

**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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## 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  $62.13 \pm 0.20$ , while in *Rhacophorus bipunctatus* it ranged between 41.13 % - 92.13 % with a mean of  $68.45 \pm 1.18$ .

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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