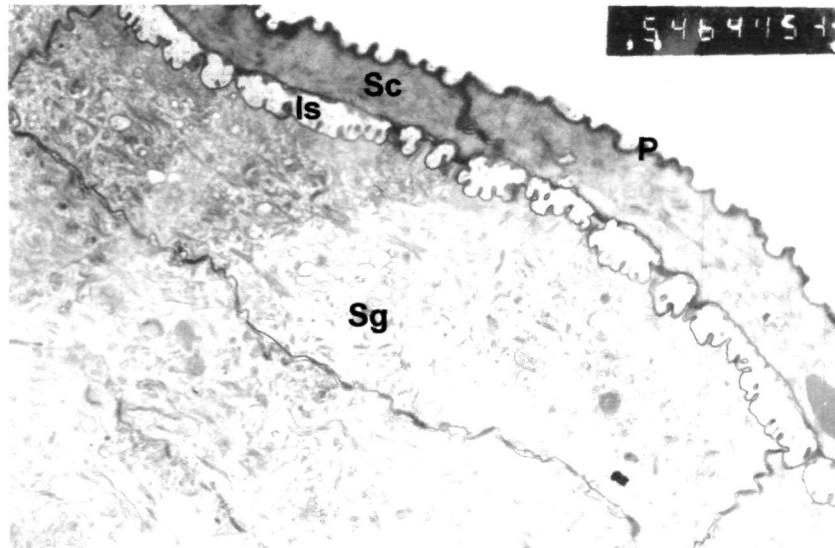


Fig. 5.2 - TEM of the male skin of *Polypedates leucomystax* showing the stratum corneum (Sc), stratum granulosum (Sg), inter cellular space (Is), protuberances (P) (X7250).

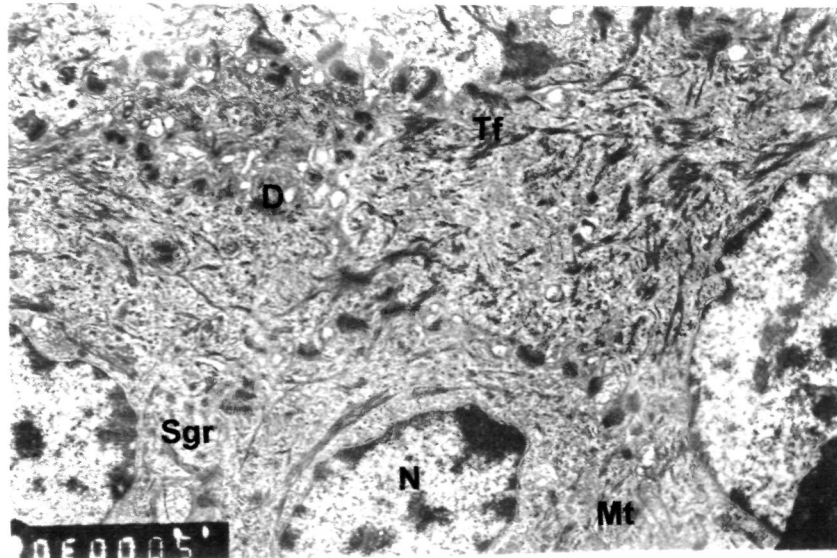


Fig. 5.3 - TEM of the female skin of *Polypedates leucomystax* showing stratum germinativum (Sgr), desmosomes (D), nucleus (N), mitochondria (Mt), tonofilaments (Tf) (X7250).

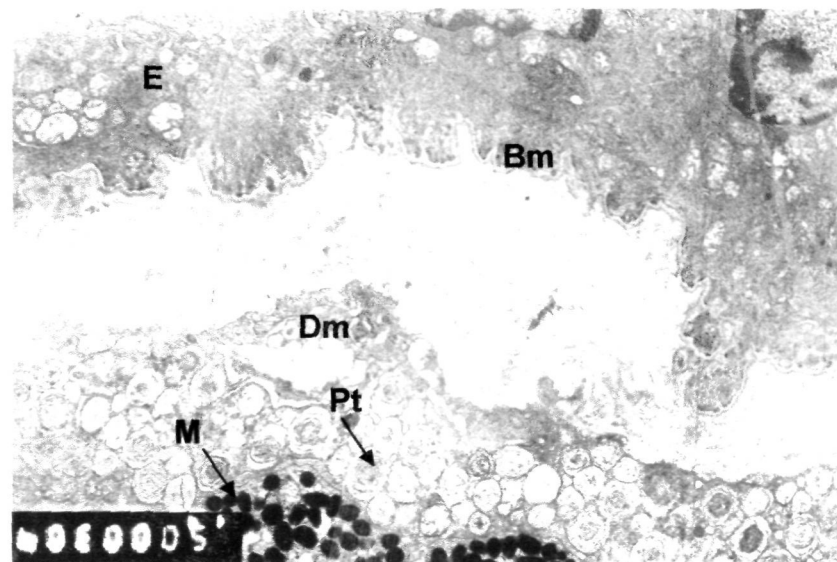


Fig. 5.4 - TEM of the female skin of *Polypedates leucomystax* showing epidermis (E), basement membrane (Bm), dermis (Dm), pterinosomes (Pt), melanophores (M) (X7250).

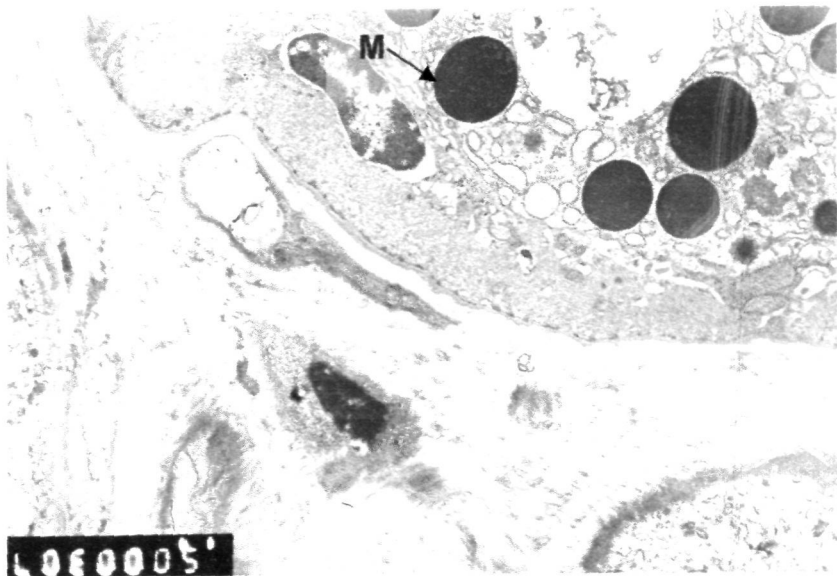


Fig. 5.5 - TEM of the female skin of *Polypedates leucomystax* showing melanophores (M) (X7250).

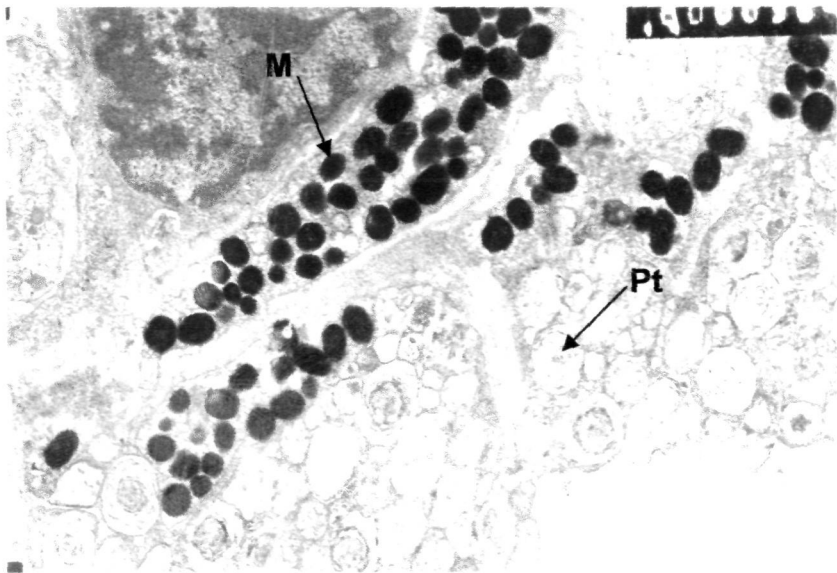


Fig. 5.6 - TEM of the female skin of *Polypedates leucomystax* showing melanophores (M), pterinosomes (Pt) (X11,600).

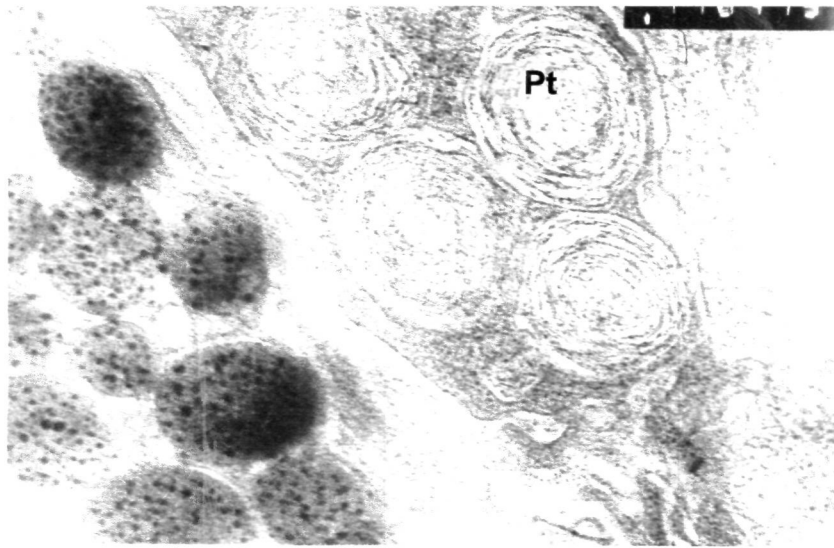


Fig. 5.7 - TEM of the male skin of *Polypedates leucomystax* showing magnified view of pterinosomes (Pt) (X58,000).

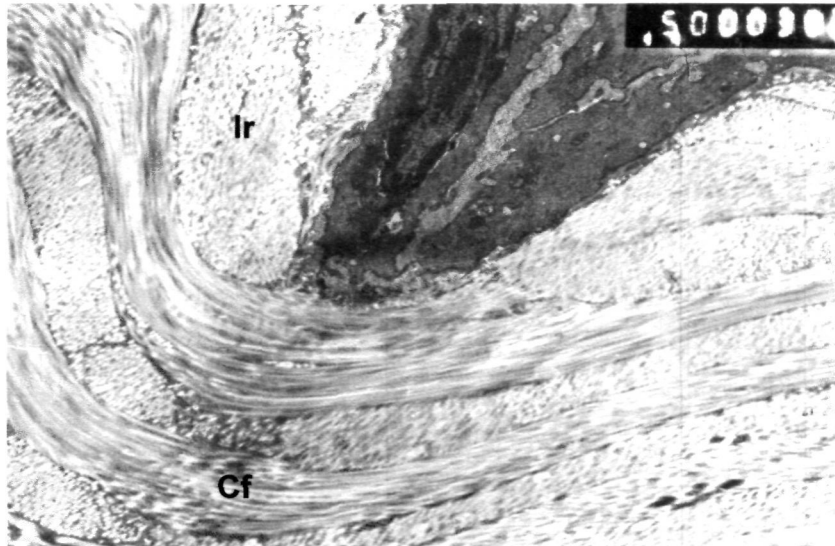


Fig. 5.8 - TEM of the female skin of *Polypedates leucomystax* showing showing alternating layer of collagenous fibres (Cf), iridophores (Ir) (X7250).

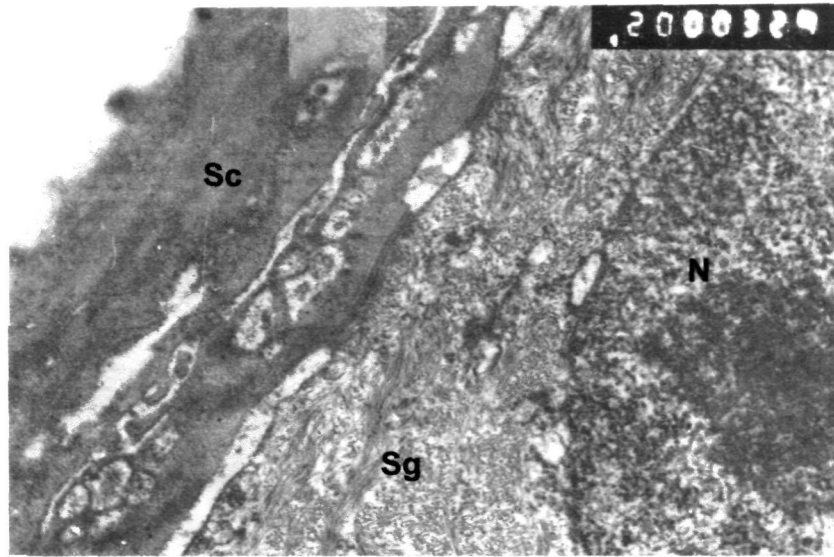


Fig. 5.9 - TEM of the male skin of *Rhacophorus bipunctatus* showing stratum corneum (Sc), stratum granulosum (Sg), nucleus (N) (X29,000).

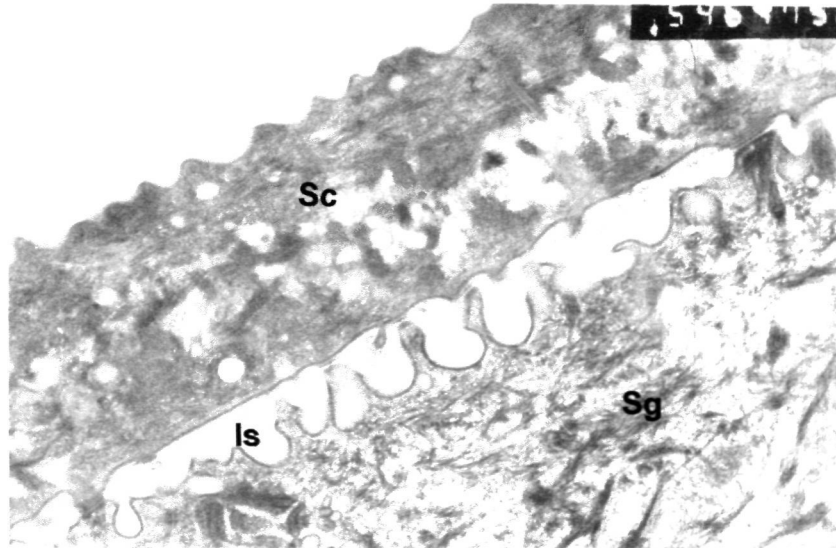


Fig. 5.10 - TEM of the female skin of *Rhacophorus bipunctatus* showing stratum corneum (Sc), stratum granulosum (Sg), inter cellular space (Is) (X20,300).

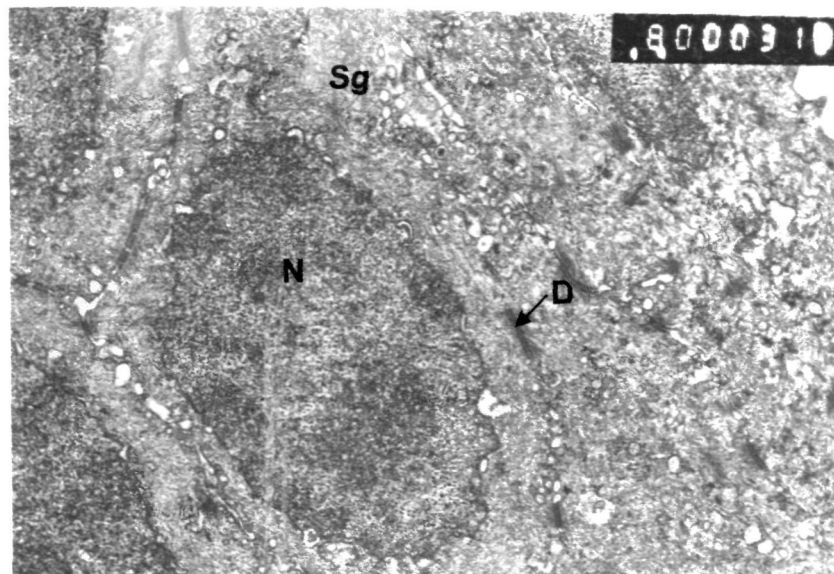


Fig. 5.11 - TEM of the male skin of *Rhacophorus bipunctatus* showing stratum granulosum (Sg), nucleus (N), desmosomes (D) (X11,600).

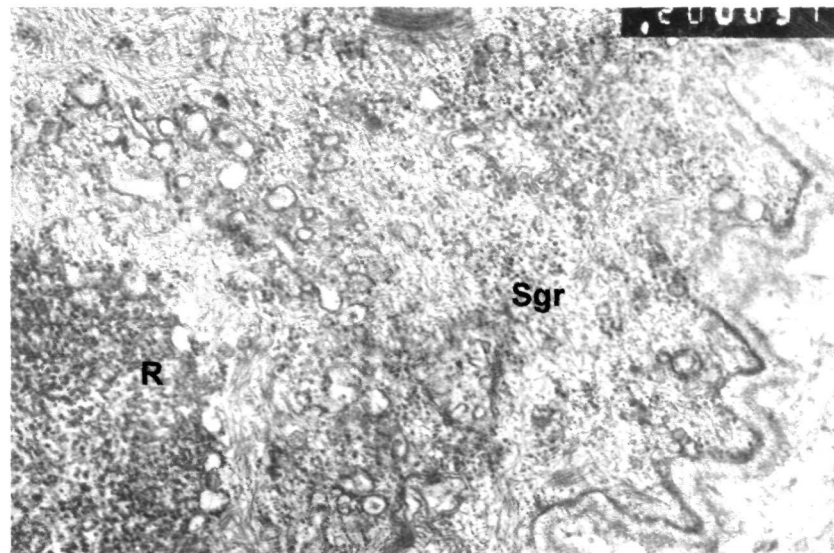


Fig. 5.12 - TEM of the male skin of *Rhacophorus bipunctatus* showing stratum germinativum (Sgr), ribosomes (R), (X29,000).

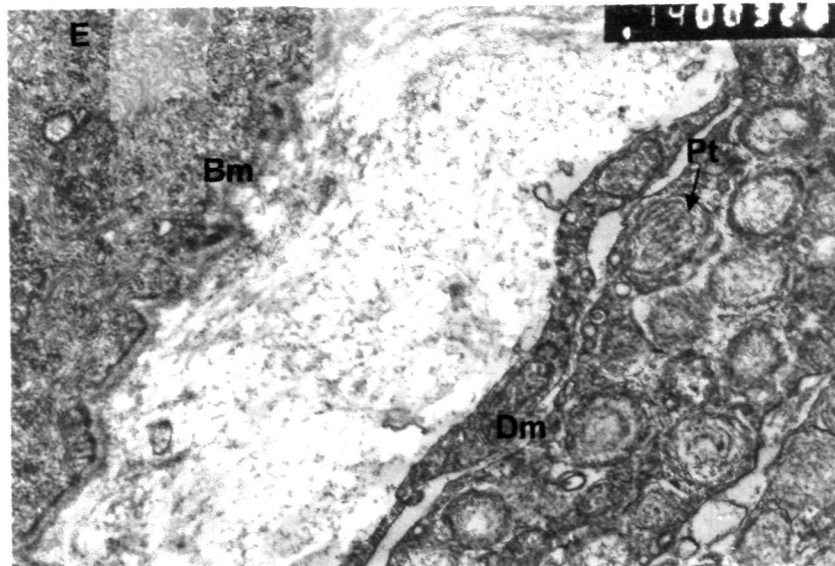


Fig. 5.13 - TEM of the male skin of *Rhacophorus bipunctatus* showing epidermis (E), basement membrane (Bm), dermis (Dm), pterinosomes (Pt) (X20,300).

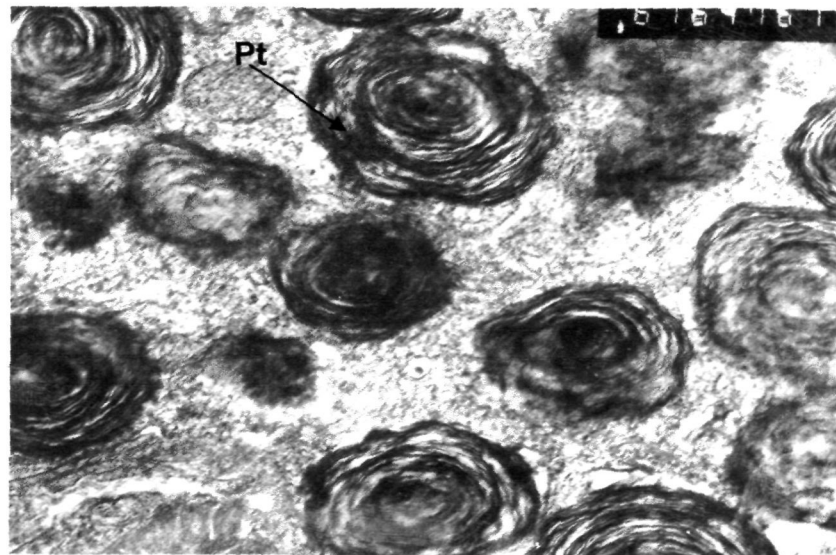


Fig. 5.14 - TEM of the male skin of *Rhacophorus bipunctatus* showing magnified view of pterinosomes (Pt) (X39,150).

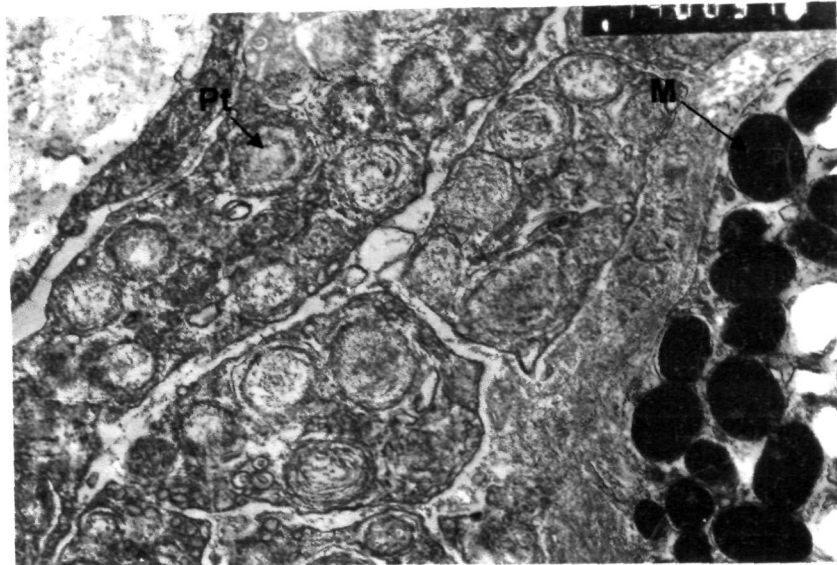


Fig. 5.15 - TEM of the male skin of *Rhacophorus bipunctatus* showing petinosomes (Pt), melanophores (M) (X20,300).

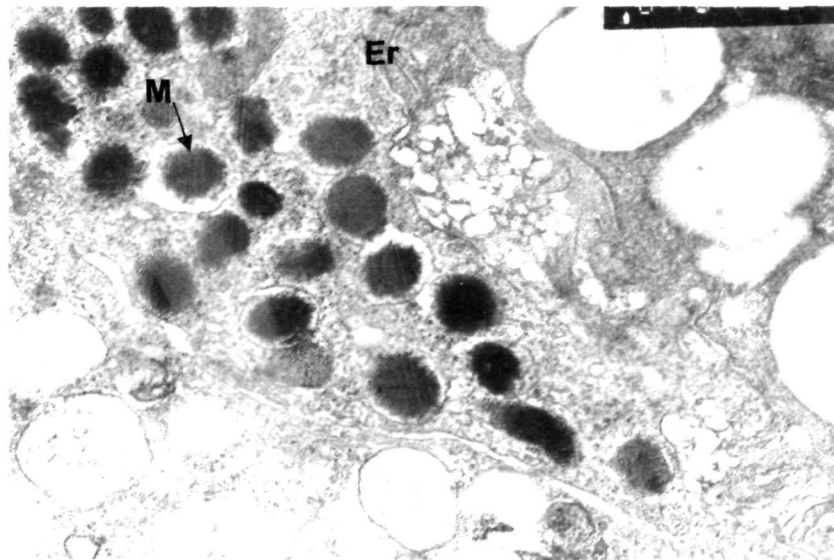


Fig. 5.16 - TEM of the male skin of *Rhacophorus bipunctatus* showing melanophores (M), endoplasmic reticulum (Er) (X29,000).

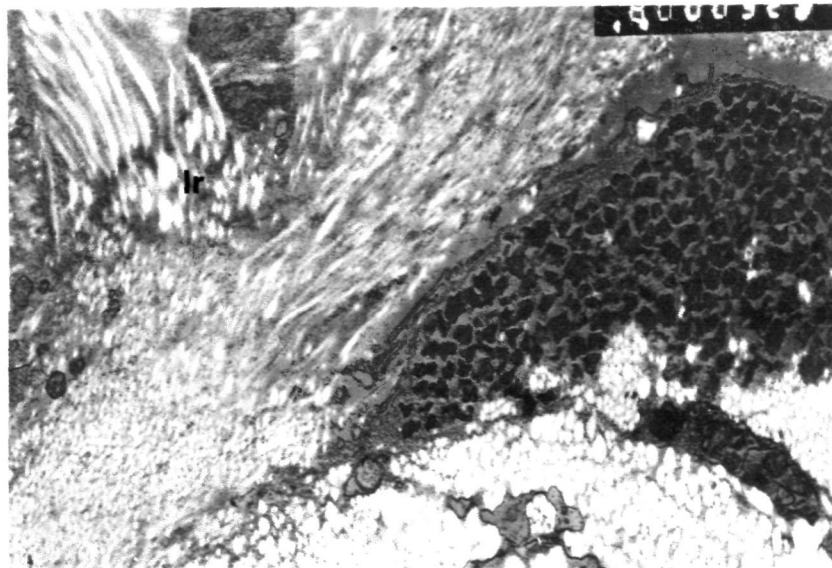


Fig. 5.17 - TEM of the female skin of *Rhacophorus bipunctatus* showing iridophores (Ir) (X11,600).

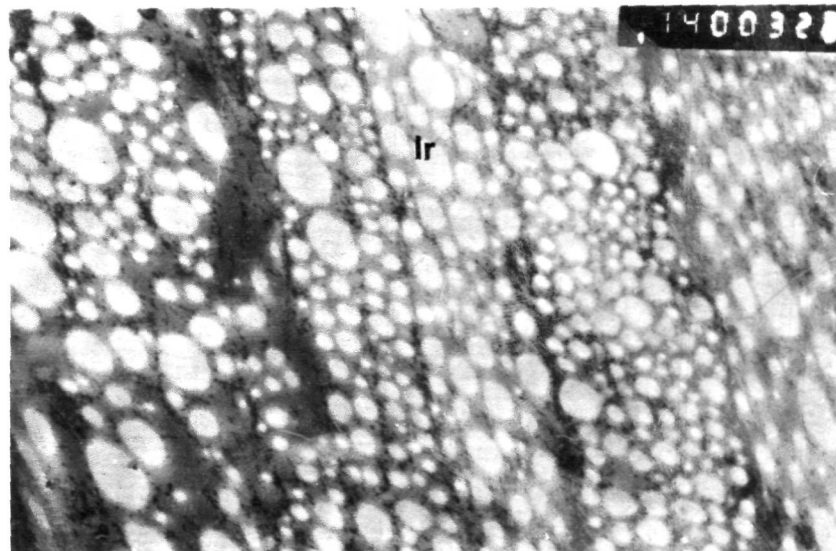


Fig. 5.18 - TEM of the male skin of *Rhacophorus bipunctatus* showing magnified view of iridophores (Ir) (X20,300).

Discussion:

Comparative studies of the skin of the two tree frog species *Polypedates leucomystax* and *Rhacophorus bipunctatus* revealed that the ultrastructure of the skin was similar and resembled the skin of other amphibian species. Goniakowska-Witalenska and Kubiczek (1998) have reported that the skin of the tree frog, *Hyla arborea arborea* has typical structure resembling that of other amphibians (Noble, 1931; Mills and Prum, 1984; Delfino, 1991; Duellman and Trueb, 1994; Frost-Mason *et al.*, 1994; Fox, 1994; Warburg *et al.*, 1994). The epidermis of these two frog species consists of a number of layers, which are similar to the other amphibians (Lavker, 1974; Fox, 1974; Whitear, 1975; Blankemeyer and Shahin, 1993). Since *Polypedates leucomystax* and *Rhacophorus bipunctatus* were adapted to arboreal mode of life, the outer most layer of the epidermis, the keratinized stratum corneum with little recognizable ultrastructure may be related to their terrestrial adaptation, as it has been suggested that keratinization of body epidermis may be a crucial adaptation for terrestrial life (Menon *et al.*, 2000). The tree frogs, *Polypedates leucomystax* and *Rhacophorus bipunctatus* leading an arboreal life style, visit the ephemeral pools solely during the breeding period. Hence, the integument as an adaptable organ system may play an important role in both aquatic and terrestrial mode of life as it has been reported that the skin of amphibian performs several functions such as mechanical protection (Farquhar and Palade, 1965; Fox, 1986), water absorption (Hoffman and Katz, 1999; Sullivan *et al.*, 2000), and respiration (Duellman and Trueb, 1994). It has been reported that the keratinized layer of the integument may be related to

the protection against friction (De Brito-Gitirana, 2005). In this context it is to be noted that keratinization and sloughing mechanism in amphibians are little understood.

As observed in the stratum granulosum the epithelial cells were devoid of nucleus suggesting that these cells prepared to move from the stratum germinativum to the stratum corneum, to be replaced and shed periodically. It was observed that the epidermal cells with nucleus in the stratum germinativum moved to the upper layer i.e., stratum spinosum and later into the stratum granulosum and finally move towards the stratum corneum. Thus, it may be suggested that the epidermis participates in the process of keratinization. It has also been reported that in the epidermis of amphibians, cells from the germinative layer move into the intermediate spinosus layer, and later differentiate into cells of the replacement layer; the latter eventually replaces the external corneous layer during molt (Lodi and Bani, 1971; Budtz and Larsen, 1973, 1975). The presence of tonofilaments in the epidermal cells of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were observed, suggesting that they provide a mechanical support to the skin. Desmosomes observed between the adjacent epidermal cells permit surface shedding by their breakdown, since it has been suggested that desmosomes may ultimately be degraded by lysosomal enzymes (Douglas *et al.*, 1970). Presence of endoplasmic reticulum and ribosomes in the epidermal cells indicates active protein synthesis and transport. The presence of mitochondria in the epidermal cells suggests that they are metabolically active.

Located beneath the epidermis, is the dermis containing chromatophores or pigment cells. During the present study, pigment cells like xanthophores, melanophores

and iridophores were observed in both the species. The xanthophores containing pterinosomes as pigmentary organelles imparts red, orange and yellow colour to the frog (Bagnara, 1966). Melanophores located in the lower layers of the dermis imparts black or brown colour to the organism. The shining appearance of the skin in *Polypedates leucomystax* and *Rhacophorus bipunctatus* might be due to the presence of iridophores in the integument as it has been suggested that these iridophores may play a role in reflection, diffraction and other optical phenomena (Menter *et al.*, 1979). It is to be noted in this context that many vertebrates exhibit complex skin pigmentation patterns achieved by differential distribution of three pigment cell (chromatophores) types - xanthophores, melanophores, and iridophores (Fernandez and Bagnara, 1993). The yellowish brown colour in dorsum of *Polypedates leucomystax* may be derived from the relative positions occupied by different types of chromatophores. The observation on the variation in skin colour from yellowish to brownish in the morning and evening in *Polypedates leucomystax* indicates pronounced colour change. It may be mentioned here that many kinds of pigment cells provide patterns and contribute colour change through the mediation of a variety of mechanisms involving both physiological and morphological colour changes. These changes occur as adaptive responses to several environmental cues, principally background colouration and the presence or absence of light. Further, the principal adaptive role of pigmentation serves to match the colour of the animal to the back ground upon which it sits (Bagnara, 1986). In particular chromatophores can effect colour changes which are a vital part of the strategy of many species to avoid attack by

predators, obtain prey more easily, and allow conspecific communication (Sugden *et al.*, 2004).

Iridophores are cells that participate in pigmentary phenomena by virtue of their physical properties. They utilize crystalline deposits of purines in organelles called reflecting platelets that produce structural colours by means of an ordered arrangement of these organelles within the cell. Thus, the reflecting platelets may be elements used in reflection, light scatter, diffraction and interferences (Menter *et al.*, 1979). Since the reflecting platelets were more prominent in *Rhacophorus bipunctatus*, they might be performing the said function of reflection, diffraction, light scattering and interferences. This is relevant in view of the observation that *Rhacophorus bipunctatus* exhibits brilliant green colour of the dorsal skin irrespective of breeding and non breeding phases. These chromatophores make up dermal chromatophore units, and the distribution of these units determines the ultimate pigment pattern of a species (Bagnara *et al.*, 1968). It has been reported that skin colouration and patterns displayed by other vertebrate species have important roles in adapting the species to their environment (Kelsh, 2004). In the present investigation it may be suggested that the chromatophores may work together to determine the varied skin colouration and patterns displayed by the two tree frog species, and may have important roles in adapting these tree frogs to their specific environment.

Conclusion

CONCLUSIONS

The present work dealt with studies on the distribution of the tree frogs *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Meghalaya. The breeding behaviour, habit and habitat, development and metamorphosis in relation to some ecological factors and water chemistry (rainfall, relative humidity, air and water temperature and water pH) was also studied at the four study sites I, II, III and IV at Cherrapunjee, Meghalaya. Food and feeding of the tadpoles and adult frogs and the morphological changes of the oral structure of the tadpoles, and the ultrastructure of the skin was carried out. The major findings of these studies on *Polypedates leucomystax* and *Rhacophorus bipunctatus* are mentioned chapter-wise.

Chapter I - Distribution of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in Meghalaya:

Extensive survey and collection were conducted during the years 2002, 2003 and 2004 through out the state of Meghalaya. The morphometric measurements of the frogs collected were taken into account following the method given by Chanda (1994). *Polypedates leucomystax* was collected from nineteen surveyed sites, namely Cherrapunjee, Shillong, Mawsynram, Mawphlang, Pynursla, Balat, Mawkyrwat, Nongstoin, Mairang, Umsning, Nongpoh, Jowai, Dawki, Baghmara, Williamnagar, Rongjeng, Tura, Garobadha and Dadenggiri. The nineteen surveyed and collection sites of *Polypedates leucomystax* ranged from higher mid-altitude between 1300 m – 1700 m asl to lower mid-altitude between 120 m – 770 m asl and lower altitude regions ranging between 15 m – 40 m asl. The morphometric measurements of the males collected from

different parts of the state ranged from 42 to 66 mm with a mean of 45.0 ± 5.23 mm, and that of the females ranged from 45 to 78 mm with a mean of 62.0 ± 7.60 mm.

While *Rhacophorus bipunctatus* was collected only from ten survey sites, namely Cherrapunjee, Shillong, Mawsynram, Pynursla, Nongstoin, Mawkyrwat, Jowai, Thadlaskein, Nongpoh and Umsning. The distribution of *Rhacophorus bipunctatus* in Meghalaya ranged from higher mid altitude regions between 1300m – 1560m asl, and also from lower mid altitude regions ranging from 560 m to 770 m. The morphometric measurements of the males collected from different parts of the state ranged from 39 to 45 mm with a mean of 43.5 ± 0.42 mm and the females ranged from 63 to 65 mm with a mean of 64.0 ± 0.21 mm. In the population of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* the males were found to be smaller than the females.

Thus, from the present study it revealed the current status on the distribution of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in exact localities collected from different parts of Meghalaya. *Polypedates leucomystax* is widely distributed than *Rhacophorus bipunctatus* and was collected from all the seven districts of Meghalaya, namely East Khasi Hills, West Khasi Hills, Jaintia Hills, Ri Bhoi, West Garo Hills, East Garo Hills and South Garo Hills districts, indicating that it has diverse adaptations to live in low and high altitudes. While *Rhacophorus bipunctatus* preferred higher and lower mid altitude regions with cooler climate and was collected from only four districts, namely East Khasi Hills, West Khasi Hills, Jaintia Hills and Ri Bhoi districts. It thus seems that the distribution of *Rhacophorus bipunctatus* in Meghalaya is influenced by regional and local factors such as seasonal distribution of rainfall, moisture, temperature, vegetation and physiological tolerances of the species. These two tree frogs preferred ephemeral

pools, which were formed during the rainy season as these pools provide suitable places for breeding, development and metamorphosis of these tree frogs.

Chapter II - Breeding behaviour, habit and habitat of *Polypedates leucomystax* and *Rhacophorus bipunctatus*:

Breeding behaviour of the tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* was studied with the help of photographic documentation and observations were based on the courtship, mating calls, foam nest construction, size of amplexing frogs, clutch size, and sex ratio at the study sites I, II, III and IV at Cherrapunjee, and is renowned for receiving maximum rainfall in the world, and rainfall seems to be the primary factor initiating breeding activity in amphibians.

These four study sites were ephemeral pools and served as good habitat for breeding, development and metamorphosis of these two tree frogs. The plants found near these study sites were identified as *Imperata cylindrical*, *Hydrilla sp.*, *Gleichenia sp.*, *Rhododendron arboretum*, *Alnus nepalensis* etc.

Breeding behaviour, habit and habitat of *Polypedates leucomystax* (Gravenhorst, 1829) and *Rhacophorus bipunctatus* Ahl, 1927 in relation to some ecological factors (temperature, relative humidity and rainfall) revealed that the breeding phase of both the tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus* at Cherrapunjee was similar and coincides with the rainy season (April to August), when air temperature and relative humidity were comparatively high. During the breeding phase (April to August) air temperature ranged between 21.3°C – 25.7°C, relative humidity between 68 % - 89 % and rainfall between 501.5 mm – 5008.2 mm.

The present study revealed that the emergence of *Polypedates leucomystax* from its hibernation in the month of March was initiated with increasing atmospheric temperature and the arrival of the rain. Spawning and foam nest construction took place from the month of April to August when there was accumulation of water in the ephemeral pools so that the frogs could carry on their breeding activity longer without fear of desiccation. After emergence, males moved towards the breeding sites and only with the arrival of the first few showers of rain started making advertisement calls, which attracted the female to come to the breeding site for spawning. It was found that the advertisement call of *Polypedates leucomystax* consisted of three long notes. Courtship in *Polypedates leucomystax* started by the male making the advertisement calls and the female responded to the calls by moving to the breeding sites. In *Polypedates leucomystax* it was observed that the male choose the oviposition sites closer to a water body for spawning and foam nest construction. This tree frog breeds in shallow ephemeral ponds and constructed its foam nest about 5-40 cm above the water surface on the margins of the pools. Some amplexing pairs constructed their foam nests in between stones or rocks, cemented tank, and some were also found to construct their foam nests about 1 m away from the water body attached to grassy edges, which served as substratum to the foam nest. The diameter of the foam nests ranged between 6-12cm and clutch size between 252-530 in different nests and the egg measured 1.5 mm.

Similar to *Polypedates leucomystax*, it was observed that *Rhacophorus bipunctatus* emerged in the month of March when it was warmer and its breeding activity coincide with the rainy season from the month of April and continued till August. Rainfall has been observed to initiate advertisement calls in *Rhacophorus bipunctatus*.

The advertisement calls attracted the females to the breeding sites and it was composed of six long notes, and it was different from that of *Polypedates leucomystax* indicating that the advertisement call was species specific. In *Rhacophorus bipunctatus*, it was found that the amplexing pair moved away from the water body and selects a suitable site for foam nest construction on the grassy edges, which served as a substratum to the foam nests closer to a water body. This species constructed its foam nest close to ephemeral and shallow pools and on branches of trees hanging over a water body. Construction of the foam nests closer to water bodies and on tree leaves above water might be an adaptation to avoid predators in the aquatic habitat during early development. The diameter of the foam nests ranged between 6-8 cm and the clutch size between 45-76 in different foam nests and were comparatively smaller than *Polypedates leucomystax*, and the size of the egg measured 3.0 mm.

In both the species, it was found that during the breeding period the males are usually more in number than the females at the breeding sites. The breeding cycle of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was similar, and it was divided into four phases - hibernating phase, emerging and pre-breeding phase, breeding and spawning phase and post-breeding phase. *Polypedates leucomystax* and *Rhacophorus bipunctatus* hibernate during the months of November to February (air temperature: 13.5°C – 20.5°C, relative humidity: 42 % - 69 %, and rainfall: 0.0 mm – 298.6 mm). During hibernating period the frogs hide themselves on the forest litters in case of *Polypedates leucomystax* where as *Rhacophorus bipunctatus* were found hiding in banana stem. Emergence of the frogs from hibernation was observed in the month of March (air temperature: 20.6°C – 23.8°C, relative humidity: 52 % - 88 %, and rainfall:

163.2 mm – 1119.6 mm). Breeding and spawning took place from the month of April to August (air temperature: 21.3°C – 25.7°C, relative humidity: 65 % - 89 %, and rainfall: 501.5 mm – 5008.2 mm). In the month of September and October (air temperature: 19.4°C – 22.6°C, relative humidity: 54 % -79 %, and rainfall: 66.3 mm – 1917.3 mm) the frogs under go post breeding phase. Histological study of the testis of *Polypedates leucomystax* and *Rhacophorus bipunctatus* showed the presence of different spermatogenic cells during the different periods of the breeding cycle. Young and mature oocytes filled up the ovary during the breeding period and during the post breeding period no mature oocytes were observed.

On the basis of the present findings, it may be concluded that there is similarity in the breeding behaviour as well as in the breeding cycle of both the species of the tree frogs *Polypedates leucomystax* and *Rhacophorus bipunctatus*. Further, ecological factors, rainfall, relative humidity and air temperature and seem to play a major role in regulation of breeding behaviour of these two tree frog species. In both the species courtship, was initiated by the advertisement calls given by the males. Both species showed diversity in the selection of the oviposition sites and foam nests construction and the preference of these two tree frogs for a particular type of breeding habitat reflects the differential fitness and adaptiveness of these species in the various habitats available in that particular natural environment. The differences observed in advertisement call, selection of oviposition site, foam nest size, clutch size and egg size in these species seem, to be species-specific adaptations.

Chapter III - Development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the ecological factors and water chemistry (e.g., temperature and pH) of the breeding sites:

Development and metamorphosis of *Polypedates leucomystax* and *Rhacophorus bipunctatus* in relation to some ecological factors (rainfall, relative humidity, air and water temperature and water pH) was studied at the study sites I, II, III and IV at Cherrapunjee, Meghalaya. Some of the foam nests collected from the study sites were brought to the laboratory and maintained in plastic trays containing pond water to allow further development and metamorphosis, and observed under a stereoscopic dissecting binocular microscope. The different developmental stages of both the species were based on new external morphological changes as per the criteria given by Gosner (1960), and photographs of the developmental and metamorphic stages were also taken.

The present investigation revealed that, the time of development and metamorphosis in both the species was similar and depends on the ephemeral pools at the breeding sites to complete their life cycle. Both in the natural environment as well as under laboratory conditions, the development and metamorphosis of *Polypedates leucomystax* was completed in about 60 – 61 days and in *Rhacophorus bipunctatus* about 59 – 60 days respectively. In both the species hatching was observed after 4 days of fertilization. The mortality percentage of both the tree frog species was also recorded under laboratory condition. In *Polypedates leucomystax* mortality percentage ranged between 34.48 % - 84.32 % with a mean of 62.13 ± 0.20 and in *Rhacophorus bipunctatus* between 41.13 % - 92.13 % with a mean of 68.45 ± 1.18 .

Each developmental stage has been based on the new external morphological changes. The eggs of *Polypedates leucomystax* measured about 1.5 mm in diameter, and were found to be comparatively smaller than those of *Rhacophorus bipunctatus* measuring about 3 mm in diameter. Association of fertilized eggs of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* with the foam nests was found to be essential up to hatching for further normal development. The foam nest of both the species seems to provide optimal conditions for the early development of the larva. Comparatively, larger size of the egg and the presence of large amount of yolk in *Rhacophorus bipunctatus* might be an adaptation to its arboreal mode of life.

Water temperature of the study sites I and II where development and metamorphosis of *Polypedates leucomystax* took place ranged between 18.4°C – 24.0°C, while that of study sites III and IV of *Rhacophorus bipunctatus* ranged between 18.2°C – 23.4°C. The pH ranged between 6.8 – 7.2 in the four study sites (I, II, III and IV) during the development of the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus*. The water temperature and water pH of the four study sites was similar and provided a suitable environmental condition for the development and metamorphosis of the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* during its life cycle. At the study sites, during the period of development and metamorphosis (April to October) atmospheric temperature ranged between 19.4°C – 25.7°C, rainfall between 66.3 mm – 5008.2 mm and relative humidity between 54% - 89%.

On the basis of the present findings, it may be concluded that there was a similarity in the development and metamorphosis of both *Polypedates leucomystax* and *Rhacophorus bipunctatus*. These tree frogs developed and metamorphosed successfully

in the water temperature range between 18°C – 24°C and in the water pH range between 6.8 – 7.2. Comparatively, larger size of the eggs and the presence of large amount of yolk in *Rhacophorus bipunctatus* might be an adaptation to its arboreal mode of life and higher altitude habitat. In both *Polypedates leucomystax* and *Rhacophorus bipunctatus*, hatching was observed after four days of fertilization indicating rapid developmental rate, which might be advantageous for these two tree frog species in ephemeral habitats, which allow larvae to metamorphose quickly and escape desiccation. The foam nests seem to provide optimal conditions for the early development and ensure successful normal hatching of the larva.

Chapter IV - Food and feeding of the tadpoles and adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus*, and the morphological changes of the oral structure of the tadpoles:

Food and feeding of the tadpoles and adults of *Polypedates leucomystax* and *Rhacophorus bipunctatus* and the oral structures of the tadpoles of both species was studied. Adult frogs and different developmental stages of the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were collected from the study sites (I, II, III and IV) at Cherrapunjee. The tadpoles were staged according to Gosner (1960) and autopsy was made for analysis of gut content. Identification of the food items present in the gut of the tadpoles was made following the works of Edmonson (1959). The oral structure of the developing tadpoles during metamorphosis were studied using

stereoscopic binocular microscope and also with the help of Scanning Electron Microscopy.

Food and feeding habits of the tadpoles and adults of *Polypedates leucomystax* and *Rhacophorus bipunctatus* studied revealed that the tadpoles of both the tree frog species started feeding from stage 25 onwards. The food items of the tadpoles of both the species was similar and the tadpoles of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were both omnivorous and microphagous feeders. During early premetamorphic stages the tadpoles of both the species fed mostly on the detritus, plant materials and phytoplankton and during the prometamorphic stages they fed both on phytoplankton and Zooplankton. The tadpoles of these two tree frogs were also observed to exhibit cannibalism in the natural environment as well as under laboratory conditions. The tadpoles stopped feeding from stage 40 onwards. The adults of both the species consumed similar food items and included mostly insects.

Under scanning electron microscopic studies it was found that development of the teeth of the tadpoles of both the species began from stage 25. Complete dental formula was observed at stages between 31-39. The number of teeth rows of the tadpoles of both the tree frog species was different, and increased and changed with the progress of development and metamorphosis and it was species specific. The dental formula of *Polypedates leucomystax* was 1:3+3/3, while that of *Rhacophorus bipunctatus* was 2:3+3/3. The dental formula of the tadpoles of both the species was different and species specific. Teeth began to shed with the onset of metamorphosis and the metamorphose froglet had a developed tongue.

On the basis of present findings, it may be concluded that the food items of the tadpoles as well as that of the adult frogs of both the species are similar. Similarity in the food items ingested by the tadpoles of both the species may be due to the availability and composition of same food items in the similar habitats occupied by the tadpoles. Further, the tadpoles of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were omnivorous and microphagous feeders. The dental formula of the tadpoles of the two species was different and species specific. The increase number in the number teeth rows during the development and metamorphosis of the tadpoles might be helpful in the feeding of a wide variety of food items consumed by the tadpoles

Chapter V - Ultrastructure of the skin of *Polypedates leucomystax* and *Rhacophorus bipunctatus*:

Ultrastructure of the skin of the adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus* was studied during the breeding phase with the help of transmission electron microscopy. Adult males and females of *Polypedates leucomystax* and *Rhacophorus bipunctatus* were collected from the study sites (I, II, III and IV) at Cherrapunjee, anesthetized and the sections of the skin from the dorsal and ventral parts of males and females of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* were processed for transmission electron microscopic studies.

Ultrastructure of the skin of the adult frogs of *Polypedates leucomystax* and *Rhacophorus bipunctatus* during the breeding phase revealed that it was similar. The epidermis of both the species was similar and consisted of four layers namely, stratum

corneum, stratum granulosum, stratum spinosum, and stratum germinativum. The outer most layer the stratum corneum was thickened and keratinized and consisted of a single layer of flattened cells. In the stratum granulosum some cells were observed to be devoid of a nucleus as these cells had prepared to move to the stratum corneum, as stratum corneum sloughed or shed periodically and have to be replaced. Desmosomes, ribosomes, tonofilaments, golgi bodies, vacuoles and collagen fibres were also observed in the epidermal cells.

The basement membrane composed of collagenous fibres separated the epidermis from the dermis. The dermis was composed of two layers, the stratum spongiosum and the underlying stratum compactum. Pigment cells like pterinosomes, melanophores and iridophores were observed in both the species and these pigments cells may work together to determine the varied skin colouration and patterns displayed in both *Polypedates leucomystax* and *Rhacophorus bipunctatus* and may have important roles in adapting these two tree frogs to their environment.

On the basis of present findings, it may be concluded that the ultrastructure of the skin of both *Polypedates leucomystax* and *Rhacophorus bipunctatus* was similar to other amphibians. The epidermis was composed of four layers. The outer most layer, the stratum corneum was keratinized, which may be related to their terrestrial adaptation. In the dermis, pigment cells like pterinosomes, melanophores and iridophores were present in both the species. The colouration seen in these two tree frogs is the result of the work of these pigment cells found in the dermis.

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BIO DATA

Name: ASORPHI JANAI IANGRAI

Date of Birth: 30th March 1979

Father's Name: (L) H.T. Warjri

Address: c/o Mrs. W. Iangrai
Lower Nongrim Hills
Laitumkhrah
Shillong 793003
Meghalaya

SC/ST: Schedule Tribe, Khasi

Academic Qualification:

Examination	Univ/Board	Year	Div/Class
H.S.L.C.	M.B.O.S.E.	1994	IInd Class
P.U. (Sc)	N.E.H.U.	1996	IInd Class
B.Sc. (Maj)	N.E.H.U.	1999	IInd Class
M.Sc. Zoo	N.E.H.U.	2001	Ist Class

List of research publications:

1. S. Khongwir, A. Iangrai, and R.N.K. Hooroo. Development of mouth parts in the tadpoles of *Rhacophorus maximus*. Uttar Pradesh Journal of Zoology 23(2): 101-104, 2004.
2. S. Khongwir, R.N.K. Hooroo, A. Iangrai, and B.B.P. Gupta. Physico-chemical conditions of the breeding site of *Rhacophorus maximus*. Geobios 31(1): 55-56, 2004.
3. A. Iangrai, R.N.K. Hooroo, B.B.P. Gupta & K. Chatterjee. Changes in the teeth row structure of the tadpoles of *Polypedates leucomystax* with reference to food and feeding habits. Uttar Pradesh Journal of Zoology 24(2): 143-146, 2004.

4. **A. Iangrai, S. Khongwir, R.N.K. Hooroo & B.B.P. Gupta.** Influence of some environmental factors on the breeding and development of *Polypedates leucomystax* (Gravenhorst) Geobios 31: 290-292, 2004.

Symposium/Seminar Attended:

1. Attended the National Academy of Sciences, India, Seventy Second Annual Session from October 25th – 27th, 2003 held at NEHU, Shillong.
2. Presented paper at the Regional Symposium on Research Thrust in Animal Sciences in N. E. Region – An Appraisal, from March 24th – 25th, 2006 organised by the Department of Zoology, North Eastern Hill University, Shillong.
3. Attended the Regional Seminar on “Biodiversity-Herbal Medicine” on 10th December 2005 organised by Synod College.
4. Attended seminar on “Human Rights: Experiences and Initiatives” on 3rd August 2006 organised by Synod College.
5. Attended the Regional Level Seminar on “ Extension and Outreach Activities of the College – New Dimensions” held on 20th – 22nd July 2006 organised by Union Christian College.

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