STUDIES ON ECOLOGY, BREEDING BEHAVIOR AND DEVELOPMENT OF *Polypedates braueri* (VOGT, 1911) (AMPHIBIA: ANURA: RHACOPHORIDAE) FROM MIZORAM, INDIA.

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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BY
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SUBMITTED
IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY OF MIZORAM UNIVERSITY, AIZAWL.

Dedicated to My Late Grandfather & My Beloved Family

SUPERVISOR'S CERTIFICATE

This is to certify that the thesis entitled "**Studies on Ecology, Breeding Behavior and Development of *Polypedates braueri* (Vogt, 1911) (Amphibia: Anura: Rhacophoridae) from Mizoram, India**" written by Vanlalsiammawii has been written under my Supervision.

She has fulfilled all the required norms laid down within the Ph.D regulations of Mizoram University. The thesis is the result of her own investigations. Neither the thesis as a whole nor part of it was su bmitted to/by any other University for any degree.

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<u>DECLARATION BY THE CANDIDATE</u>

Mizoram University

JUNE, 2025

I, **Vanlalsiammawii**, hereby declare that the subject matter of the thesis entitled "**Studies on Ecology, Breeding Behavior and Development of *Polypedates braueri* (Vogt, 1911) (Amphibia: Anura: Rhacophoridae) from Mizoram, India**" is the record of work done by me, that the contents of this thesis did not form 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/Institutes.

This is being submitted to the Mizoram University for the degree of Doctor of Philosophy in Zoology.

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VANLALSIAMMAWII

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

Amphibians represent an ancient and diverse group of vertebrates, extensively studied across various scientific disciplines, including evolution, ecology, engineering, and medicine. Despite being considered highly sensitive organisms, amphibians have survived four of the Earth's major mass extinction events and are distributed across nearly every continent (Wake & Vredenburg, 2008; Carroll, 2009; Barnosky et al., 2011; Alroy, 2015). Many species exhibit biphasic life cycles, acting as crucial connectors between terrestrial and aquatic ecosystems by facilitating energy and nutrient transfer (Finlay & Vredenburg, 2007). With rapid growth rates and large population sizes, amphibians play a significant ecological role—both as prey and as regulators of prey populations—contributing to functional diversity in food webs (Colón-Gaud et al., 2009; Zipkin et al., 2020). Nevertheless, fundamental data on natural history and species distribution remain unknown for many amphibians; the IUCN lists 16.4% (1,185 species) as Data Deficient—the highest proportion among vertebrate classes (IUCN, 2025).

Amphibians were the first vertebrates to successfully transition from aquatic to terrestrial environments, roughly 350 million years ago. While they generally follow a biphasic life cycle—living first in water and then on land—some species remain fully aquatic or terrestrial. The term "amphibian" originates from the Greek word *amphibios*, meaning "double life." Modern amphibians fall into three orders: **Anura** (frogs and toads), **Caudata** (salamanders), and **Gymnophiona** (caecilians), each exhibiting a variety of adaptations and habitat preferences. Most amphibians develop through four stages—egg, larva (tadpole), juvenile (froglet), and adult—though some bypass the larval stage entirely. Generally, amphibians are ectothermic, quadrupedal animals with smooth or glandular skin, lacking scales, hair, or feathers (Duellman & Trueb, 1994). Their skin must remain moist, making them highly sensitive to environmental conditions, especially temperature and humidity. This sensitivity also limits their geographical distribution, as they thrive best between 20°C and 30°C.

The group **Lissamphibia** includes all living amphibians, comprising around **8,885** recognized species. The order Anura accounts for about **88%** of these species, encompassing approximately **7,836** species, 54 families, and roughly 4,568 genera globally (Frost, 2025). Since 1985, the number of described amphibians has increased by over 60%, with **2180** new species identified between 2011 and 2025 (AmphibiaWeb, 2025). Amphibians inhabit nearly all terrestrial habitats except extreme environments such as Arctic, Antarctic regions, waterless deserts, and select Pacific islands. Their distribution is influenced by environmental factors like rainfall, temperature, water availability, vegetation, and terrain. While most amphibians cannot tolerate saltwater, some anurans have demonstrated a limited tolerance to low salinity (Balinsky, 1981). Consequently, long-distance oceanic dispersal is rare, and their global distribution patterns are largely shaped by historical vicariance (Duellman & Trueb, 1994). Many species enter hibernation during colder months (Ahmed et al., 2009).

Global amphibian decline became a serious conservation issue in 1989. Habitat loss and degradation—especially due to urbanization—pose severe threats to amphibian survival (Wake, 1991; Blaustein, 1994; Alford & Richards, 1999). Given their ecological roles as predators, prey, and herbivores, the decline of amphibians could lead to broader ecosystem imbalances. Their usefulness as bioindicators is a pressing concern due to their sensitivity to environmental stressors (Blaustein, 1994). Amphibians play keystone roles in forest ecosystems, influencing vital ecosystem processes and contributing to system resilienceresistance (=stability) (Davic and Welsh, 2004). Adult amphibians are important predators as well as prey and larval amphibians may be important herbivores (Blaustein et al., 1994). Therefore, a world-wide decline of amphibian populations could have a significant and detrimental impact on both natural ecosystems and human welfare (Gardner, 2001). According to the second Global Amphibian Assessment (GAA), amphibians are the most threatened vertebrate class (40.7% of species are globally threatened). While habitat loss and degradation remain the most common threat to amphibians, an increasing number of species are being pushed to the brink of extinction by disease and climate change effects. The urgency to act is becoming increasingly critical (IUCN, Luedke et al., 2023). Amphibians' unshelled eggs, dual habitats, small home ranges, and low dispersal ability make them reliable indicators of ecosystem health. Thus, widespread population declines could significantly impact both biodiversity and human well-being. India, recognized for its amphibian diversity, is particularly rich in endemic species, especially in the **Western Ghats** and **Northeast India**—two global biodiversity hotspots (Ahmed et al., 2009).

Amphibian research in India dates back to Günther (1858), with Boulenger (1882, 1920) considered a pioneer in Indian herpetology. The first comprehensive checklist of Indian amphibians, listing 181 species, was published by Inger and Dutta (1986). Subsequent revisions and taxonomic updates increased the number to 216 species by the late 1990s (Chanda & Ghosh, 1988; Das & Whitaker, 1990; Dutta, 1992, 1997; Deuti, 1996). Since then, regular compilations by researchers such as Daniels (2001, 2005), Chanda (2002), and Dinesh et al. (2009–2023) have advanced documentation efforts. Currently, India is home to **472 amphibian species**, nearly half of which are endemic. Despite this richness, detailed knowledge on their biology, taxonomy, and conservation remains limited (Gosavi et al., 2022).

Amphibian species richness and abundance are strongly linked to microenvironmental factors such as **rainfall, temperature, elevation**, and **habitat integrity** (Andrew et al., 2005; Sajjan et al., 2017; Sengupta et al., 2010). Due to their dual dependency on aquatic and terrestrial ecosystems, amphibians are especially prone to local extinctions compared to birds and mammals (Alcala et al., 2004). Their interactions with symbiotic microbes also play vital roles in health, reproduction, and adaptability (Wang et al., 2025).

Northeast India comprises Assam, Arunachal Pradesh, Meghalaya, Nagaland, Manipur, Mizoram, Tripura, and Sikkim, covering an area of **262,397 km²**. Situated at the confluence of the Indo-Chinese, Indo-Malayan, and Indian biogeographic realms, the region is part of the Eastern Himalayas and Indo-Burma biodiversity hotspots, hosting unique flora and fauna (Mittermeier et al., 2004; Conservation International, 2005). Its diverse climate and terrain have fostered high biodiversity and endemism. Over **105 amphibian species** have been documented from this region

(Ahmed et al., 2009). However, many areas remain poorly surveyed, and evidence suggests that much of the region's biodiversity is at risk of vanishing unrecorded (Pawar, 1999).

The region includes three main biogeographic zones: the **Eastern Himalaya** (north), the **Northeast Hills** (south), and the **Brahmaputra River Basin** (center) (Mani, 1974; Olson & Dinerstein, 2002). Northeast India's unique geological history, elevation range (100–7000+ m), and habitat diversity—from tropical to alpine—create a complex biogeographic landscape. Its biological affinities are most closely aligned with Southeast Asia (Mani, 1974). Despite its amphibian richness, endemism within the Indian context remains relatively low due to political boundaries and faunal continuity across borders.

Mizoram, part of this region, lies between 21°56′N to 24°31′N and 92°16′E to 93°26′E, covering 21,081 km². It shares international borders with Bangladesh and Myanmar, making it strategically important. Bounded by Assam and Manipur to the north, Chin Hills (Myanmar) to the east and south, and Tripura and Chittagong Hills (Bangladesh) to the west, Mizoram comprises 11 districts. The state is characterized by tropical evergreen and semi-evergreen forests, interspersed with grasslands. Classified as Assam Subtropical Pine Forest (9/C2) by Champion & Seth (1968), it experiences mild temperatures (10–20°C in winter, 15–30°C in summer) and average annual rainfall of 2037.6 mm. Of Northeast India's amphibians, 46 species are reported from Mizoram (Ahmed et al., 2009), including 43 anurans across six families: Bufonidae, Dicroglossidae, Megophrydae, Microhylidae, Ranidae, and Rhacophoridae.

While several researchers have studied Mizoram's amphibians, much remains unknown, especially in protected and community forest areas. Additionally, limited research exists on the breeding behavior and development of Rhacophoridae species, particularly *Polypedates braueri*.

Polypedates braueri, described by Vogt in 1911, belongs to the family Rhacophoridae. It is a small-sized arboreal frog (SVL: 45–65 mm) with granular dorsal skin, coarse ventral skin, and distinct coloration patterns, including a triangular interorbital line and transverse limb bands (Kuraishi et al., 2011). The species occurs up to **2200 m** elevation and is found in Taiwan, tropical and subtropical China, Myanmar, Vietnam, Thailand, Northeast India, and Guam (Frost, 2025; IUCN, 2025). It inhabits forested environments and breeds in natural and artificial water bodies such as tanks and rain-collection reservoirs (Hsu et al., 2012). In Taiwan, the breeding season spans March to September; in Mizoram, it extends from April to September (Siammawii et al., 2024). The species deposits foam nests on land, with clutch sizes ranging from **215–500 eggs** (Yang, 1998; Siammawii et al., 2024). Listed as **Least Concern** by the IUCN, *P. braueri* is widely distributed and adapts well to various habitats. However, in Mizoram, data on its ecology, reproduction, and development remain scarce, necessitating further research. Therefore, the present work is conducted for better understanding and contribution towards conservation of a poorly known Upland Treefrog, *Polypedates braueri*.

SYSTEMATIC POSITION

Phylum : Chordata

Kingdom : Vertebrata

Class : Amphibian

Order : Anuran

Family : Rhacophoridae

Genus : *Polypedates*

Species : *braueri*

The objectives of this work are as follows:

- To study the distribution and habitat of *Polypedates braueri* in Mizoram.
- To analyse the phylogenetic position and genetic divergence using partial 16S rDNA.
- To study the breeding biology with respect to environmental factors of *P. braueri*.
- To study the embryonic and larval development of *P. braueri*.
- To study oral morphology, food and feeding behavior of tadpoles and adults of *P.braueri.*

CHAPTER I

TO STUDY THE DISTRIBUTION AND HABITAT OF *Polypedates braueri* IN MIZORAM.

INTRODUCTION

Amphibians are ancient creatures valued by biologists and naturalists around the world. Globally, amphibians are distributed in cosmopolitan except for extreme northern latitudes, Antarctic and most oceanic islands (Frost, 2024). 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. According to Dinesh et al. (2024), India harboured 454 amphibians, out of which 411 represent anurans belonging to 12 families. The family Rhacophoridae being largest group, comprising 120 species. They are represented by small to large sized frogs distributed from Subsaharan Africa; southern Asia from Sri Lanka, Nepal, and India, to Japan, the Philippines and Sulwesi (Indonesia). In India, Rhacophorids inhabit 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. In India, the family Rhacophoridae comprises of two subfamilies (Rhacophorinae and Philautinae) and five genera *Rhacophorus*, *Polypedates*, *Philautus*, *Chirixalus* and *Theloderma* (Iangrai, 2007). The genus *Polypedates* (Tschudi, 1838) was distributed in Eastern India, southeastern Asia, Philippines, and Borneo.

Different *Polypedates* sp. and their distributions are as follows: *P. braueri* found in tropical and subtropical China (including parts of Tibet), Thailand, Myanmar, Vietnam and northeastern India (Mizoram) (Fig. 1.1). *P. assamensis* known only from type locality (Dhemaji District) in Assam, northeastern India; *P. bengalensis* from eastern India (West Bengal, Odisha), southwestern Bangladesh; *P. colletti* (Boulenger, 1890) from Peninsular Thailand through peninsular Malaysia to Sumatra, Borneo, Natuna Islands, and islands of the South China Sea; southern Vietnam (Minh Hai); *P. cruciger* Blyth, 1852 endemic to Sri Lanka; *P. discantus* Rujirawan, Stuart, and Aowphol, 2013 known from the type locality in southern peninsular Thailand, including adjacent Phi Phi Archipelago, southern Tanintharyi, peninsular Myanmar, and Peninsular Malaysia; *P. hecticus* Peters, 1863 endemic to Philipines, *P. himalayensis* in northeastern India (Assam, Arunachal Pradesh, Meghalaya), extreme northern West Bengal and central Bhutan; *P. impresus* Yang, 2008 known definitely only from a few localities in Yunnan and Guangxi, China, Laos, peninsular Thailand and northeastern Vietnam; *P. insularis* found in Nicobar Islands, India (below 500 m elevation); *P. iskandari* Riyanto, Mumpuni, and McGuire, 2011 endemic to Indonesis; *P. leucomystax* nominally from India, Bangladesh, eastern Nepal, Myanmar, southern China, Philippines, Thailand, Cambodia, peninsular Malaysia, Sumatra, Sulawesi, Borneo and Japan. *P. macrotis* (Boulenger, 1891) from Central peninsular Thailand into peninsular Malaysia; Sumatra, Borneo, western Java, and Natuna Island, Sulu Archipelago, Palawan, Busuanga, Calauit, Jolo, and Dumaran islands, Philippines; *P. maculatus* reported from Bangladesh, Bhutan, India (except northeastern states, Haryana, Rajasthan and Punjab), Myanmar, Nepal and Sri Lanka; *P. megacephalus* from subtropical and tropical China (excluding Taiwan), Northeast India, Laos, Myanmar and Sri Lanka; *P. mutus* from northeastern India (Mizoram), southern and northern China, and Myanmar; *P. occidentalis* known from type locality Thrissur and Palghat District, Kerala, South India; *P. otilophus* (Boulenger, 1893) distributed in eastern, western and northern Borneo; *P. pseudocruciger* found in Western Ghats of Tamil Nadu, Karnataka and Kerela, southwestern India; *P. pseudotilophus* Matsui, Hamidy, and Kuraishi, 2014 endemic to Indonesia; *P. ranwellai* Wickramasinghe, Munindradasa, and Fernando, 2012 known only from the type locality (Gilimale Forest, Ratnapura, Sabaragamuwa Province, 150 m elevation, Sri Lanka); *P. subanseriensis* known only from the type locality Arunachal Pradesh, northeastern India; *P. teniatus* from Bengal region to Assam, southern Nepal and Bhutan; *P. teraiensis* recorded in Eastern Nepal, Bhutan, eastern, peninsular and northeastern India, Bangladesh , Myanmar and China; *P. zed* (Dubois, 1987) known only from the type locality, Narayanghat, Chitwan District, central Nepal (Frost, 2025).

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 (Iangrai, 2007).

Globally, there are 25 nominal species under the genus *Polypedates*, out of which 14 species are reported from India, and 3 species from Mizoram Northeast India namely *P braueri, P. mutus* and *P. teraiensis* (Frost, 2025). Amphibians are often difficult to detect, as many are small, nocturnal, and cryptic (Tanadini & Schmidt, 2011; Durso & Seigel 2015; Hammond et al., 2021), which can make determining their distributions difficult. Yet, this baseline information is crucial for conservation, especially in light of contemporary anthropogenic pressures (Luedtke et al., 2023). Climate is the major determinant of the distribution of terrestrial organisms and richness patterns at large scales (e.g., Nix, 1982; Currie, 2001; Guisan & Thuiller, 2005). Projections of future climate conditions predict that major changes will take place in most subtropical regions globally, including an increase in average global temperature, a decrease in precipitation and changes in their annual patterns (Brinson, 2006; Christensen et al., 2007; IPCC, 2008; Allison et al., 2009; Zank et al., 2014). As a result, species are expected to modify their geographic range (Hughes, 2000; Parmesan & Yohe, 2003; Parmesan, 2006). The study of distribution patterns have a high potential to provide relevant information that can be used to understand the evolutionary history of a species (e.g. Richards et al., 2007) and guide species management and conservation policies (e.g. Guisan & Thuiller, 2005; Baldi et al., 2017).

Habitat destruction and fragmentation are considered major threats to amphibian populations (Marsh & Trenham, 2001; Chanson et al., 2008). In particular, habitat fragmentation can reduce connectivity among local populations (Reh & Seitz, 1990; Fahrig, 2003; Cushman, 2006), which can affect long-term population persistence by increasing inbreeding (Sjögren-Gulve, 1994). Amphibians are often considered as species with low dispersal ability (Smith & Green, 2005; Boissinot, 2009) and are frequently specialised to a particular habitat. Thus, habitat preferences, and particularly terrestrial habitats, of amphibian species require further investigation (Trochet et al., 2016).

It is a fact that “structure is the first thing that we notice whenever looking at organisms” (Gans, 1985). The description and analysis of structure is the province of the science of morphology. Morphological analysis is a well-established and cost-effective technique for the taxonomic identification of wildlife remains. Morphological study is routinely used for species identification in a wide variety of disciplines, including paleozoology, botany, entomology, ichthyology, herpetology, ornithology, and mammalogy (Trail, 2021). Morphological characters formed the exclusive basis for species definitions from the time of Linnaeus until recent decades. The detailed morphological “diagnosis” remains a required and essential element for new species descriptions, even as behavioral, ecological, and genetic characters are now often included (Sornoza-Molina et al., 2018).

REVIEW OF LITERATURE

Polypedates braueri was first described by Vogt in 1911 from Taiwan, China. Stejneger (1925), Ahl (1931), Wolf (1936), and Zhao & Adler (1993) placed *P. braueri* as *P. megacephalus*. Okada (1931) review (as *Rhacophorus leucomystax*) Taiwan (China) population. Lue, Tu, & Hsiang (1999) provided a brief account (as *Polypedates megacephalus*) for Taiwan. Introduced populations from Okinawa (Japan) and Guam may be of this species rather than *Polypedates megacephalus*. Records of *Polypedates megacephalus* from Taiwan apply to this species. Yang (1998) report the breeding biology. It was removed from the synonymy of *P. megacephalus* by Kuraishi et al. (2011), and also provided the detailed descriptions of adult frog from Hong Kong and Taiwan population. Kuraishi et al (2013) discussed the taxonomic status of this member of the *Polypedates leucomystax* group. Pan et al. (2013) provided a phylogeny reconstruction of the *Polypedates leucomystax* that suggested that this species extends to Yunnan, Sichuan, Guangxi, Guangdong, Fujian, Jiangxi, Zhejiang, and Anhui. Zug (2013) provided a brief discussion of the introduced Guam population. Jiang law & Chou (2015) reported from Yuchih, Taiwan. Rahman et al. (2020) genetically confirmed from northern Myanmar and Xizang. Lalmuansanga (2020) reported as a new country report from Mizoram. Che et al. (2020) provided a detailed account for Tibet, China. Yuan et al. (2021) provided a genetically-confirmed locality from Phu Hin Rong, Phitsanulok, north-central Thailand. Zug (2022) briefly discussed habitat, range, and identification in Myanmar. Siammawii et al. (2023) reported colour changing behaviour in *P. braueri*. Kohler et al. (2024) provided new records from Jiangsu, China, as well as a dot map for the species range.

MATERIALS AND METHODS

Distribution

Extensive survey and collection were conducted during the years 2021, 2022 and 2023 at 25 localities located in 9 Districts of Mizoram during both day and night by Visual Encounter Survey (VES) and Acoustic Encounter Survey (AES). These surveyed sites ranged from an altitude between 80–1429 m asl (Table 1.1; Fig. 1.2–1.8). GPS coordinates were taken using Garmin Etrex 10 (Fig. 1.11). Distribution map were constructed using QGIS Version 3.10.8.

Collection of specimens

The specimens were collected using hands and nets. Identification of the specimen was based on characters given by Vogt (1911) and Kuraishi et al. (2011). We euthanized the live specimens using MS-222 following the standardized protocol of Conroy et al. (2009), preserved in 70% ethanol, catalogued and deposited in the Departmental Museum of Zoology, Mizoram University **(MZMU)**, Aizawl (Fig. 1.9).

Morphometric measurements

Morphometric measurements were taken using Dial calliper (Mitutoyo 505-507) accurate to 0.01 mm (Fig. 1.10) following Yang and Poyarkov (2021) and Garg et al. (2021). We examined a total of 64 individuals, 5 juvenile (2 males and 3 females), 37 males and 22 females.

Abbreviations used for morphometric parameters:

SVL: Snout-vent length;

HW: Head width;

HL: Head length;

IN: Internasal space;

SL: Distance from the front of the eye to the tip of the snout;

SN: Distance from the nostril to the tip of the snout;

IUE: Minimum distance between upper eyelids;

HAL: Hand lenth (from the base of the outer palmer tubercle to the tip of the toe);

FLL: Forelimb length (from the elbow to the base of the outer tubercle);

TFL: Third Finger length(from the base of the first subarticular tubercle);

FL: Femur length (from vent to knee);

TL: Tibia length;

TFOL: Length of the tarsus and foot (from the heel to tip of toe IV);

FOL: Foot length (from the base of the inner metatarsal tubercle to the tip of the toe);

TRL: Trunk length (from the underarm to groin region)

In the analysis, we included only the adult specimen. Data analysis were done using Graphpad prism ver. 10 And IBM SPSS Ver. 25

RESULTS

Distribution

A brief description of the survey areas and sites of collections of *Polypedates braueri*

A significant part of the Indo-Myanmar biodiversity hotspot, Mizoram is situated in northeastern India and is home to a wide variety of wildlife and plants. It lies in the latitude range of 21°56' N to 24°31' N and the longitude range of 92°16' E to 93°26' E. Its location is strategically important both politically and physically, since it is sandwiched between Bangladesh and Myanmar, with a common international boundary of around 585 kilometers. Mizoram is 21,081 square kilometers in total size. The Cachar district of Assam and the state of Manipur border it on the north, the Chin Hills of Myanmar border it on the east and south, and the Chittagong Hill tracts of Bangladesh and the state of Tripura border it on the west.. The state is divided into eleven administrative divisions/districts – Aizawl district, Champhai district, Kolasib district, Lawngtlai district, Lunglei district, Mamit district, Saiha district, Serchhip district, Hnahthial district, Saitual district and Khawzawl district. Mizoram's vegetation consists primarily of tropical evergreen and semi-evergreen forests, with grasslands spanning considerable regions.

From 25 surveyed sites, *Polypedates braueri* was collected from 15 different localities, located in 8 Districts, namely Damparengpui (23.711596°N 92.424412°E), Lallen (23.612109°N 92.455065°E), Reiek (23.672313°N 92.604645°E), Mamit District; Sihzau (23.466658°N 93.385358°E), Champhai (23.455156°N 93.360233°E), Murlen National Park (23.658700°N 93.284391°E), Champhai District; Sailam Community Reserved Forest (23.3497 °N, 92.7981°E), Hmuifang Community Reserved Forest (23.454040°N 92.757425°E), Sihphir (23.81536°N 92.74953°E), Aizawl District; Sialhawk (23.293368°N 93.102044°E), Khawzawl District; Zobawk (22.852062°N 92.821195°E), Lunglei Ditrict; Lungdai (23.862725°N 92.743827°E), Pualreng Wildlife Sanctuary (24.193622°N 92.809551°E), Kolasib District; Chakhei (22.288333°N 93.136666°E), Siaha District, and Tamdil National Wetland (23.737682°N 92.952983°E), Sailtual

District. The elevation ranged between 312–1429 m asl. According to Singh et al. (2002), the forest type can be classified into three types Bamboo Forest (tropical evergreen and sub-tropical mixed deciduous forests), Montane sub-tropical forests and Tropical wet evergreen and semi-evergreen forests as given in the table below (Table 1.2; Fig. 1.12).

64 individuals were collected from 15 different study areas. The details of the collection sites of the study frog species were given below:

1. Damparengpui (23.711596°N 92.424412°E) is a Village in West Phaileng Block in Mamit District of Mizoram State, India. It is located 29 KM towards South from District head quarters Mamit. 12 km from West Phaileng. 36 KM from State capital Aizawl. A thorough survey was conducted in this area, three males and one female *Polypedates braueri* were collected from the grasses and vegetation. individuals were found among leaf litters and grasses close to water body.
2. Lallen village is located in West Phaileng subdivision of Mamit district in Mizoram, India. It is situated 14 km away from sub-district headquarter W. Phaileng and 58 km away from district headquarter Mamit. On 24th March, 2021 c.a 1650 h, two males were found among grasses, branch of trees close to water body.
3. Reiek is a mountain and tourist spot 29 km from Aizawl, Mizoram, India. It rests at an elevation of 1,465 metres overlooking Aizawl and offers a view of the surrounding valleys and hills. On a clear day, the plains of Bangladesh can be seen from the top of the hill. Reiek Hill is surrounded by thick lush green temperate trees and bushes.Three males were found sitting beside rock pools and among grasses.
4. Sihzau, Zotlang is a fish farming area only about 9 km from Champhai. Two males and one female were found hiding among the vegetations, leaf litters, and pebbles close to water body.
5. Champhai is the third largest town in Mizoram. Unlike most urban places in Mizoram, Champhai is situated in a valley and on top of a small hill. It is

situated at an elevation of approximately 1,678 meters (5,505 feet) above sea level. A single male was encountered among tree trunk on 1st May 2022 c.a 1800 h, and on 9th December, 2021 c.a 1745 h, a single female frog was collected perching on bushes.

6. Murlen National Park is a national park located in the Champhai district Mizoram in India. The size of the park area is 200 km2 (77 sq m). The park is situated about 245 km east of Aizawl, and is close to the Chin Hills. The tropical, semi-evergreen and sub montane Forests of Murlen are home to a rich variety of flora and fauna. A single female individual was found among leaf litters close to water body on 30th April, 2022 c.a 1700 h.
7. Sailam Community Reserved Forest is well known for hotspot of different species of birds located in Sailam village, about 76 kilometers from Aizawl, remotely snuggled inside a virgin forest. The reserved forest is about 20 acres in size. Thirteen males and twelve females of *Polypedates braueri* were found among grasses, branch of trees, leaf litters and in the pond.
8. Hmuifang Community Reserved is a tourist spot about 50 km from Aizawl, Mizoram, India. With an elevation of 1,619 metres, the mountain is covered with forest reserves since all the Mizo Chief's time. Two males and three females were found perching on grasses.
9. Sihphir is one of the biggest villages in Mizoram, located 9 km towards North from District headquarters Aizawl. A single female specimen was found among leaf litters close to water body on 5th October, 2021 c.a 1930 h.
10. Sialhawk is a village in Khawzawl Block in Khawzawl District of Mizoram State, India. It is located 49 km towards south from District headquarters, Khawzawl and c.a. 180 km from Aizawl. On 11th May, 2022 c.a 1630 h, a single female *P. braueri* was found among grasses closed to human settlement.
11. Zobawk is a Village in Lunglei District of Mizoram State, India. It is located 9 km towards East from District headquarters Lunglei. On 5th July, 2021 c.a 1900 h, one male individual was found among pebbles beside fish pond.

12. Lungdai is a Village in Thingdawl Block in Kolasib District of Mizoram State, India. It is located 46 km towards South from District headquarters Kolasib and 20 KM from State capital Aizawl. Eight males and two females were found among grasses and shrubs on the bank of artificial pond.
13. Pualreng Wildlife Sanctuary in Kolasib District covered an area of 50 Sq. Km at an elevation of 750 m asl. It was located 115 km from the state capital Aizawl. It harbour important flora and fauna especially birds and wild animals. On 20 July, 2020 c.a 1740 h, a single male frog was found among leaf litters on roadside of forested area.
14. Tamdil National Wetland, a 4 sq km wetland in Mizoram, India, that's known for its biodiversity and eco-tourism. It is surrounded by natural forests with a composition of indigenous bamboo species, trees of various species. It is located in the Saitual sub-division of Aizawl district, about 85 km from Aizawl city and 10.5 km from Saitual town. On 5th July, 2021 c.a 1730 h, two males *P. braueri* were found settling alongside the bank of a stream.
15. Chakhei or Chakhang is one of the villages/towns in Southern part of Mizoram at an elevation of 888 m asl. It was located 74 KM from District capital Siaha and 347 km from state capital Aizawl. On 25th September, 2024 c.a 1740 h, two male individuals were collected among grasses and bushes close to water body.

Habitat

P. braueri was found to be nocturnal arboreal frog (Fig. 1.13), found in forested area as well as broadly in disturbed habitats with a shrub or tree border. They were also collected from grasslands, among leaf litters, muddy paths, and roadsides of secondary forested area. They can live in natural as well as artificial ponds, running water, streams, creeks, and brooks. (Fig. 1.14–1.17).

Morphology

The diagnostic features of *P. braueri* include:

Body

Head as broad as body; snout blunt anteriorly; nostrils nearer to snout tip than to eye; interorbital space 11/3 times as wide as upper eyelid; tympanum diameter 2/3 of eye diameter; skin smooth, granulated very finely on back, and coarsely on belly and underside of limbs; a narrow, but sharp fold beginning at lower corner of eyelid, extending deeply over upper arm.

Color

Dorsal bright to dark brownish gray with irregular dark markings; ventral yellowish white; numerous dark dots unevenly scattered on the body; transverse bands on limbs fairly developed; dark marking running from the tip of snout to the lateral side below the armpit.

Limbs

Disks in fingers larger than those in toes, but smaller than tympanum; tibiotarsal articulation reaching anterior border of eye; toes up to 3/4 of their length webbed; subarticular tubercles well developed; inner metatarsal tubercle relatively large, oval, and protruded; a very characteristic marking on posterior side of thigh, numerous white/yellow spots bordered by often totally deep black color; first finger is shorter than the second, third finger longest, longer than snout, hind limbs longer than the total length. Foot is provided with inner metatarsal tubercle but outer metatarsal tubercle is absent. First toe smaller than second toe; fourth toe longest; finger and toe are in the formula 1<2<4<3 and 1<2<3<5<4 respectively (Fig. 1.18).

Description:

A medium sized frog, snout-vent length (SVL) of males ranges from 40.30 to 55.36 mm with a mean of 49.3±3.89 mm in males (N=37), and females ranged from 51.10 to 72.60 mm with a mean of 65.57±5.66 mm (N=22) (Table 1.3–1.7). Head length is almost same to head width, snout pointed and snout length about half of head length, nostril closer to tip of snout than to eyes, tympanum is distinct and round and internarial space is smaller than interorbital space. Vomerine teeth small, in oblique series, originating from the anterior border of choanae. Forelimbs moderately long, fingers free and long, tips of fingers dilated into disc shaped and provided with pad's circumferential groove and pad's basal groove.

***Sexual dimorphism*:** Sexual dimorphism was represented by their sizes where females being larger than males.

During herpetofaunal survey in the Sailam Community Reserve Forest, near Sailam Village, Aizawl District, Mizoram. At ca. 1800 h on 23 March 2022, we encountered an adult and a subadult *Polypedates braueri* emerging from vegetation near a seasonal lake in the Community Reserve Forest (23°20'58.92"N, 92°47'53.16"E; elev. 1,370 m asl). When initially encountered, the adult was grayish brown but began changing to a dark yellowish brown after being disturbed. The next day, the nocturnal coloration changed again to light yellowish brown and finally to pale yellowish brown (Fig. 19). The frog was collected and deposited in the Departmental Museum of Zoology (MZMU), Mizoram University (MZMU1998).

Based on the box plot analysis (Fig. 1.20), the morphology between the male and female *P. braueri* showed significant variations ($p<0.05$) in all the compared characters such as Snout Vent Length (SVL), Head Length (HL), Head Width (HW) etc where the female specimens showed higher values in all the parameters. This analysis showed that the sexual dimorphism in this species is evident where the adult females are comparatively larger than the adult males.

The morphological characters, when plot along the first two principal component axes PC1 (92 %) and PC 2 (3 %) showed distinct clustering between the sexes (Fig. 1.22). This proves to show that the morphological variations, when taken for each sex are interdependent to each other, whereby, an increment in the SVL is related to an increase in all measured characters (Fig 1.21).

Based on the box plot analysis (Fig. 1.23), the morphology between the male and female *P. braueri* showed significant variations ($p<0.05$) only in the ration of the compared characters such as Head width (HW)/Head Length (HL) and Trunk length(TRL)/Snout-vent length (SVL), where the female specimens showed higher values in these parameters.

The ratio of the given morphological characters, when plot along the first two principal component axes PC1 (27 %) and PC2 (19 %) showed clustering between the sexes are overlapped (Fig. 1.25). This proves to show that the ratio of given characters like HL/SVL, IN/IUE, IN/SL, FLL/HAL, TFL/HAL, TL/FL, and TFOL/FOL are not enough to predict sexual dimorphism in *Polypedates braueri* unless it is for HW/HL and TRL/ SVL (Fig. 1.24).

DISCUSSION

The present study revealed the current status on the distribution, habitat and morphology of *Polypedates braueri* collected from different parts of Mizoram. From 24 surveyed sites, we collected 64 *Polypedates braueri* from 15 different localities (Eight Districts) of Mizoram. The elevation ranging from 312 m asl which is Damparengpui located in Mamit District to 1429 m asl as Hmuifang Community Reserved Forest, Aizawl District. This observation on the altitudinal distribution of *P. braueri* agreed with the investigation carried out by other authors as given in the table below (Table 1.8). The lowest and highest elevation was reported to be 0 and 2200 m asl respectively from Taiwan, China (Kuraishi et al., 2011; Hsu et al., 2012; Jang-Liaw & Chou, 2015; Yang, 2019).

The wide altitudinal distributional range might be due to physiological, behavioural and morphological plasticity that enables them to survive in different environments (Ultsch et al., 1999).

Individuals of *P. braueri* were observed on tree trunk, grass, leaf litters, vicinity of pond etc. of forested as well as disturbed area as reported by earlier workers (Hsu et al., 2012; Zug, 2013; Lalmuansanga, 2020; Zug, 2022). Many factors have been suggested to influence patterns of species distribution and assemblage composition at specific sites, such as competition (Morin, 1983), predation (Gascon, 1991; Eterovick & Sazima, 2000), morphological and behavioural attributes (Crump, 1974; Toft, 1985).

Our observation reports biggest sizes in both male (SVL= 55.56 mm; MZMU 2538) and female (SVL= 72.60 mm; MZMU 1776) *P. braueri* compared to the existing literatures (Table 1.9).Vogt (1911) reported SVL to be 49 mm of single individual from Taiwan. Kuraishi et al. (2011) reported the size of female ranges from 63–65 mm and male 45–48 mm from China. Muansanga (2020) reported a single female with SVL of 65 mm from Mizoram. Jang-law & Chou (2015) reported 70 mm from Taiwan, China.

Most studies reveal that the size of males is important during the breeding season as in the case of temperate and tropical anurans as large males have more access to females since they are better fighters or are more attractive to females (Wilbur et al., 1978; Ryan, 1980; Berven, 1981; Duellman & Trueb, 1994).

Apart from their size differences between males and females, sexual dimorphism was not observed in *P. braueri*. But from our observations during the breeding season, the amplecting pairs showed that males were darker compared to females. Color plasticity has been described in several rhacophorids (Lillywhite et al. 1998; Kuramoto & Joshy, 2003; Rowley et al., 2011; Poyarkov et al., 2018; Deepak et al., 2019; Pandey et al., 2021), with the responses attributed to stress, reproductive activity, diel period, microhabitat (i.e., background matching), and disturbances by observers (Bennet et al., 1998; Molur & Molur, 2010; Marcus, 2017; Poyarkov et al., 2018; Deepak et al., 2019). Lillywhite et al. (1998) also reported color change in a *Polypedates maculatus*, a congener of *P. braueri* that was light in color (light gray, tan, or yellow) when placed in sunlight and changed to a darker color in shaded areas.

Table 1.1: Survey sites of *Polypedates braueri* at an elevation between 80–1429.

Sl/No	District	Locality	GPS	Elevation (m)
1	Kolasib	Buhchangphai	24.325914°N 92.652408°E	59
2		Pualreng Wildlife Sanctuary	24.193622°N 92.809551°E	617
3		Lungdai	23.862725°N 92.743827°E	1240
4	Mamit	Damparengpui	23.711596°N 92.424412°E	312
5		Teirei	23.691489°N 92.452713°E	265
6		Lallen	23.612109°N 92.455065°E	722
7		Reiek	23.672313°N 92.604645°E	917
8	Saitual	Tamdil National Wetland	23.737682°N 92.952983°E	801
9	Aizawl	Sihfa	23.735406°N 92.969435°E	890
10		Sihphir	23.81536°N 92.74953°E	928
11		Hmuifang Community Reserve Forest	23.454040°N 92.757425°E	1429
12		Sailam Community Reserve Forest	23.3497 °N 92.7981°E	1400
13	Khawzawl	Tlangpui	23.281356°N 93.067133°E	611
14		Sialhawk	23.293368°N 93.102044°E	1314
15	Serchhip	Biate	23.250188°N 93.117349°	1463

Table 1.1: Continued-

16	Champhai	Champhai	23.455156°N 93.360233°E	1369
17		Sihzau	23.466658°N 93.385358°E	1168
18		Murlen National Park	23.658700°N 93.284391°E	1410
19	Lunglei	Putlunga sih	22.950172°N 92.612042°E	86
20		Zobawk	22.852062°N 92.821195°E	1140
21	Siaha	Phura	22.233875°N 92.901426°E	201
22		Palak dil	22.205329°N 92.888561°E	316
23		Khaikhy	22.018049°N 92.919383°E	82
24		Lungpuk	22.059502°N 92.922873°E	92
25		Chakhei	22.288333°N 93.136666°E	1408

Table 1.2: Collection sites of *Polypedates braueri* at an elvation between 312–1429 m asl.

S/N	District	Locality	Elevation (m)	GPS	Forest type (Acc. to Singh et al 2002)
1	Mamit	Damparengpui	312	23.711596°N 92.424412°E	Bamboo Forest (tropical evergreen and sub-tropical mixed deciduous forests)
2		Lallen	722	23.612109°N 92.455065°E	
3		Reiek Community Reserved Forest	917	23.672313°N 92.604645°E	Montane sub-tropical forests
4	Champhai	Sihzau	1168	23.466658°N 93.385358°E	
5		Champhai	1369	23.455156°N 93.360233°E	
6		Murlen National Park	1410	23.658700°N 93.284391°E	
7	Aizawl	Sailam Community Reserved Forest	1400	23.3497 °N 92.7981°E	
8		Hmuifang Community Reserved Forest	1429	23.454040°N 92.757425°E	
9		Sihphir	928	23.81536°N 92.74953°E	
10	Khawzawl	Sialhawk	1314	23.293368°N 93.102044°E	
11	Lunglei	Zobawk	1140	22.852062°N 92.821195°E	
12	Siaha	Chakhei	1408	22.288333°N 93.136666°E	
13	Kolasib	Lungdai	1240	23.862725°N 92.743827°E	
14		Pualreng Wildlife Sanctuary	617	24.193622°N 92.809551°E	Tropical wet evergreen and semi-evergreen forests
15	Saitual	Tamdil National Wetland	801	23.737682°N 92.952983°E	

Table 1.3: Morphometric measurements of *Polypedates braueri* (N=16).

MZMU	2623	2376	1992	2295	2650	729	1991	2261	2435	2453A	2453B	2453C	24253D	2453F	2453I	2453J
Sex	Juvenile	Juvenile	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
Location	Hmuifang	Sihzau	Sailam	Lalllen	Lunglei	Lallen	Sailam	Dampa	Tamdil	Sailam	Sailam	Sailam	Sailam	Sailam	Sailam	Sailam
Date	27/2/21	25/4/21	16/10/20	24/3/21	16/5/21	24/3/21	16/10/20	24/3/21	2/7/21	15/7/21	15/7/21	15/7/21	15/7/21	15/7/21	15/7/21	15/7/21
SVL	24.60	38.80	46.20	45.1	50.18	40.30	51.10	50.80	50.10	52.84	47.50	48.20	54.30	46.70	54.60	54.40
HW	7.80	14.10	15.00	14.8	15.82	14.70	17.10	15.70	15.80	17.84	17.00	15.10	15.70	16.80	16.10	15.90
HL	8.80	15.40	16.80	16.3	18.04	16.20	18.50	17.70	18.00	19.16	18.30	16.00	19.40	18.10	19.70	19.60
IN	2.70	3.60	3.80	4.00	3.84	3.70	4.20	3.80	3.80	4.60	4.00	4.00	4.50	4.30	4.50	4.50
SL	4.40	6.00	6.70	6.60	7.94	6.50	7.90	8.00	7.90	7.10	6.00	6.10	8.00	6.10	8.10	8.00
SN	1.80	2.10	2.40	2.40	2.62	2.30	2.60	2.50	2.60	2.30	2.20	2.20	2.30	2.20	2.50	2.40
IUE	3.20	4.80	5.20	5.30	5.54	5.10	6.10	5.50	5.50	5.60	5.30	5.50	6.30	5.40	6.40	6.30
FLL	5.00	7.80	8.90	9.10	9.64	8.30	9.70	9.60	9.60	10.00	9.20	9.30	10.00	9.00	10.20	10.10
HAL	8.70	12.5	13.10	12.20	14.12	12.60	14.40	14.40	14.10	14.60	12.90	12.80	14.50	14.30	14.50	14.50
TFL	5.00	7.82	8.26	7.80	9.06	7.84	9.24	9.20	9.00	9.30	7.90	7.90	9.40	8.40	9.50	9.50
FL	12.50	20.30	24.00	22.40	23.24	22.40	26.20	24.30	23.20	26.60	23.80	23.90	26.20	23.70	26.40	26.20
TL	13.30	20.60	24.40	23.20	23.82	22.90	26.90	24.50	23.80	26.80	24.60	24.80	27.10	24.30	27.40	27.30
TFOL	16.80	28.40	32.20	29.40	31.66	29.70	35.00	32.30	31.60	33.80	32.70	32.60	33.60	31.50	34.00	33.70
FOL	10.90	17.70	20.20	19.50	19.96	20.10	23.00	20.30	19.90	22.50	21.30	21.20	22.30	20.50	23.10	22.50
TRL	11.02	17.32	22.34	21.80	21.94	18.00	22.58	22.50	21.92	25.60	23.58	22.00	26.16	19.20	26.80	26.14
HL/SVL	0.35	0.39	0.36	0.36	0.38	0.40	0.36	0.34	0.35	0.36	0.38	0.33	0.35	0.38	0.36	0.36
HW/HL	0.88	0.91	0.89	0.90	0.88	0.90	0.92	0.88	0.87	0.93	0.92	0.94	0.80	0.92	0.81	0.81
IN/IUE	0.84	0.75	0.73	0.75	0.68	0.72	0.68	0.69	0.69	0.82	0.75	0.72	0.71	0.79	0.70	0.71
IN/SL	0.61	0.60	0.56	0.60	0.54	0.56	0.54	0.47	0.48	0.64	0.66	0.65	0.56	0.70	0.55	0.56
FLL/HAL	0.57	0.62	0.67	0.74	0.68	0.66	0.67	0.66	0.68	0.68	0.71	0.72	0.68	0.62	0.70	0.69
TFL/HAL	0.57	0.62	0.63	0.63	0.63	0.64	0.64	0.63	0.63	0.63	0.61	0.61	0.64	0.58	0.65	0.65
TL/FL	1.06	1.01	1.01	1.03	1.02	1.04	1.02	1.01	1.02	1.01	1.03	1.037	1.03	1.02	1.03	1.04
FOL/TFOL	0.64	0.62	0.62	0.66	0.62	0.68	0.65	0.62	0.62	0.66	0.65	0.65	0.66	0.65	0.67	0.66
TRL/SVL	0.44	0.44	0.48	0.48	0.43	0.46	0.44	0.44	0.43	0.48	0.49	0.45	0.48	0.41	0.49	0.486

Table 1.4: Morphometric measurements of female *Polypedates braueri* (N=16).

MZMU	2517	3147	2621	3410D	3410E	3410C	3410A	3410B	2953	2956	3145	2303	3149	2913	3531	2852
Sex	Male	Male	male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
Location	Sailam	Lungdai	Dampa	Lungdai	Lungdai	Lungdai	Lungdai	Lungdai	Hmuifang	Reiek	Lungdai	Tamdil	Lungdai	Reiek	Chakhei	Champhai
Date	4/9/21	1/5/23	11/4/21	7/7/23	7/7/23	7/7/23	7/7/23	7/7/23	20/7/21	26/7/21	1/5/23	5/7/21	1/5/23	13/6/21	25/9/24	1/5/22
SVL	50.10	52.06	41.50	47.62	54.50	43.00	44.30	47.92	46.60	50.88	53.50	53.32	46.74	50.94	54.40	50.76
HW	16.80	17.32	14.60	15.64	17.36	15.26	15.58	16.24	16.24	16.46	18.52	17.48	14.58	16.02	17.32	16.04
HL	17.90	18.30	16.20	17.88	18.72	16.44	17.16	17.10	18.10	17.88	19.82	18.62	17.08	17.98	18.70	17.66
IN	4.30	4.56	3.60	4.60	4.00	4.16	4.46	4.36	4.48	4.28	5.10	4.60	4.20	4.40	4.00	4.82
SL	7.40	7.90	5.90	7.46	8.36	7.28	7.76	7.80	7.46	7.60	9.04	8.34	7.54	8.00	8.32	7.46
SN	2.60	3.34	2.20	3.10	2.78	3.48	3.00	2.70	2.90	2.76	3.38	3.26	3.10	2.98	2.74	2.68
IUE	5.30	5.74	5.00	4.98	5.80	4.80	4.72	5.16	5.32	5.36	5.56	5.26	4.84	5.24	5.80	5.00
FLL	9.90	10.50	8.70	10.20	10.86	8.88	9.62	10.40	10.32	10.62	10.84	11.48	9.66	10.02	10.82	9.34
HAL	14.00	16.00	11.80	13.70	16.00	13.66	14.12	14.46	14.56	15.46	15.68	16.24	13.92	14.64	16.20	14.08
TFL	8.10	8.98	7.20	8.20	8.34	7.92	8.00	8.66	8.00	8.20	8.10	7.84	8.06	7.26	8.24	6.66
FL	24.60	25.80	21.90	23.26	25.60	22.78	21.90	23.44	25.00	25.64	26.86	26.00	23.30	26.68	25.62	25.98
TL	25.10	27.52	22.70	23.00	26.60	22.14	22.48	23.24	23.34	25.12	26.58	26.20	22.80	25.00	26.60	24.22
TFOL	33.00	38.20	28.90	31.40	35.86	29.76	29.66	32.52	31.82	34.24	36.20	35.44	30.78	34.88	35.76	33.96
FOL	22.10	24.76	19.00	19.84	22.52	19.40	19.44	21.52	20.94	21.26	23.74	22.26	20.14	21.22	22.50	20.82
TRL	25.46	26.24	19.64	21.68	26.66	19.48	20.32	21.98	21.20	24.24	25.90	26.42	21.80	24.46	26.62	23.88
HL/SVL	0.35	0.35	0.39	0.37	0.34	0.38	0.38	0.35	0.38	0.35	0.37	0.34	0.36	0.35	0.32	0.34
HW/HL	0.93	0.94	0.90	0.87	0.92	0.92	0.91	0.94	0.89	0.92	0.93	0.93	0.85	0.89	0.90	0.91
IN/IUE	0.81	0.79	0.72	0.92	0.68	0.86	0.94	0.84	0.84	0.79	0.91	0.87	0.86	0.83	0.64	0.96
IN/SL	0.58	0.57	0.61	0.61	0.47	0.57	0.57	0.55	0.60	0.56	0.56	0.55	0.55	0.55	0.44	0.64
FLL/HAL	0.71	0.65	0.73	0.74	0.67	0.65	0.68	0.71	0.71	0.68	0.69	0.71	0.69	0.68	0.66	0.66
TFL/HAL	0.57	0.56	0.61	0.59	0.52	0.57	0.56	0.59	0.54	0.53	0.51	0.48	0.57	0.49	0.52	0.47
TL/FL	1.02	1.06	1.03	0.98	1.03	0.97	1.02	0.99	0.93	0.97	0.98	1.01	0.97	0.93	1.00	0.93
FOL/TFOL	0.66	0.64	0.65	0.63	0.62	0.65	0.65	0.66	0.65	0.62	0.65	0.62	0.65	0.61	0.58	0.61
TRL/SVL	0.50	0.50	0.47	0.45	0.48	0.45	0.45	0.45	0.45	0.47	0.48	0.49	0.46	0.48	0.46	0.47

Table 1.5: Morphometric measurements of *Polypedates braueri* (N=16).

MZMU	2622	2378	2260	1998	2890	2688	2453E	2453G	2453H	2518	2613	1031	1776	3146	2954	2687
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female
Location	Hmuifang	Sihzau	Dampa	Sailam	Sailam	Champhai	Sailam	Sailam	Sailam	Sailam	Sihphir	Hmuifang	Pualreng	Lungdai	Hmuifang	Champhai
Date	27/2/21	25/4/21	24/3/21	23/3/23	24/7/21	9/12/21	15/7/21	15/7/21	15/7/21	4/9/21	5/10/21	5/5/16	20/7/20	1/5/23	13/5/21	9/12 /22
SVL	28.80	34.10	39.00	67.40	55.00	53.90	70.40	66.60	69.00	51.10	64.40	69.30	72.60	60.54	68.90	64.46
HW	14.20	11.90	13.80	23.30	16.20	16.10	23.90	22.60	24.20	17.40	23.01	23.70	23.00	21.04	23.63	22.96
HL	10.90	13.20	14.80	23.50	19.80	19.60	25.50	23.00	23.90	18.60	24.50	25.30	24.60	21.62	24.68	24.20
IN	2.70	3.40	3.60	5.40	4.50	4.10	6.10	5.10	5.60	4.70	5.09	5.60	5.70	5.66	5.94	5.96
SL	4.90	5.30	6.10	9.20	8.10	8.00	9.70	9.10	9.60	7.50	9.09	9.40	9.50	9.70	10.26	10.78
SN	2.00	1.70	2.20	3.30	2.40	2.50	3.40	3.20	3.10	2.50	3.10	3.30	3.30	3.68	4.22	4.30
IUE	3.30	4.50	4.90	7.30	6.50	6.30	9.40	7.50	6.50	5.20	7.20	8.90	7.40	6.30	7.50	6.58
FLL	5.80	6.30	8.00	14.80	10.40	9.80	16.10	14.60	16.00	10.20	15.30	15.80	16.30	12.92	15.00	13.90
HAL	9.10	10.80	12.80	22.60	14.70	14.50	22.90	22.30	22.90	14.80	22.02	22.80	23.10	18.86	24.58	20.82
TFL	5.00	6.24	7.56	13.00	8.98	8.22	13.60	12.80	13.50	8.40	12.70	12.30	13.46	11.56	13.06	13.20
FL	13.90	17.30	20.80	35.30	26.70	24.70	37.50	35.60	37.30	24.70	37.00	35.80	36.40	30.72	37.36	35.08
TL	14.30	17.60	21.30	36.40	27.80	27.40	40.10	36.00	38.60	25.20	38.00	36.70	36.80	31.20	37.38	35.80
TFOL	18.80	21.70	28.70	48.40	34.30	32.60	50.02	48.30	49.80	33.70	51.10	48.90	49.30	41.36	50.58	49.38
FOL	12.30	14.50	18.00	31.10	23.50	20.50	32.70	30.70	32.00	22.70	34.50	32.10	32.90	26.80	30.60	31.76
TRL	12.68	15.38	17.74	33.32	26.76	26.64	36.42	34.76	35.94	24.78	30.66	36.46	37.84	30.44	35.86	35.38
HL/SVL	0.37	0.38	0.37	0.34	0.36	0.36	0.36	0.34	0.34	0.360	0.38	0.36	0.33	0.35	0.35	64.46
HW/HL	1.30	0.90	0.93	0.99	0.81	0.82	0.93	0.98	1.01	0.93	0.93	0.93	0.93	0.97	0.95	22.96
IN/IUE	0.81	0.75	0.73	0.73	0.69	0.65	0.64	0.68	0.86	0.90	0.71	0.62	0.77	0.89	0.79	24.20
IN/SL	0.55	0.64	0.59	0.58	0.55	0.51	0.62	0.56	0.58	0.62	0.55	0.59	0.60	0.58	0.57	5.96
HAL/FLL	1.56	1.71	1.60	1.52	1.41	1.47	1.42	1.52	1.43	1.45	1.40	1.44	1.41	1.45	1.63	10.78
TFL/HAL	0.54	0.57	0.59	0.57	0.61	0.56	0.59	0.57	0.58	0.56	0.67	0.53	0.58	0.61	0.53	4.30
TL/FL	1.02	1.01	1.02	1.03	1.04	1.11	1.06	1.01	1.03	1.02	1.02	1.02	1.01	1.01	1.00	6.58
FOL/TFOL	0.65	0.66	0.62	0.64	0.68	0.62	0.65	0.63	0.64	0.67	0.67	0.65	0.66	0.64	0.60	13.90
TRL/SVL	0.44	0.45	0.45	0.49	0.48	0.49	0.51	0.52	0.52	0.48	0.47	0.52	0.52	0.51	0.52	20.82

Table 1.6: Morphometric measurements of *Polypedates braueri* (N=16).

MZMU	3185C	2955	3185B	3185A	2881	2891	2867	2453K	2884	2731	2538	2863	2914	2377	3531	2269
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female	Male	Male	Male	Male	Male	Male	Male
Location	Sailam	Hmuifang	Sailam	Sailam	Sialhawk	Sailam	Murlen	Sailam	Lungdai	Sailam	Sailam	Sailam	Reiek	Sihzau	Chakhei	Dampa
Date	29/5/23	20/7/21	29/5/23	29/5/23	11/5/22	2/3/22	30/4/22	15/7/21	20/5/22	16/7/22	6/6/22	12/9/22	13/6/21	25/4/21	25/9/24	23/3/21
SVL	67.12	68.78	67.50	63.16	68.86	69.04	64.46	69.62	69.30	46.10	55.36	46.70	47.76	50.58	50.94	53.82
HW	24.08	23.00	24.56	23.40	23.42	24.14	22.96	23.06	23.84	14.36	18.86	14.50	14.52	18.10	17.22	16.00
HL	24.4	23.64	24.96	23.74	24.60	24.00	24.20	23.70	24.82	16.44	21.86	17.22	17.32	19.66	18.52	19.80
IN	5.88	5.92	5.90	5.76	5.98	6.00	5.96	6.00	5.90	3.80	4.68	4.20	4.18	4.82	4.64	4.10
SL	9.82	9.90	10.08	9.70	10.18	10.26	10.78	10.40	11.10	7.24	8.58	7.52	7.54	8.74	7.42	7.94
SN	3.28	3.76	3.50	3.62	3.88	3.28	4.30	3.92	3.96	2.88	3.11	3.32	3.18	3.34	2.46	4.98
IUE	7.16	8.10	7.44	7.16	7.54	6.40	6.58	6.70	7.20	4.62	5.54	4.76	4.98	5.00	5.14	5.68
FLL	14.38	14.74	15.80	15.62	15.20	15.60	13.90	15.02	14.4	9.02	12.80	9.34	9.76	10.98	10.16	10.70
HAL	20.72	22.00	23.26	20.36	24.42	22.44	20.82	21.00	21.00	13.40	16.34	13.20	13.80	15.34	14.64	13.94
TFL	12.58	13.60	13.44	11.20	13.24	13.50	13.20	12.60	12.64	7.68	9.50	8.00	7.88	8.92	8.36	8.30
FL	37.40	37.72	36.88	36.50	37.46	40.06	35.08	37.34	36.62	24.64	30.56	22.98	23.60	26.72	24.68	27.14
TL	37.56	37.74	37.50	36.60	37.54	38.38	35.80	35.36	36.36	24.12	29.36	22.68	25.50	25.02	25.22	27.1
TFOL	46.54	49.16	50.94	48.60	50.58	50.00	49.38	47.20	50.52	31.50	38.00	30.78	25.00	33.12	33.64	31.90
FOL	28.82	34.16	34.42	30.30	30.60	32.70	31.76	29.04	31.72	20.76	25.36	19.86	25.32	23.32	22.66	21.72
TRL	36.34	36.46	36.24	33.54	35.86	39.10	35.38	34.68	36.40	19.40	27.90	21.60	22.04	22.76	24.72	27.1
HL/SVL	0.36	0.34	0.36	0.37	0.35	0.34	0.37	0.34	0.35	0.35	0.39	0.36	0.36	0.38	0.36	0.36
HW/HL	0.98	0.97	0.98	0.98	0.95	1.01	0.94	0.97	0.96	0.87	0.86	0.84	0.83	0.92	0.33	0.81
IN/IUE	0.82	0.73	0.79	0.80	0.79	0.93	0.90	0.89	0.81	0.82	0.84	0.88	0.83	0.96	0.90	0.72
IN/SL	0.59	0.59	0.58	0.59	0.58	0.58	0.55	0.57	0.53	0.52	0.54	0.55	0.55	0.55	0.62	0.51
HAL/FLL	1.44	1.49	1.47	1.30	1.60	1.43	1.49	1.39	1.45	0.67	0.78	0.71	0.71	0.71	1.44	0.76
TFL/HAL	0.61	0.61	0.57	0.55	0.54	0.60	0.63	0.60	0.60	0.57	0.58	0.61	0.57	0.58	0.57	0.59
TL/FL	1.00	1.00	1.01	1.00	1.00	0.95	1.02	0.94	0.99	0.97	0.96	0.98	1.08	0.93	1.02	0.99
FOL/TFOL	0.61	0.69	0.67	0.62	0.60	0.65	0.64	0.61	0.62	0.65	0.66	0.64	1.01	0.70	0.67	0.68
TRL/SVL	0.54	0.53	0.53	0.53	0.52	0.56	0.54	0.49	0.52	0.42	0.50	0.46	0.46	0.44	0.48	0.50

Table 1.7: Range, Mean and Standard deviation of morphometric measurements of *Polypedates braueri.*

Characters	Female (N=22)		Male (N=37)	
	Range (mm)	Mean±SD	Range(mm)	Mean±SD
SVL	51.10–72.60	65.57±5.66	40.30–55.36	49.3±3.89
HW	16.10–24.56	22.41±2.49	14.36–18.86	16.11±1.19
HL	18.60–25.50	23.46±1.88	16.00–21.86	18.02±1.30
IN	4.10–6.10	5.56±0.54	3.60–5.10	4.27±0.34
SL	7.50–11.10	9.58±0.86	5.90–9.04	7.52±0.77
SN	2.40–4.30	3.40±0.51	2.20–4.98	2.82±0.54
IUE	5.20–9.40	7.15±0.89	4.62–6.40	5.35±0.44
FLL	9.80–16.30	14.37±1.89	8.30–12.80	9.91±0.87
HAL	14.50–24.58	21.05±2.91	11.80–16.34	14.21±1.09
TFL	8.22–13.60	12.26±1.64	6.66–9.50	8.32±0.70
FL	24.70–40.06	34.95±4.26	21.90–30.56	24.81±1.85
TL	25.20–40.10	35.44±3.91	22.14–29.36	24.93±1.80
TFOL	32.60–51.10	46.72±5.76	25.00–38.20	32.61±2.61
FOL	20.50–34.50	30.17±3.74	19.00–25.36	21.52±1.66
TRL	24.78–39.10	33.99±3.8	18.00–27.90	23.19±2.62
HL/SVL	0.33–0.38	0.35±0.01	0.33–0.40	0.36±0.01
HW/HL	0.81–1.01	0.95±0.04	0.81–0.94	0.89±0.04
IN/IUE	0.62–0.93	0.78±0.09	0.68–0.96	0.80±0.08
IN/SL	0.51–0.62	0.58±0.02	0.47–0.70	0.57±0.05
FLL/HAL	1.30–1.63	1.46±0.06	0.62–0.78	0.69±0.03
TFL/HAL	0.53–0.63	0.58±0.02	0.47–0.65	0.58±0.04
TL/FL	0.94–1.11	1.01±0.03	0.93–1.08	1.01±0.03
FOL/TFOL	0.60–0.69	0.64±0.02	0.61–1.01	0.66±0.06
TRL/SVL	0.47–0.56	0.51±0.02	0.41–0.51	0.46±0.02

Table 1.8: Comparison of the altitudinal rang of *Polypedates braueri* with the existing literature.

Locality	Elevation (m asl)	References
Mizoram	312–1429	This study
Reiek, Mizoram	1200	Lalmuansanga (2020)
Sichuan, China	502	Huang et al. (2019)
Lao cai, Vietnam	80	Frost (2025)
Myanmar	212–446	Rahman et al. (2020)
Guam	45	Zug (2013)
China	11–1640	Pan et al. (2013)
(Medog)Tibet, China	1931	Che et al. (2020)
Thailand	51–1167	Yuan et al. (2021)
Taiwan, China	0–2200	Kuraishi et al. (2011); Hsu et al. (2012); Jang-Liaw & Chou2(015); Yang (2019)

Table 1.9: Comparision of the size of *Polypedates braueri* from this study with existing literatures.

S/N	SVL (mm)	Locality	Reference
1	51.10-72.60 (F) 41.30-55.36 (M)	Mizoram	This Study
2	49	Taiwan	Vogt (1911)
3	63-65 (F) 45-48 (M)	Taiwan, China	Kuraishi et al. (2011)
4	70	Taiwan	Jang-law & Chou (2015)
5	65 (F)	Mizoram	Lalmuansanga (2020)

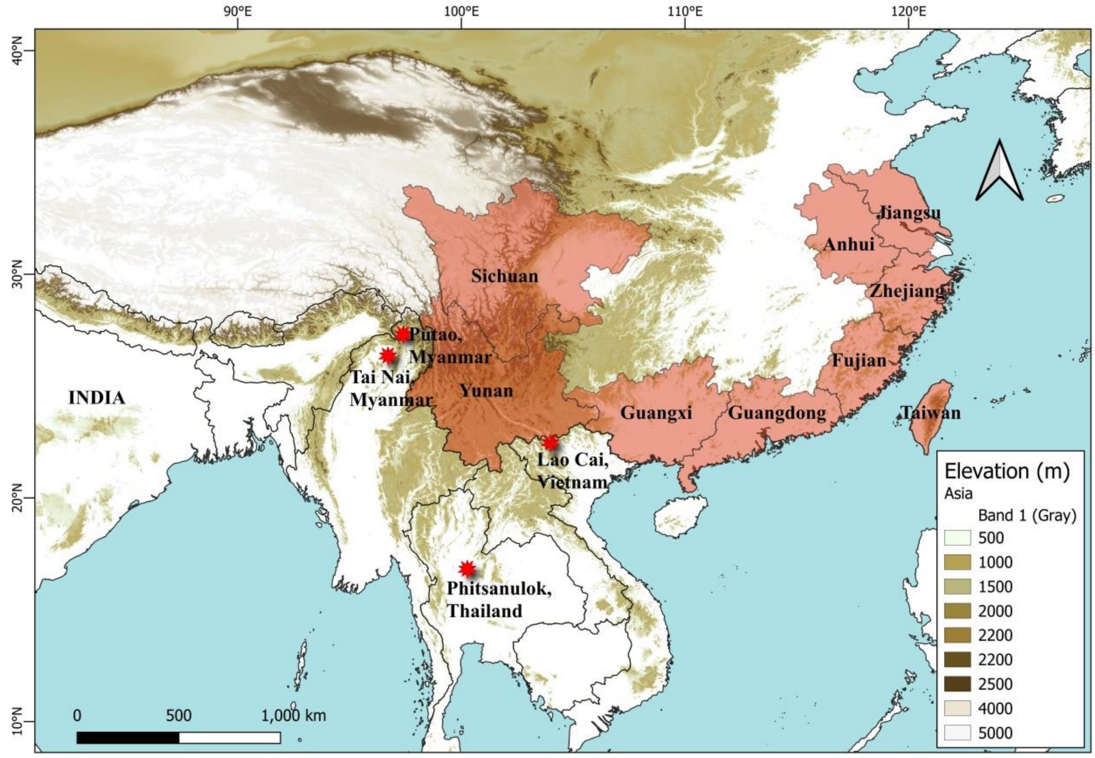


Figure 1.1: Distriution of *Polypedates braueri.* The red mark on the map represent the distribution of *Polypedates braueri.* Taiwan, tropical and subtropical China, Thailand, Myanmar, Northeast India and Guam.

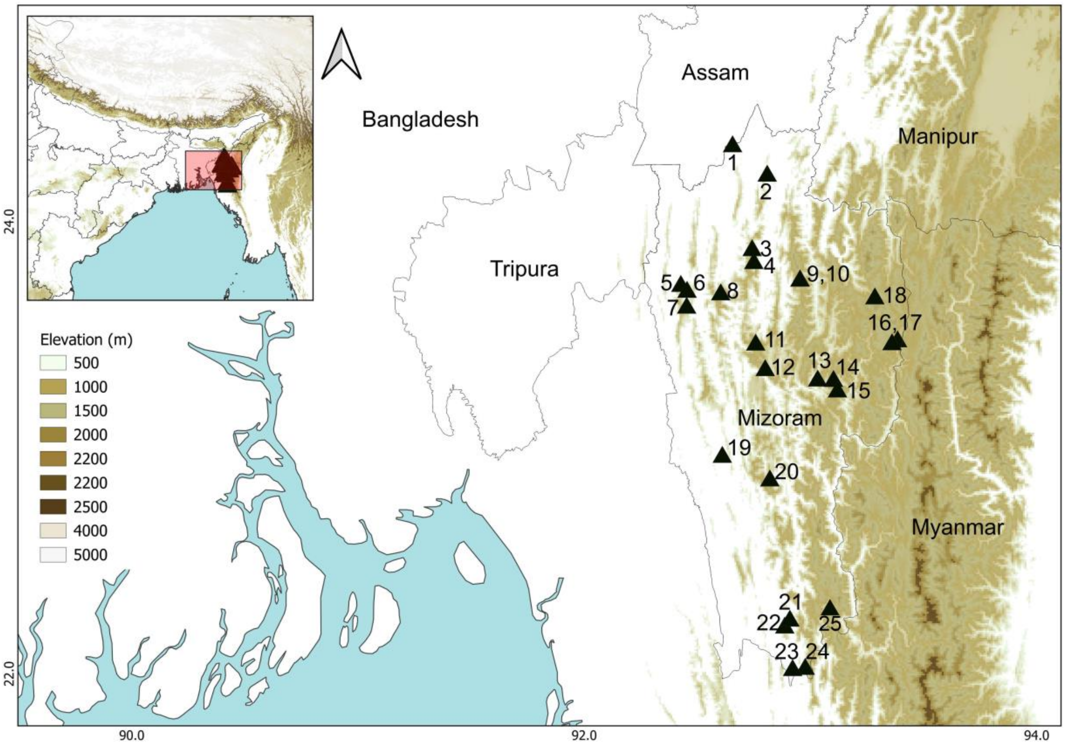


Figure 1.2: Survey sites of *Polypedates braueri* 1. Buhchangphai, 2. Pualreng, 3. Lungdai, 4. Damparengpui, 5. Teirei, 6. Lallen, 7. Reiek, 8. Tamdil National Wetland, 9. Sihfa, 10. Sihphir, 11. Hmuifang Community Reserved Forest, 12. Sailam Community Reserved Forest, 13. Tlangpui, 14. Sialhawk, 15. Biate, 16. Champhai, 17. Sihzau, 18. Murlen National Park, 19. Putlunga sih, 20. Zobawk, 21. Phura, 22. Palak dil, 23. Khaikhy, 24. Lungpuk and 25.Chakhei.

Figure 1.3: Survey site of *Polypedates braueri* at Damparengpui, Mamit District.

Figure 1.4: Survey site of *Polypedates braueri* at Tamdil National Wetland, Saitual District.

Figure 1.5: Survey site of *Polypedates braueri* at Sailam Community Reserved Forest, Aizawl District.

Figure 1.6: Survey site of *Polypedates braueri* at Reiek Community Reserved Forest, Mamit District.

Figure 1.7: Survey site of *Polypedates braueri* at Phura, Siaha District.

Figure 1.8: Survey site of *Polypedates braueri* at Buhchangphai, Kolasib District.

Figure 1.9: Collected specimens of *Polypedates braueri* deposited in Departmental Museum of Zoology, Mizoram University.

Figure 1.10: Dial calliper (Mitutoyo 505-507) accurate to 0.01 mm.

Figure 1.11: Garmin Etrex 10.

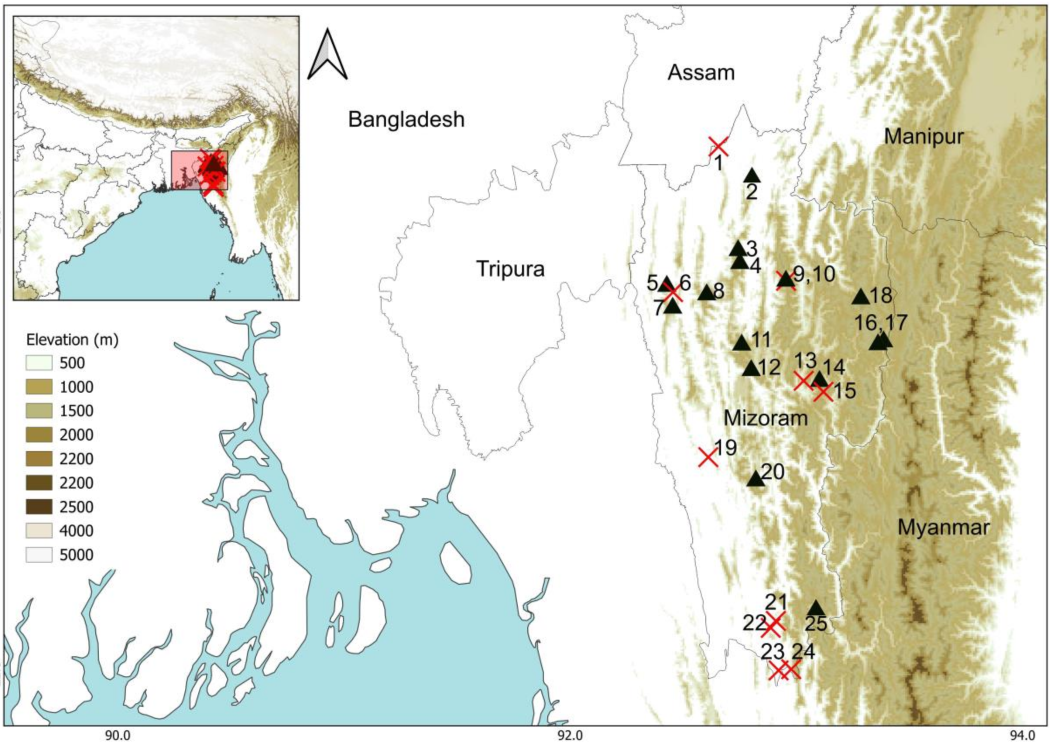


Figure 1.12: Mizoram map showing collection sites of *Polypedates braueri*. The black triangles represent the collected sites of the specimens. 2. Pualreng, 3. Lungdai, 4. Damparengpui, 6. Lallen, 7. Reiek, 8. Tamdil National Wetland, Sihphir, 11. Hmuifang Community Reserved Forest, 12. Sailam Community Reserved Forest, 14. Sialhawk, 16. Champhai, 17. Sihzau, 18. Murlen National Park, 20. Zobawk, and 25.Chakhei. The red marks represent the surveyed sites where specimens were not found. 1. Buhchangphai, 5. Teirei, 9. Sihfa, 10. 13. Tlangpui, 15. Biate, 19. Putlunga sih, 21. Phura, 22. Palak dil, 23. Khaikhy, and 24. Lungpuk 25. Chakhei.

Figure 1.13: *Polypedates braueri* calling from branch of shrubs c.a 1.5 m above the ground.

Figure 1.14: Habitat of *Polypedates braueri* at Sailam Community Reserved Forest, Aizawl District.

Figure 1.15: Habitat of *Polypedates braueri* at Reiek Community Reserved Forest, Mamit District.

Figure 1.15: Habitat of *Polypedates braueri* at Reiek Community Reserved Forest, Mamit District.

Figure 1.16: Habitat of *Polypedates braueri* at Lungdai, Kolasib District.

Figure 1.17: Habitat of *Polypedates braueri* at Dampa Tiger Reserve (Damparengpui), Mamit District.

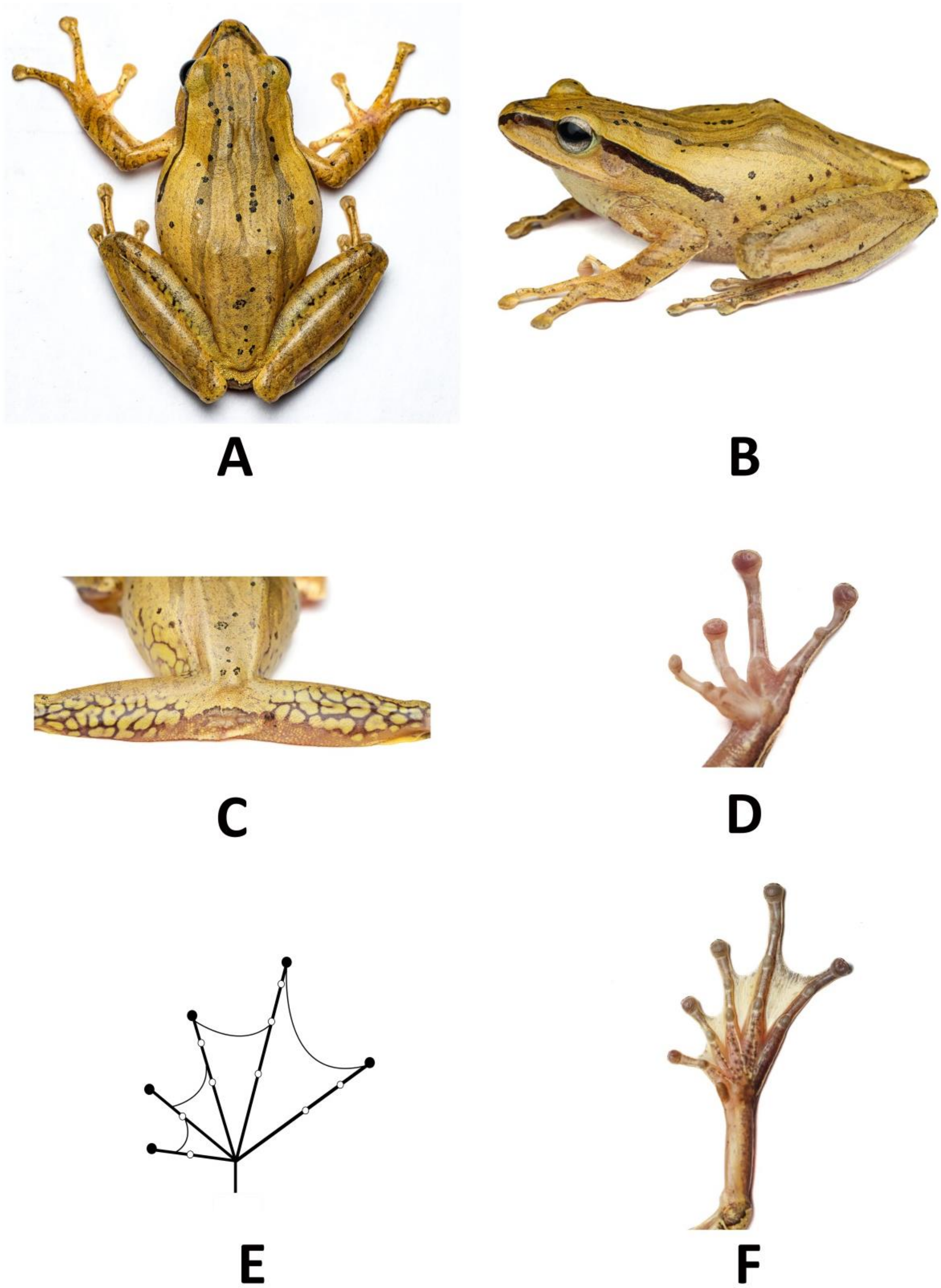


Figure 1.18: Morphology of *Polypedates braueri.* (A) Dorsal view (B) Lateral view (C) distinguishing marks on the posterior hindlimbs (D) Finger formula 1<2<4< 3 (E, F) Toe formula 1<2<3<5<4.

Figure 1.19: Color changes in an Upland Treefrog (*Polypedates braueri*) from Sailam Village, Mizoram, India: Grayish brown at the time of the initial evening encounter (A), dark yellowish brown after being disturbed by njkm observers (B), and light yellowish brown (C) and pale yellowish brown (D) the next day.

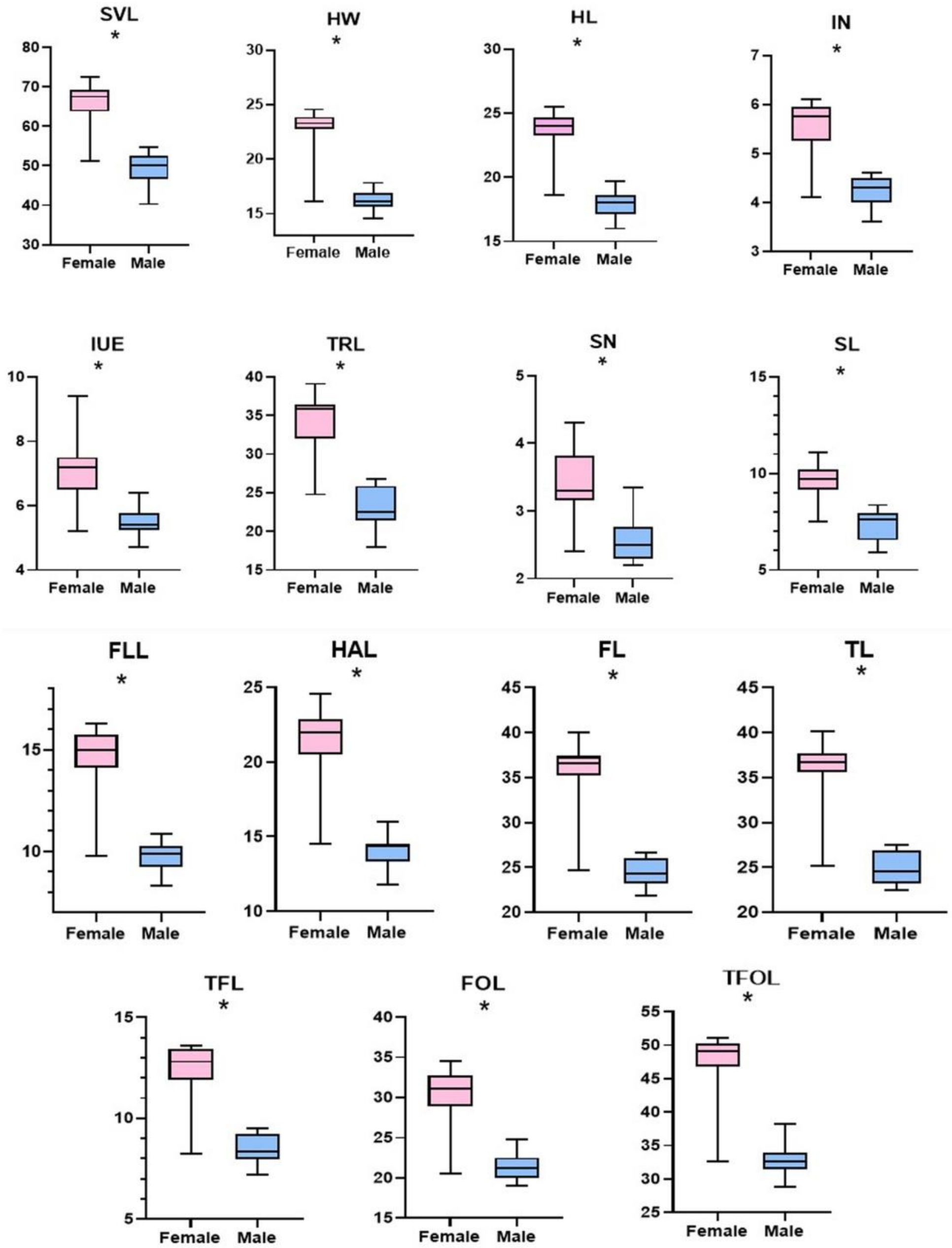


Figure 1.20: Boxplot diagram showing sexual dimorphism among the given characteristics in *Polypedates braueri* ($p<0.05$) (* indicates $p<0.001$).

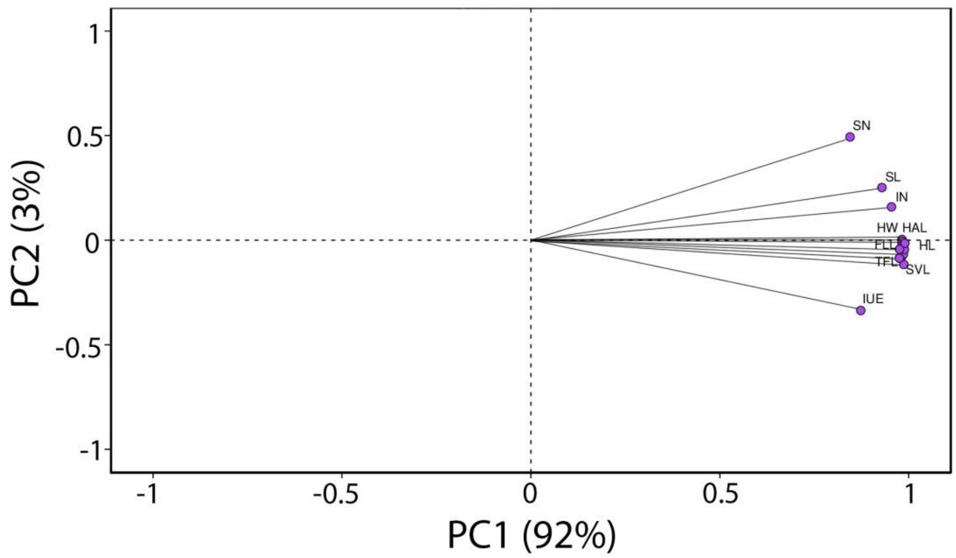


Figure 1.21: Fig: PCA loading plot showing the distribution of the analysed morphological characters along the first two principle components.

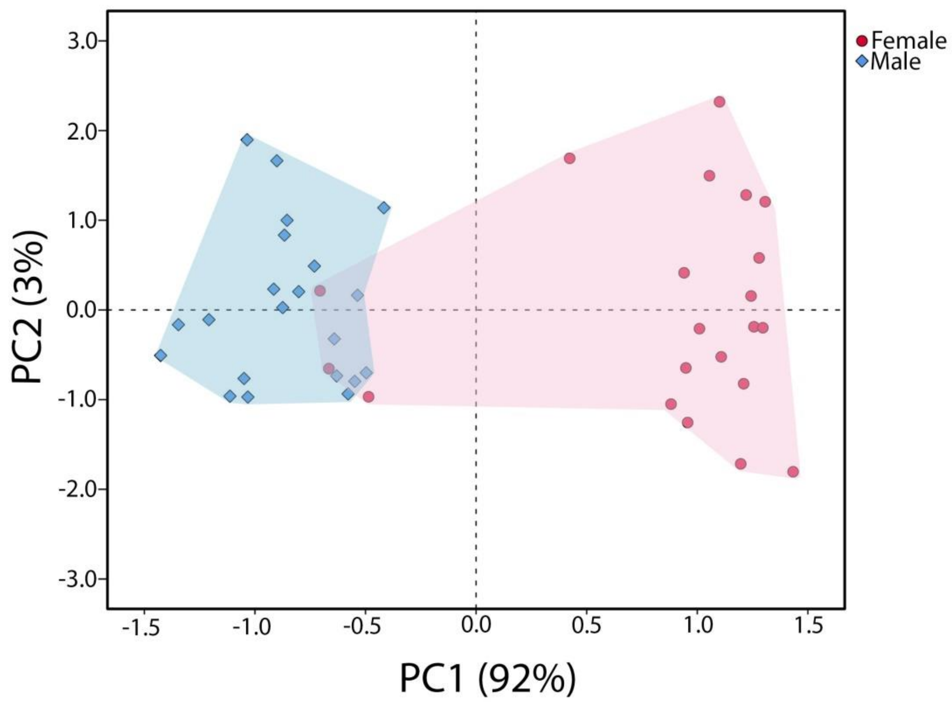


Figure 1.22: PCA score plot of *Polypedates braueri* along the first two components based on the standardised meristics including SVL, HW, HL, IN, IUE, SL, SN, TFL, FLL, HAL, TL, FL, FOL, TFOL and TRL.

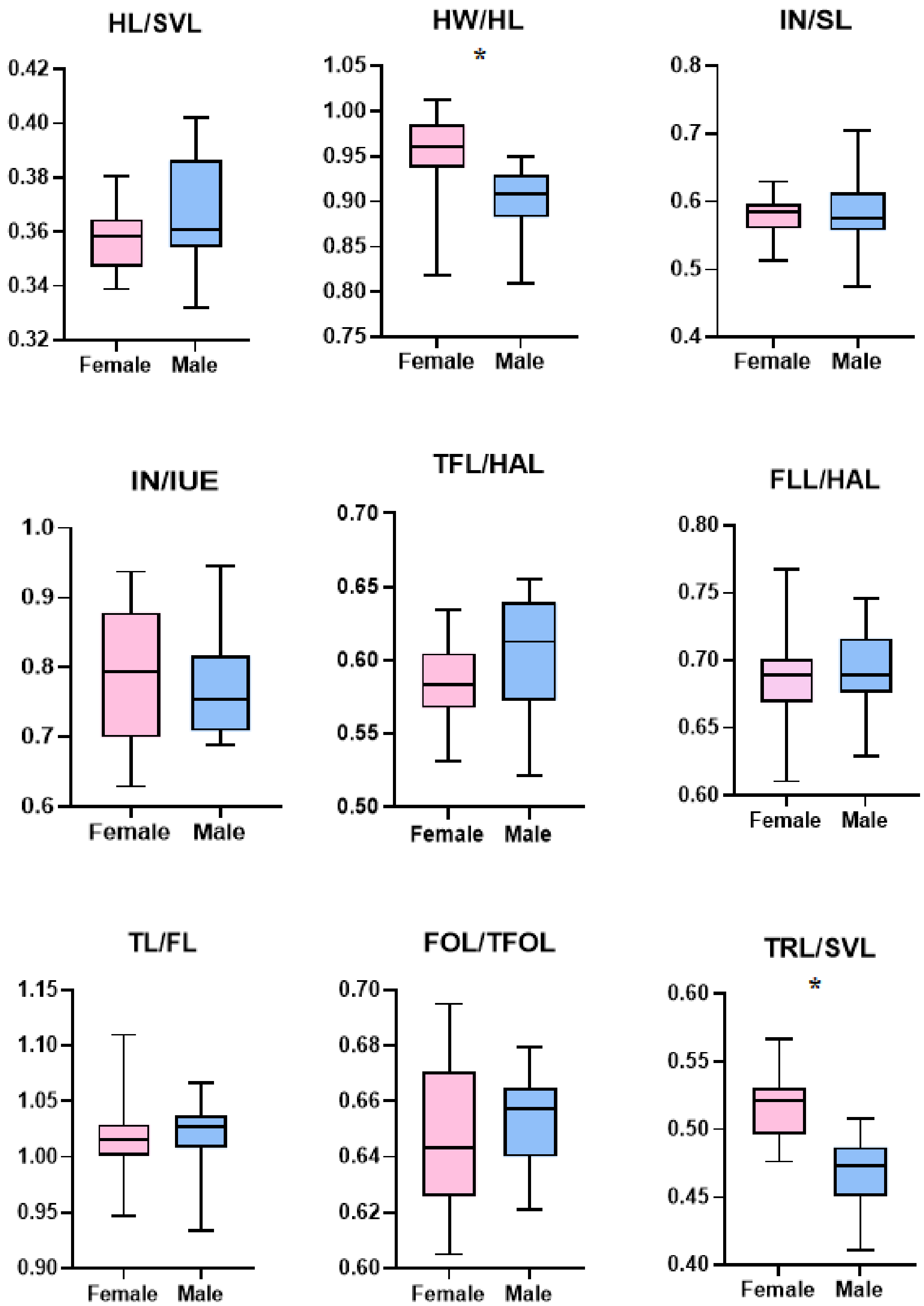


Figure 1.23: Boxplot showing ratio of the given characteristics in *Polypedates braueri* (* indicates p<0.001).

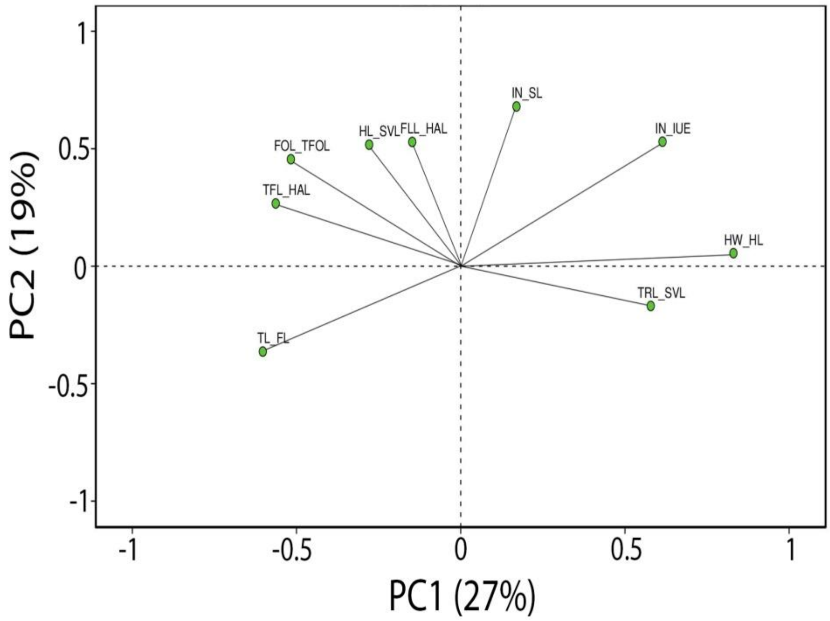


Figure 1.24: PCA loading plot showing the distribution of the analysed variables along the first two principle components.

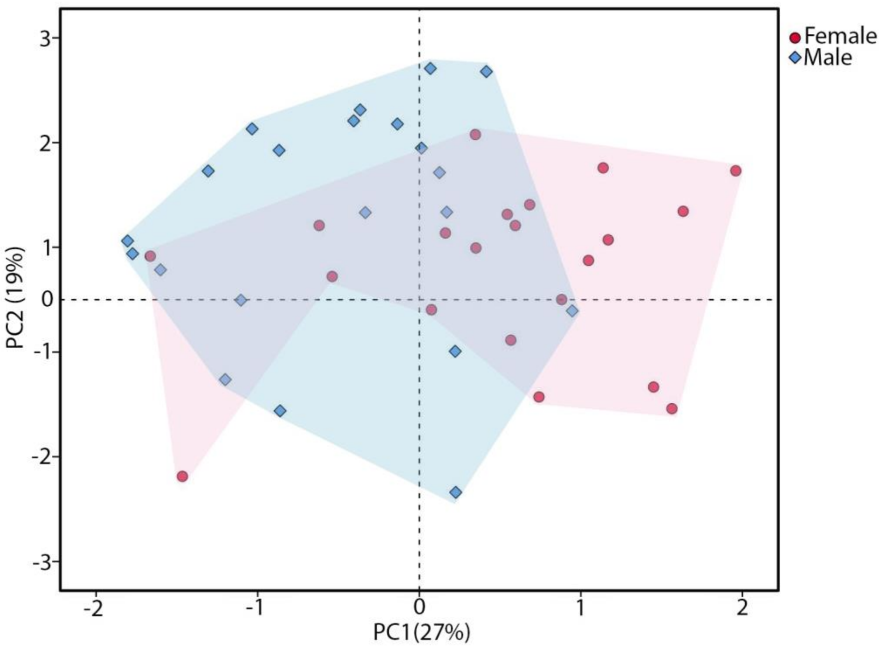


Figure 1.25: PCA score plot of *Polypedates braueri* along the first two components based on the standardised meristics including HL/SVL, HW/HL, IN/IUE, IN/SL, FLL/HAL, TFL/HAL, TL/FL, TFOL/FOL, TRL/SVL.

CHAPTER II

TO ANALYSE THE PHYLOGENETIC POSITION AND GENETIC DIVERGENCE USING PARTIAL 16S RDNA

INTRODUCTION

The ability to recognize species is a key component of biological science and has important implications for conservation and ecology (Pan et al., 2013). It can be challenging and lead to misidentifications to identify species solely by looking at physical characteristics (Stuart et al., 2006). Molecular taxonomy and phylogeny play a major role in modern amphibian taxonomy (Frost et al., 2006). Integrated taxonomic methods have been effectively used recently to address the difficulties related to species identification (Phuge et al., 2020). Khatiwada et al. (2017) highlighted that cryptic frogs with high morphological similarity further create uncertainty in species identification, especially in the field. With the advancement of molecular technology in taxonomic research, identifying cryptic taxa has become easier (Taylor & Harris, 2012). Recently, molecular phylogenetics has become requisite tool for the identification of species origins, demographic changes, species migration patterns and conservation (Yang & Rannala, 2012). The continuing description of new amphibians is the result of the introduction of molecular methods in the taxonomic field (Khatiwada et al., 2017; Khatiwada et al., 2019; Pyron & Beamer, 2022; Saikia et al., 2023; Miralles et al., 2023; Vences et al., 2024; Purser et al., 2025). Extracted DNA amplification by polymerase chain reaction (PCR) is an extremely sensitive and specific tool which has been used for molecular analysis of amphibians to identify taxon (Harris, 2001; Jayawardena et al., 2017, Al-Qahtani & Amer, 2019). Farnsworth et al. (2013) highlighted that correct identification and delimitation of organisms is the first important step in documenting, managing and sustaining biodiversity and can be aided by DNA barcoding approaches. For amphibians, the mitochondrial gene, 16S ribosomal RNA (16S rRNA) has been used successfully for species identification and for detection of cryptic species (Vences et al., 2005; Hasan et al., 2012; Rockney et al., 2015; Trofimets et al., 2024). Various authors used 16S rRNA to examine the evolutionary connections among species, which is widely accepted as a suitable phylogenetic marker for resolving vertebrate relationships (Nakahara et al., 2004, Al-Qahtani and Amer, 2019, Metallinou et al., 2012; Katiwada et al., 2021; Siammawii et al., 2022; Alrefaei et al., 2022; Siammawii et al., 2023a; Naveen et al., 2024). This gene has highly conserved and reliable molecular markers that have evolved slowly and are functionally preserved (Eardly & Van Berkum, 2005). DNA barcoding is an important tool for identification of organisms when dealing with cryptic species (Floyd et al. 2002). Phylogenetic trees are important tools for organizing knowledge of biological diversity, and they communicate hypothesized evolutionary relationships among nested groups of taxa (monophyletic groups) that are supported by shared traits (Novick and Catley, 2007). However, species taxonomy using molecular methods in Northeast India is greatly lacking (Siammawii et al., 2022; Siammawii et al., 2023b; Naveen et al., 2024).

Matsui et al. (1986) stated that the genus *Polypedates* sp. is a complex species which has been thought to include many cryptic species but is notoriously difficult to classify. According to Fei et al. (2009), *P. leucomystax* complex are the most common and most widely distributed species group among Asian rhacophorids, occurring from Nepal through Southeast Asia and continental China to Taiwan. In addition, they were also occured in several islands of the Ryukyus, Japan, and Guam as a result of recent unintentional introductions (Christy et al. 2007; Kuraishi et al. 2009). Grosjean et al. (2008) distinguished *Polypedates* from *Rhacophorus* and found them to be sister taxa. The *P. leucomystax* complex: *P. braueri*, *P. megacephalus*, *P. mutus*, *P. impresus*, and *P. leucomystax* are a group notoriously difficult to identify because of their very similar color pattern and body size (Kuraishi et al., 2012). Brown et al. (2010) studied the range extension of *P. leucomystax* than in the past in Southeast Asia and provided the evidence of recent human-mediated range expansion, using mitochondrial gene sequences (16S rDNA) from samples of geographically widespread tree frogs in Southeast Asia, including *P. leucomystax* and its close relatives. Kuraishi et al. (2011) was the first to genetically confirmed and removed *P. braueri* from *P. megacephalus* from Taiwan. Furthermore, Kuraishi et al. (2012) reported on the molecular phylogenetics of the *Polypedates leucomystax* complex. Blair et al. (2013) investigated the evolutionary history of the *P. leucomystax* complex from Vietnam and Laos and tested the hypotheses pertaining to climate, geology, and dispersal patterns.

REVIEW OF LITERATURE

Grosjean et al. (2008) reported on larval and molecular characters that serve to distinguish *Polypedates* from *Rhacophorus* and found them to be sister taxa. On the basis of a somewhat larger dataset Li et al. (2008) found *Polypedates* (including *Taruga*) to be the sister taxon of *Feihyla* and *Rhacophorus*. Pyron & Wiens (2011) confirmed this result and provided a tree of exemplar species. Kuraishi et al. (2012) reported on the molecular phylogenetics of the *Polypedates leucomystax* complex. Li et al. (2013) confirmed *Polypedates* as the sister taxon of *Taruga* and recovered substantial phylogenetic structure within their relatively dense taxon sampling. Sutthiwisesa et al. (2020) reported the molecular identification of the morphologically cryptic Asian common treefrogs (Anura: Rhacophoridae, *Polypedates leucomystax* complex) in Thailand. Yuan et al. (2021) reported on molecular phylogenetics with a special focus on geographical range evolution of the genus *Polypedates* (Anura: Rhacophoridae) from the Oligocene to present.

Kuraishi et al. (2011) genetically confirmed *P. braueri* and removed from *P. megacephalus* from Taiwan population. They present molecular, acoustic, and morphological evidence for the heterospecific relationships of the populations from Taiwan and Hong Kong. Pan et al. (2013) provided a phylogeny reconstruction of the *Polypedates leucomystax* that suggested that this species extends to Yunnan, Sichuan, Guangxi, Guangdong, Fujian, Jiangxi, Zhejiang, and Anhui. Zug (2013) provided a brief discussion of the introduced Guam population. Huang et al (2019) reported on the complete mitochondrial genome. Rahman et al. (2020) genetically confirmed from northern Myanmar and Xizang. Lalronunga et al. (2020), provided genetically confirmed from Mizoram, northeastern India. Che et al. (2020) provided a detailed account including genomics for Tibet, China. Yuan et al. (2021) provided a genetically-confirmed from Phu Hin Rong, Phitsanulok, north-central Thailand.

MATERIALS AND METHODS

Genomic DNA was isolated from 15 individual liver tissues using DNeasy Blood and Tissue Kit (Qiagen™, Valencia, California, USA) following the procedure given by the manufacturer. The partial mitochondrial 16S rRNA was amplified using the primers: forward (L02510 — CGCCTG TTT ATC AAA AAC AT, Palumbi, 1996) and reverse (H03063 — CTC CGG TTT GAACTC AGA TC, Rassmann, 1997). The PCR was performed in a 20 µL reaction mixture containing 2 µL of 1X amplification buffer, 14.8 µL of water, 1 µL of 0.25 mM dNTPs, 0.5 µL of 0.2 pM each of forward and reverse primers, 1µL of extracted DNA (approximately 50 ng/µL), and 0.2 µL of 1U Taq DNA polymerase at the thermal regime of 94˚C for 3 min, followed by 35 cycles of 94˚C for 30 sec, 49˚C for 40 sec, 72˚C for 30 sec, and with a final extension at 72˚C for 5 min. The amplified PCR products were further processed for purification and sequencing using Sanger's dideoxy method at Barcode BioSciences, Bangalore, India. The newly generated DNA sequences were deposited in GenBank repository under accession numbers: PP858626, PV523859, MW757225, MW165467, PP858623, PP858624, PP858619, PP858622, PV523858, PP858625, PP858620, PV523856, PV523857, PP858627 and PP858621.

A dataset was created using the newly generated sequences of *P. braueri* combined with their congener sequences obtained from the NCBI database: *P. braueri* KR827990 from Thailand, OL377829 and OL377860 from China, KR827988 and KR827989 from Vietnam, MN511834, MN511835 and MN511836 from Myanmar, *P. himalayensis* MT983151, *P. teraiensis* (OP741139), *P. mutus* (ON072235), *P. taeniatus* (MT983168), *P. discantus* (KF303483), *P. megacephalus* (MT609234), and *P. pseudocruciger* (OL907107), with *Raorchestes rezakhani* (OQ780545), *Chiromantis doriae* (OQ780555) and *Zhangixalus smaragdinus* (MW658922) as the outgroups (Table 2.1).

The phylogenetic relationship analyses were carried out in PhyloSuite v1.2.3 (Zhang et al., 2020, Xiang et al., 2023). Sequence alignment was performed using MAFFT v7.505 (Katoh & Standley, 2013) using '--auto' strategy and normal alignment mode. Gap sites were then removed with trimAl v1.2rev57 (Capella-Gutiérrez et al., 2009) using "-automated1" command. ModelFinder v2.2.0 (Kalyaanamoorthy et al., 2017) was used to select the best-fit model using BIC criterion.

Maximum likelihood phylogenies were inferred using IQ-TREE v2.2.0 (Nguyen et al., 2015) under the GTR+I+G4+F model for 10000 ultrafast bootstraps (Minh et al., 2013). The phylogenetic tree was annotated using iTOL v6 (Letunic & Bork, 2024). The uncorrected genetic distance was calculated in Mega 11 (Tamura et al., 2021).

RESULTS

The phylogenetic analysis of the *Polypedates* species complex was performed using the partial mitochondrial 16S rRNA. Based on the topology of the maximum likelihood phylogenetic tree (Fig 2.1), the studied population of *P. braueri* from Mizoram, India formed a distinct clade (highlighted in red). The samples studied formed a sister clade with *P. braueri* sequences from Vietnam (KR827988, KR827989), Thailand (KR827990) and China (OL377829, OL377860). Within the studied dataset, a distinct clade consisting of three *P. braueri* (MN511834, MN511836, MN511835) representing populations from Myanmar and one *P. himalayensis* (MT983151) arose which could be an indicator of misidentification as the species of *Polypedates* (within the *P. leucomystax* complex) showed high uniformity to the eye in their morphological features. The uncorrected pairwise genetic distance (k2p distance) was also studied for the dataset (Table 2.2). The population within Mizoram, India showed 0.03% variation. The genetically closest population is represented by a specimen from Thailand (KR827990) with a distance of 0.21% followed by specimens from China (OL377829, OL377860) with 0.25%. Specimens from Vietnam (KR827988, KR827989) showed 0.28% and specimens from Myanmar (MN511834, MN511836, MN511835) showed 0.40% to 0.44%. *P. himalayensis* (MT983151) has the closest genetic distance among its congener (0.47 %) while *P. pseudocruciger* (OL907107) is the farthest (1.07 %).

DISCUSSION

The issue of species delimitation has long been a confusing topic often leading to controversies concerning both the definition of the species category and methods for inferring the boundaries between them. Moreover, the very difinition of a species has yet been a subject of debate and scrutiny, which is reflected by the existence of more than twenty species concepts. From the biological species concept (Mayr, 1942) to the development of the genotypic cluster concept for species (Mallet, 1995) has done nothing but shown the incompatibility as well as the degree of acceptance by contemporary biologists. However, in this thesis, the delimitation of the studied species was performed to fulfil the criteria and the definitive terms of the said concepts. The study on their morphology and ecology (ecological species concept; Van Valen, 1976) has put significantly weight on the demarcation. This coupled with the analysis on their phylogeny (phylogenetic species concept; Cracraft 1983) was accepted to diagnose the studied population of *Polypedates* from Mizoram to be *P. braueri* as well as to distinguish them from *P. teraiensis*, its sympatric congener within the state.

Table 2.1: Accession of *Polypedates sp.* and outgroup species from different localities.

S/N	Species	Accession No.	Locality	References
1	*P. braueri*	PP858626	Lunglei, Mizoram	This study
2	*P. braueri*	PV523859	Pualreng , Mizoram	This study
3	*P. braueri*	MW757225	Champhai, Mizoram	This study
4	*P. braueri*	MW165467	Sihzau, Mizoram	This study
5	*P. braueri*	PP858623	Hmuifang, Mizoram	This study
6	*P. braueri*	PP858624	Lallen, Mizoram	This study
7	*P. braueri*	PP858619	Sialhawk, Mizoram	This study
8	*P. braueri*	PP858622	Sailam, Mizoram	This study
9	*P. braueri*	PV523858	Reiek, Mizoram	This study
10	*P. braueri*	PP858625	Sihphir, Mizoram	This study
11	*P. braueri*	PP858620	Murlen, Mizoram	This study
12	*P. braueri*	PV523856	Chakhei, Mizoram	This study
13	*P. braueri*	PV523857	Tamdil, Mizoram	This study
14	*P. braueri*	PP858627	Lungdai, Mizoram	This study
15	*P. braueri*	PP858621	Dampa, Mizoram	This study
16	*P. braueri*	KR827988	Vietnam	Grosjean et al. (2015)
17	*P. braueri*	KR827989	Vietnam	Grosjean et al. (2015)

Table 2.1: Continued-

18	*P. braueri*	OL377829	China	Goa & Hu (2021)
19	*P. braueri*	OL377860	China	Goa & Hu (2021)
20	*P. braueri*	MN511834	Myanmar	Rahman et al (2020)
21	*P. braueri*	MN511835	Myanmar	Rahman et al (2020)
21	*P. braueri*	MN511836	Myanmar	Rahman et al (2020)
23	*P. himalayensis*	MT983151	Nepal	Khatiwada (2020)
24	*P. teraiensis*	OP741139	Bangladesh	Hossain et al. (Unpub.)
25	*P. mutus*	ON072235	Mizoram	Siammawii et al. (2023a)
26	*P. taeniatus*	MT983168	Nepal	Khatiwada (2020)
27	*P. discantus*	KF303483	Thailand	Kujirawan (2013)
28	*P. megacephalus*	MT609234	Myanmar	Mulcahy et al (2020)
29	*P. pseudocruciger*	OL907107	Kerala	Sandeep et al. (2021)
30	*Raorchestes rezakhani*	OQ780545	Mizoram	Lalremsanga et al. (2020)
31	*Chiromantis doriae*	OQ780555	Mizoram	Lalremsanga et al. (2020)
32	*Zhangixalus smaragdinus*	MW658922	Mizoram	Lalremsanga et al. (2020)

Table 2.2: The uncorrected K2P distances of 16S rRNA of *Polypedates braueri* estimate using the mitochondrial 16S gene of *Polypedates braueri* (PB), *P.himalayensis* (PH), *P. teraiensis* (PT), *P. maculatus* (PM), P. teniatus (PTA), *P.* discuntus (PD), *P. megacephalus* (PME), *P. pseudocruciger* (PP), *Chiromantis doriae* (CD), *Zhangixalus smaragdinus* (ZS), and *Raorchestes rezakhani* (RR). Genbank accession numbers are listed after the names of the taxa.

S/N	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*PB* PP858626																
2	*PB* PV523859	0.000															
3	*PB* MW757225	0.000	0.000														
4	*PB* MW165467	0.000	0.000	0.000													
5	*PB* PP858623	0.000	0.000	0.000	0.000												
6	*PB* PP858624	0.000	0.000	0.000	0.000	0.000											
7	*PB* PP858619	0.000	0.000	0.000	0.000	0.000	0.000										
8	*PB* PP858622	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
9	*PB* PV523858	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000								
10	*PB* PP858625	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000							
11	*PB* PP858620	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
12	*PB* PV523856	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
13	*PB* PV523857	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
14	*PB* PP858627	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003			
15	*PB* PP858621	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000		
16	*PB* KR827990	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.025	0.025	
17	*PB* OL377829	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.028	0.028	0.021

Table 2.2: Continued–

S/N	Species	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
18	*PB* OL377860	0.000															
19	*PB* KR827988	0.024	0.024														
20	*PB* KR827989	0.024	0.024	0.000													
21	*PB* MN511834	0.037	0.037	0.043	0.043												
22	*PB* MN511835	0.050	0.050	0.053	0.053	0.041											
23	*PB* MN511836	0.050	0.050	0.053	0.053	0.041	0.000										
24	*PH* MT983151	0.053	0.053	0.060	0.060	0.044	0.028	0.028									
25	*PT* OP741139	0.063	0.063	0.073	0.073	0.080	0.063	0.063	0.069								
26	*PMU* ON072235	0.070	0.070	0.080	0.080	0.076	0.066	0.066	0.086	0.044							
27	*PTA* MT983168	0.070	0.070	0.080	0.080	0.086	0.070	0.070	0.076	0.006	0.047						
28	*PD* KF303483	0.087	0.087	0.095	0.095	0.080	0.070	0.070	0.094	0.094	0.080	0.094					
29	*PME* MT609234	0.073	0.073	0.083	0.083	0.090	0.083	0.083	0.079	0.047	0.040	0.053	0.108				
30	*PP* OL907107	0.125	0.125	0.121	0.121	0.114	0.103	0.103	0.107	0.129	0.147	0.132	0.121	0.150			
31	*CD* OQ780555	0.215	0.215	0.203	0.203	0.206	0.215	0.215	0.210	0.233	0.225	0.237	0.212	0.245	0.244		
32	*ZS* MW658922	0.245	0.245	0.228	0.228	0.243	0.244	0.244	0.248	0.250	0.241	0.255	0.228	0.262	0.239	0.158	
33	*RZ* OQ780545	0.270	0.270	0.247	0.247	0.268	0.268	0.268	0.282	0.279	0.265	0.274	0.265	0.283	0.255	0.181	0.213

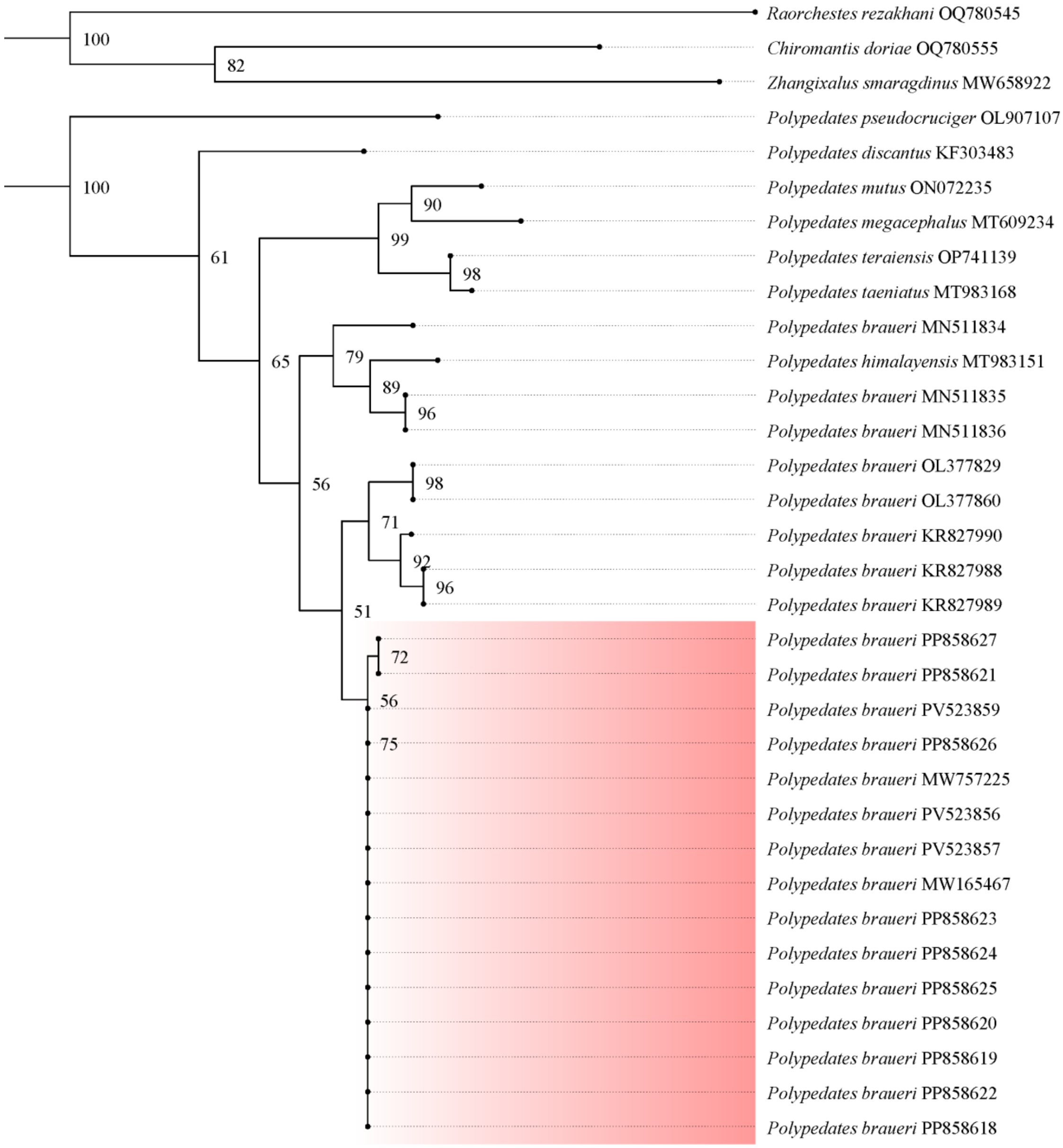


Figure 2.1: Phylogenetic tree of *Polypedates braueri*.

CHAPTER III

TO STUDY THE BREEDING BIOLOGY WITH RESPECT TO ENVIRONMENTAL FACTORS OF *P. braueri*

INTRODUCTION

The breeding biology of anurans is the most diverse among vertebrates and is directly dependent on temperature and the onset of rainfall (Duellman and Trueb, 1994; Humtsoe et al., 2020). Most tropical frogs exhibit seasonality in reproduction where rainfall is one of the major determinants for its timing. Rainfall intensity and timing play a major role in determining the initiation of breeding in anurans (Zug et al., 2001). Humidity exerts a greater effect over temperature and rainfall in the breeding activity of anurans (Roy et al., 2004). Oviposition site selection, along with other factors, such as the fidelity to breeding sites, can have a strong influence on anuran larval development (Resetarits and Wilbur, 1989; Wheeler et al., 2006). With the destruction or modification of habitats, absence of breeding or unsuccessful breeding may occur, which in turn, has the potential to dramatically reduce population fitness and long-term survival (Lind et al., 1996; Wheeler et al., 2006). Modern method like bioacoustics allowed recognizing cryptic species (e.g., Matsui et al., 1986; Narins et al., 1998; Kuraishi et al., 2011). Rhacophorid frogs are known to build foam or gel nests on various substrates to protect their embryos from desiccation, and some of these frogs undergo direct development (Biju, 2009; Khongwir et al., 2016; Rangad et al., 2022).The lack of natural history information for rhacophorids from less-explored regions, such as Northeast India, precludes a better understanding of their biology, ecology, and taxonomy.

Our survey on the existing literatures showed that studies on the breeding behaviour of *Polypedates* in Northeast India have been limited, including only *P. leucomystax* (Iangrai, 2007), P. *teraiensis* (Dubois, 1987) (Lalramdinfeli, 2017; Lalchhanhimi and Lalremsanga, 2017) and *P. himalayensis* (Annandale, 1912) (Rangad et al., 2022). We investigated the breeding behaviour, including vocalization and oviposition, of *P. braueri* in Mizoram, India. Identifying anuran breeding sites and understanding the breeding biology, along with the specific ecological requirements of *P. braueri*, can help promote realistic conservation strategies to ensure the species' long-term survival and the health of the anuran community as a whole.

REVIEW OF LITERATURE

Alcala & Brown (1956) and Alcala (1962) worked on early life history and breeding behaviour of *P. leucomystax* from Philippines. Matsui et al. (1986) worked on the acoustic and karyotypic evidence for specific separation of *Polypedates megacephalu*s from *P. leucomystax* in Taiwan. Narins et al. (1998) reported the morphological, behavioral, and genetic divergence of sympatric morphotypes *P. leucomystax* in peninsular Malaysia. Mohanty and Dutta (1988) and Girish & Saidapur (1999) worked on the breeding biology of *P. maculatus*. Iangrai (2007) studies the ecology, breeding behaviour and metamorphosis of tree frogs *P. leucomystax* and *Rhacophorus bipunctatus* of Meghalaya. Kuraishi et al. (2011) compared call of *P. megacephalus* from Hong Kong and Taiwan population and confirmed to be different species. He concluded that the Taiwan population was *P. braueri* and was genetically confirmed. Lalchhanhimi & Lalremsanga (2017) reported details on the breeding biology of *P. teraiensis* from Mizoram, Northeast India. Gogoi & Sengupta (2017) reported call of male *P. himalayensis, P. leucomystax, P. maculatus* and *P. teraiensis* from different parts of India. Tesia et al. (2017) reported breeding season and clutch size of *P. megacephalus* in Arunachal Pradesh. Liu et al. (2018) compared call characteristics for *P. megacephalus* and *P. mutus* from Meghalaya.Tapley & Girgin (2015) and Galunder & Rodder (2018) reported the clutch size and types of egg deposition of *P. otilophus*. Lalramdinfeli (2020) worked on the comparative study on the breeding and development of *P. teraiensis* and *Zhangixalus smaragdinus* (earlier *Rhacophorus maximus*) of Mizoram. Haas et al. (2022) published guide to the tadpoles of Borneo which summarized the knowledge of habitat, reproduction, larval morphology and coloration of *P. otilophus*. Rangad et al. (2022) worked on a brief account on the breeding biology of *P. himalayensis* from Meghalaya.

P. braueri has been reported to breed from March–September in Taiwan (Yang, 1998; Hsu et al., 2006; Hsu et al., 2012) and from May–September in Mizoram (Muansanga, 2020). Yang (1998) reported the number of eggs. Yang (2020) provided a brief description on female call from Taiwan. Siammawii et al. (2024a) reported a brief study on the breeding biology of from Mizoram, Northeast India.

MATERIALS AND METHODS

Field surveys were conducted during 2021, 2022 and 2023 in Mizoram State, India (Fig. 3.1) at a seasonal pond in Sailam Community Reserved Forest, Aizawl District (23.3497°N, 92.7981°E, elevation 1370 m; Fig. 3.2, 3.4A, B) and in the Reiek Community Reserved Forests of Mamit District (23.6772°N, 92.6030°E, elevation 1229 m; Fig. 3.3, 3.4C, D). The Reiek study site encompasses rain-fed, small, rocky pools surrounded by vegetation. Both study sites are in montane subtropical forest (Singh et al., 2002), with an annual precipitation of 2593 mm at Sailam and 2460 mm at Reiek. The driest period was during November–March and the highest precipitation occurs during June–September (Guhathakurta et al., 2020). During the survey period, rainfall, relative humidity, ambient temperature, and types of flora at the study sites were recorded. Rainfall data was obtained from the State Meteorological Centre, Directorate of Science and Technology, Government of Mizoram. Humidity and air temperature were recorded using a digital thermo-hygrometer (KM 918). Surveys were carried out employing standard audio and visual encounter methods. Searches for frogs and oviposition sites were done from 16:00–02:00 h using dim flashlights and headlamps along the edges of ponds and the surrounding vegetation. The male calls were recorded using handheld recorder (Zoom H4N Pro) and analysed using standard software (Raven Pro 1.6 and Sound Ruler 0.9.6). To verify spawning characteristics, we placed a single pair in amplexus into a water-filled 2000-ml plastic container with a water depth of about 5 cm and ca. 20 cm of air. In order to determine clutch size, 20 egg masses, along with amplecting pairs, were collected and brought to the laboratory. We measured nest diameters and counted the number of eggs per clutch and the SVL of females using Mitutoyo digital callipers. We used Pearson's correlation coefficient to test the possible relationship between SVL of 20 individual females and their respective number of eggs. For histological study, ovaries from the females and testes from the males were excised and immediately fixed in Bouin's solution (24 hours). 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 7-8μ thickness with the help of Microtome, and stained with haematoxylin and counter-stained with eosin. After mounted with DPX, the sections were then observed under the microscope.

RESULTS

During this study, we observed that *Polypedates braueri* was a seasonal breeder and its breeding activity coincides with the southwest monsoon season from April–September in Mizoram.

Breeding season. Following the dry season, monsoon showers in Mizoram begin in April. Spawning was triggered by the onset of the heavier monsoon rains in late April (118 mm and 180 mm precipitation in 2020 and 2021, respectively) and higher air temperatures, with a maximum of 33.5°C at both the study site Reiek and Sailam. Breeding activity continued till mid-September with daily air temperature ranging from 14°C–33.5°C, relative humidity 24–100% and rainfall 73.2–460.2 mm in Sailam Community Reserved Forest (Table 3.1; Fig. 3.5) and Kruskal Wallis H test breeding season against environmental factors (air temperature, relative humidity, rainfall) showed significant in rainfall and min temp where $p<0.005$ (Table 3.2). At the Reiek Community Reserved Forest, daily air temperature ranging from 15°C–33.5°C, relative humidity 52–97% and rainfall 0.0–478.7 mm (Table 3.3; Fig. 3.6) and Kruskal Wallis H test breeding season against environmental factors (air temperature, relative humidity, rainfall) showed significant in rainfall and air-temp where $p<0.005$ (Table 3.4). Peak breeding activity occurred in July and ceased at the end of September. Floating foam nests on the seasonal pond were suspended from overhanging vegetation and floating on the water, moving with water level changes. Despite the availability of water in the study sites, breeding activity was ceased by the end of September. 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 vegetations around the water body.

Mating behaviour. On 7 July 2021 at Sailam, adult male frogs were observed to enter the breeding ground first, shortly after dark around 17:30 h, and began calling. Calling males were observed among twigs, small shrubs, and bushes surrounding the pond and on vegetation floating on the water surface; calls couldbe heard from a distance of 25–30 m away (Fig. 3.7). In response to advertisement calls, three gravid females were seen in different spots entering the breeding site from the surrounding habitat and slowly approaching the males. One male then clasped a female in axillary amplexus (Fig. 3.9), and subsequently three amplecting pairs settled on the available substrates (logs, grasses, pebbles) on the ground near the water until eggs were laid. Non-mating calls were usually heard from nightfall until the next morning. Group mating and male-male combats were not observed, and frogs appeared to select nest sites with a minimum distance of 3 m apart. Amplexus took place during both day and night and lasted for 25–85 min before foam nest construction began and egg deposition took place over a period of 30–55 min. Thus, the total amplecting duration lasted for 55–140 min.

***Acoustic analysis*.** The call of *P. braueri* (Fig. 3.8) was found to include 1–5 notes with 3–8 pulses per note. The mean call duration was 43 ± 3 ms (mean ± SD) for calls with a single note, 276 ± 35 ms for calls with two notes, 422 ± 39 ms for calls with three notes, 494 ± 16 ms for calls with four notes, and 598 ms for calls with five notes. A single note duration was 25 ± 2 ms. The interval between subsequent notes was 128 ± 24 ms. The frequency spectra for ten calls ranged from 843.75–1406.25 Hz. The dominant pulse frequency was 515.63–1031.25 Hz and the fundamental pulse frequency was 421.88–515.63 Hz (n = 5). The maximum amplitude of the pulse was 0.9 V and dominant frequency at peak amplitude was 843.75 Hz. The amplitude peaked in the first quarter of each note and decreased gradually towards the end.

***Oviposition*.** Terrestrial oviposition was observed from April and throughout the breeding season. Amplexus was axillary, where the male placed his forelimbs under the armpits or, in some cases, over the forelimbs of the female (Fig. 3.9), and lasted for one to two hours (55–140 min). Cloacal fluid was then secreted from the gravid female along with eggs. At this time the male also started to release seminal fluid to fertilize the eggs. The female first kicked her hind legs in an up and down movement, which led to the construction of the foam nest. At the same time, the male also stimulated the female and contributed to foam nest building by moving his hind legs at regular intervals. The duration of foam nest construction along with egg deposition took place within about 30–55 min. Foam nests were unpigmented whitish or sometimes pale yellow in colour, more or less spherical in shape. The foam nests were constructed on wet forest floors, damp ditches, under vegetation, and leaf litter cover located about 0.5–3 m away from the water body (Fig. 3.10) in the case of Sailam, whereas at Reiek, foam nest were seen among leaf litter submerged in water. After foam nest construction, the male slowly disengaged from the female and left the oviposition site. The female remained with the foam nest, continued to gently move her legs back and forth until she detached from the sticky foam, leaving after about 30 minutes. After this, no sign of parental care was observed. The dominant flora at both breeding sites included *Ageratina adenophora*, *Commelina* sp., firewood (*Crassocephalum crepidioides*), ferns (*Dryopteris* sp.), and other herbs, such as *Cynodon* sp., *Cyperus* sp., *Dicrocephala* sp., *Leucas* sp., *Acmella calva*, *Eleusine indica*, *Persicaria* sp., *Lobelia nummalaria*, *Crassocephalum crepidioides*, *Urena lobata*, *Seline* sp., *Rungia* sp., *Drymaria diandra*, *Prunella vulgaris*, and *Polygonum* sp.(Fig. 3.9).

Clutch size vs. female SVL. *Polypedates braueri* exhibited distinct sexual size dimorphism with males smaller (SVL 46.0 ± 2.2 mm, n = 37) than females (SVL 54.5 ± 4.5 mm, n = 21). Clutch size varied from 215–408 eggs (306 ± 17, n = 20) with foam nest sizes from 7–10 cm in diameter. Twelve of the twenty clutches examined were infested by dipteran larvae (Fig 3.12). The Pearson Correlation estimated between female SVL and clutch size was found to be significantly positive ($r = 0.55$, $p = 0.05$) (Table 3.5).

Histology of gonads of P. braueri

Testis and ovary of *Polypedates braueri* were studied and observed histologically during the breeding season.

The weight of the ovaries ranged between 0.81gm and 1.38 gm (N=21). The sections of the ovary during the breeding period showed the presence of outer peritoneal covering, the theca externa and inner covering, the theca interna, and growing as well as mature oocytes. The theca interna surrounds each egg except for the limited area bulging toward the body cavity, where it is covered by only the theca externa. Each mature oocyte was found to be covered by a transparent vitelline membrane and had large amount of yolk distributed in the cytoplasm (Fig 3.13).

During the post breeding phase all the eggs were released.

The testis weighed between 0.004 gm – 0.018 gm. During pre-breeding period (March), the spermaatocytes undergo further development into spermatids during the emergence phase. The seminiferous tubules and connective tissue were distinct. The spermatids appeared in a cluster and their number increased (Fig. 3.14). During the breeding season (April-September), the histological sections of the testis observed showed the presence of different stages of spermatogenic cells and sertoli cells. Spermatogonia are spherical in shape and uniformly distributed around the periphery of the seminiferous tubule. Spermatocytes can be classified into primary and secondary. Primary spermatocytes are irregularly oval or spherical in shape, possessing large and vesicular nuclei, and they are larger than the spermatogonia. Secondary spermatocytes are oval in shape and are smaller than the primary spermatocytes, and are lie toward the lumen of the tubule. Numerous spermatids are also more concentrated around the lumen. The tubule was filled with slender and filamentous spermatozoa. Mature spermatozoa occur in bundles (Fig. 3.15).

The seminiferous tubule during the post breeding phase (October) 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 (Fig. 3.16).

DISCUSSION

Our observation shows that the breeding behaviour of *Polypedates braueri* coincides with changes in environmental factors including rainfall, air temperature, and relative humidity. The breeding season of *P. braueri* starts with the onset of the southwest monsoon and lasts from April–September. Its congener *P. leucomystax* in Meghalaya which breed between April through August (Iangrai, 2007), *P. teraiensis* from Mizoram, breed from March–August (Lalchhanhimi and Lalremsanga, 2017), and *P. himalayensis*, which starts their breeding activity from March till July (Rangad et al., 2022). According to Willaert (2016), the interaction between climate-specific physical factors is important for the initiation of breeding activity in anurans. Many authors have also reported that rainfall is the primary extrinsic factor mediating reproductive activity in anurans in tropical regions (Crump, 1974; Aichinger, 1987; Donelly and Guyer, 1994; Neckel-Oliveira et al., 2000, Lalchhanhimi and Lalremsanga, 2017). Rainfall not only triggers breeding behaviour, but also aids in survival of the tadpoles by carrying the developing larva from their breeding sites to larger water bodies for completion of their development, as observed in *P. himalayensis* (Rangad et al., 2022).

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 which was also found in *P. leucomystax* (Iangrai, 2007). Further, the females were found to be larger in size than the males. The observed sexual dimorphism in size may help in increasing mating success.

Our results showed that *P. braueri* amplexus was in axillary mode. The duration of foam nest construction along with egg deposition took place within about 30–55 min. In *P.leucomystax* axillary amplexus lasted for about 2–3 hours or sometimes even longer (Iangrai, 2007), in *P. teraiensis* axillary amplexus was observed to last for 40-90 minutes before the oviposition took place, and in *P.himalayensis* amplexus was found to be of axillary mode which lasted for about two to three hours, both in the natural as well as laboratory condition. After amplexus, foam nest construction occurred which lasted for about 2.5–3 hours (Rangad, 2022).

Oviposition took place on land close to water bodies. Foam nests were spotted partially hidden among grasses and were often half buried. Few nests were found far from water with potentially no chance of being submerged even with a rise in the water level. Nests farther away from water were more prone to desiccation and predation. The selection of appropriate oviposition sites is particularly important for amphibians that lack parental care (Hooroo et al., 2017).

The results of our study showed that the courtship calls emitted by males of *P. braueri* are distinct from the calls of congeneric species, in various parameters (Table 3.6). These differences in vocal characters can be a potential diagnostic tool in the field of taxonomy since the validity of unique vocalizations has been establishedto clarify the taxonomic differences between closely related amphibian species (Gogoi and Sengupta, 2017; Rangad et al., 2022). Several factors have been invoked to explain geographic variation in frog calls including reinforcement changes in the acoustic environment or a divergence associated with morphological changes over the geographic range of the species (Martin, 1972; Butlin, 1987; Loftus–Hills and Littlejohn, 1992). In this study no female calls were observed, as was reported for *P. braueri* in Yilan County, Taiwan (Yang, 2020).

At our study sites, all observed clutches were laid outside the main water bodies, sometimes adhering to vegetation and among litter in the vicinity of the pond. Changes in water level likely helps the hatchlings from the foam nest to complete their development in the aquatic habitat. Unlike the sympatric species *P. leucomystax*, *P. teraiensis* and *P. otilophus* build foam nest above the ground, attached to rocks and stones near the pond, some hanging on branch of trees, logs or walls of human habitation or sometimes deposited eggs on walls above 15–30 cm in captivity (Iangrai, 2007; Lalchhanhimi & Lalremsanga, 2017; Galunder & Rodder, 2018), while *P. braueri* and *P. himalayensis* (Rangad et al., 2022) constructed their nest on the forest floor (Fig. 3.17). They preferred ephemeral pools with some vegetation surrounding it as their habitat. These temporary pools and water holes which are formed during the monsoon with vegetation provides excellent habitat for breeding and development. The individuals of the species climbs well and often seen 1-2 m above surface. The isolated vernal pools adopted by *P. braueri* in this observation might be due for the survival of their offsprings from the predators Other sympatric species found in both study sites include *P. teraiensis*, *Raorchestes* *rezakhani*, *Rhacophorus bipunctatus*, *Rohanixalus senapatiensi*, *Minervarya asmati*, *Zhangixalus smaragdinus*, *Sylvirana lacrima*, *Kurixalus yangi* and *Duttaphrynus melanostictus* (Fig. 3.18).

The observed clutch size in the studied species ranged from 215–408 eggs, which was lower than the clutch size of 400–500 eggs reported from Taiwan by Yang (1998) and also lower than that of *P. teraiensis* (600–650 eggs; Lalchhanhimi and Lalremsanga, 2017), *P. himalayensis* (500–600 eggs), *P. megacephalus* (500–600; Tesia et al., 2017) and *P. himalayensis* (274–750 eggs; Rangad et al., 2022) from Northeast India but higher than *P. otilophus* (19–119 eggs; Galunder & Rodder, 2018) (Table 3.7). However, clutch size and breeding phenology may vary over the geographic range of a wide-ranging species which may lead to variation in population dynamics (Ritke et al., 1990; Morrison and Hero, 2003). Our observation shows that there is a significant, positive correlation between gravid female body size and clutch size ($r = 0.55$). Large females can produce more eggs than small females, and this positive relationship between clutch size and female size is known for several anuran species (Kusano and Hayashi, 2002; Wogel et al., 2002; Dziminski and Alford, 2005). Additionally, Mohanty-Hejmadi et al. (1983) reported that there is a correlation between the number of eggs and female's nutritional state. However, our sample size is not large enough and this relationship needs to be investigated further using a larger sample size.

The histological study of the testis and ovaries of *P. braueri* showed different phases of spermatogenesis and oogenesis during the different phases of the breeding cycle. Presence of numerous spermatids and few spermatozoa seems to suggest that the males of these tree frogs remained prepared for reproduction. Our observations also suggested that while the process of spermatogenesis is independent of rainfall. According to Iangrai (2007) spermatogenesis in *P. leucomystax* is dependent on increased environmental temperature, however, rainfall seem to be essential for spermatogenesis (Iangrai, 2007). He also mentioned 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).

The histological studies on the testis and ovaries of *Polypedates braueri* showed different stages of development of the spermatozoa and the ovum during spermatogenesis and oogenesis respectively. In ovaries, mature oocytes filled up the ovary during the pre-breeding period and breeding phase. In contrast, 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. According to Saidapur (1989), ovarian cycle in all amphibians is regulated by both intrinsic and extrinsic factors. The intrinsic factors mainly include the hypophysial gonadotrophins and ovarian estrogen. The extrinsic factors are temperature, light, rainfall and relative humidity which serve as the environmental synchronizers of gametogenetic and breeding activities. Adequate supply of food and water is however, essential. In males, the transverse section of testis during the pre-breeding, breeding and post-breeding season season showed seminiferous tubules, spermatocytes and bundles of spermatozoa. But seminiferous tubules were not clearly distinct as compared to pre breeding and post breeding season. Spermatids and spermatozoa were more abundant in pre breeding and breeding season compared to post breeding season, which might be due to the realeased of sperm during mating season. Similar cases were also reported by Iangrai (2007) in *P. leucomystax* and Rangad (2021) in *P. himalayensis.* The presence of different stages of spermatogenic cells and mature spermatozoa indicates that the testes of *P. braueri* are actively involved in process of spermatogenesis. Lofts (1964) and Jorgensen et al. (1979) also stated that the high spermatogenic activity during the breeding season where the seminiferous tubules become rapidly stocked with spermatozoa, which was later released during the breeding period.

Most of the time we observed that *P. braueri* foam nests were hidden among grasses of *Cyperus* sp. The preference of these plant species might be due the most dominant species of vegetation in this area close to the water body which can easily be submerged by the rise in pond water, also the structures of leaves provided a better hidden places which is important for avoidance of predators resulting in higher successful rates of development for the developing embryos. According to Hale et al. (2019) plants can interact with amphibian behaviour and physiology to affect all aspects of amphibian ecology including foraging, growth, reproduction, interactions with competitors and predators, and disease dynamics, emphasizing the influence of plants on amphibians needs greater attention. Because plant communities are often the most direct target of restoration and management efforts, it it is crucial to have knowledge and understand how plants affect amphibians for effective management of amphibian populations (Burrow & Maerz, 2022). They also added that in aquatic environments, plant structure including living plants, detritus and downed woody debris creates surfaces for the growth of biofilms, structures for calling and attaching eggs, and refuge from predation. Though, greater structural complexity can reduce larval performance if that structure increases predator attack success. Plant structure also regulates light penetration, which in turn influences water temperatures and dissolved oxygen, which can have strong effects on larval growth and development. Animal variety and distribution have long been known to be influenced by vegetation (Grinnell, 1917; Lindeman, 1942; MacArthur, 1958). However, with regard to amphibian diversity, the mechanical significance of vegetation is a topic of emerging research interest.

The reproductive biology provided here broadens the information on natural history of Mizoram populations of *P. braueri*. Further research on the rhacophorids of north-eastern India is needed so that more information about their evolution, ecology, and reproductive strategies could be obtained.

Table 3.1: Environmental factors at Sailam Community Reserved Forest, Aizawl District. Red box indicate the breeding season.

Month	2021					2022					2023				
	Rainfall	Tem-min	Tem-max	Hum-min	Hum-max	Rainfall	Tem-min	Tem-max	Hum-min	Hum-max	Rainfall	Tem-min	Tem-max	Hum-min	Hum-max
January	3.0	9	27	55	96	23.9	8	23	33	96	0.0	10	26	36	68
February	0.5	9	30	59	86	17.2	10	24	32	94	0.0	13	28	35	68
March	61.0	13	33.5	40	85	53.3	15	30	30	95	78.0	13	29	30	90
April	85.5	16	33.5	24	90	73.2	16	32	35	94	98.0	14	33	30	88
May	164.2	15	34	30	85	327.8	17	31	45	98	102.8	16	32	30	96
June	336.1	17	32.5	40	95	355.3	17	29	50	100	381.7	18	32	40	98
July	360.3	17	32.5	40	90	460.2	19	31	50	98	291.0	21	31	50	95
August	353.5	16.5	32.7	45	95	277.8	19	31	50	95	427.0	20	32	40	96
September	249.3	15.7	32.7	37	87	195.0	20	30	50	96	321.0	20	31	45	96
October	155.2	16	31	50	96	211.1	17	29	50	100	80.0	18	28	21	96
November	72.0	15	27	40	99	2.0	16	27	45	96	108.0	16	27	40	95
December	77.0	11	24	40	97	18.3	12	25	40	88	83.0	12	26	50	97

Table 3.2: Kruskal Wallis H test breeding season against environmental factors (air temperature, relative humidity, rainfall) showed significant in rainfall and min temp where $p<0.005$.

	Rainfall	Min_Tem	Max_Tem	Min_Hum	Max_Hum
Kruskal-Wallis H	15.384	12.322	2.634	1.817	.040
df	1	1	1	1	1
Asymp. Sig.	.000	.000	.105	.178	.841

Table 3.3: Environmental factors at Reiek Community Reserved Forest, Mamit District. Red box indicate the breeding season.

Month	2021					2022					2023				
	Rainfall	Tem-Min	Tem-Max	Hum-min	Hum-max	Rainfall	Tem-Min	Tem-Max	Hum-min	Hum-max	Rainfall	Tem-Min	Tem-Max	Hum-min	Hum-max
January	0	10.5	26	66	91	0	8	24.5	85	98	0	10	27	73	96
February	0	10.5	29	66	92	19.1	19	27.5	84	96	0	13	27	86	96
March	64	15	33	71	95	59	20	32.5	62	96	114.5	15	31	65	96
April	84.1	15.5	33.5	76	96	115	15.5	32.5	84	96	79.5	15	32	52	95
May	191.5	20	33	82	96	0	16.5	31	87	96	129	18	32	67	96
June	341.6	18	31	80	95	263.9	17.5	28.5	87	96	315.5	18	31	75	96
July	345	19	32	81	96	478.7	21	31.5	89	96	444	20	32	78	96
August	321.5	20	31.5	82	96	443	20.5	30.5	88	96	286.8	21	31.5	82	96
September	299	21	24.5	80	96	333.2	20	32	87	96	375	20.5	33.5	74	96
October	71.4	20	34	84	96	105	17.5	33	88	97	105	19.5	32	65	96
November	36	15	29	80	96	0	16	32	85	97	98	16	30	66	95
December	0	12	27	82	96	0	12	26	82	96	9	12	31.5	61	95

Table 3.4: Kruskal Wallis H test breeding season against environmental factors (air temperature, relative humidity, rainfall) showed significant in rainfall and air-temp where p<0.005.

	Rainfall	Min_Tem	Max_tem	Min_Hum	Max_Hum
Kruskal-Wallis H	20.759	15.787	18.956	.031	.102
df	1	1	1	1	1
Asymp. Sig.	.000	.000	.000	.860	.749

Table 3.5: Size of female (SVL) and their respective number of eggs.

Sl.No	**SVL (mm)**	**Clutch size**
1	70.40	366
2	66.70	287
3	69.00	408
4	64.40	320
5	67.40	307
6	58.70	288
7	57.90	296
8	69.30	324
9	62.50	215
10	64.60	250
11	60.54	296
12	68.90	278
13	67.12	351
14	68.78	314
15	67.50	269
16	63.16	322
17	68.86	376
18	69.04	347
19	64.46	309
20	69.62	358

Table 3.6: Comparison of call parameters among congeners of *Polypedates braueri*.

Sl. no.	Species	Call Duration (ms)	Notes	Pulse fundamental frequency (Hz)	Frequency spectrum (Hz)	References
1.	*Polypedates braueri*	43–598	1–5	421.875–515.625	843.75–1406.25	This study
2.	*P. himalayensis*	4700	6–7	1139–2211	1306.03–3819.52	Gogoi & Sengupta (2017); Rangad et al. (2022)
3.	*P. leucomystax*	454	3	–	–	Iangrai (2007)
4.	*P. leucomystax*	1700	1	1148.4–1176	–	Gogoi and Sengupta (2017)
5.	*P. maculatus*	114 or 6500	9	388–1249	–	Gogoi & Sengupta (2017)
6.	*P. teraiensis*	1750	1	990.5–1205.9	–	Gogoi & Sengupta (2017)

Table: 3.7: Comparison of clutch size of *P. braueri* with congener species.

Species name	Number of eggs	References
P. braueri	215–408	This study
P. braueri	400–500	Yang (1998)
P. himalayensis	274–750	Rangad et al (2022)
P. leucomystax	254–530	Iangrai (2007)
P. maculatus	275–719	Mohanty & Dutta (1988)
P. maculatus	210–448	Girish & Saidapur (1999)
P. megacephalus	500–600	Tesiaet al. (2017)
P. otilophus	19–119	Galunder & Rodder (2018)
P. teraiensis	600–650	Lalchhanhimi & (2017)

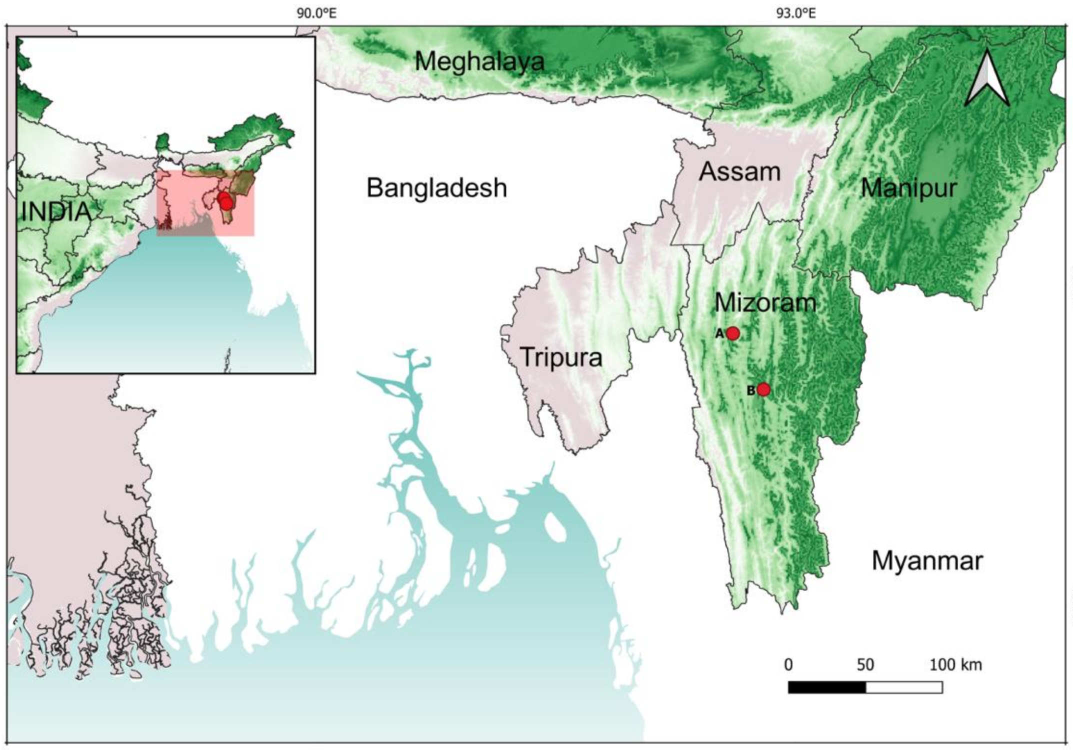


Figure 3.1: Elevation map showing study sites at A) Reiek Community Reserved Forest, Mamit District. B) Sailam Community Reserved Forest, Aizawl District, Mizoram, Northeast India.

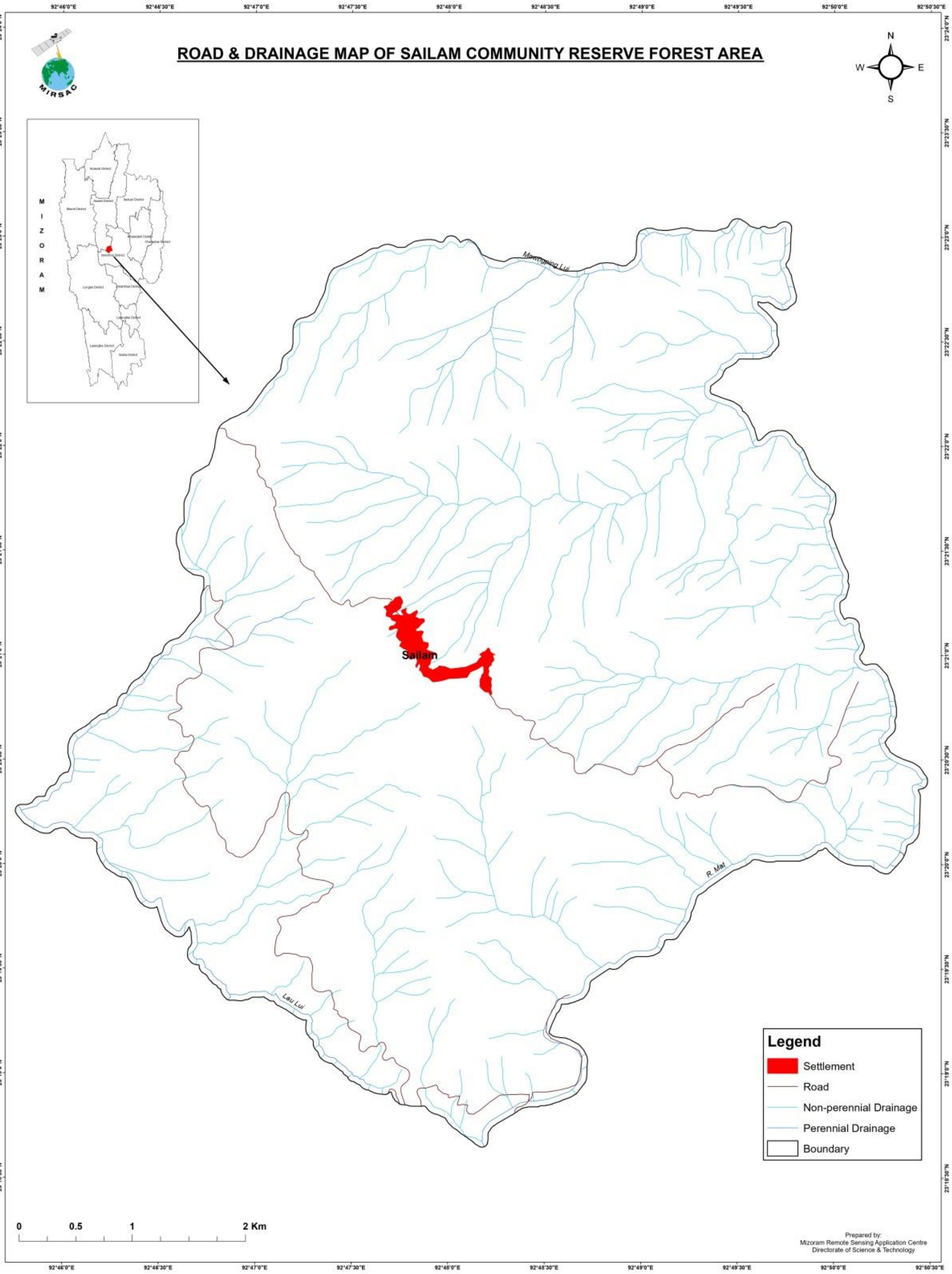


Figure 3.2: Road and drainage map of Sailam Community Reserved Forest Area.

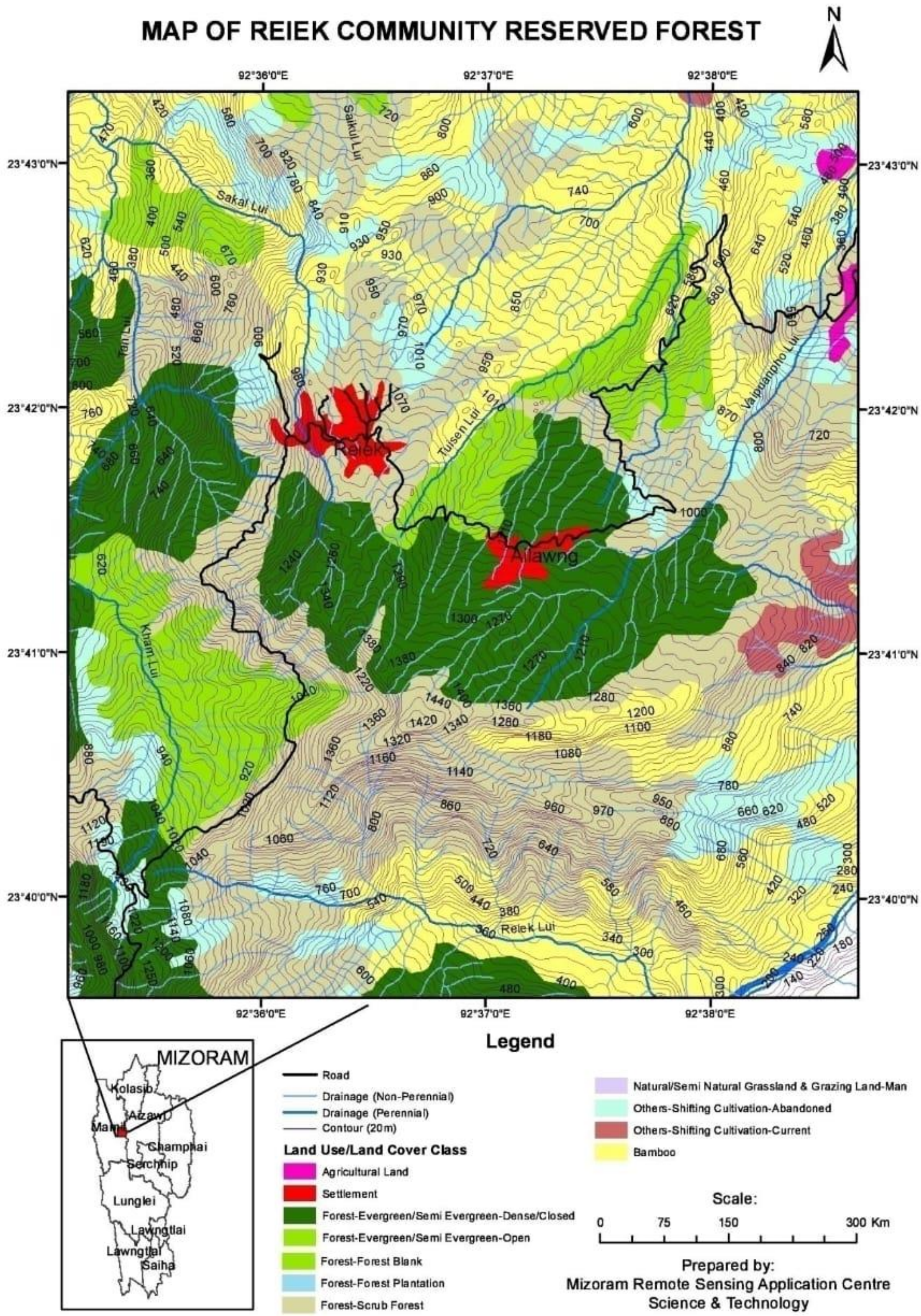


Figure 3.3: Land use/ land cover map of Reiek Community Reserved Forest Area.

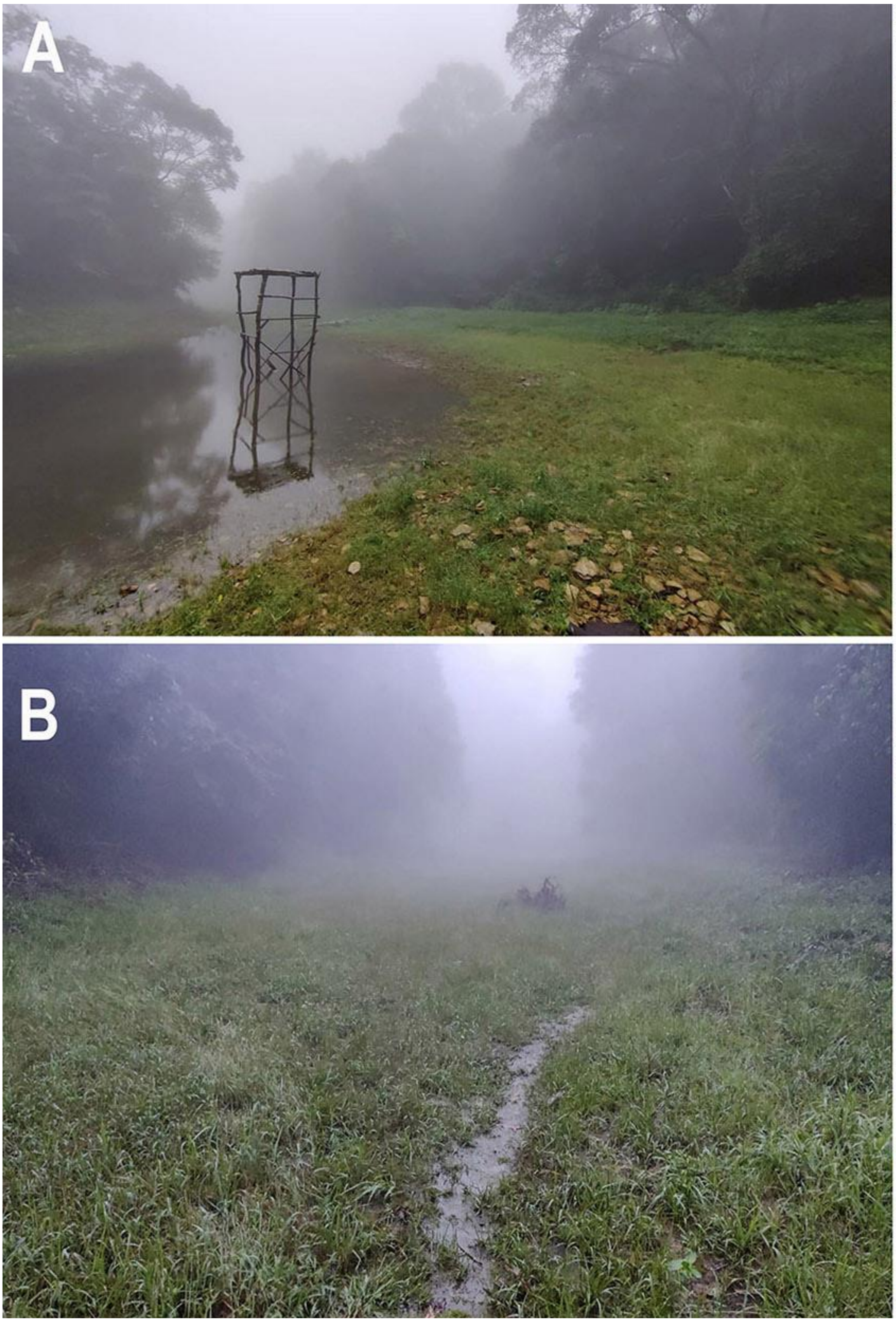


Figure 3.4: Breeding ground of *Polypedates braueri* at Sailam Community Reserved Forest, Aizawl District.

Figure 3.5: Breeding ground of *Polypedates braueri* at Reiek Community Reserved Forest, Mamit District.

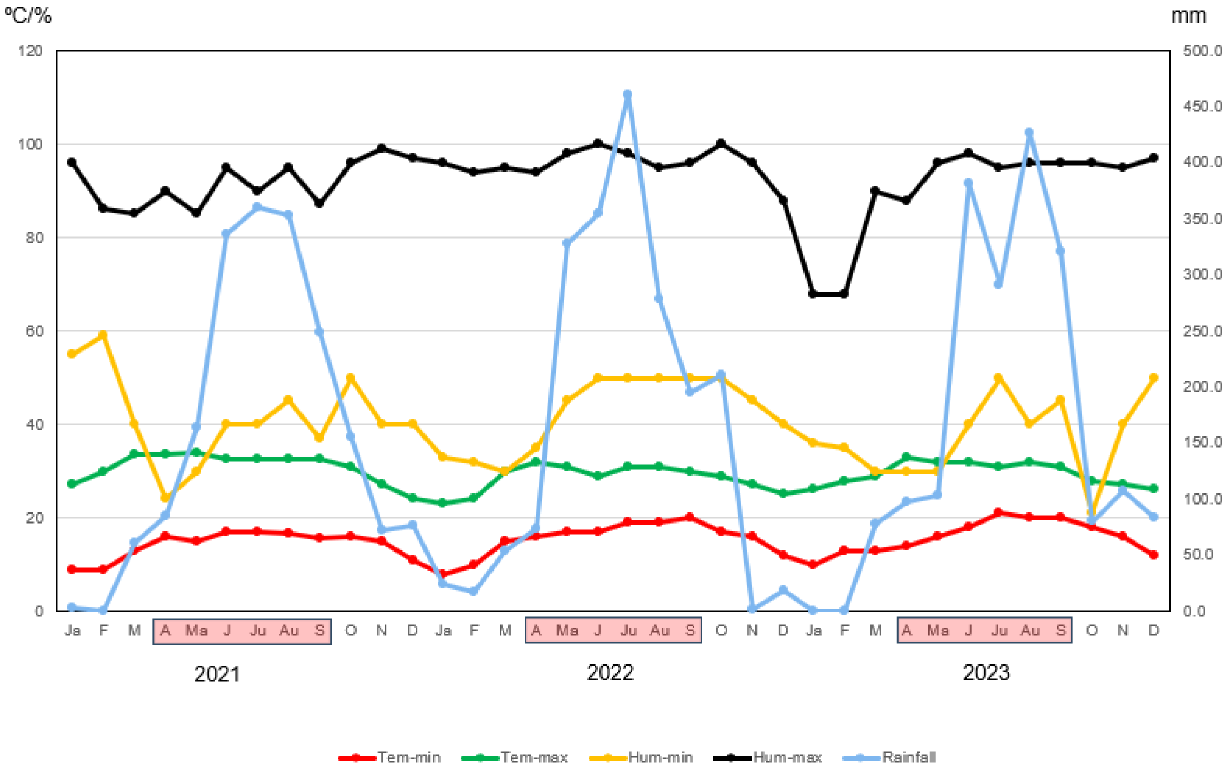


Figure 3.6: Graph showing ecological parameters during breeding and non breeding season at Sailam Community Reserved Forest, Aizawl District. Pink box indicates the breeding season.

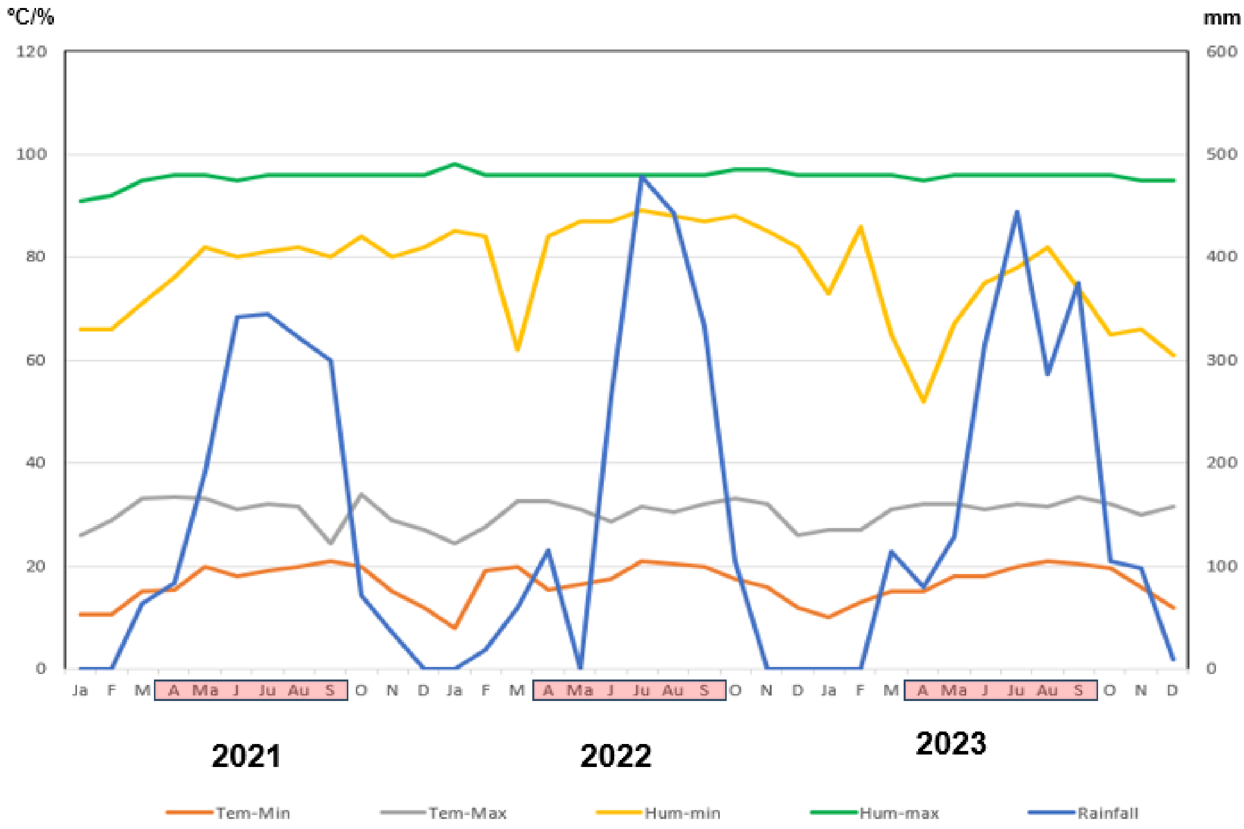


Figure 3.7: Graph showing ecological parameters during breeding and non breeding season at Reiek Community Reserved Forest, Mamit District. Pink box indicates the breeding season.

Figure 3.8: (A) Emerging male *Polypedates braueri* calling from vegetation floating on the water surface. (B) *Polypedates braueri* on a shrub branch beside a pool at Sailam Village, Mizoram, India.

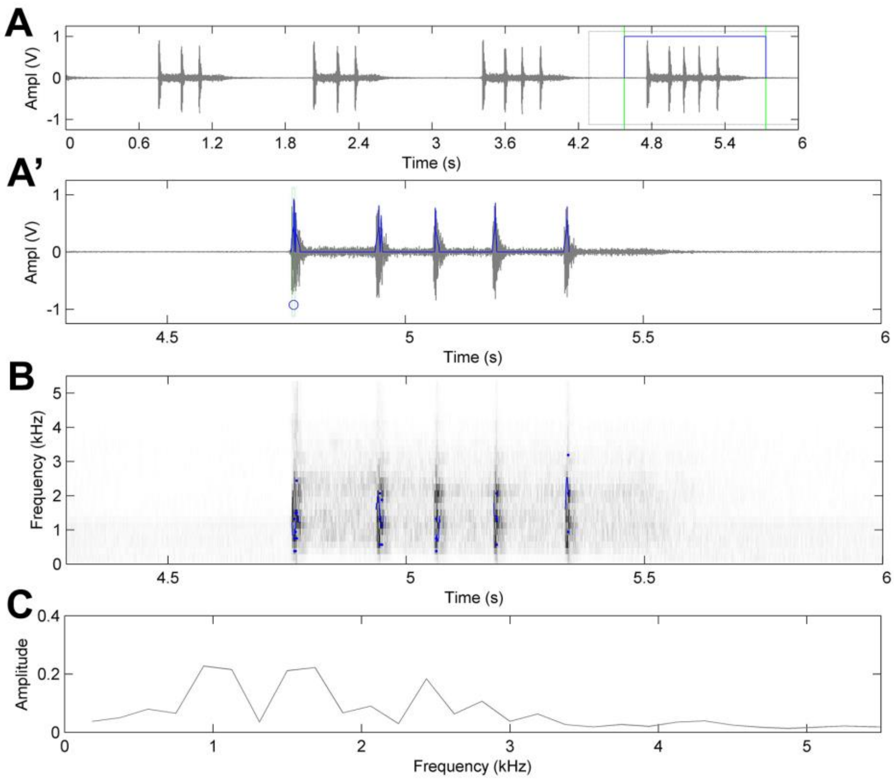


Figure 3.9: Advertisement call of *Polypedates braueri* shown as oscillograms (A, A'), a spectrogram (B), and by its frequency spectrum (C).

Figure 3.10: Axillary amplexus in *Polypedates braueri* with formation of foam nest.

Figure 3.11. Constructed foam nests of *Polypedates braueri* at Sailam Village, Mizoram, India. (A) A nest in damp ditches. (B, C) Nests under vegetation cover. (D) A nest partially buried among leaf litter close to water body.

Figure 3.12: The dominant flora at both breeding sites A. *Leucas sp.* B. *Dichrocephala integrifolia* C. *Pseudognaphalium affine* D. *Acmella calva* E. *Drymaria diandra* F. *Ageratinaadenophora* G. *Dryopteris sp*. H. *Eleusine indica* I. *Polygonum* sp. J. *Persicaria chinensis* K. *Lobelia nummalaria* L. *Crassocephalum crepidioides* M. *Urena lobata* **N.** *Seline* sp. O. *Cyperus* sp. P. *Torenia* sp. Q. *Persicaria* sp. R. *Commelina* sp S. *Persicaria* sp. T. *Rungia* sp.

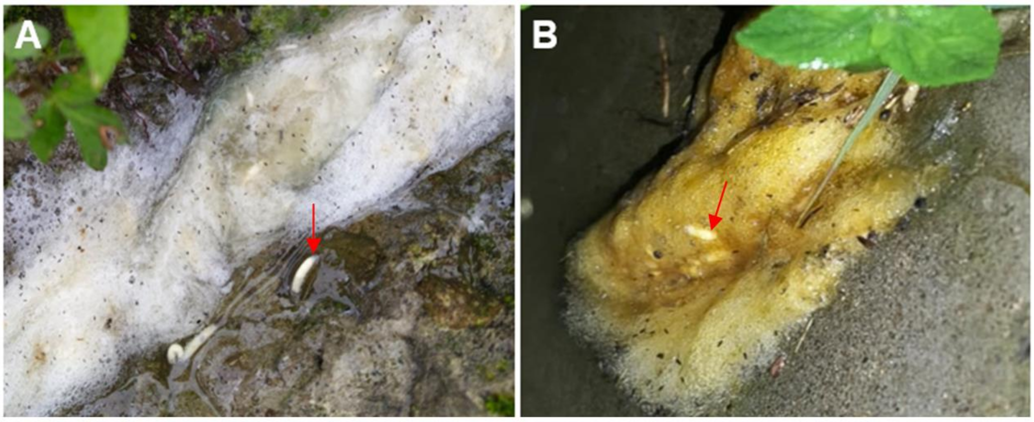


Figure 3.13: Foam nest infected by a dipteran larva

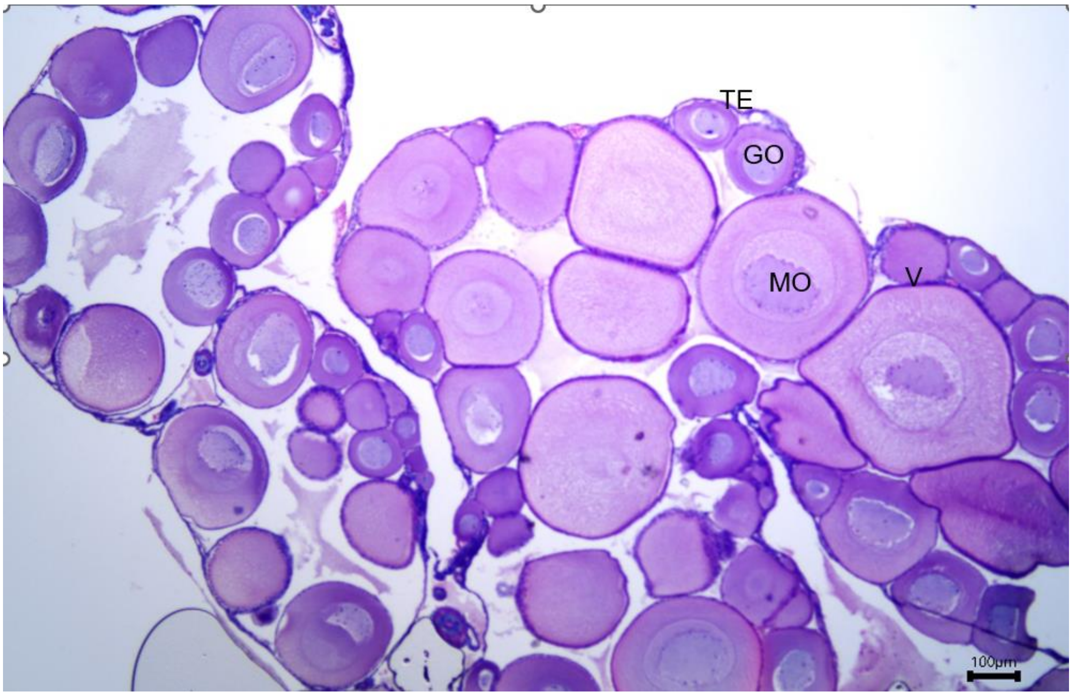


Figure 3.14: T.S of ovary of *Polypedates braueri* during breeding season. TE- Theca externa, GO- Growing oocyte, MO- Mature oocyte, V- Vitelline membrane (10X).

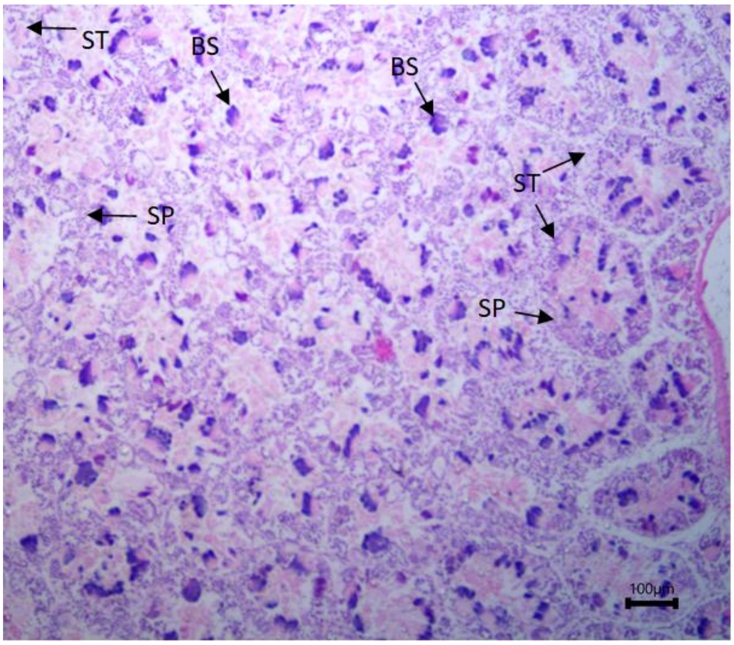


Figure 3.15: T.S of testis of *Polypedates braueri* during pre-breeding season showing ST= Seminiferous tubules; SP= Spermatocytes; BS= Bundles of Spermatozoa (10X).

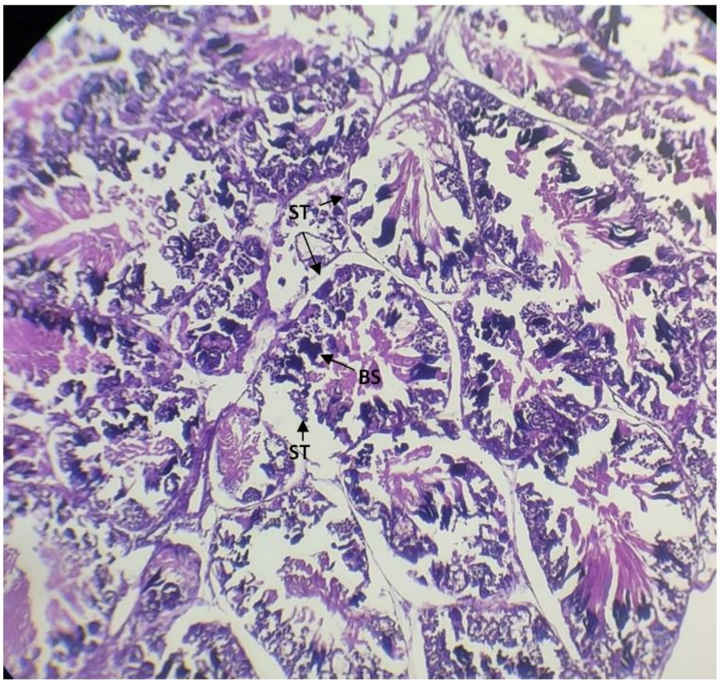


Figure 3.16: T.S of testis of *Polypedates braueri* during breeding season.

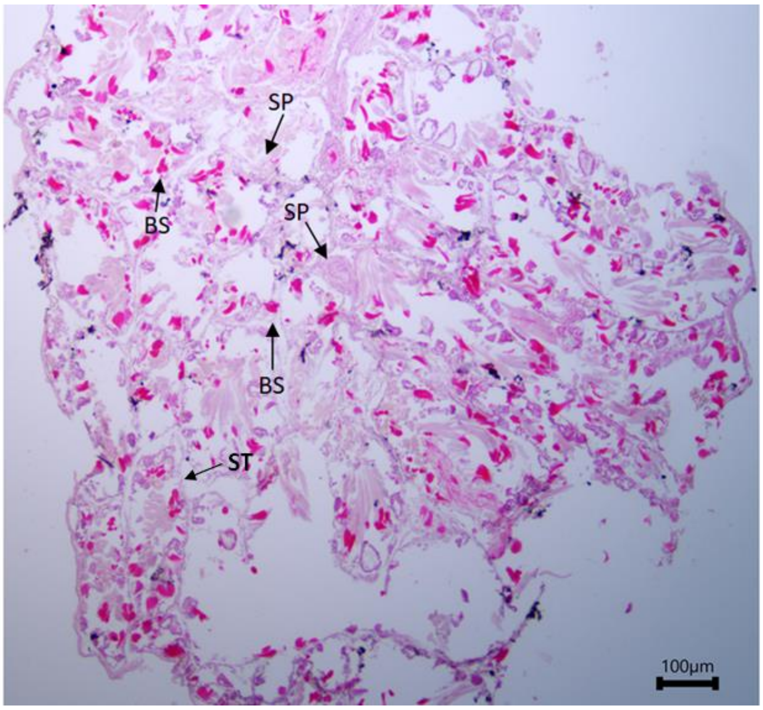


Figure 3.17: T.S of testis of *Polypedates braueri* during post breeding season showing ST= Seminiferous tubules; SP= Spermatocytes; BS= Bundles of Spermatozoa (10X).

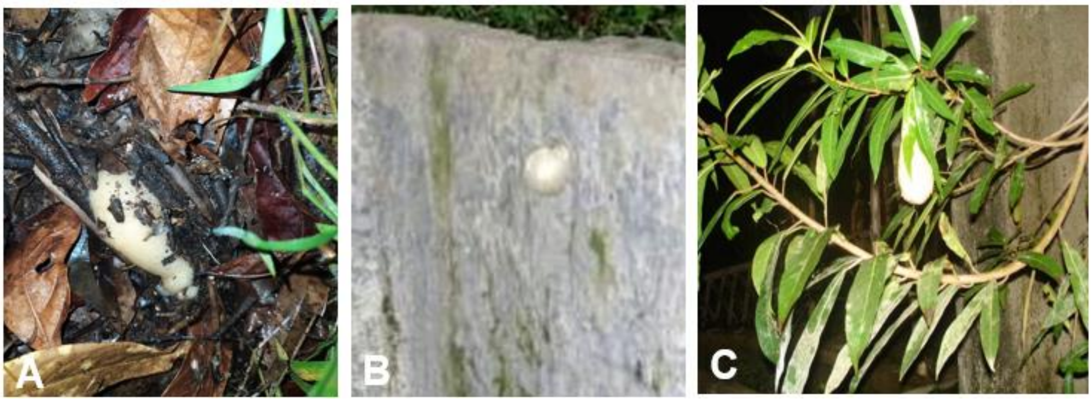


Figure 3.18: Foam nest of (A) *Polypedates braueri* deposited on forest floor (B, C) *Polypedates teraiensis* deposited on wall and tree trunk.

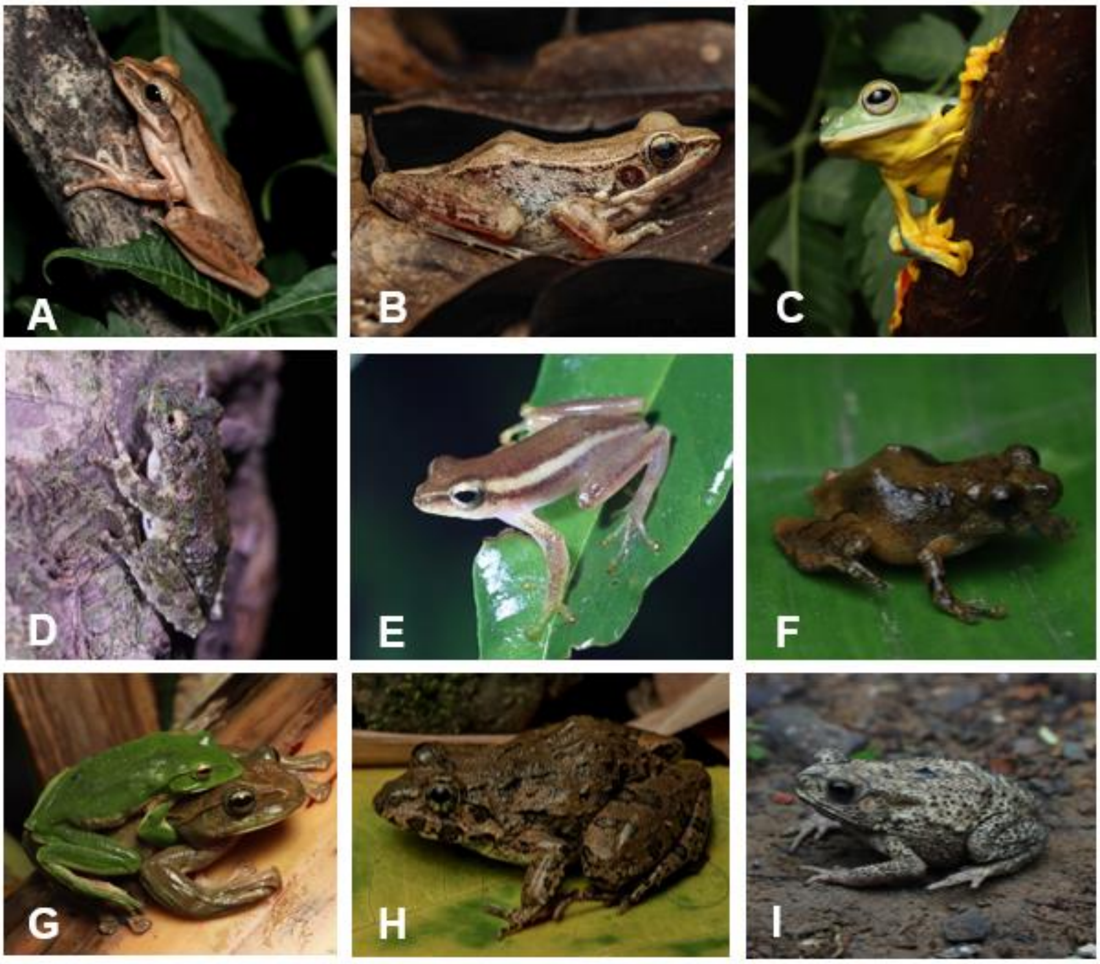


Figure 3.19: Other sympatric species found in both study sites A. *P. teraiensis*B. *Sylvirana lacrima* C. *Rhacophorus bipunctatus* D. *Kurixalus yangi* E. *Rohanixalus senapatiensis* F. *Raorchestes rezakhani* G. *Zhangixalus smaragdinus* H. *Minervarya asmati* and I. *Duttaphrynus melanostictus.*

CHAPTER IV

TO STUDY THE EMBRYONIC AND LARVAL DEVELOPMENT OF *P. braueri*

INTRODUCTION

The descriptions of anuran embryos and larvae are requisite to the study of frog taxonomy, phylogeny, life history, and behaviour (Gosner, 1960). Comprehensive ontogenic descriptions are vital for relating the growth patterns of amphibian taxa (Costa et al., 2010). Morphometric comparisons of amphibians at different developmental stages obtained from different habitat types may indicate the consequences of food shortages or environmental contamination due to pesticides, fertilisers or other pollutants (Khan & Law, 2005; Stephens et al., 2017; Jilani et al., 2018). The ancestral life history of most amphibians is biphasic, meaning that embryos develop into a feeding larva that swims in an aquatic habitat and undergoes metamorphosis to reach the adult form (Callery, 2006; Duellman & Trueb, 1994).

Gosner's (1960) developmental stage is the most famous developmental staging system for Anura and has been used in various studies of this animal group (e.g., Wassersug & Duellman, 1984; Dayton & Fitzgerald, 2001), but there is considerable interspecific variation in developmental patterns and larval morphological change during development (Sayim & Kaya, 2008). The morphology of tadpoles has long received too little attention in taxonomic and phylogenetic contexts, beyond the use of Orton's general tadpole types, despite the potential of larval characters for resolving problems in systematics. A possible explanation for this neglect is the ontogenetic variation of external morphology. In order to understand the value of larval characters in taxonomy and systematics, it is necessary to determine the developmental stage at which characters reach their definitive size, form and colour before meaningful comparisons can be made within and between species (Grosjean, 2005). The increasing number of tadpole descriptions and the 'discovery' of new characters (e.g. buccopharyngeal features) proved to be relevant in resolving taxonomic problems (e.g. Wassersug, 1980; Lannoo et al., 1987; Grillitsch et al., 1993; Chou & Lin, 1997; Altig & McDiarmid, 1999a; Iskandar et al., 2014).

Duellman & Trueb (1986) recognized 29 types of reproductive modes in anurans that lead from eggs laid in water and free living tadpoles to terrestrial eggs with direct development and ovo-viviparity. Haddad & Prado (2005) added 10 more types and Iskandar et al. (2014) added one more (giving birth to tadpoles instead of eggs), which highlights that there may be still more amphibian reproductive modes to be discovered. These adaptations should protect clutches and larvae from terrestrial predators. They also added that most tree frogs seem to have lower dispersal abilities than many other anurans, such as ranids and dicroglossids. The developmental speed of amphibians is strongly negatively correlated with temperature (Duellman and Trueb, 1986).

The family Rhacophoridae are known for their variation in life history traits concerning larval development (Grosjean et al., 2008; Li et al., 2009). The Anuran family Rhacophoridae shows a wide variety of egg-laying strategies, three types of larval modes, herbivorous/detritophagous, oophagous, and direct development without feeding are known in Rhacophoridae (Meegaskumbura et al., 2015; Kishimoto & Hayashi, 2017). The first one is common, most of the anurans that belongs to these family including *Polypedates* sp. deposit eggs in a foam nest above the water surface, and hatched tadpoles fall into the water and feed on planktons and/or detritus (Iangrai, 2007; Chakravarty et al., 2011; Meegaskumbura et al., 2015; Biju et al., 2016; lalramdinfeli, 2020; Rangad 2020). In contrast, some other species for eg., *Leptomantis harrissoni* and *Vampyrius vampyrus*, deposit eggs in a foam nest placed on the wall of a tree hole and the tadpoles develop there until metamorphosis is complete (Malkmus & Dehling, 2008; Rowley et al., 2012; Vassilieva et al., 2013; Haas et al., 2022). The second larval mode, oophagy, is seen in tadpoles of those of the genera *Frankixalus*, *Gracixalus*, and *Kurixalus* (but excluding *K. idiootocus*). They feed on unfertilized trophic eggs that the parent female periodically deposits (Ueda, 1986; Kuramoto & Wang, 1987; Kam et al., 1996, 1997; Rowley et al., 2012; Vassilieva et al., 2013; Biju et al., 2016; Shangpliang, 2021). The third larval mode, direct development, is reported in the genera *Pseudophilautus*, *Raorchestes*, and *Philautus*. Their embryos initiate metamorphosis inside the gelatinous covering without feeding (Meegaskumbura et al., 2015; Biju et al., 2016; Siammawii et al., 2024b). Crump (2015) reported that the evolution of characters has been seen as a tendency to be independent of water bodies by direct development.

The external fertilization and development of their eggs make them a prime candidate for investigations involving biological and mechanical manipulations. Early embryonic and larval development is considered as a critical factor for understanding phylogeny, comparative embryology, and molecular mechanisms of development in amphibians (Hurney et al., 2015). Only 1.29% normal developmental stages are available for the total 7730 species of anurans found in the world (Wang et al., 2017; Frost, 2025). Therefore studies on the successive ontogenic stages to record the normal developmental table are crucial in understanding the ecology of a species and for planning conservation measures. No studies have reported the development of *P. braueri*, a rhacophorid species with data deficient in the IUCN red list category.

REVIEW OF LITERATURE

The life history, breeding biology and developmental stages of the genus *Polypedates* were studied by various workers. Flower (1896) reported notes on tadpoles of *P. leucomystax* from the Malay Peninsula. Annandale (1912) provided tadpole morphology and oral morphology of *P. himalayensis* and *P. maculatus* from Assam, India. Smith (1917) studied tadpoles of *P. leucomystax* from Thailand. Pope (1931) and Liu (1940; 1950) worked on tadpoles of *P. megacephalus* from China. Inger (1956) worked on *P. leucomystax* from the lowlands of North Borneo. Alcala & Brown (1956) and Alcala (1962) highlighted early life history, breeding behaviour and early development of *P. leucomystax* from Philippines. Yorke (1983) reported survival of embryos and larvae of the frog *P.leucomystax* in Malaysia. Mohanty-Hejmadi & Dutta (1988) studied the life history of *P. maculatus*. Girish & Saidapur (1999) worked on mating and nesting behaviour and early development of *P. maculatus*. Chou & Lin (1997) provided notes on tadpoles of *P. megacephalus* from Taiwan. Grosjean (2004) also provided tadpole descriptions of *P. mutus*, *P. maculatus*, *P. megacephalus* and *P. himalayensis* from China, Nepal and Vietnam. Sheridan (2008) reported that the larval life of *P. leucomystax* from Sakaerat (northeastern Thailand). Chakravarty et al. (2011) worked on tadpole morphology and table of developmental stages of *P. teraiensis* from Assam, India. Pearlindah et al. (2012) presented embryo development of *P. leucomystax* at Campus of State University of Malan. Tamuly and Dey (2014) studied larval morphology and development of *P. teraiensis* from Assam. Tapley & Girgin (2015) studied tadpoles of *P. otilophus* from Hungary. Galunder & Rodder (2018) worked on tadpoles of *P. otilophus*. Lalramdinfeli (2017) compared the breeding and development of two rhacophorids, *Zhangixalus smaragdinus* and *P*. teraiensis from Mizoram, India. Tesia et al. (2017) worked on development of *Polypedates megacephalus* from Arunachal Pradesh. Rangad (2021) studied the developmental stages of *P. himalayensis* from Meghalaya. Iangrai (2022) reported the stages of development and metamorphosis of tree frog *Polypedates leucomystax* from Meghalaya. Raj (2023) provided larval descriptions of *P. pseudocruciger* from Wayanad and Vagamon hills, Western Ghats, India. Raj et al. (2023) also studied the detailed larval external morphology of *P. maculatus*, *P. megacephalus* and *P. teraiensis* from India.

MATERIALS AND METHODS

Embryonic and larval developments were observed during three breeding seasons (April–September 2021, 2022 and 2023) both in the fields and in the laboratory. Amplecting pairs from the study sites were observed from the month of late April which were collected and brought to the laboratory and allowed them to lay their eggs and maintained in a terrarium to allow further development and metamorphosis. The eggs laid in the field were left to develop naturally. Some egg masses were fixed immediately in the field in 8% formaldehyde and 70% ethanol at a ratio of 1:1. Temperature and pH of water were maintained as in the natural condition and water will be changed every alternate day. The rate of development was observed under a stereoscopic dissecting binocular microscope (CETI 9554.1200). The time of onset of each new stage was noted and some developmental stages were fixed in a mixture of 70% alcohol and 4% formaldehyde in the ratio of 1:1. Staging of the embryos and larvae of the species were 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 microscope (CETI 9554. 1200). The hatched tadpoles reared in a captivity containing pond water with algae as natural food collected from the study sites and fed daily with supplement fish food. In order to know the stages attained by the developing tadpoles, regular observation were conducted depending on the rate of development.

Developing embryos were monitored in natural conditions by recording water temperature, humidity and pH using thermometer, hygrometer and pH meter, respectively.

The specimens that were preserved comprised all of the developmental stages of tadpoles based on tadpole staging of Gosner (1960). Embryos and tadpoles were photographed and staged according to their corresponding developmental stages. Specimens were also measured following preservation through standard tadpole body measurement by Altig et al. (2007) and Grosjean (2005), specifically, Body Length (BL), Tail Length (TaL), Musculature Height (MH), Maximum Tail Height and Total Length (TL) depending on developmental phases. Vernier caliper was used to measure the morphometric measurements (Widholzer & Castroviejo-Fisher 2018) and a stereomicroscope was used to identify their labial tooth row formula (Gamal

2014). Abbreviations used in morphometric measurement of developing embryos and tadpoles (N = 10) are as follows:

TL: Total Length; TAL: Tail Length; BL: Body Length; BW: Body Width; IOD: Interorbital Distance; IND: Internarial Distance; SO: Snout – Orbit Distance; SN: Snout – Naris Distance; MTH: Muscle Tail Length; TMW: Tail Muscle Width.

RESULTS

The embryonic development was studied in the laboratory where water temperature ranged between 12.5°C–24°C and pH=5.78–8.88 (Table 4.1–4.3). The completion of metamorphosis from the newly laid egg was also observed in the natural environment at the Reiek Community Reserved Forest (water temperature 14.2°C–30.3 °C; pH= 6.1–7.4) and Sailam Community Reserved Forest (water temperature 10.6°C–29.8°C; pH= 6.0–7.4). The duration of metamorphosis from the time of fertilization is about 75 days. During winter season, when the temperature is low overwintering tadpoles were observed during the month of December–March where water temperature ranged between 7.2°C –26.2°C. The tadpole does not reach the minimum size for metamorphosis before the onset of winter. Overwintering *P. braueri* tadpoles continued to grow and develop, but at a slow rate due to the low winter temperatures. Tadpole metamophosed only after the temperature rised i.e late February to March where water temperature ranged from 12.5°C–29.8°C and some never did.

A brief account of complete normal developmental stages (46 stages) of *P. braueri* were distinguished as follows and illustrated in Figures and table 4.4. Stages of development and metamorphosis were divided into 11 major developmental terms consisting of 46 developmental stages, each consisting of a number of successive stages defined by Gosner (1960). The time in brackets represents the approximate time elapsed after oviposition. Measurements are given as mean±SD.

Phase I. Fertilization stages

Stage 1: Fertilized eggs. The freshly layed egg was spherical in shape. The time of fertilization was defined as 0 h measured about 1.83±0.01mm in diameter (Fig. 4.1a).

Stage 2: Gray crescent. A faintly gray crescent is visible on the animal pole of the egg. The vegetal hemisphere is more prominent. It measures about 1.83±0.01mm. This stage is observed within 0:30 hr after fertilization (Fig. 4.1b).

Phase II. Cleavage Stages

Stage 3: 2-cell. First cleavage starts at 1:50 h after fertilization. A furrow appears in the animal hemisphere extends down through the vegetal hemisphere, dividing the egg into two blastomeres measuring 1.83±0.01mm diameter (Fig. 4.1c).

Stage 4: 4-cell. Second division begins and formed four blastomeres at 2:50 h, the cell barely increased in size measured 1.84±0.01 mm in diameter (Fig. 4.1d).

Stage 5: 8-cell. Third division begins after 3:45 h with four small blastomeres in the animal hemisphere and four large blastomeres formed in the vegetal hemisphere. The eggs measured 1.85±0.01mm in diameter (Fig 4.1e).

Stage 6: 16-cell. Fourth division marked by last regular cleavage occurred around 5:50 h measured 1.87±0.01mm. The fourth sets of cleavage planes are holoblastic and vertical extending from the animal pole and progress downwards dividing the egg into 16 blastomeres (Fig. 4.1f).

Stage 7: 32-cell. Fifth division begins at around 7:00 h, by the starts of irregular egg cleavage. The fifth cleavage furrows of the micromeres and macromeres take place resulting in formation of smaller micromeres and macromeres. The cells which are in the animal hemisphere are smaller than the cells at the vegetal hemisphere. The eggs measured 1.92±0.01mm in diameter (Fig. 4.1g).

Stage 8: Mid-cleavage (morula). Irregular cleavages continue at this stage at around 9:15 h where the smaller micromeres are more numerous than the larger macromeres measured 1.95±0.01mm (Fig. 4. 1h).

Stage 9: Late-cleavage (blastula). The last stage of cleavages where blastomeres are smaller in the animal hemisphere than in the vegetal hemisphere. A series of repeated mitotic divisions of the fertilized egg results in the formation of late blastula which appeared like a ball of cells consisting of many smaller blastomeres measured 1.98±0.01mm in diameter at around 10:30 h (Fig. 4.1i).

Phase III. Gastrula

Stage 10: Dorsal lip. Gastrulation occurred, there is invagination of the cells at the dorsal lip of the blastopore at around 12:10 h, measured 2.06±0.03mm. There is inward migration of the micromeres (Fig. 4.2a).

Stage 11: Mid gastrula. The dorsal lip of blastopore expands into semicircle and the invaginated blastomeres are now exposed within a ring form, called the yolk plug at around 13:25 h, measured 2.14±0.02 mm in diameter (Fig. 4.2b).

Stage 12: Late gastrula. At around 15:15h, the blastomeres invaginated inside the embryo through the blastopore. Now, the yolk plug become smaller in size. The eggs measured 2.17±0.04 mm (Fig. 4.2c).

Phase IV. Neurulation Stages

Stage 13: Neural plate. Neurulation begins around 16:50 h where there is gradual disappearance of the yolk plug. The dorsal surface is flattened to form the tubular area, i.e., the neural plate measured 2.27±0.01mm in diameter (Fig. 4.2d).

Stage 14: Neural fold. At this stage elevation of two lateral ridges separated by the neural groove forms the neural fold at around 17:40h. This stage is marked by a distinct neural fold with broad cerebral, narrow spinal cord regions, and slight elongation of the embryo measured 2.32±0.01mm (Fig. 4.2e).

Stage 15: Rotation and closure of neural fold. At around 18:10h there is closure of the cerebral and spinal cord regions as the neural folds approach each other at close proximity. The embryo within the capsule begins to rotate at this stage. The embryo measured 2.36±0.02 mm (Fig. 4.2f).

Stage 16: Neural tube. Elongation of the embryo with distinct gill plates is well marked at around 21:10 h measured 2.55±0.03mm in total length (TOL) (Fig. 4.2g).

Phase V. Tail bud stages

Stage 17: Tail bud stage. This stage marks the beginning of tail formation at around 23:40 h. The tail bud appears at the posterior end of the embryo. there is a distinct and recognizable head on the anterior region. The embry measured 2.68±0.02 mm (Fig. 4.2h).

Stage 18: Muscular response stage. The embryo shows muscular response to external stimuli after 1 day and 3 hour. The embryo is elongated and the gill plates are observed. The total length of tadpoles measured 2.77±0.04 mm (Fig. 4.2i).

Stage 19: Heart beat. The heart pulsation is visible at this stage around 1d 20 h. External gill buds are conspicuous. The total length of the embryo measured 5.748±0.2 mm (Fig. 4.3a).

Stage 20: Gill circulation. The gills are clearly visible and circulation of corpuscles through the external gill filaments is observed. The base of the gills is covered with opercular fold and the oral sucker is well developed at this stage around 2d 19 h (TOL 7.32±0.24 mm) (Fig. 4.3b).

Phase VI. External gill stages

Stage 21: Cornea transparent. Cornea becomes transparent, and oral suckers and nasal pits become prominent. The embryo increased in length measuring 8.46±0.18mm at 3d 7 h (Fig. 4.3c).

Stage 22: Tailfin circulation. After 4d 3h, tail fins become transparent, and mouth becomes slightly wider measuring 9.64±0.13 mm in total length (Fig. 4.3d).

VII. Operculum, oral disc stages

Stage 23: Operculum covered gill base. The left and right external gills become clearly visible after 5d 18h. The lengths of the gills become comparatively reduced. There is an appearance of pigments over the whole body. The total length of the tadpoles were 11.15±0.33 mm (Fig. 4.3e).

Stage 24: Operculum closed on right. Day eight is marked by shortening of the left external gills, while the right operculum fold closes measured 11.6±0.11 mm (Fig. 4.3f).

Stage 25: Operculum closed on both sides. At this stage, the gills disappear and are being reabsorbed into the body. This stage marked the completion of early embryonic development which allows free exit of the developing free-swimming tadpole ready for formation of the hind limb bud. Spiracle appeared on the left lateral side of the body. The tadpoles measured 14.72±0.53 mm in TOL around day 10 (Fig. 4.3g).

VIII. Hind limb bud formation stages

Stage 26: Hind limb bud appeared. This stage starts by the appearance of hindlimb bud at around 22 days measured 25.19±0.53 mm in TOL. The tail increases in length. Limb bud appears at the junction of tail and trunk and is less than half of its diameter. Pigmentation increased from the head to the tail region of the body. (Fig. 4.3h).

Stage 27: Length of hindlimb bud equal to half of its diameter. After 24 days, the hindlimb bud increases in size, the length of the limbbud is equal to half of its diameter. Patches of dark pigmentation in the tail region become more prominent than before. The tadpoles measured 29.13±0.46 mm in TOL (Fig. 4.3i).

Stage 28: Length of limb bud equal to its diameter. The total length of the tadpoles measured 31.08±0.68 mm after 26 days onwards. This stage is marked where the hind limb bud is slightly conical at its free distal end (Fig. 4.4a).

Stage 29: Length of limb bud equal to one and half times its diameter. After 30 days the size of hindlimb bud become more prominent, and the distal end of the hind limb bud is oarshaped. The tadpoles measured 30.66±1.41mm (Fig. 4.4b).

Stage 30: Length of limb bud equal to twice its diameter. At this stage after 36 days, the distal half of the conical hind limb bud is slightly bent ventrally, and the distal end of hind limb bud is equal to twice its diameter. The total length of the tadpoles measured 32.1±0.65mm (Fig. 4.4c).

IX. Toe differentiation and development stages

Stage 31: Foot paddle shaped stage. The developing tadpoles possess paddle shaped hind limb at this stage. Toe differentiation begins with the appearance of a dorsal shallow indentation on the toe. Body pigmentation is well marked on the tail and body. The tadpoles measured 33.09±0.67 mm at 39 days (Fig. 4.4d).

Stage 32: First indentation. After day 43, the head and trunk are well developed. The margin of the foot-paddle becomes slightly indented on the dorsal side which marks the prominence of the future fourth and fifth toes. Now, the tadpoles total length were about 34.82±0.54 mm (Fig. 4.4e).

Stage 33: Second indentation. The margin of the footpaddle becomes indented on the ventral side behind the prominence of fourth toe, which marks the prominence of the third toe after 45 days. The tadpoles measured 35.57±0.47 mm (Fig. 4.4f).

Stage 34: Third indentation. At this stage around 48 days, the margin of foot paddle becomes indented on the ventral side behind the prominence of third toe, which marks the prominence of second toe. The tadpoles measured 38.8±1.05 mm (Fig. 4.4h).

Stage 35: Fourth indentation. After 50 days the tadpoles now measured

40.67±0.48mm. At this stage the first toe of the hind limb is prominent. All the five toes are visible and fourth toe is very long. The margin of foot paddle becomes indented on the ventral side behind the prominence of second toe, which marks the prominence of first toe (Fig. 4.4i).

Stage 36: 3-5 toes are separated. At day 51 all five toes are clearly visible and there is proper separation of the toes from the third to the fifth. Maximum incurvation between fourth and fifth toe directed towards the tip of second toe. The tadpoles now measured 42.29±0.26mm (Fig. 4.4g, j).

Stage 37: All toes separated. Margin of fourth and fifth toe directed towards the tip of first toe. Pigmentation appears in the fourth and fifth toes along the foot. Toes are longer and all toes were separated. The total length of the tadpoles were 42.82±0.46 mm after 52days). (Fig. 4.5a).

Stage 38: Appearance of inner metatarsal tubercle. After day 54 the tadpoles now measured 43.21±0.11mm. The tadpoles developed a small outgrowth on the inner side of the toe called metatarsal tubercle. Limbs are creamy white with dark patches of pigmentation that spread along the toes. The structures of the oral disc are more prominent and well develop at this stage (Fig. 4.5b).

Stage 39: Appearance of subarticular tubercles. At this stage subarticular tubercles are prominent and the inner metatarsal tubercles appear as shovel shaped at around 55 days. The hind limbs become very prominent marked with dark patches of pigmentation all over that speads along the upper toes portion of toes. Now the tadpoles measured 43.93±0.33 mm (Fig. 4.5c).

X. Well developed hind limb stages

Stage 40: Appearance of toe pads. After day 58, toe pads are completely developed, and hind limbs are well developed with differentiated toes. Mouthparts are well developed and vent tube is still visible. The tadpoles measured 45.43±0.26 mm (Fig. 4.5d).

Stage 41: Vent tube gone. Cloacal tail piece is reduced and the forelimbs are visible through the epidermis. The tadpoles attained maximum length at this stage measured 47.28±0.38 mm after 61days (Fig. 4.5e).

XI. Metamorphosis

Stage 42: Forelimbs emerge. After day 67 drastic changes of metamorphosis begins. The emergence of both forelimbs takes place. Right limb emerges first by rupturing opercular fold and left forelimb emerges later through spiracle opening. Mouth restructuring takes place anterior to nostril. The keratinised mouthparts started to degenerate. Body stripes clearly visible at this stage. The tadpoles measured 42.85±0.34 mm (Fig. 4.5f).

Stage 43: Mouth between nostril and eye, tail atrophies. This stage is marked by angle of mouth become extended between nostril and the anterior margin of the eye at around 69 days. The dorsal and ventral fins start shrinking. The tail resorption starts. The tadpole now looks like miniature of frog with tail. At this stage, they started to live outside the water body. The total length of the froglets measured 30.97±0.62mm (Fig. 4.5g).

Stage 44: Mouth beneath eye, tail greatly reduced. Angle of mouth reaches the middle of the eye at 71 days. After the completion of forelimb and hindlimb development, tail resorption is very fast. The dorsal and ventral fins have disappeared. The froglets measured 24.72±0.91mm (Fig. 4.5h).

Stage 45: Angle of mouth reached posterior margin of the eye. At 73days the tail reabsorption continues, a dark triangular stub still remains. The mandible extended beyond the eye. Tongue was fully developed. The froglets measured 18.03±0.49 mm (Fig. 4.5i).

Stage 46: Metamorphosed into froglet. Finally after 75 days the process of metamorphosis is completed and the froglet appears to be a miniature of adult frog. The tail reabsorption is still in the process with small dark triangular stub remains intact. The froglets measured 16.6±0.42 mm (Fig. 4.5j).

As developmental stage progress, it was observed that the tadpole’s total length also grows and there are morphological differences between different life stages of the tadpoles. Total length of tadpole continues increasing till Gosner stage 41, then there is a gradual decreased in the total length from Gosner stage 42 till completion of metamorphosis which is due to reabsorption of the tail (Fig. 4.6 & 4.7). Measurements (in mm) of tadpoles of various Gosner stages (25, 28, 30, 34, 38, 40 42, 44, 46) are given in Table 4.6.

Pearson correlation between Body length (BL) and Tail length (TL) of *P. braueri* tadpoles from Goner stage 40–46 shows significant correlation ($p<0.001$) (Table 4.5). This shows that with every decreased in body length tail length also decreased and vice versa.

Description of tadpoles

Based on Gosner stage 34 tadpoles, nostril was rounded with faint dark rim, positioned dorsolaterally, closer to snout than eyes (END/NSD = 1.39 ± 0.38). Eyes lateral, relatively small; pupils oriented laterally, interorbital distance wider than internasal distance (IOD/ IND = 1.64 ± 0.11). Spiracle single sinistral, positioned laterally in the form of a tapering tube; vent tube medial, dextral, oblique; vent opening located at the ventral edge of the ventral fin; tail moderately long, represents approximately two thirds of the total length (TAL/TL = 0.67 ± 0.02), lanceolate, elongate narrow tapering tip. Tail higher than body, caudal musculature accounting for 52.77% of height of tail (TAH/TMH = 1.93 ± 0.16). Mouth positioned anteroventrally.

Description in dorsal view

The tadpole of *P. braueri* at Gosner stage 34 has an elongated broadly ovoid body from dorsal view with a long tail, snout bluntly rounded, body longer than wide (BL/BW = 1.82 ± 0.09), body wider posteriorly (Fig. 4.8a). Eyes are dorso-laterally located while nostrils are positioned antero-posteriorly, and both are visible from dorsal view while spiracle is not visible. Body almost uniformly light-brown or dark-greyish-brown.

Description in ventral view

In ventral view (Fig. 4.8b), body pigmentation is darker at the anterior region while abdomen is whitish/ greyish with dark patches more to the lateral side compared to the medial region. Both eyes and mouthparts are clearly visible while spiracle is problematic to locate from the ventral view. Intestinal coil is slightly visible. The junction of the tail is broad at body and tapered towards the end. Vent tube is dextral, the opening lying to the right of the plane of the ventral fin.

Description in lateral view

In lateral view (Fig. 4.8c), body depressed dorsoventrally, especially in its rostral part, with a flat dorsum. Spiracle present on the left lateral side of the body. Tail fins slightly transparent, whitish in colour, musculature strongly developed, broad at body tail junction tapering beyond, tail fins relatively tall; dorsal fin originating slightly anterior to the body-tail junction, nearly equal in height to the ventral fin.

Colouration

Colouration in life

Vital colouration is almost uniformly light-brown or darkgreyish-brown above, sometimes almost black, with delicate light brown/black marble speckling on the tail fins; belly slightly paler than the dorsal surface, spiracle and vent tube grey and translucent. The proximal quarter of the tail is coloured similarly to the body, and the rest of the tail is markedly paler. The iris is dark grey or black, with a thin golden rim around the pupil. A small white patch is present at the tip of the snout from Gosner stage 24 till stage 40 (Fig. 4.8c).

Colouration in preservative

In preservative, the colouration fade to light grey, and the tail colouration has less contrast. Intestinal coil visible on the abdomen. The iris is black, a thin golden rim around the pupil disappeared. A small white patch at the tip of the snout is slightly visible (Fig. 4.8a & b).

DISCUSSION

From this work, it was found that *P. braueri* completed its life cycle within 75 days, in the laboratory conditions as well as in the natural condition where water temperature 12.5–30.3℃ and pH 5.78–8.88 likely to be optimal ranged. Tadpole hatched at stage-20 and left the foam nest at stage 22 after 4 days and 3 hours (9.64±0.13 mm). Gosner (1960) reported that the embryos of most species hatch between the stages 17–20. The larva attain maximum size at stage 41 (47.28±0.38 mm) after 61 days. According to Chakravarty et al. (2011) the foam nest is essential for development of this rhacophorid species, when eggs were removed from the foam nest before hatching the embryo did not develop further. The difference in the duration of embryonic and larval period may also be depending on species as reported by Rowe et al. (2001).

Different species belonging to the genus *Polypedates* showed variances on their size of eggs, time of hatching, size of tadpoles and time of metamorphosis as shown in the table given below (Table 4.7). The current study showed that *P. braueri* completed metamorphosis after 75 days of fertilization at atmospheric temperature between 14–33.5℃. Tadpole hatched at stage 20 after 2days 19 hours. *P. maculatus* completed metamorphosis within 50–70 days at temperature ranging between 30˚C–35˚C (Mohanty- Hejmadi & Dutta, 1988; Girish & Saidapur, 1999; Saidapur, 2001). Tesia et al. (2017) reported that *P. megacephalus* completed metamorphosis after 45 days at water temperature 18–20℃. *P. teraiensis* completed metamorphosis within 58–64 days at air temperature between 26ºC–38ºC. Tadpole hatched at stage 20 after 3 day 4 hour/4 day 8 hour (Chakravarty et al., 2011; Lalramdinfeli, 2020), *P. himalayensis* within 71 days at temperature between 8.5–23.7 ˚C. The tadpoles hatched at stage 19 after 3 and half days (Rangad, 2020). *P. leucomystax* metamorphosed within 67–100 days at temperature between 24˚C–30˚C (Alcala, 1962; Kiyasetuo & Khare, 1986; Iangrai, 2022). *P. otilophus* completed within 74–131 days at temperature between 19˚C–30˚C. The tadpole hatched at stage 25 after 10 days (Tapley & Girgin, 2015; Galunder & Rodder, 2018). Comparing the size of newly metamorphosed froglet with the existing literature, *P. teraiensis* is the smallest with SVL 15.55 mm (Lalramdinfeli, 2020), 15.6 mm (Chakravarty et al., 2011) and 16.2 mm (Tamuly & Dey, 2014), followed by *P. braueri* (16.6 mm)(This study), *P.*

himalayensis (17.57 mm) (Rangad, 2020), *P. leucomystax* 19.4 mm (Sheridan, 2008) and 20 mm (Iangrai, 2022), and *P. maculatus* (21– 23 mm) (Saidapur, 2001).

In the present study overwintering tadpoles were also observed during the month of December-March, probably did so because they did not reach the minimum size for metamorphosis before the onset of winter (Wilbur & Collins, 1973; Collins, 1979). Overwintering *P. braueri* tadpoles continued to grow and develop, but at a slow rate due to the low winter temperatures. Some individuals reached metamorphosis during the winter, while some of the tadpole metamophosed only after the temperature rised i.e late February to March and some never did. Our observations were also supported by Hsu et al. (2012). Temperature, length of growing season, timing of breeding, and resource availability can influence whether tadpoles overwinter (Collins, 1979; Brown, 1990; Bury & Adams, 1999).

According to Raj et al. (2023), tadpoles of *Polypedates* have a compressed and rounded body; eyes mostly lateral and in some cases anterolateral; tail long and flagellum common; caudal fins high, extending sometimes anterior to body tail junction; tail muscle not massive; feeding by rasping surfaces with anteroventral mouth. Light point on snout was present in *P. braueri*, which was also reported in *P. leucomystax* (Flower, 1896; Alcala & Brown, 1956; Alcala, 1962; Smith, 1917), *P. megacephalus* (Pope, 1931; Liu, 1940; 1950; Chou & Lin, 1997; Grosjean, 2004) and *P. mutus* (Grosjean, 2004); but absent in *P. maculatus* (Mohanty-Hejmadi & Dutta, 1988; Raj et al., 2023), *P. himalayensis* (Annandale, 1912; Grosjean, 2004), *P. himalayensis* (Rangad, 2021); *P. megacephalus* (Raj et al., 2023); *P. leucomystax* (Inger, 1956); *P. pseudocruciger* (Raj, 2023) and *P. teraiensis* (Chakravarty et al., 2011; Lalramdinfeli, 2017).

The planning of the frog's conservation in its native environments can make use of these insights. Larval characteristics are sometimes a very useful tool for anuran taxonomy delimitation. Taxonomically, an integrated strategy that combines morphological and molecular data, as in the current work, may be helpful. To comprehend the phylogenetic and evolutionary importance of larval anurans across different lineages, more thorough research is required. According to Sheratt et al. (2018), tadpoles with comparable ecological niches and feeding habits also tend to have similar body features, which provide credence to the idea of "guild".

Convergence in morphologies between lineages can be explained by the fact that tadpoles' external morphology is known to be adaptable to the various ecological settings in which they live (Orton, 1953, Altig & Johnston, 1989). Likewise, anuran larvae's buccopharyngeal and oral morphology are tailored to their eating habits (van Buskirk, 2009; Haad et al., 2011).

Table 4.1: Monthly variation in water temperature and pH during 2021–2023 at Reiek Community Reserved Forest.

2021			2022			2023		
	Water temperature			Water temperature			Water temperature	
pH	Min	Max	pH	Min	Max	pH	Min	Max
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	7.2±0.46	14.2	29.4
*	*	*	7.2±0.40	14.9	30.1	6.9±0.04	14.5	29.8
7.4±0.26	18.6	30.3	6.9±0.34	15.2	29.8	7.1±0.26	17.2	29.8
7.2±0.32	17.2	29.6	6.9±0.24	16.4	27.6	6.6±0.42	17.2	29.6
7.1±0..30	18.2	29.4	6.8±0.44	19.9	29.8	7.2±0.24	19.4	29.6
7.2±0.24	19.3	28.8	6.6±0.32	18.8	29.8	7.1±0.46	19.8	28.9
6.6±0.31	19.5	23.8	6.9±0.36	19.2	29.9	6.6±0.14	19.8	30.1
6.9±0.27	18.6	30.3	7.1±0.26	16.4	30.2	7.1±0.26	18.6	29.8
*	*	*	*	*	*	6.8±0.53	15.2	29.4
*	*	*	*	*	*	*	*	*

Table 4.2: Monthly variation in water temperature and pH during 2021–2023 at Sailam Community Reserved Forest.

2021			2022			2023		
	Water temperature			Water temperature			Water temperature	
pH	Min	Max	pH	Min	Max	pH	Min	Max
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
6.9±0.32	15.1	28.4	6.8±0.53	14.5	29.8	6.9±0.51	12.5	29.6
6.9±0.42	13.4	29.8	6.9±0.67	15.4	29.9	6.7±0.65	15.4	29.8
7.2±0.24	16.2	28.6	7.1±0.24	16.2	27.7	7.2±0.24	16.2	28.6
7.3±0.42	16.8	29.3	7.2±0.46	17.1	28.4	7.1±0.46	19.6	28.2
7.1±0.36	15.1	29.7	6.9±0.04	16.5	29.6	6.6±0.14	18.5	29.6
7.2±0.42	13.4	28.8	7.1±0.26	18.6	28.8	7.1±0.26	18.6	28.8
6.9±0.41	15.2	29.5	6.6±0.42	16.2	27.8	6.4±0.02	16.8	26.8
6.9±0.34	14.3	26.4	6.9±0.32	14.4	25.7	6.89±0.12	14.6	25.8
6.8±0.43	10.6	23.4	6.9±0.24	10.8	24.2	6.9±0.44	11.6	24.4

Table 4.3: Monthly variation in water temperature and pH during 2021–2023 in the Laboratory.

2021			**2022**			**2023**		
	Water temperature			**Water temperature**			**Water temperature**	
pH	**Min**	**Max**	**pH**	**Min**	**Max**	**pH**	**Min**	**Max**
7.4±0.36	7.2	19.5	7.2±0.34	7.5	19.8	6.5±0.67	8.6	19.4
7.2±0.42	8.6	22.1	6.4±0.33	9.3	21.2	6.8±0.53	10.4	22.2
7.1±0.34	12.5	22.5	6.5±0.37	13.7	26.1	6.9±0.27	14.5	26.2
6.9±0.46	15.1	28.4	6.8±0.53	16.5	29.6	7.1±0.34	16.1	29.6
6.7±0.45	15.4	30.2	6.9±0.27	15.4	29.9	7.2±0.34	17.4	29.6
7.2±0.34	16.2	29.6	7.1±0.34	16.8	29.7	6.4±0.33	19.2	30.1
7.3±0.43	16.8	29.3	7.1±0.26	17.1	28.2	6.9±0.02	19.8	28.6
7.1±0.26	18.1	29.7	6.8±0.04	16.5	29.6	6.9±0.04	18.1	30.2
7.2±0.34	17.4	28.8	7.1±0.06	17.1	29.8	7.1±0.34	18.6	29.8
6.9±0.42	18	29.5	6.1±0.02	17.2	28.8	7.1±0.66	18.4	28.8
6.7±0.54	16.3	27.4	6.9±0.02	18.4	25.5	6.8±0.53	16.3	25.4
6.8±0.53	10	25.4	6.9±0.04	9.8	24	6.9±0.67	9.2	24.4

Table 4.4: Developmental stages of *Polypedates braueri* (Measurements are given as mean±SD) (N=10).

	NO	STAGE	AGE	SIZE (mm)
EMBRYOS	1	Fertilization	0 h	1.83±0.01
	2	Grey crescent	0:30 h	1.83±0.01
CLEAVAGES	3	2-cell	1:50 h	1.83±0.01
	4	4-cell	2:50 h	1.84±0.01
	5	8-cell	3:45 h	1.85±0.01
	6	16-cell	5:50 h	1.87±0.01
	7	32-cell	7:00 h	1.92±0.01
	8	Mid cleavage	9:15 h	1.95±0.01
	9	Late cleavage	10:30h	1.98±0.01
GASTRULA	10	Dorsal lip	12:10 h	2.06±0.03
	11	Yolk plug	13:25 h	2.14±0.02
	12	Late Gastrula	15:15h	2.17±0.04
NEURULATION	13	Neural plate	16:50 h	2.27±0.01
	14	Neural folds	17:40h	2.32±0.01
	15	Elongation, rotation	18:10 h	2.36±0.02
	16	Neural tube, gill plates	21:10 h	2.55±0.03
TAIL BUD STAGES	17	Tail bud, adhesive gland	23:40 h	2.68±0.02
	18	Muscular response, olfactory pits	1d 3h	2.77±0.04
	19	Heartbeat, gill buds	1d 20h	5.748±0.2
	20	Gill circulation, tail elongation	2d 19 h	7.32±0.24
EXTERNAL GILLS	21	Cornea transparent, mouth opens	3d 7h	8.46±0.18
	22	Tail fins transparent, fin circulation	4d 3h	9.64±0.13
OPERCULUM, ORAL DISC	23	Labia and teeth differentiation, operculum covers gill bases	5d 18 h	11.15±0.33
	24	External gills atrophy, operculum closes on right	8	11.6±0.11
	25	Mouthparts obvious, spiracle forms on left	10	14.72±0.53
HIND LIMB BUD FORMATION	26	L< ½ D	22	25.19±0.53
	27	L>1 ½ D	24	29.13±0.46
	28	L> D	26	31.08±0.68
	29	L>1 ½ D	30	30.66±1.41
	30	L=2D	36	32.1±0.65

Table 4.4: Continued-

TOE DIFFERENTIATION AND DEVELOPMENT	31	Foot paddle	39	33.09±0.67
	32	Indentation 4-5	43	34.82±0.54
	33	Indentation 3-4	45	35.57±0.47
	34	Indentation 2-3	48	38.8±1.05
	35	Indentation 1-2	50	40.67±0.48
	36	3-5 toes are separated	51	42.29±0.26
	37	All toes separated	52	42.82±0.46
	38	Metatarsal tubercle	54	43.21±0.11
	39	Sub-articular tubercle	55	43.93±0.33
DEVELOPED HIND LIMB METAMORPHOSIS	40	Foot tubercles	58	45.43±0.26
	41	Forelimbs visible, vent tube gone	61	47.28±0.38
	42	Forelimbs emerge, mouth anterior to nostril	67	42.85±0.34
	43	Mouth between nostril and eye, tail atrophies	69	30.97±0.62
	44	Mouth beneath eye, tail greatly reduced	71	25.96±2.04
	45	Mouth exterior to eye	73	18.03±0.49
	46	Metamorphosis complete	75	16.6±0.42

Table 4.5: Pearson correlation between Body length (BL) and Tail length (TL) of *P. braueri* tadpoles from developmental stages 40–46 showing significant correlation ($p<0.001$).

		BL	TaL
BL	Pearson Correlation	1	.690**
	Sig. (2-tailed)		.000
	N	70	70
TaL	Pearson Correlation	.690**	1
	Sig. (2-tailed)	.000	
	N	70	70

Table 4.6: Measurements of tadpoles of *Polypedates braueri* in various Gosner stages (25, 28, 30, 32, 34, 38, 40, 42, 44 & 46). Mean ± standard deviation and range of 10 measured individuals per stage.

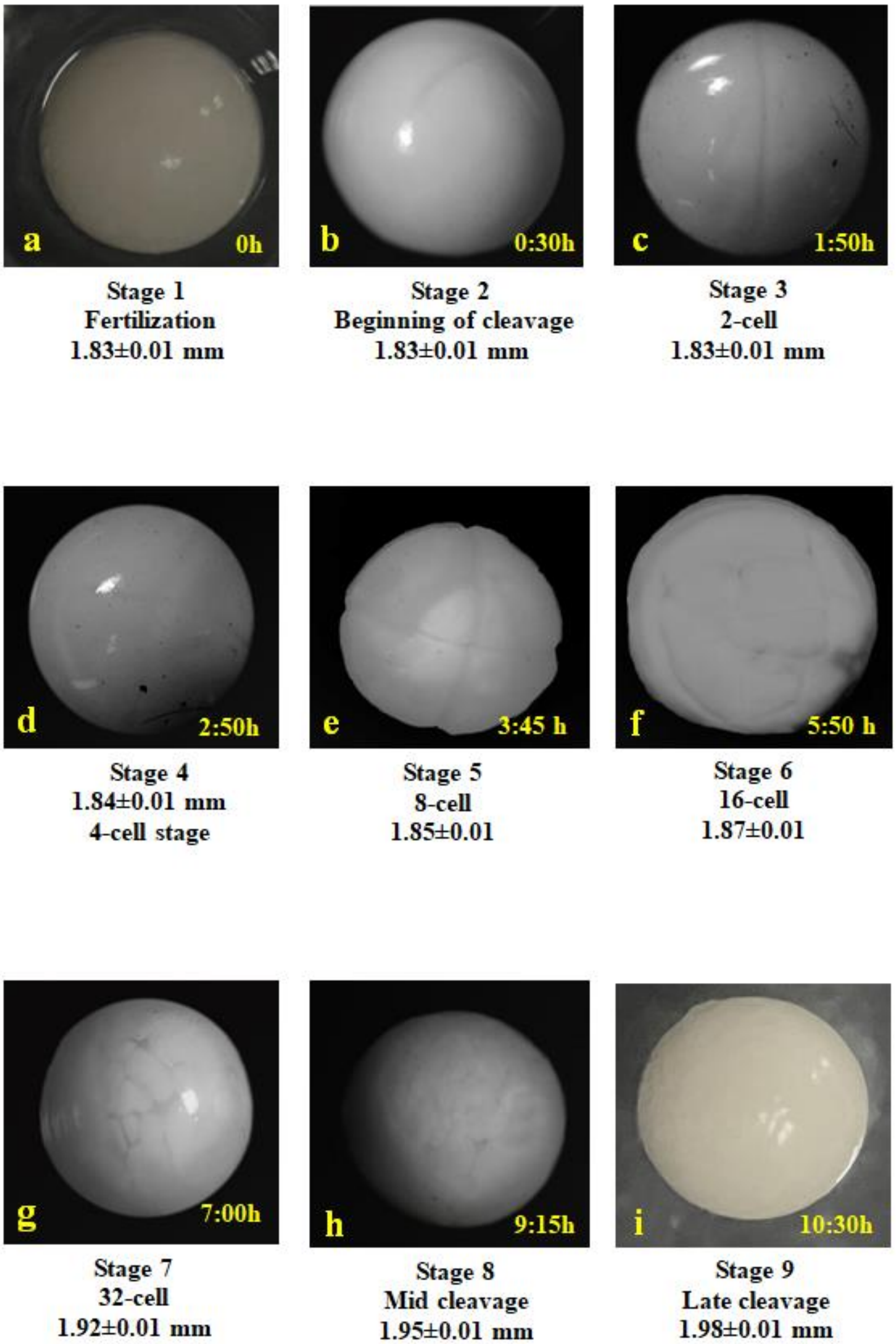
	Oral Disc	**Hind limb bud development**		**Toe differentiation**			**Developed hind limb**	**Metamorphosis**		
Characters	**Stage 25**	**Stage 28**	**Stage 30**	**Stage 32**	**Stage 34**	**Stage 38**	**Stage 40**	**Stage 42**	**Stage 44**	**Stage 46**
TL	14.72±0.5	30.70±1.19	31.62±0.74	34.82±0.54	38.80±1.05	43.28±0.21	45.43±0.26	42.85±0.31	25.96±2.04	16.59±0.42
BL	10.05±0.25	11.04±0.68	11.56±0.74	12.82±0.39	13.50±0.21	14.62±0.16	15.12±0.30	16.82±0.93	14.82±0.69	14.32±0.50
BW	4.97±0.22	6.38±0.28	6.55±0.21	6.46±0.48	7.42±0.11	8.44 ±0.25	8.49±0.35	6.71 ±0.26	7.34±0.91	5.53±0.30
IOD	2.99±0.18	3.96±0.65	4.3 ±0.75	4.24±0.47	4.66±0.11	5.22 ±0.38	5.08±0.31	3.52 ±0.07	5.58±0.94	3.81±0.04
IND	1.77±0.05	3.11±1.33	2.56± 0.24	2.76±0.19	2.90±0.05	3.1 ±0.18	3.09±0.23	2.42 ±0.14	2.91±0.11	1.93±0.11
END	0.95±0.27	2.56±0.33	2.63±0.24	2.59±0.26	2.65±0.07	3.33 ±0.36	2.61±0.05	2.47 ±0.21	2.70±0.15	1.84±0.03
SOD	1.73±0.03	3.78±0.21	3.8 ±1.12	3.89±0.29	4.32±0.07	4.92 ±0.25	4.97±0.15	3.74± 0.47	3.84±0.48	2.88±0.03
NSD	0.82±0.07	1.77±0.05	1.76± 0.03	1.93±0.14	2.11±0.16	2.38± 0.19	2.36±0.03	1.67 ±0.13	1.29±0.19	0.56±0.05
TAL	10.15±0.31	19.16±0.95	20.25±1.50	24.46±0.89	24.48±0.37	29.92±0.61	30.66±0.30	29.21±0.83	9.07±1.47	3.85±0.55
TAH	2.98±0.37	5.64±0.17	5.85 ±0.33	6.47±0.29	8.30±0.06	9.85± 0.27	8.44±0.06	5.1 ±0.34	3.04±0.59	1.60±0.04
TMH	1.56±0.12	3.02±0.42	3.32 ±0.35	3.78±0.13	4.37±0.09	5.19± 0.41	4.53±0.09	3.24 ±0.20	3.04±0.59	1.60±0.04
TMW	0.81±0.05	1.744±0.16	2.05 ±.0.43	2.84±0.21	3.42±0.05	3.86 ±0.22	4.18±0.18	3.21 ±0.53	3.21±0.52	1.88±0.09
UF	1.14±0.21	1.82±0.07	2.04 ±0.33	2.72±0.14	3.01±0.02±	3.23 ±0.17	3.08±0.04	1.16±0.13	-	-

Table 4.7: Comparison of development of *P. braueri* with congener species.

Species	Size of eggs (mm)	Hatching stage and age	Size of hatching tadpole (mm)	Metamorphosis (Days)	Size of newly metamorphosed froglet (mm)	Air Temperature (ºC)	Water Temperature (ºC)	References
Polypedates braueri	1.83±0.01	20(24d 19 h)	9.64±0.13	75	16.6±0.42	14–33.5	10.6–30.3	This Study
Polypedates himalayensis	1.87 ± 0.01	19 (3½ days)	3.2 ± 0.02	71	17.57 ± 0.25	10.3– 25.8	8.5–23.7	Rangad (2020)
Polypedates otilophus	-	-	-	74-84	-	22–26	-	Tapley & Girgin (2015)
	-	25 (10d)	-	120-131	-	19–27	-	Galunder & Rodder (2018)
Polypedates megacephalus	2.0	22(8.09)	-	89	16.4–16.6	-	18–20	Tesia et al. (2017)
Polypedates teraiensis	1.34±0.07	20 (3d 4h)	7.18 ±0.49	61– 64	15.55 ± 0.37	26–38	24–28	Lalramdinfeli (2020)
	2.0–2.2.	20 (4d 8h)	4.7–5.3	58	15.6–17.4	26–32	-	Chakravrty et al. (2011)
		22 (4d)	7.8	42	16.2	26–33	-	Tamuly & Dey (2014)

Table 4.7: Continued-

Species	Size of eggs (mm)	Hatching stage and age	Size of hatching tadpole (mm)	Metamorphosis (Days)	Size of newly metamorphosed froglet (mm)	Air Temperature (ºC)	Water Temperature (ºC)	References
Polypedates maculatus	-	-	-	55	-	30–35	-	Mohanty-Hejmadi & Dutta (1988)
	-	23(2–3d)	-	60	-	-	-	Girish & Saidapur (1999)
	-	-	-	50–70	21-23	-	-	Saidapur (2001)
Polypedates leucomystax	-	-	-	70–100	-	-	24–30	Alcala (1962)
	-	-		57–58	-	15–24	-	Kiyasetuo & Khare (1986)
	-	-	-	42	19.4	-	-	Sheridan (2008)
	1.5	21(4)	-	6–61	20	21.3–25.7	18.2– 24.0	Iangrai (2022)



Stage 1
Fertilization
1.83±0.01 mm

Stage 2
Beginning of cleavage
1.83±0.01 mm

Stage 3
2-cell
1.83±0.01 mm

Stage 4
1.84±0.01 mm
4-cell stage

Stage 5
8-cell
1.85±0.01

Stage 6
16-cell
1.87±0.01

Stage 7
32-cell
1.92±0.01 mm

Stage 8
Mid cleavage
1.95±0.01 mm

Stage 9
Late cleavage
1.98±0.01 mm

Figure 4.1: Developmental stages of *Polypedates braueri* from Gosner stage 1 to 9.

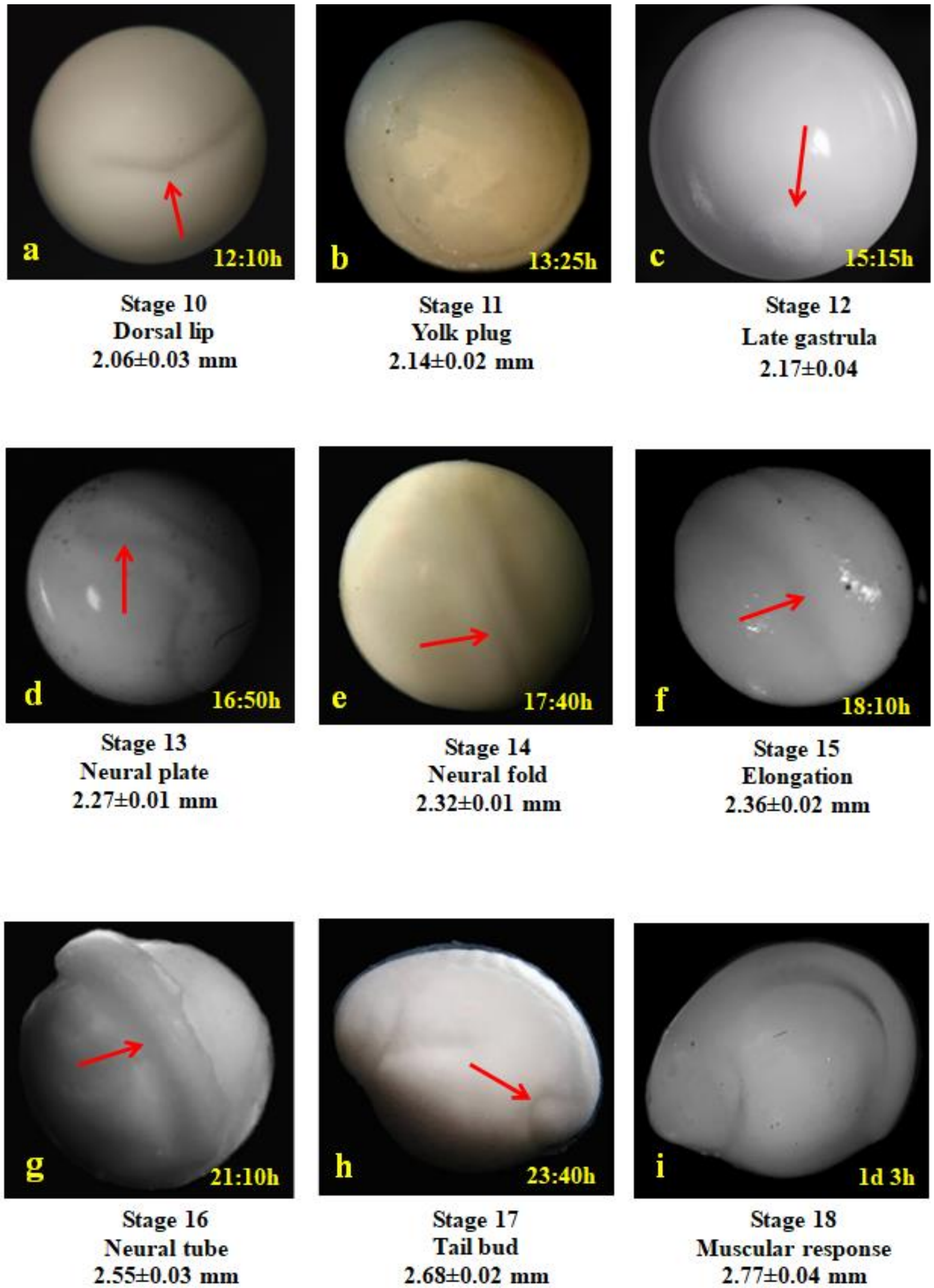


Figure 4.2: Developmental stages of *Polypedates braueri* from Gosner stage 10 to 18.

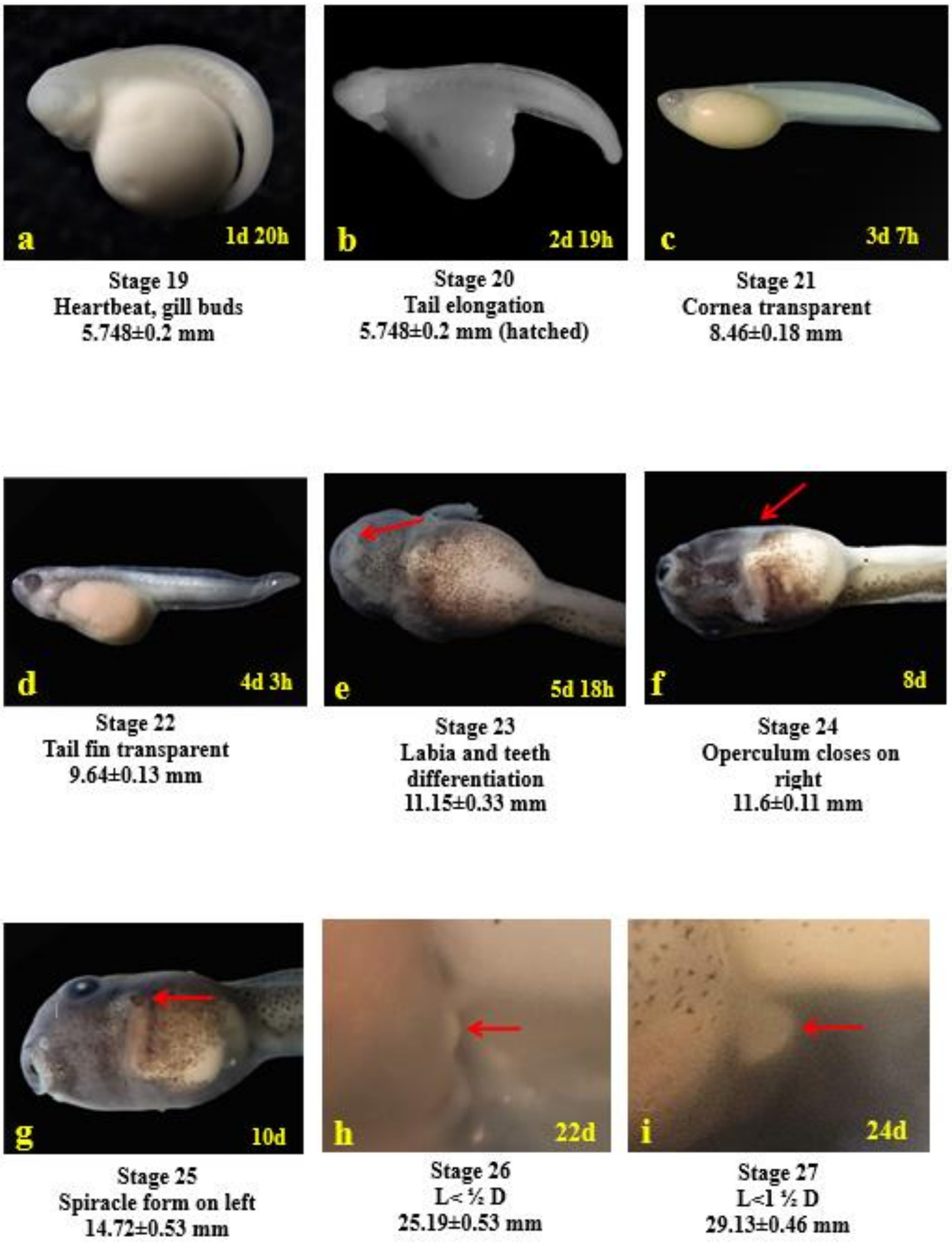


Figure 4.3: Developmental stages of *Polypedates braueri* from Gosner stage 19 to 27.

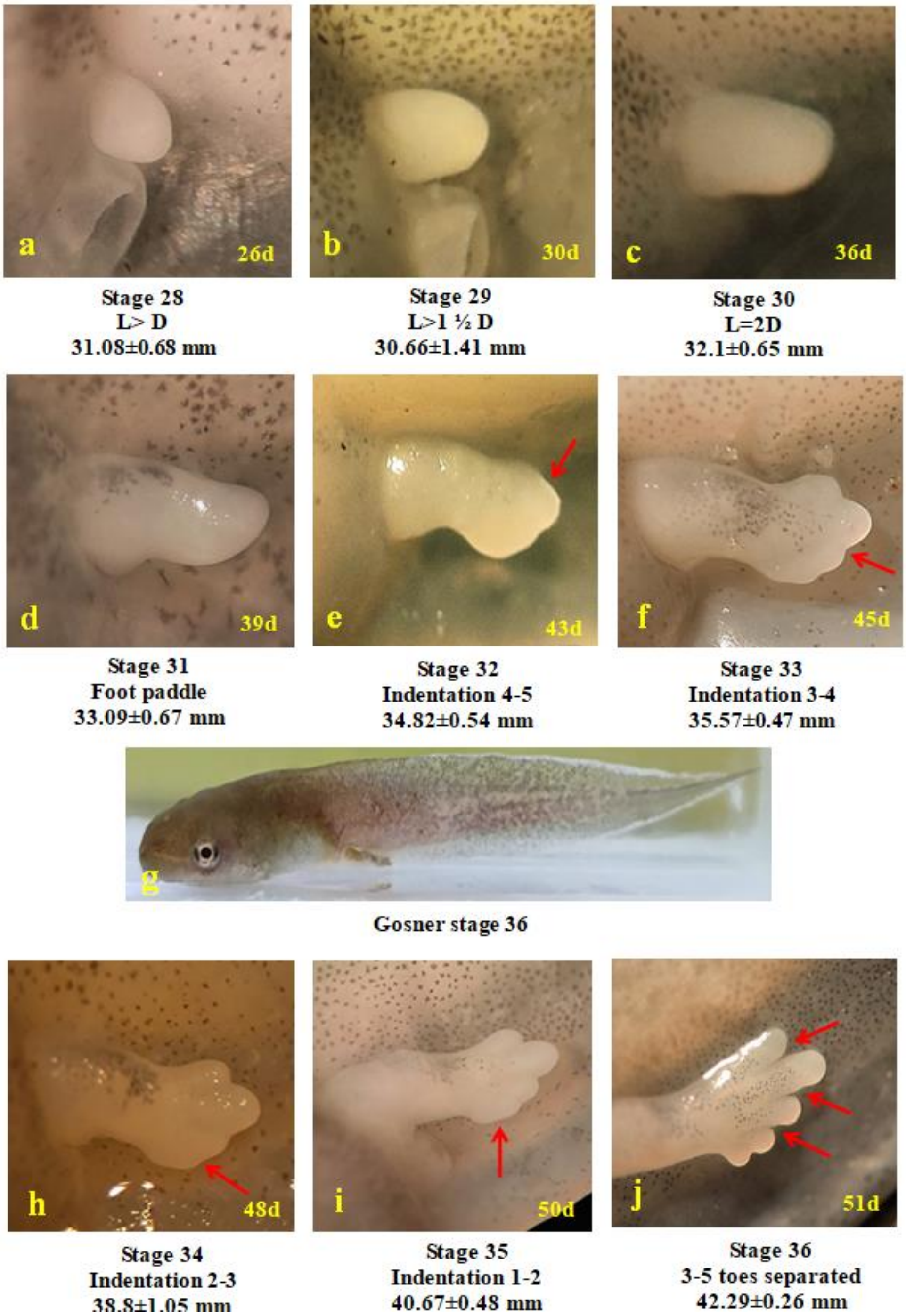


Figure 4.4: Developmental stages of *Polypedates braueri* from Gosner stage 28 to 36.

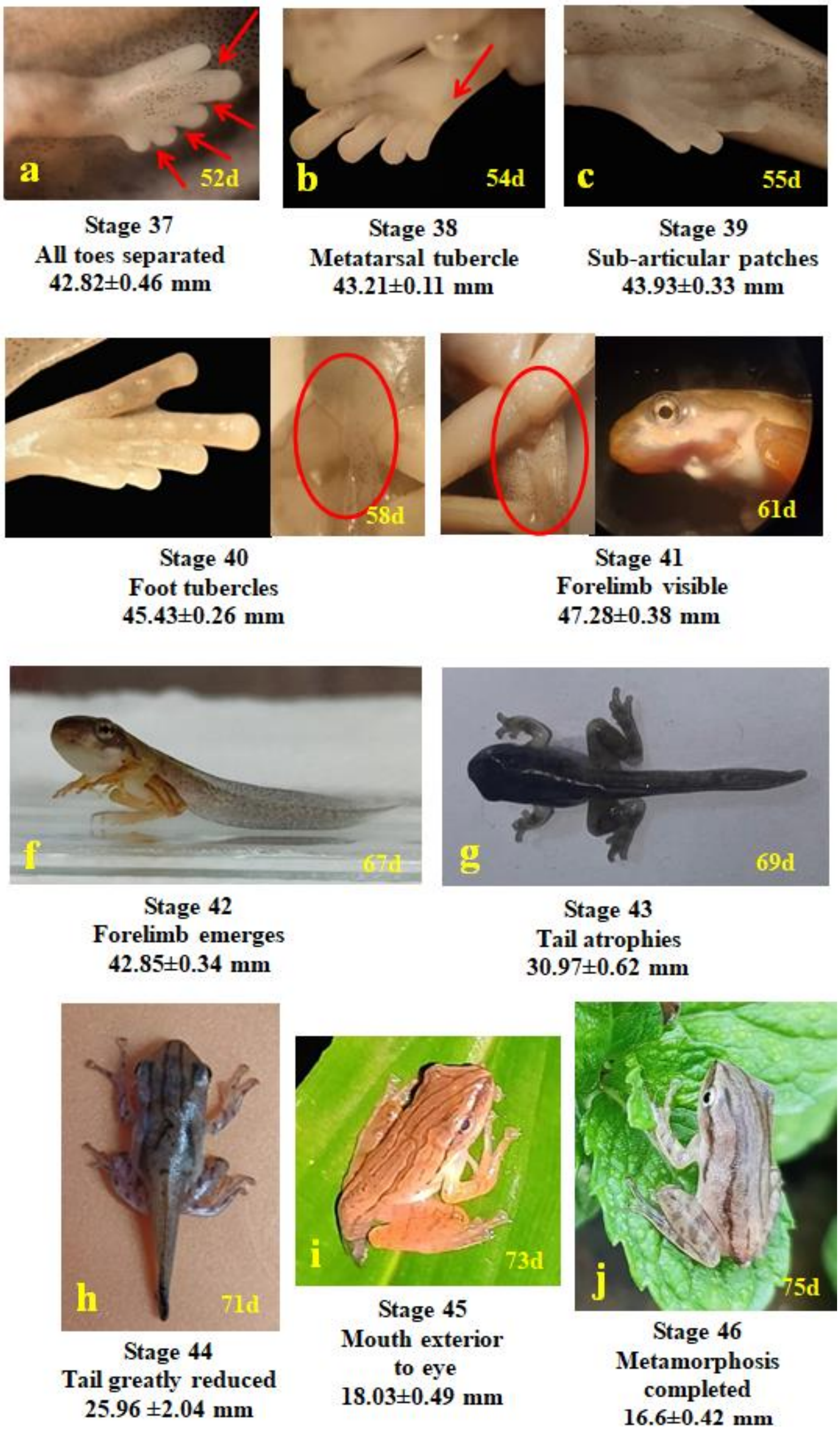


Figure 4.5: Developmental stages of *Polypedates braueri* from Gosner stage 37 to 46.

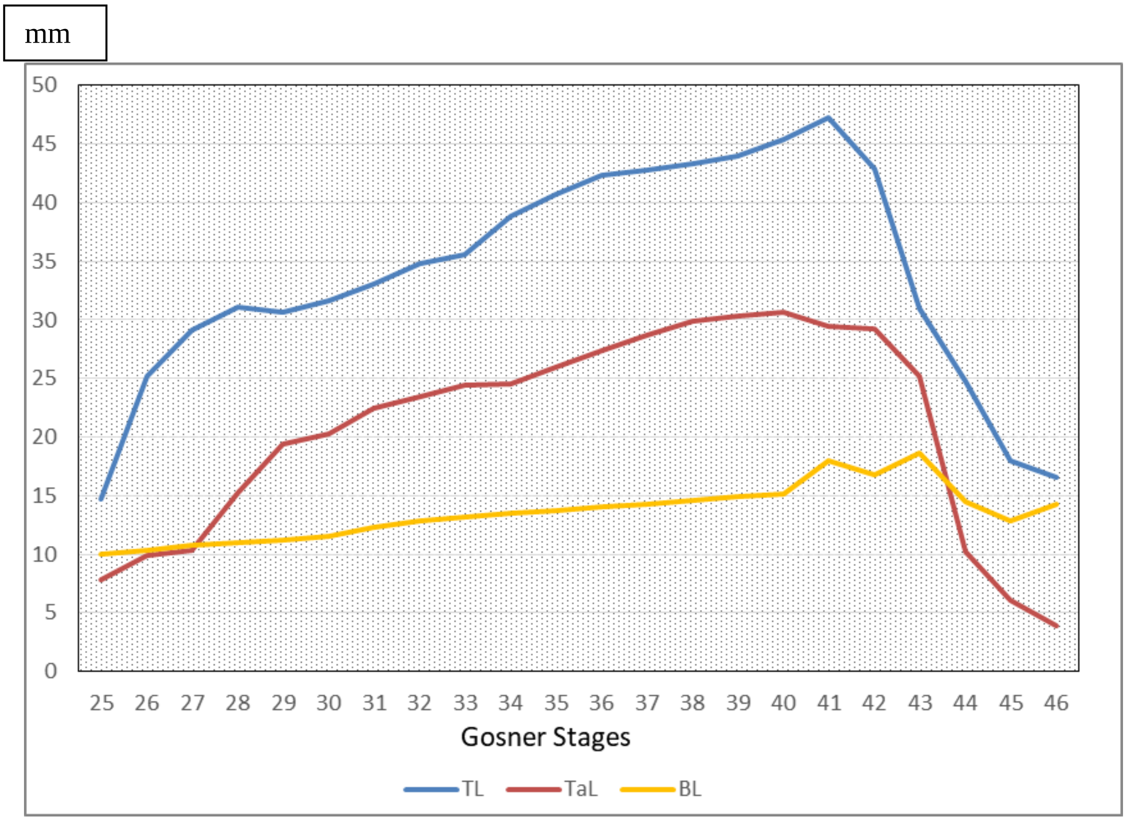


Figure 4.6: Ontogenic changes in the Total length (TL), Tail Length (TaL) and Body Length (BL) of P. *braueri* at each Gosner stages from 25–46.

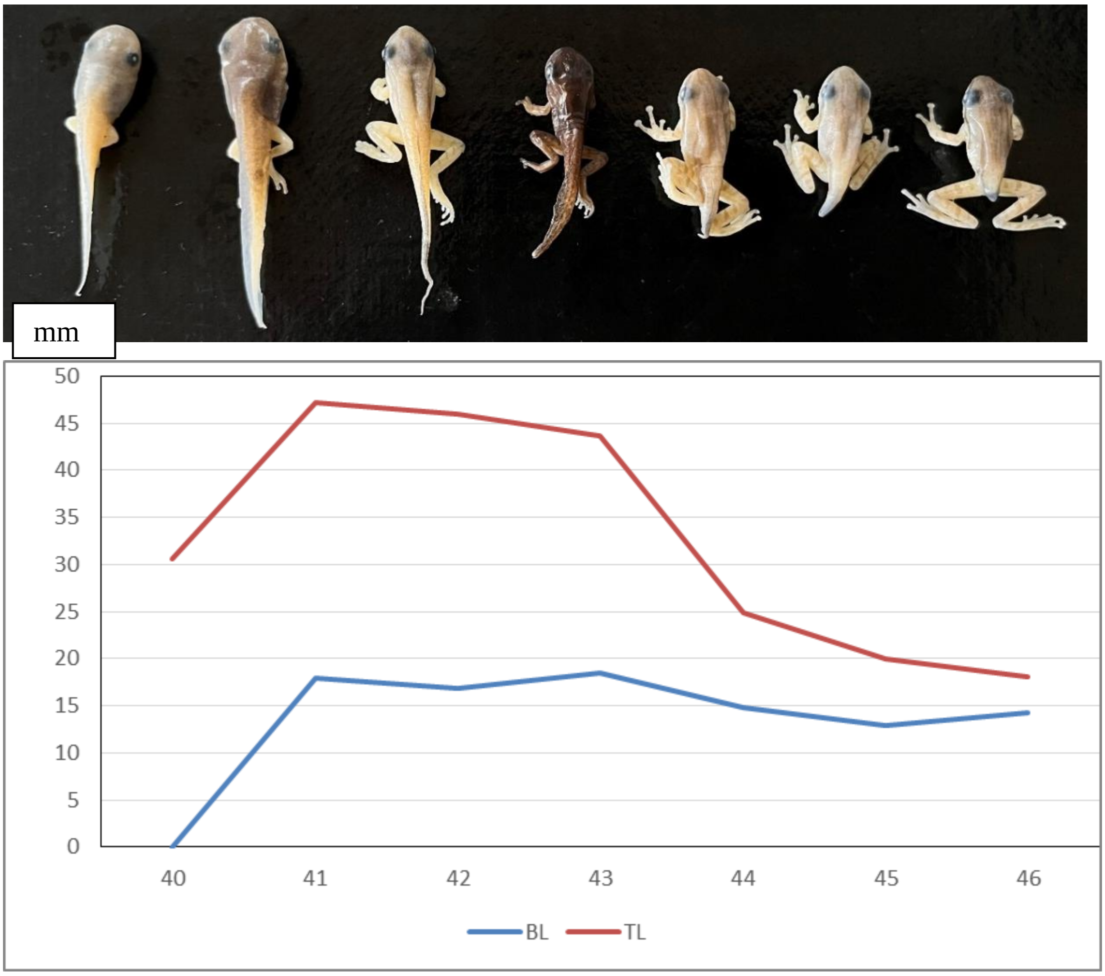


Figure 4.7: Ontogenic change in Body length (BL) and Tail length (TaL) at late pre-metamorphic (40) to metamorphic climax (46).

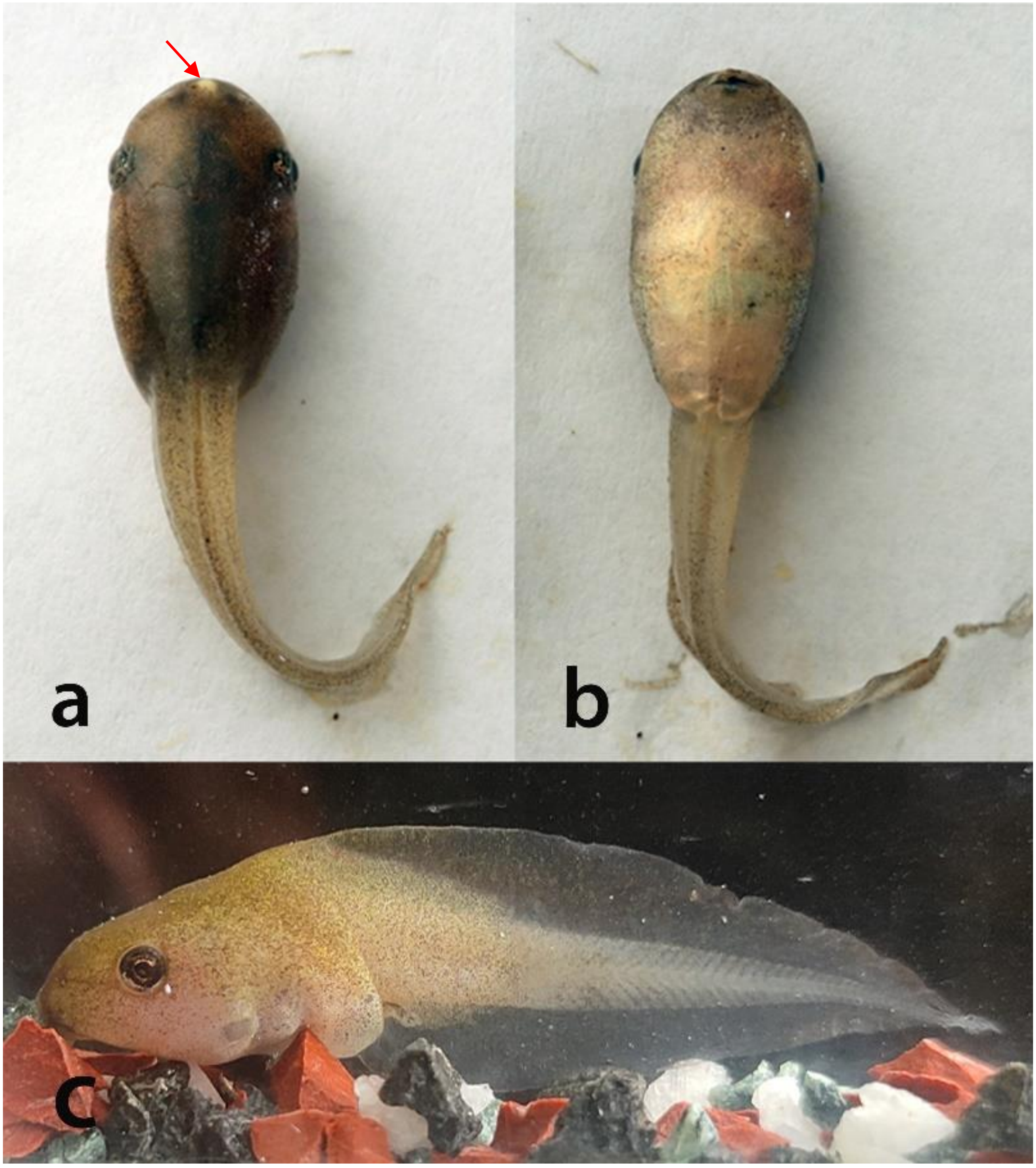


Figure 4.8: Gosner stage 34 tadpole of *Polypedates braueri* (a) Dorsal view (b) Ventral view (c) Lateral view.

CHAPTER V

TO STUDY ORAL MORPHOLOGY, FOOD AND FEEDING BEHAVIOR OF TADPOLES AND ADULTS OF *P.braueri*

INTRODUCTION

Understanding the functional roles of tadpoles within aquatic habitats requires knowledge of their feeding ecology and trophic status (Montana et al., 2019), with studies on their oral features providing information on both morphological adaptations to diverse larval habitats and higher taxonomic connections among frogs (Wassersug, 1980; Inger, 1983; Wassersug & Heyer, 1983; Khan & Mufti, 1994). Tadpoles are considered one of the most effective indicators of ecological health (Guzy et al., 2012). However, ecological and dietary specialisations of tadpoles have been shown to cause extensive changes over time in their oral apparatus, which is reflected in their adaptations to diverse habitats and phylogeny (Wassersug, 1980; Inger, 1985; Duellman & Trueb, 1994; Altig & Johnson, 1999; Khan, 1991). In addition, tadpole growth frequently results in morphological changes in its feeding equipment, including the number of teeth and gap size, allowing a larger variety of food items (Toft, 1980; Christian, 1982; Iangrai, 2007). It has been suggested that variations in the size and shape of the oral apparatus, the papillae at the margins of the oral disc, the shapes of the jaw sheaths, and the number of tooth rows and gaps in rows of teeth are all important features in identifying tadpoles and the ontogenic variation in labial teeth structure (Duellman & Trueb, 1994; James et al., 2000; Khan & Mufti, 1994; Iangrai, 2007; Li et al., 2011). More than half of the anuran species described to date still lack information on their embryonic/larval stages (Candioti et al., 2023). Further large-scale analyses on tadpole biology are crucial for their impact in varied scientific disciplines including anuran conservation. Conceptually, the discussion of the anuran biphasic life cycle is relevant in the context of shortfalls of biodiversity knowledge and their interrelationships (Flores et al., 2023; Candioti et al., 2023).

According to Wells (2007) and Rowland et al. (2016), amphibians are crucial predators of small species that make up a sizable amount of the biomass found in ecosystems. Although anurans are recognized for their generalist eating habits and prey on a wide range of invertebrate groups, the majority of amphibian species eat nearly solely arthropods (Duellman, 2005). However, some species have been reported to exhibit herbivory as in the case of *Siren lacertian*, which feeds on aquatic macrophytes (Ultsch, 1973), *Bufo marinus*, which feed on vegetable scraps

(Alexander, 1964) and *Hyla truncata*, which is frugivorous in nature (da Silva et al., 1989).

Given their evolutionary history, diet is acknowledged as one of the most significant elements of amphibians' natural history (Duellman & Trueb, 1994; Da Rosa et al., 2002). In majority of the adult anurans, the prey is captured by employing the sticky projectile tongue which is fixed at the anterior part of the mouth while the posterior part is usually free. Specialization in the diet is often found to be associated with morphological, physiological as well as behavioral characteristics that help in the location, identification, capture, ingestion and finally digestion of the captured prey items (Rangad, 2021).

Analysis of the food spectrum of anuran tadpoles is therefore very essential since amphibians are considered to play an important role as indicators of ecosystem deterioration (Wake, 1991). According to Alford (1999), information on the diets of tadpoles and field studies of tadpoles are relatively few and uncommon. Anuran tadpoles are considered important primary consumers in many freshwater habitats and play a crucial role in the food chain. Their growth is directly influenced by the amount of food consumed (Kiffney & Richardson, 2001) and the quality of that food (Kupferberg et al., 1994). Wassersug (1980) reported that differences in the internal oral features among species may be linked to tadpoles' preferences for food particles of varying sizes. Additionally, the number and density of labial teeth, as well as the arrangement of tooth rows, may represent adaptation to different foraging modes (Altig, 2006).

Dietary data for anuran larvae from India are relatively limited (Khare & Sahu, 1984; Ao & Khare, 1986; Sekar, 1990; Saidapur, 2001; Sinha et al., 2001; Khongwir et al., 2003; Dey & Goswami, 2015; Asrafuzzaman et al., 2018). The majority of research has been focused on the Western Ghats (biodiversity hotspot) and the west coast of India and other areas remain understudied (Aravind & Gururaja, 2011). Few works on anuran dietary data are available in northeast India (Iangrai, 2007; Sailo & Lalremsanga, 2011; Sengupta et al. 2013; Tamuly & Dey, 2013; Singh et al., 2014; Dey & Goswami, 2015, Lalramdinfeli, 2017, Rangad, 2021). At both the larval and adult stages, amphibians contribute significantly to ecosystems. As they develop into terrestrial adults, larval amphibians transport energy from aquatic habitats to the land and affect the variety and availability of primary consumers (Ranvestel et al., 2004). The energy from invertebrates is then transferred to higher trophic levels by adult amphibians (Burton & Likens, 1975; Duellman & Trueb, 1994; Wells, 2007; Frauendorf et al., 2013). Determining how amphibian trophic patterns impact ecosystem structure and function hence requires an understanding of these patterns (Ranvestel et al., 2004). Kowledge of food and feeding behavior of both tadpoles and adults frog is very essential for their successful breeding and survival. Dramatic declines in amphibian populations have been noted since 1980 from all over the world and are thus perceived to be one of the most critical threats to global biodiversity (Dey & Goswami, 2015).

REVIEW OF LITERATURE

The oral structure and food items of tadpoles of the genus *Polypedates* were studied by various workers. Flower (1896) reported notes on tadpoles of *P. leucomystax* from the Malay Peninsula. Annandale (1912) provided tadpole morphology and oral morphology of *P. himalayensis* and *P. maculatus* from Assam, India. Smith (1917) studied tadpoles of *P. leucomystax* from Thailand. Pope (1931) and Liu (1940; 1950) worked on tadpoles of *P. megacephalus* from China. Inger (1956) worked on *P. leucomystax* from the lowlands of North Borneo. Alcala & Brown (1956) and Alcala (1962) studied the mouthparts of *P. leucomystax* from Philippines. Mohanty-Hejmadi & Dutta (1988) studied the life history of *P. maculatus*. Chou & Lin (1997) presented notes on tadpoles of *P. megacephalus* from Taiwan. Grosjean (2004) also provided tadpole descriptions of *P. mutus*, *P. maculatus*, *P. megacephalus* and *P. himalayensis* from China, Nepal and Vietnam. Iangrai (2007) worked on the oral parts and diet of *P. leucomystax* from Meghalaya. Chakravarty et al. (2011) highlighted on tadpole oral apparatus of *P. teraiensis* from Assam, India. Lalramdinfeli (2017) compared the breeding and development of two rhacophorids, *Zhangixalus smaragdinus* and *P. teraiensis* from Mizoram and reported the details of oral morphology and diet of tadpoles of *P. teraiensis*. Le et al. (2018) reported the diet composition of *P. leucomystax* in Vietnam. Pham et al. (2019) worked on diet of *P. megacephalus* from Sin Ho District, Lai Chau Province. Rangad (2021) studied the amphibian fauna in select forests of Meghalaya with special reference to oral apparatus and diet of both tadpole and adult *P. himalayensis*. Rout et al. (2022) worked on the oral and buccophryngeal morphology of the tadpoles of *Polypedates maculatus* with notes on their diet pattern. Pham et al. (2023) provided feeding ecology of *P megacephalus* from Northwestern Vietnam. Raj (2023) observed larval descriptions and oral ultrastructures of *Polypedates pseudocruciger* from Wayanad, Western Ghats of India. Nakamura & Tominaga (2024) studied diet of *P. leucomystax* on Okinawajima, Ryukyu Archipelago, Japan. Atiqah & Sarman (2014) reported diet of *P. otilophus* during wet and dry periods from Malaysia (Unpublished). Siammawii et al. (2025) presented the details of tadpole oral morphology of *P. braueri* from Mizoram, India.

MATERIALS AND METHODS

Tadpole preservation and morphometry

All the collected tadpole specimens subjected to study were euthanized using tricane methane sulfonate-MS 222 (Galex et al., 2020) and preserved in 1:1 ratio of 70% alcohol and 4% formalin, catalogued in Departmental Museum of Zoology, Mizoram University-*Polypedates braueri* tadpoles as MZMU-PBT. Measurements of tadpoles were taken following Tolledo & Toledo (2010) using Mitutoyo dial vernier caliper, 505–731, to the nearest 0.1 mm: total length (TL), body length (BL), body width at the level of spiracle (BW), internarial distance (IND), interorbital distance (IOD), eye-naris distance (END), tip of snout-orbital distance (SOD); naris-snout distance (NSD), tail length (TAL), tail height (TAH), tail muscle height at base of tail (TMH), tail muscle width at base of tail (TMW), upper tail fin height (UTF).

Light microscopy

For light microscopy, five specimens each from developing stages 25 till metamorphosis were euthanised with tricane methane sulfonate-MS 222. Staging followed Gosner (1960). Following the anesthesia, they were transferred to 1:1 ratio of 70% alcohol and 4% formalin. Then the oral appaaratus were observed under the light microscope by placing the specimens dorsoventrally. Pins and forceps were used to adjust the position of the mouthparts. Finally the images were taken using camera.

Scanning electron microscopy (SEM)

For scanning electron microscopy, one specimen at each of the following Gosner stages 25, 30, 38 and 42 were euthanised with tricane methane sulfonate-MS 222 and decapitated for further study. The MS-222 solution was prepared by dissolving 5 g of powdered MS-222 in 1 litre of deionised water. The pH of the solution was adjusted to 7 using sodium bicarbonate (2 g increment). The tadpoles were well submerged in this solution for roughly 20 minutes. Following the anesthesia, they were transferred to 1:1 ratio of 70% alcohol and 4% formalin. Staging followed Gosner (1960). The specimens were later dried and carefully mounted on metal stubs, and then sputter coated with gold in ion sputtering chamber (Hitachi MC1000Plus II, Tokyo, Japan). Finally, the images were taken with a scanning electron microscope (Hitachi TM4000Plus II., Tokyo, Japan) at an electron accelerating voltage of 15 kV.

The terminology and abbreviations of oral apparatus were based on Lalremsanga et al. (2013). A-1 = first anterior tooth row, A-2 = second anterior tooth row, A-3 = third anterior tooth row, A-4 = fourth anterior tooth row, A-5 = fifth anterior tooth row, P-1 = first posterior tooth row, P-2 = second posterior tooth row, P-3 = third posterior tooth row, UJS = upper jaw sheath, LJS = lower jaw sheath, MP = marginal papillae, SM = submarginal papillae, TR = tooth ridge for tooth rows, b = base, n = neck, h = head.

Food analysis

Tadpoles

To study the food and feeding habitats of the species, different developmental stages of the tadpoles were collected from the natural habitat and preserved in 4% formaldehyde and autopsied for qualitative analysis of the gut contents. The gut of each tadpoles (N = 10 individuals per developmental stage) were dissected, the contents were transferred to a watch glass and mixed with 0.5 ml of water. One drop of gut content were placed on glass slide, covered by coverslip and examined under microscope (10x, 40x) following the method done by Dey and Goswami (2015). All food items were expressed in terms of percent abundance and percent frequency of occurrence.

The degree of dominance of food items in stomach sample was calculated by Berger- Parker Diversity Index which is as follows:

$$D = N_{max} / N$$

where, N= the total no. of individuals & Nmax= the no. of individual in the most abundant resource.

The species diversity is calculated by Shannon-Weiner Index

$$H' = -S\ p_i \ln p_i$$

Where, pi= proportional abundance of the ith species= (ni/N).

The species evenness is calculated by

$$E = H' / \ln S$$

Where S= total no. of individual.

Identification on the food items of the tadpoles were made following the methods of Edmonson (1959) and Smith (1994). Food items were identified upto genus level. The oral structures of the tadpoles were also studied using stereoscopic binocular microscope (CETI 9554. 1200) following the criteria of Altig and McDiarmid (1999) and Altig (2007). Description of oral disc and labial tooth raw formula (LTRF) were made according to the method provided by Altig (2007). Lengths of the intestines were measured for different tadpole stages in order to know their rate of shortening of intestines in relation to developing tadpoles.

Adult frogs

Frogs were collected manually in their habitats and stomach flushing was carried out immediately following the procedure of Secor & Faulkner (2002), Sole et al. (2005) and Bahuguna et al. (2019). Flushing was applied as soon as possible after capturing anurans, in order to precede digestion. Some individuals were immediately released into their habitats ensured that the current activity of the treated specimens was not essentially disturbed by the stomach-flushing while some of the individuals were collected for further studies. Flushing is a reliable method to recover stomach contents. In most of the anurans the contents were recovered completely after the second flushing and only seldom did the procedure need to be repeated (maximum of six times) before all contents were recovered. If individuals are captured during or shortly after their foraging activity and flushed, no contents will be undigested and can subsequently be identified without problems (Sole et al, 2005). The stomach contents were picked up with forceps and fixed in 70% ethanol in a vial. All contents were analyzed under a stereo microscope. Identifications of food items were possible up to the order level. The food contents were then identified with the aid of keys provided by Ward & Whipple (1959).

RESULTS

Oral disc

The light micrograph showed that *P. braueri* has oral disc oriented anteroventrally and demarginated, marginal papillae of anterior labium is largely interrupted medially while the marginal papillae on the posterior labium is interrupted very shortly at the centre, a row of submarginal papillae is also present in the posterior labium and at the angle of the mouth but is also interrupted in the medial region, papillae rounded in form. Keratodont jaw starts developing at stage 25. Labial papillae and beak disappearing by stage 42. The upper jaw sheath has slightly M-shaped arch and the lower jaw sheath is V-shaped, both jaw sheaths are serrated, black/dark brown in colour in their entirety. Well-developed keratinised rows of teeth are present on both the upper and lower labia. The keratinised teeth, upper and lower labium are dark brown/black in colour (Fig. 5.1). The labial tooth row formula (LTRF) of P. *braueri* changes at different Gosner stages during development. The numerator in LTRF shows tooth rows in anterior labium while denominator shows tooth rows in posterior labium. The range/number in parenthesis shows number of interrupted rows (Altig, 2007). The differentiation of the oral apparatus in the tadpoles of P. *braueri* started at stage 23. The appearance of the keratodonts as distinct minute horny teeth rows started at stage 25 which is when tadpoles begin to feed. The 1st anterior tooth rows A-1 are continuous whereas the 2nd and 3rd rows A-2, A-3 are interrupted and all the posterior labium P-1, P-2 and P-3 are continuous making LTRF: 3(2–3)/3 (Fig. 4a). Order of lengths of labial tooth rows A1 > P1 > P2 > P3 > A2 > A3. The average total length (TL) during stage 25 was 14.72 ± 0.5 mm (N = 10).

Between stages 26 and 34 LTRF changes to 4(2–4)/3 or 4(2–4)/3(1). At these stages, the oral apparatus is more prominent, the upper labium has now four rows of labial teeth interrupted medially from the 2nd row to 4th row. Three tooth rows are present on the lower labium where the first row is interrupted or continuous depending on the individual (Fig. 5.2b & c). Order of lengths of labial tooth rows A1 > P3 > P2 > P1 > A2 > A3 > A4.

Between stage 35 and 41 LTRF changes to 5(2–5)/3, 5(2–5)/3(1) or sometimes 4(2–4)/3. Five labial tooth rows are present on the upper labium, interrupted from 2^{nd} to 5^{th} rows and three tooth rows on the lower labium. LTRF are at its maximum during this stage. The mouth is widened and more prominent than the previous stage (Fig. 5.2b, d & e). Order of lengths of labial tooth rows A1 >P3 > P2 > P1 > A2 > A3 > A4>A5.

From stage 42 onwards, the oral apparatus starts degenerating and gradually transformed into adult mouth (Fig. 5.2f). After attaining stage 46, the froglet feeds on a carnivorous diet, e.g. insects. The average TL was 42.85 ± 0.32 mm (N = 10).

The scanning electron microscope (Fig. 5.2) revealed the keratinised labial tooth of the oral apparatus is a derivative of tooth ridge cells and consists of three different regions: a curved spatulate apical head portion bearing pointed marginal denticles or serrations ranging from 8–14, laterally tapered region from the middle neck, and a thin base embedded in the labial tissue. The serrations on the upper jaw sheath faintly blunt medially and fairly pointed towards the lateral edges while the serrations on lower jaw sheath uniformly pointed along the entire length.

Diet of tadpoles

Qualitative analysis of gut contents of the tadpoles of *Polypedates braueri* revealed that the larvae started feeding from stage 25 onwards. They mainly feed on planktons belonging to different families as well as detritus (Fig. 5.3, 5.4). The list of all the food items in the gut of tadpoles and its percent abundance and percent frequency occurrence are shown in table.

Stage 25: From this stage it was observed that tadpoles start feeding. The gut contents of the tadpoles contain phytoplankton consisting of *Stauroneis*, *Pinnularia*, *Gomphonima*, *Navicula*, *Synedra*, *Eunotia*, *Cocconeis* and *Cyclotella* (Bacillariophyceae), *Phacus, Euglena* (Euglenophyceae), *Mougeotia*, *Staurastrum*, *Closterium* (Zygnematophyceae), *Difflugia* (Tubulinea), *Oedogonium*, *Chlamydomonas* (Chlorophyceae) and *Chlorella* (Chlorellaceae). Among phytoplanktons, 15 unidentified food item was observed (Table 5.1)

Stage 26–30: At these stages, the gut content of the tadpoles is similar to that of Stage 25 except in additions of *Cylindrocystis* (Zygnematophyceae), *Arcella* (Tubulinea), *Exerohylum*, *Myxocyclus* (Dothiomycetes), *Ankistrodesmus*

(Selenastraceae) and zooplanktons like *Lecane*, *Notholca* (Monogononta)(Table 5.2).

Stage 31–41: Analysis of the gut content of the tadpoles at these stages reveals that the tadpoles also feed on phytoplanktons similar to the previous stage (Table 5.3).

Stage 42–46: At these stages tadpoles still feed on phytoplanktons and Zooplanktons but few in numbers. The tadpoles at these stages start to lose its feeding larval structures. From Stage 46 onwards, the froglet starts feeding on carnivorous diet (Table 5.4).

The above observations clearly showed that Bacillariophyceae (65.95%) were most frequent among the food items found in the gut of tadpoles of *Polypedates braueri* which is followed by Zygnematophyceae (16.21%), Trebouxiophyceae (5.53), Chlorophyceae (4.79%), Euglenophyceae (4.67%), Tubulinea (2.39%) and Unidentified organisms (1.96%), Zooplanktons Monogonontia (1.23%), Cyanophyceae (0.24), Dothiomycetes (0.18) and Selenastraceae (0.06%). In Bacillariophyceae, the most frequently occurring species was *Pinnularia* comprising 24.45% of the diet followed by *Gomphonima* comprising 13.15% (Table 5.5).

Estimates of food diversity based on the reciprocal form of the Berger-Diversity Index (1/d) and Shannon-Wiener Diversity Index (H') reveals that the greatest degree of dominance in food items occurs at stage 25 and the highest species diversity found at stage 26–30. Species Evenness (E) constrained between 0.81 – 0.91 reveals that the species are not equally abundant (Table 5.6).

Diet of adults

Because anurans were stomach-flushed within three hours after capture, some of the items were totally intact, most were partially digested. However, parts with heavily sclerotised cuticle remained undigested so that single wings, heads, thorax and abdominal segments of arthropods allowed an identification of the item, at least to order level.

Food items of adult frogs of *P braueri* includes grasshopper belonging to order Orthoptera, Earwig (Dermaptera), Soil mite (Oribatida), Carpenter ant

(Hymenoptera), Stick insect (Phasmida), Hister beetle (Coleoptera), Streptaxid Land snail (Stylommatophora), Eruciform larva (Lepidoptera), Vermiform Larva (Diptera) as well as plant debris.

DISCUSSION

Oral apparatus

In the tadpoles of *P. braueri,* the marginal papillae started developing at stage 23. The appearance of the keratodonts as distinct minute horny teeth rows started at stage 25 which was marked as the beginning stage of feeding activity. The larval oral structure observed showed variations in LTRF at different developmental stage which has also reported in its congener species. More than one tooth row formula was observed in *P. braueri,* it was not the only case reported among the genus *Polypedates*: in terms of LTRF, a lot of variations were observed among the tadpoles of other *Polypedates* sp. from different geographical regions. A summary of these comparisons is provided in Table 5.6. The structure of the mouth and buccal cavities of anuran larvae are highly adaptive and are correlated with their feeding ecology (Wassersug, 1976).

According to Altig & McDiarmid (1999), a gap in the tooth row is due to a physical break in the tooth ridge. Altig & Johnston (1989) proposed that a tadpole with more tooth rows on the upper labium than the lower labium has a positive imbalance, and assumed that the differences in balance values reflected differences in feeding mode.

Several authors have reported that labial teeth are some of the most distinguishing features for identifying amphibian larvae and that there is ontogenic variation in labial teeth structure (Dutta & Mohanty-Hejmadi, 1984; James et al., 2000; Khan & Mufti, 1994; Altig, 2007), because the dental formula is one of the most important characteristics of tadpoles for species identification (Sahu & Khare, 1983).

Tadpoles of *P. braueri* have a single tooth row per ridge (uniserial) throughout their development. Altig & McDiarmid (1999) reported that most tadpoles have a single tooth row per ridge (uniserial) but tadpoles with bi- (e.g. discoglossids) and even tri- or multi-serial rows have also been observed. Tadpoles of *P. braueri* are nektonic, LTRF greater than 2/3 and their mouthparts possess a number of keratinised teeth rows developed by epidermal tissue. It was reported that tadpole jaw sheaths are formed of many compact layers of keratinised cells formed in the mitotic zone at their bases, which is used for scraping the food items (Grosjean et al., 2011).

The tadpoles of *P. braueri* can be classified under Orton (1953) type IV tadpoles where the spiracle is present on the left side of the body, having well-developed mouthparts with keratinized jaw sheaths. According to Altig and Johnston (1989), it can also be categorised as necktonic exotroph type of ecomorphology having lateral eyes with high fins, rasping food from submerges surfaces within the water column whereas *P. himalayensis* which are bottom dwellers and they ingest the food material by scrapping the food items available at the bottom of the shallow water body with the help of their keratinized mouthparts (Rangad, 2021).

Tadpoles are good biological indicators. Since life history patterns for much of the anuran fauna from these regions remain insufficiently understood, larval descriptions from the current study make a significant contribution to the knowledge of the biology of this species, in terms of microhabitats, ecomorphology, natural history and distribution. The morphological data from this study could be taxonomically informative, as larval features are frequently a valuable tool for accurately defining the taxonomic boundaries of anurans. Comprehending the morphology and ecology of larval forms can help assess the significance of the conservation of the anuran biodiversity, which would help maintain the biota of Northeast India.

Diet of tadpoles

Gut content analysis of the tadpoles of *P. braueri* showed that the feeding activity of the tadpoles started from stage 25 onwards till completion of metamorphosis. But during memorphic climax (42–46), food items were fewer comared to other developmental stage. In 2007, Iangrai reported in her thesis that the food spectrum of *P. leucomystax* from Meghalaya was found to stop feeding at stage 40. Chakravarty et al. (2011) and Rangad (2020) reported that the tadpoles of *Polypedates* teraiensis from Assam and P. hamalayensis from Meghalay stopped their feeding activity at stage 42 whereby the larvae undergoes fasting during the period of intestinal emodeling. Rangad (2021) suggested that local environmental factors play a very important role inregulating the onset of metamorphosis and it may be due to this reason that there occurs a difference in the stages at which feeding activity stops since this phenomena is clear indication of the start of metamorphosis.

Diet composition of *P. braueri* tadpoles revealed members of class Bacillariophyceae to be the most important prey category, an observation similar to Sinha et al. (2001). The importance of Bacillariophyceae as a food source has also been reported for other anuran genera such as *Lithobates, Dendrosophus, Eupemphix, Scinax* and *Microhyla berdmorei* (Hendricks, 1973; Kupferberg, 1997a; Rossa-Feres et al., 2004; Lalremsanga & Muansanga, 2022; Rout et al., 2022). Bacillariophyceae can be richer in calories, mainly as a form of lipids and they are more easily accessible for consumption than filamentous algae (Kupferberg et al., 1994). Being a source of carbohydrates, chlorophytic algae also form another important food source (Bold & Wynne, 1985). The diet preference and choice of algae as food indicates that the conservation of habitat in terms of algal diversity is essential for the survival and successful completion of life cycle of amphibian tadpoles.

As they were mainly collected from small rock-pools and seasonal ponds within a short period, Bacillariophyceae, Chlorophyceae and Zygnematophyceae are comparatively more abundant than other food items. The preference of Bacillariophyceae, Chlorophyceae and Zygnematophyceae in the food habits of this species may be that the only available food items in their microhabitat that enhanced the faster developmental rate within a short duration. The food items of tadpoles are not diversified as expected, it is suggested that this might be due to the breeding ground as well as tadpoles habitat are mainly temporary or ephemeral isolated water bodies that harbours less diversified food items (phytoplanktons and zooplanktons). From the observation of Pryor (2003), it was evident that diatoms alone are sufficient for the growth and development of tadpoles and diatoms contain more calories (in the form of fat and protein) than other algae and hence they are a preferred food (Kupferberg 1997b).

Diet of adult Polypedates braueri

We used the stomach-flushing method to obtain the stomach contents from 60 individuals. Food items of adult frogs of *P braueri* includes Orthoptera, Dermaptera, Oribatida, Hymenoptera, Phasmida, Coleoptera, Stylommatophora, Lepidoptera, Diptera as well as plant debris. Like its congener species *P. himalayensis* (Rangad, 2021), *P. leucomystax* (Iangrai, 2007; Le et al, 2018; Nakamura & Atsushi, 2024) and *P. megacephalus* (Pham et al., 2019, Pham et al., 2023), *P. braueri* can also considered carnivorous non selective feeder. Their stomach contents showed a wide food spectrum and the dominant prey items for both the species were Arthopod specially Orthoptera. Other item includes Araneae, Opiliones, insect larvae, Coleoptera, Blattodea, Hemiptera, Hymenoptera, Isoptera and Lepidoptera. In addition to these Collembola, Diplopoda and Gastropda were ingested by *P. leucomystax* and Diptera by *P. himalayensis*. In all of these species Arthropods specially Orthoptera were dominant prey items. Prey compositions vary locally according to the composition of local prey guilds, as Berry (1965) predicted.

According to Almeida et al. (2019), the diet of anuran species is composed of a wide range of prey items, which may be connected to the availability of prey in the habitat. Anurans' food habits are thought to be influenced by gape, according to Silva et al. (2024). (Araújo et al., 2007; França et al., 2004; Maneyro et al., 2004) Gape is a limiting factor in prey selection, whereas the latter impacts the capacity to subdue animals. The size and, thus, the diversity of prey that can be consumed can be limited by gape limits (Huckembeck et al., 2018). The diversity of prey devoured and mouth width have a positive and linear association because animals with larger mouth openings can eat a wider variety of prey categories, which would include prey items of all sizes (De Carvalho Batista et al., 2011; Sales et al., 2011). Since tadpoles may only survive successfully in a habitat with enough food, knowledge of feeding behavior is essential from a conservation perspective. The number of anurans in the wild will be impacted by low survival due to habitat destruction and food scarcity. (Dey & Goswami, 2015).

Table 5.1: Qualitative and quantitative list of food items found in the gut of tadpoles of *Polypedates braueri* and percent relative abundance and percent frequency of occurrence at stage 25 (N10).

Food items	No. of organisms	Percent frequency	Abundance percentage
Pinnularia	104	100	21.18
Gomphonima	49	100	9.97
Difflugia	9	50	1.83
Closterium	41	100	8.35
Staurastrum	12	70	2.44
Cyclotella	8	50	1.62
Unidentified	15	80	3.05
Mougeotia	14	70	2.85
Phacus	24	80	4.88
Chrollera	19	80	3.86
Stauroneis	58	100	11.81
Polycystis	11	50	2.24
Euglena	17	70	3.46
Oedogonium	3	30	0.61
Chlamydomonas	4	30	0.81
Eunotia	43	100	8.75
Synedra	51	100	10.38
Cocconeis	7	50	1.42
Zygnema	2	20	0.40

Table 5.2: Qualitative and quantitative list of food items found in the gut of tadpoles of *Polypedates braueri* and percent relative abundance and percent frequency of occurrence at Stage 26–30.

Food items	No. of organisms	Percent frequency	Abundance percentage
Pinnularia	68	100	14.25
Gomphonima	59	100	12.36
Difflugia	9	60	1.88
Closterium	50	100	10.48
Staurastrum	12	60	2.51
Cyclotella	7	50	1.46
Zygnema	7	40	1.46
Mougeotia	14	70	2.93
Phacus	14	70	2.93
Chrollera	17	70	3.56
Stauroneis	50	100	10.48
Polycystis	17	80	3.56
Euglena	11	70	2.30
Oedogonium	2	20	0.41
Chlamydomonas	7	50	1.46
Eunotia	31	100	6.49
Synedra	46	100	9.64
Cocconeis	12	20	2.51
Lecane	2	20	0.41
Notholca	15	80	3.14
Arcella	9	60	1.88
Cylindrocystis	6	50	1.25
Ankistrodesmus	2	20	0.41
Exerohylum	2	10	0.41
Myxocyclus	2	10	0.41
Unidentified	6	40	1.25

Table 5.3: Qualitative and quantitative list of food items found in the gut of tadpoles of *Polypedates braueri* and percent relative abundance and percent frequency of occurrence at Stage 31–41 (N=10).

Food items	No. of organisms	Percent frequency	Abundance percentage
Pinnularia	80	100	13.69
Gomphonima	62	100	10.61
Difflugia	20	90	3.42
Closterium	52	100	8.90
Staurastrum	44	100	7.53
Cyclotella	5	30	0.85
Zygnema	17	90	2.91
Mougeotia	49	100	8.39
Phacus	21	100	3.59
Chrollera	41	100	7.02
Stauroneis	44	100	7.53
Polycystis	9	50	1.54
Euglena	7	60	1.19
Oedogonium	16	90	2.73
Chlamydomonas	14	70	2.39
Eunotia	26	80	4.45
Synedra	37	100	6.33
Cocconeis	4	30	0.68
Lecane	5	30	0.85
Notholca	7	40	1.19
Arcella	3	10	0.51
Cylindrocystis	17	70	2.91
Unidentified	4	30	0.68

Table 5.4: Qualitative and quantitative list of food items found in the gut of tadpoles of *Polypedates braueri* and percent relative abundance and percent frequency of occurrence at Stage 42–46 (N=10).

Food items	**No. of organisms**	**Percent frequency**	**Abundance percentage**
Pinnularia	85	100	25.07
Gomphonima	60	100	17.69
Difflugia	19	90	5.60
Closterium	30	100	8.85
Staurastrum	3	20	0.88
Cyclotella	4	40	1.18
Unidentified	6	80	1.77
Mougeotia	15	30	4.42
Phacus	5	60	1.47
Chlorella	16	60	4.72
Stauroneis	23	100	6.78
Anabaena	39	30	11.50

Table 5.5: **Diet composition (%) of tadpoles of *Polypedates braueri*.**

Family	Name of the species	No. of organisms	Percent frequency
Chlorophyceae	*Oedogoinium* *Chlamydomonas* *Polycystis*	21 20 37	4.79
Zygnematophyceae	*Cylindrocystis* *Mougeotia* *Staurastrum* *Closterium* *Zygnema*	11 45 23 159 26	16.21
Tubulinea	*Arcella* *Difflugia*	4 35	2.39
Bacillariophyceae	*Stauroneis* *Synedra* *Pinnularia* *Gomphonima* *Navicula* *Eunotia* *Coconeis* *Cyclotella*	159 107 398 214 107 74 11 20	65.95
Euglenophyceae	*Euglena* *Phacus*	21 51	4.67
Trebouxiophyceae	*Chlorella*	90	5.53
Cyanophyceae	*Anabaena*	4	0.24
Selenastraceae	*Ankistrodesmus*	1	0.06
Dothiomycitis	*Exerohylum* *Myxocyclus*	2 21	0.18
Monogonontia	*Notholca* *Lecane*	12 8	1.23
Unidentified	–	32	1.96

Table 5.6: Estimates of Berger - Parker Diversity Index (1/d = reciprocal form), Shannon-Wiener Diversity Index (H') and Evenness (E) in the gut of *Polypedates braueri.*

Gosner Stages	**1/D**	**H'**	**Evenness**
25	4.63	2.52	0.87
26–30	4.01	2.65	0.81
31–41	4.47	2.63	0.86
42–46	3.9	2.20	0.91

Table 5.7: Comparison of Labial Tooth Row Formula (LTRF) among congeners of *Polypedates braueri*.

Name of Species	LTRF	Order of lengths of LTR	Locality	References
Polypedates braueri	Gosner stage 25 3(2–3)/3	A1 > P1 > P2 > P3 > A2 > A3	Mizoram, Northeast India	This study
	Stage 26–34 4(2–4)/3 or 4(2–4)/3(1)	A1 > P3 > P2 > P1 > A2 > A3 > A4		
	Stage 35–41: 5(2–5)/3, 5(2–5)/3(1) or 4(2-4)/3	A1 > P3 > P2 > P1 > A2 > A3 > A4		
Polypedates himalayensis	St 25–32: 4(2–4)/3	-	Meghalaya, Northeast India	Rangad (2021)
	St 33–36: 4(2–4)/3(1)	-		
	St 37–39: 5(2–2)/3(1)	-		
	40: 3(2–3)/3(1)	-		
	St 41: 1/3(1)	-		
	4(2–4)/3(1), 1–4/3(1) or 5(3–5)/3(1)	-	India, Nepal	Annandale (1912); Grosjean (2004)
Polypedates leucomystax	St 25: 2(2)/3 St 26–39: 4(3–4)/3 St 40: 3(2–3)/3 St 41: 1(1)/2	-	Meghalaya, Northeast india	Iangrai (2007)
	5(2–5)/3(1)	-	Borneo	Inger (1956)
	4(2–4)/3 or 4(2–4)/3(1)	-	Philipines	Alcala & Brown (1956); Alcala (1962)
	4(2–4)/3	-	Malay Peninsula, Singapore	Flower (1896) Smith (1917)
	5(2–5)/3(1)	-	Mainland Thailand, Bangkok	Flower(1899); Smith (1917)

Table 5.7: Continued-

Polypedates maculatus	4(2–4)/3, 4(2–4)/3(1) or 1–4/3(1)	-	China, Vietnam	Grosjean (2004)
	4(2–4)/3	-	India	Mohanty-Hejmadi & Dutta (1988)
	4(3)/3(1)	A2>P1>P2>A1>P3>A3>A4	Odisha, India	Rout et al. (2022)
	4(2–4)/3(1)	A2 > P1 > P2 > A1 > P3 > A3 > A4	Western Ghats, India	Raj et al. (2023)
P olypedates megacephalus	5(2–5)/3(1)	A1 > P1 > A2 > P2 > P3 > A3 > A4 > A5.	Western Ghats, India	Raj et al. (2023)
	4(2–3)/3, 4(2–4)/3(1) or 1–4/3(1)	-	China, Vietnam	Pope (1931), Liu (1940, 1950); Chou & Lin (1997); Grosjean (2004)
Polypedates mutus	5(2–5)/3(1)	-	Vietnam	Grosjean (2004)
Polypedates pseudocrucige r	5(2–5)/3(1)	P-1 > P-2 > P-3 > A-1 > A-2 > A-3 > A-4 > A-5	Western Ghats, India	Raj (2023)
Polypedates teraiensis	4(2–4)/3	A1 > P1 > P2 > P3 > A2 > A3 > A4	Western Ghats, India	Raj et al. (2023)
	St 25: 3(3–3)/3(1) St 26–41: 4(2–4)/3(1)	-	Assam and Mizoram, Northeast India	Chakravarty et al. (2011); Lalramdinfeli, (2017)

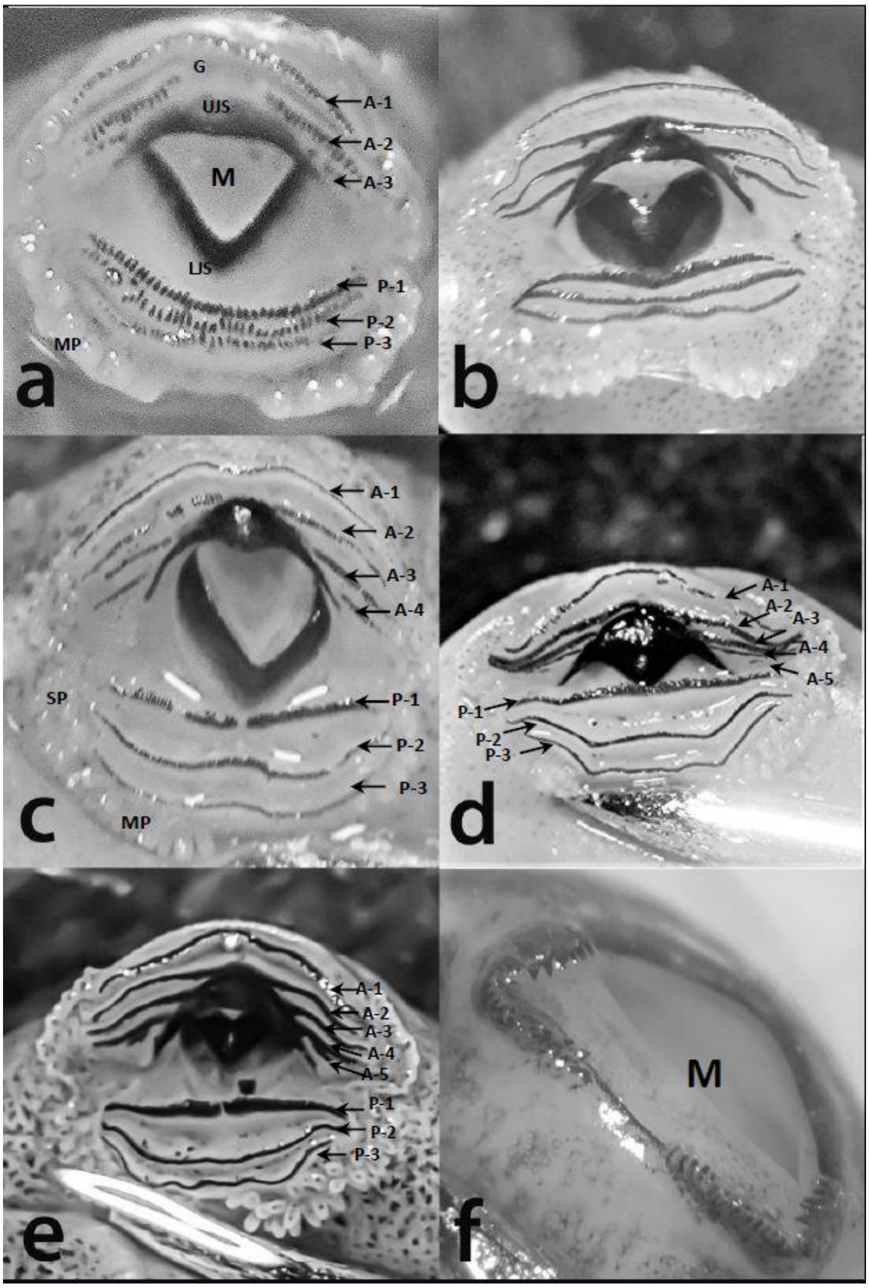


Figure 5.1. Oral apparatus of *Polypedates braueri* under light microscopy (a) at Gosner stage 25 (1.3 mm) (b & c) Gosner stage 30 (3.32 mm) (d & e) Gosner stage 38 (3.36 mm) (f) Gosner stage 42 (2.84 mm). A-1=First anterior tooth row, A-2=Second anterior tooth row, A-3=Third anterior tooth row, A-4=Fourth anterior tooth row, A-5=Fifth anterior tooth row, P-1=First posterior tooth row, P-2=Second posterior tooth row, P-3=Third posterior tooth row, UJS=Upper jaw sheath, LJS=Lower jaw sheath, MP=Marginal papillae, SM=Submarginal papillae.

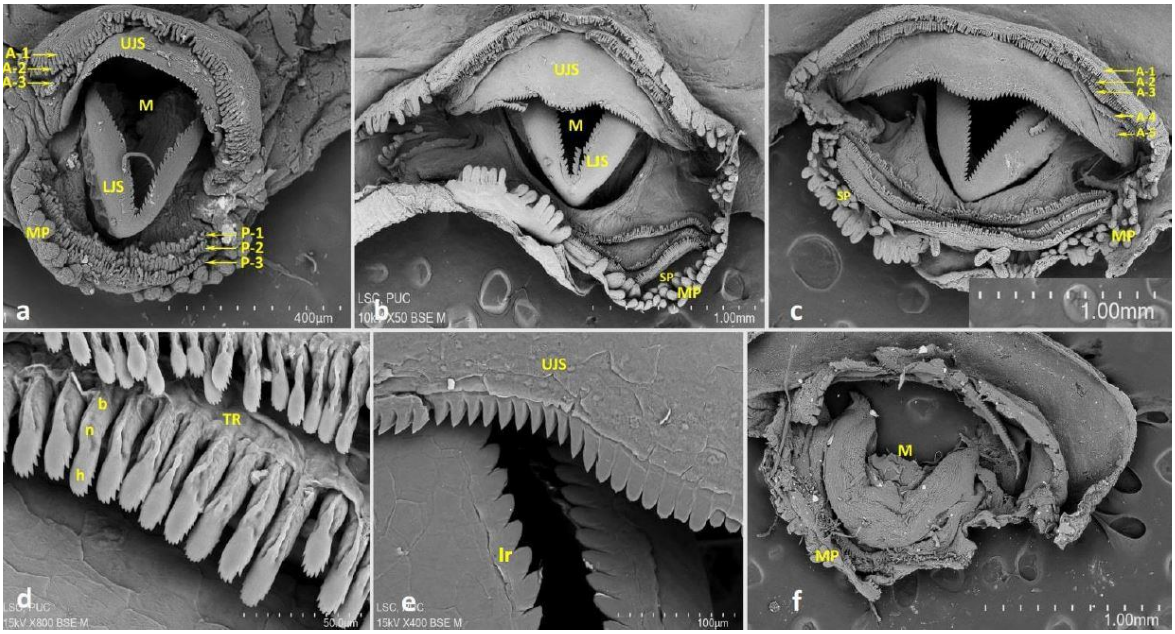


Figure 5.2: Scanning electron micrograph (SEM) of the oral apparatus of *Polypedates braueri* (a) at stage 25 (b) stage 30 (c, d, e) stage 38 (f) stage 42. Upper Jaw Sheath; LJS, Lower Jaw Sheath; G, Gap; SM, Submarginal Papilla; MP, Marginal Papilla; AL, Anterior Labial; A-1, First anterior tooth row, A-2, Second anterior tooth row; A-3=Third anterior tooth row, A-4=Fourth anterior tooth row, A-5=Fifth anterior tooth row; P-1, First posterior tooth row; P-2, Second posterior tooth row; P-3, Third posterior tooth row; M, Mouth; TR, Tooth Ridge; Ir, infrarostrodont; h, head; n, neck; b, base.

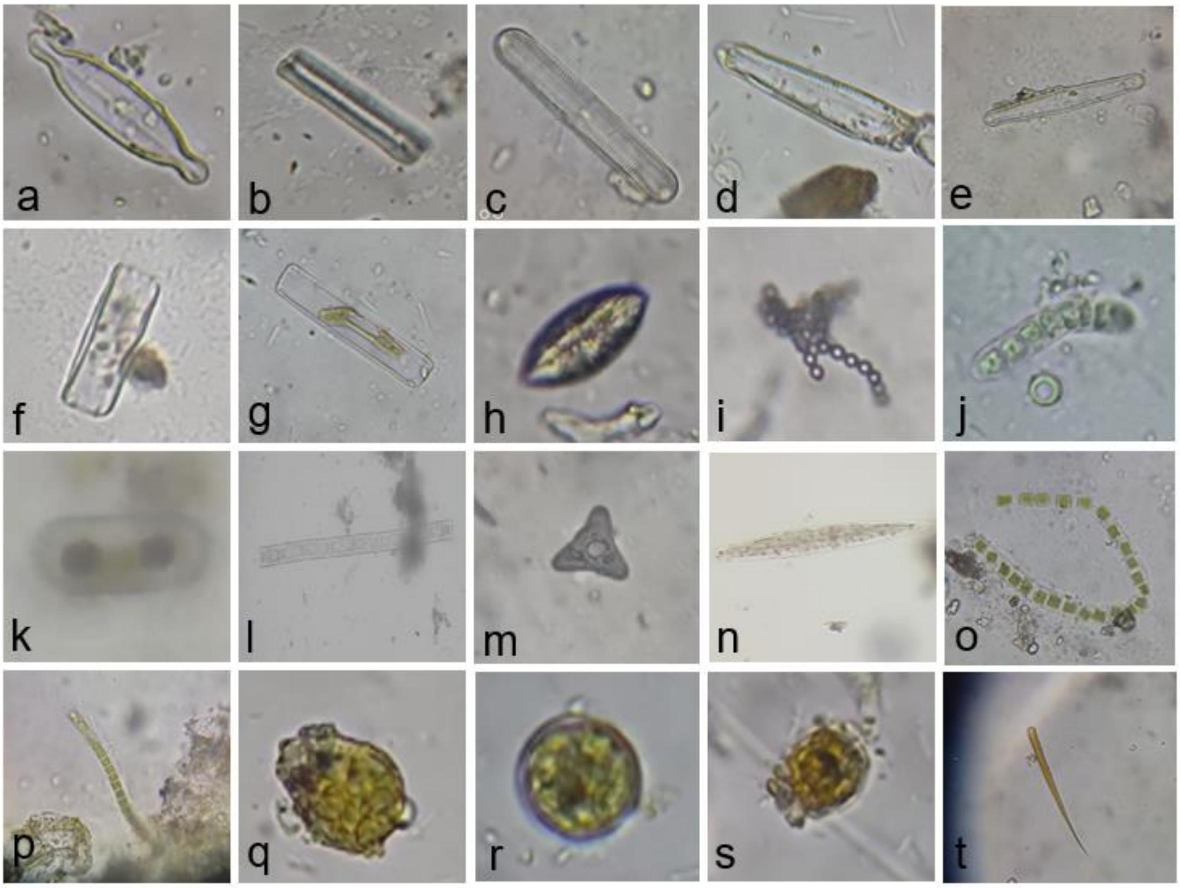


Figure 5.3: Food items in the gut of tadpoles of *Polypedates braueri.* (a) *Stauroneis* sp. (b) *Pinnularia* sp. (c) *Pinnularia* sp. (d) *Gomphonima* sp. (e) *Navicula* sp. (f) *Eunotia* sp. (g) *Eunotia* sp. (h) *Anabaena* sp. (i) *Cocconeis* sp. (j) Z*ygnema* sp (k) *Cylindrocystis* sp. (l) *Mougeotia* sp. (m) *Staurastrum* sp. (n) *Closterium* sp. (o) *Zygneman* sp. (p) *Oedogonium* sp. (q) *Chlamydomonas* sp. (r) *Chlamydomonas* sp. (s) *Chlamydomonas* sp. (t) *Ankistrodesmus* sp.

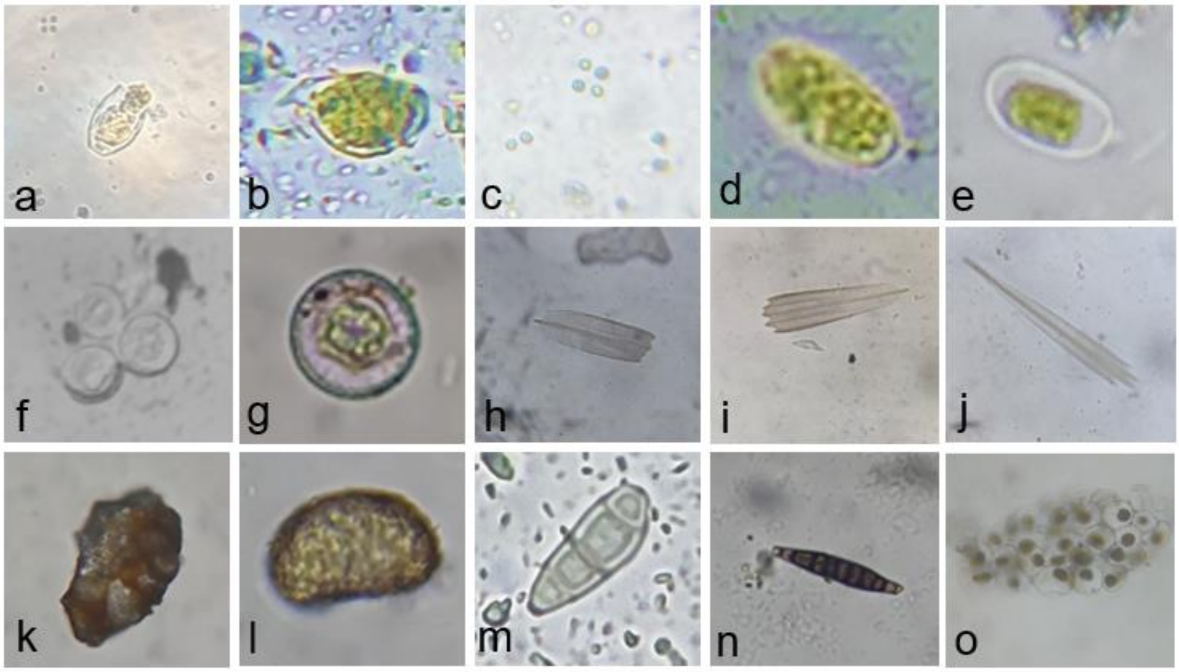


Figure 5.4: Food items in the gut of tadpoles of *Polypedates braueri.* (a) *Lecane* sp. (b) *Phacus* sp. (c) *Chlorella* sp. (d) *Polycystis* sp. (e) *Polycystis* sp. (f) *Arcella* sp. (g) *Arcella* sp. (h) *Notholca* sp. (i) *Notholca* sp. (j) *Notholca* sp. (k) *Difflugia* sp. (l) *Difflugia* sp. (m) *Exerohylum* sp. (n) *Myxocyclus* sp. (o) *Polycystis* sp.

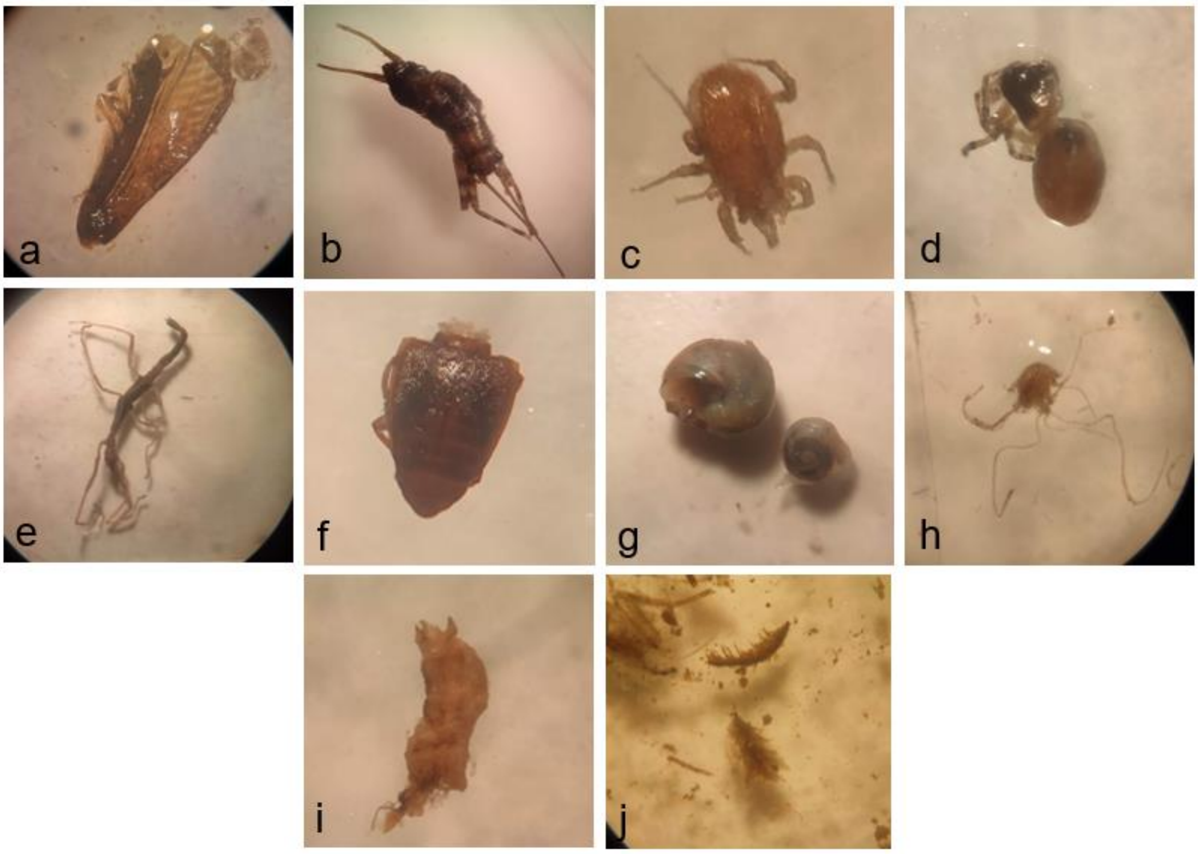


Figure 5.5: Food items inside the gut of adult *Polypedates braueri*. (a) Grasshopper (b) Earwig (c) Soil mite (d) Carpenter ant (e) Stick insect (f) Hister beetle (g) Streptaxid Land snail (h) Eruciform larva (i) Vermiform Larva (j) Plant debris.

SUMMARY

SUMMARY

In Chapter I, *Polypedates braueri* was recorded from 15 localities across 8 districts in Mizoram, suggesting a fairly wide distribution within the state. Out of 25 surveyed sites for suitable habitat for the studied species, the species was found at 15, indicating a detection rate of 60% across sampled areas. The species was observed between 312 meters (Damparengpui, Mamit District) and 1429 meters (Hmuifang Community Reserve Forest, Aizawl District) above sea level. It was found in various forest types of the state including bamboo forests, tropical evergreen, sub-tropical mixed deciduous, and montane sub-tropical forests. *P. braueri* was located in both pristine and disturbed environments, including forest edges, secondary growth, near streams, artificial and natural ponds, muddy paths, and roadside vegetation. The species exhibited nocturnal and arboreal behavior, often calling from shrubs or perching on tree branches at heights of ~1.5 meters. A total of 64 individuals were collected — 37 adult males, 22 adult females, and 5 juveniles — during the survey period from 2021 to 2023. Females were significantly larger than males across all morphometric parameters. Mean SVL was 49.3 ± 3.89 mm in males and 65.57 ± 5.66 mm in females. Observed individuals exhibited dynamic color changes in response to disturbance, changing from grayish brown to yellowish brown tones. The species is characterized by a blunt snout, enlarged toe disks, webbed toes (up to ¾ length), granular skin, and distinct yellow or white-spotted markings on the posterior thigh. Box plots showed significant sexual dimorphism in SVL, HL, HW, FLL, TL, and other parameters ($p < 0.05$), with females consistently larger. Principal Component Analysis (PC1 = 92%) revealed clear clustering by sex, indicating strong correlations among morphometric traits, especially size-related features. Ratio-based PCA (PC1 = 27%) showed weaker clustering and overlap between sexes, except for HW/HL and TRL/SVL ratios, which still differed significantly. The largest male (55.36 mm) and female (72.60 mm) specimens from Mizoram exceeded maximum

sizes reported in previous studies from China and Taiwan. The species' ability to thrive across a wide range of altitudes, habitats, and environmental conditions underscores its ecological adaptability and potential resilience to habitat changes.

In Chapter II, the *P.braueri* population from Mizoram, India formed a genetically distinct clade in the phylogenetic tree based on 16S mitochondrial DNA, indicating clear genetic divergence from other regional populations. The Mizoram *P. braueri* population is most closely related to specimens from Thailand (0.21% genetic distance), followed by China (0.25%) and Vietnam (0.28%). A separate clade consisting of three specimens from Myanmar and one *P. himalayensis* suggests potential misidentification due to high morphological similarity within the *P. leucomystax* complex. The genetic variation within the Mizoram *P. braueri* population was minimal (0.03%), indicating strong genetic consistency among local samples. Advanced tools like MAFFT, IQ-TREE, and Mega 11 were used to align sequences, infer phylogenies, and calculate genetic distances, ensuring robust analysis. Integrating morphological, ecological, and genetic data supported the identification of the Mizoram specimens as *P. braueri* and distinguished them from sympatric species like *P. teraiensis.*

In Chapter III, we observed that *P. braueri* is a seasonal breeder, with breeding activity occurring from April to September, triggered by the onset of the southwest monsoon.Breeding activity showed a significant correlation with rainfall and temperature, confirmed by Kruskal-Wallis H tests ($p < 0.005$). The peak breeding activity occurred in July, tapering off by the end of September despite the presence of water. Males were the first to arrive at breeding sites around 17:30 h, calling from vegetation, with calls audible from 25–30 m away. Axillary amplexus was observed, lasting 55–140 minutes, followed by foam nest construction and egg deposition. Nests were whitish or pale yellow, spherical, and constructed on damp forest floors or submerged litter within 0.5–3 m of water. Male advertisement calls consisted of 1–5 notes, each with 3–8 pulses; dominant frequencies ranged from 843.75–1406.25 Hz. The call parameters of *P. braueri* are distinct from its congeners**,** useful for species identification and taxonomic studies. Clutch sizes ranged from 215–408 eggs (mean = 306), and foam nests measured 7–10 cm in diameter**.** A positive correlation ($r = 0.55$, $p = 0.05$) was found between female SVL and clutch size, indicating larger females lay more eggs. Males were significantly smaller (SVL = 46.0 mm) than females (SVL = 54.5 mm), indicating sexual size dimorphism**.** 60% of foam nests examined were infested by dipteran larvae, posing a risk to embryo survival. Histological section of ovaries during the breeding season contained mature oocytes with yolk and vitelline membranes, indicating active oogenesis. Testes showed all stages of spermatogenesis during breeding, including spermatogonia, spermatocytes, spermatids, and mature spermatozoa. Post-breeding gonads showed reduced activity, with absence of mature oocytes and minimal sperm production.

In Chapter IV, *P. braueri* completed metamorphosis in about 75 days under lab and natural conditions with water temperatures between 10.6°C and 30.3°C. A total of 46 distinct developmental stages were observed and categorized following Gosner's (1960) staging system. Fertilized eggs were spherical with a diameter of approximately 1.83±0.01 mm. Tadpoles hatched at Gosner stage 20 (around 2 days and 19 hours post-fertilization) with a size of 9.64±0.13 mm. The largest size recorded was 47.28±0.38 mm at Gosner stage 41 before tail resorption began. Tadpoles overwintered when metamorphosis was delayed due to low temperatures; some completed development in late February to March, while others did not metamorphose at all. A significant positive correlation ($p < 0.001$) was found between body length and tail length from stages 40–46. Tadpoles had a dorsoventrally compressed, broadly ovoid body with lanceolate tails and distinctive oral discs positioned anteroventrally. Tadpoles were generally light-brown or greyish with pigmentation increasing over time, especially from stage 26 onwards. Development rate was influenced by ambient temperature, with slower development during winter months. Tadpoles developed functional oral structures by stage 23 and began feeding on detritus and algae. Detailed morphometric measurements (e.g., total length, tail length, body width) were recorded across developmental stages. Compared to other *Polypedates* species, *P. braueri* had an intermediate egg size and developmental period. Stage 34 tadpoles had rounded nostrils, laterally placed eyes, and a spiracle on the left lateral side—important features for taxonomy. Larval characteristics proved valuable for species identification and phylogenetic studies; findings support the importance of documenting ontogenic stages for conservation planning

In Chapter V, tadpoles of *Polypedates braueri* begin feeding at Gosner stage 25, with keratinized tooth rows developing simultaneously. Oral apparatus evolves through stages—marginal papillae, labial tooth rows, and jaw sheaths develop by stage 25, and begin to degenerate by stage 42 as metamorphosis nears completion.Changes dynamically during development: Stage 25: 3(2–3)/3; Stages 26–34: 4(2–4)/3 or 4(2–4)/3(1) and Stages 35–41: 5(2–5)/3, 5(2–5)/3(1), or 4(2–4)/3. SEM revealed that each tooth has a pointed head, tapered neck, and embedded base, suitable for scraping food. Their food preferences, which was dominated by Bacillariophyceae (65.95%), especially *Pinnularia* (24.45%) and *Gomphonima* (13.15%). Highest species diversity (Shannon-Wiener Index = 2.65) occurred during stages 26–30, with evenness values ranging between 0.81–0.91. Stage 25 showed initiation of feeding, primarily phytoplankton. During stages 26–30, fungi and zooplankton were added; Stages 31–41: Continuation with similar diet. As metamorphosis begins during stages 42–46, reduced feeding activity between was observed. Adult frogs are carnivorous generalists, consuming insects (e.g., Orthoptera, Coleoptera, Hymenoptera), snails, and some plant debris. Tadpoles were collected from seasonal ponds and rock pools, with limited food diversity likely due to the ephemeral nature of these habitats. At stage 46, froglets shift to a carnivorous diet post-metamorphosis. *P. braueri* shows similar oral developmental patterns as other *Polypedates* species but with distinct LTRFvariations. LTRF and oral morphology correlate with tadpole feeding behavior—nektonic exotrophs rasping food from submerged surfaces. The diet and development insights are critical for habitat conservation and understanding amphibian ecological roles. Tadpoles serve as reliable indicators of ecosystem health due to their sensitive feeding ecology and developmental patterns. This study adds valuable data to the limited larval biology knowledge for northeast Indian amphibians, contributing to biodiversity understanding and species identification.

CONCLUSION

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From our observations, it can concluded that *Polypedates braueri* exhibits a broad geographic and altitudinal range within Mizoram, thriving in diverse habitats from bamboo to montane forests. Its presence in both pristine and disturbed environments highlights the species' strong ecological flexibility and resilience.

Significant sexual dimorphism was observed, with females being consistently larger than males. Morphometric and PCA analyses confirmed this pattern and revealed that the largest individuals for both sexes from Mizoram exceeded known size records from other regions.

Phylogenetic analysis confirmed that the Mizoram population forms a distinct genetic clade, showing clear divergence from populations in Thailand, China, and Vietnam. This supports its classification as *P. braueri* and underscores the genetic uniqueness of the region's population.

Breeding activity is closely linked to the monsoon season in the state, peaking in July. Environmental cues like rainfall and temperature significantly influence reproductive timing, foam nest formation, and acoustic signaling — all of which are distinct from other congeners and taxonomically informative.

Female was observed to be selective in oviposition site. No parental care was observed.

Metamorphosis spans approximately 75 days, influenced by ambient temperature. Detailed documentation of larval stages and morphology, including oral structure and pigmentation changes, provides critical taxonomic and ecological insights.

Tadpoles begin feeding at stage 25 and exhibit stage-specific dietary shifts. Their diet is dominated by diatoms, indicating adaptation to food availability in temporary aquatic habitats. Adults are generalist carnivores, reinforcing the species' trophic adaptability.

The species' adaptability, distinct phylogenetic identity, and ecological role as both predator and prey make it a valuable bioindicator. Detailed knowledge of its life history, especially larval development and diet, is essential for regional amphibian conservation and habitat management.

This study fills critical gaps in the larval and reproductive biology of *P. braueri*, providing foundational data on distribution, habitat, behavior and normal table for embryonic and larval development for future taxonomic, ecological, and conservation efforts in a biologically rich yet under-studied region.

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PUBLICATIONS

Sl.No.	Authors	Title	Journal	Year, Volume (Issue): Page	IF	Index	Publisher & Country
1.	**Vanlalsiammawii**, Vanlal Remruatpuii, Malsawmhriatzuali, Lalmuansanga, Gospel Zothanmawia Hmar, Saisangpuia Sailo, Ht. Decemson, Lal Biakzuala & Hmar Tlawmte Lalremsanga	An additional record of the Tamdil leaf litter frog *Leptobrachella tamdil* (Sengupta et al, 2010) from Dampa Tiger Reserve, Mizoram, India.	*Journal of Threatened Taxa*	2020, 12(8):15951–15954	-	SCOPUS	Wildlife Information Liaison Development Society: India
2.	**Vanlal Siammawii,** Malnica Vanlal Malsawmtluangi, Lal Muansanga, Lal Biakzuala & Hmar Tlawmte Lalremsanga.	Brachyphalangy in a Tamenglong Horned Frog, *Xenophrys numhbumaeng* (Mahony, Kamei, Teeling, and Biju 2020)(Megophryidae), from	*Reptiles & Amphibians*	2021, 28: 322–323	-	SCI	International Reptile Conservation Foumdation (IRCF): USA

		Mizoram, India.					
3.	**Vanlal Siammawii,** Malnica Vanlal Malsawmtluangi, Lal Muansanga, Lal Biakzuala & Hmar Tlawmte Lalremsanga.	Adactyly in a Mawphlang Odorous Frog, *Odorrana mawphlangensis* (Pillai and Chanda 1977) (Ranidae), from Mizoram, India.	*Reptiles & Amphibians*	2021, 28: 324–325	-	SCI	International Reptile Conservation Foumdation (IRCF): USA
4.	**Vanlal Siammawii,** L. Biakzuala & H.T. Lalremsanga.	*Duttaphrynus chandai* (Nagaland Montane Torrent Toad). Hindlimb malformation.	*Herpetological Review*	2021, 52: 823	-	SCOPUS	Society for the Study of Amphibiana and Reptiles: USA
5.	Ht. Decemson, Vabeiryureilai Mathipi, **Vanlal Siammawii**, Lal Biakzuala & Hmar Tlawmte Lalremsanga.	A new record of the Banladeshi Cricket Frog, *Minervarya asmati* (Howlader, 2011), from Manipur State, with comments on the occurrence of the Paddy Frog,	*Reptiles & Amphibians*.	2021, 28(2): 250–254	-	SCI	International Reptile Conservation Foumdation (IRCF): USA

		Fejervarya multistriata (Hallowell, 1861)(Anura: Dicroglossidae), in Mizoram, India					
6.	Ht. Decemson, Vabeiryureilai Mathipi, **Vanlal Siammawii**, Lal Biakzuala & Hmar Tlawmte Lalremsanga.	First record of *Duttaphrynus chandai* (Anura: Bufonidae) from Manipur State, northeastern India, with updated information on its distribution and natural history.	*Herpetology Notes*	2021, 14: 1219–1223	0.679	SCI	Societas Europaea Herpetologica (SEH): Germany
7.	Ht. Decemson, **Vanlal Siammawii, V**. & Hmar Tlawmte Lalremsanga.	New Geographic Distribution. *Fejervarya multistriata* (Paddy frog). India. Manipur.	*Herpetological Review*	2021, 52(2): 342	-	SCOPUS	Society for the Study of Amphibiana and Reptiles: USA
8.	Lal Muansanga, Malnica Vanlal Malsawmtluangi, Gospel Zothanmawia	The first record of *Kurixalus yangi* Yu, Hui, Rao and Yang, 2018 (Anura:	*Journal of Animal Diversity*	2021, 3(2): 9–17.	0.230	SCI	Lorestan University Press: Iran

	Hmar, Lal Biakzuala, **Vanlal Siammawii,** Mathipi Vabeiryureilai, M. & Hmar Tlawmte Lalremsanga.	Rhacophoridae) from Mizoram State, India, with a reassessment of previous records of its congeners from Mizoram.					
9.	Lal Muansanga, **Vanlal Siammawii,** Gospel Zothanmawia Hmar, G.Z., Fanai Malsawmdawngliana, Lal Biakzuala, Ht. Decemson & Hmar Tlawmte Lalremsanga.	New elevation and locality records and notes on the natural history of the Tamdil Leaf-Litter Frog, *Leptobrachella tamdil* (Sengupta, Sailo, Lalremsanga, Das, and Das 2010) *Reptiles & Amphibians* 28(2): 295–297. (Megophryidae), in Mizoram, India.	*Reptiles & Amphibians*	2021, 28(2): 295–297	-	SCI	International Reptile Conservation Foumdation (IRCF): USA
10.	Hmar Tlawmte Lalremsanga, Hauzel Chinliansiama, Sanath Chandra Bohra, Lal	A new bent-toed gecko (*Cyrtodactylus* Gray: Squamata: Gekkonidae) from the state of Mizoram,	*Zootaxa*	2022, 5093(4): 465–482.	1.091	Scopus	Magnolia Press: New Zealand

	Biakzuala, Mathipi Vabeiryureilai, Lal Muansanga, Fanai Malsawmdawngliana, Gospel Zothanmawia Hmar, Ht. Decemson, H., **Vanlal Siammawii**, Das, Madrurima & Jayaditya Purkayastha.	India.					
11.	Ht. Decemson, **Vanlal Siammawii,** Mathipi Vaberryureilai, Lal Biakzuala, & Hmar Tlawmte Lalremsanga.	*Amolops indoburmanensis*.	*Herpetological Review*	53(1)	-	SCOPUS	Society for the Study of Amphibiana and Reptiles: USA
12.	**Vanlal Siammawii, V.**, Lal Muansanga, Fanai Malsawmdawngliana, C.	Partial albinism in a Malaysian Painted Frog, *Kaloula pulchra* Gray 1831,	*Reptiles & Amphibians*	2022, 29(1): 348–350.	-	SCI	International Reptile Conservation

	Lalhmingmawii & Hmar Tlawmte Hmar Tlawmte Lalremsanga.	from Mizoram, northeast India.					Foumdation (IRCF): USA
13.	**Vanlal Siammawii,** Ht. Decemson, Fanai Malsawmdawngliana, Lal Muansanga, Lal Biakzuala, Mathipi Vabeiryureilai & Hmar Tlawmte Lalremsanga.	DNA barcoding elucidates the range-extension of the Bangladesh Skittering Frog, *Euphlyctis kalasgramensis* (Dicroglossidae), in northeast India.	*Reptiles & Amphibians*	2022, 29(1), 321–325.	-	SCI	International Reptile Conservation Foumdation (IRCF): USA
14.	**Vanlal Siammawii**, Angshuman Das Tariang, Lal Mansanga, Fanai Malsawmdawngliana, Debashish Khanikar, Anand Shanker Dixit & Hmar Tlawmte Lalremsanga.	Larval oral morphology of *Fejervarya multistriata* (Hallowell, 1861) from Mizoram, Northeast India.	*Sauria*	2022, 44(2): 61–66.	-	-	Terrariengem einschaft Berlin e.V. (TGB): Berlin

15.	**Vanlal Siammawii,** Fanai Malsawmdawngliana, Lal Muansanga & Hmar Tlawmte Lalremsanga.	Hindlimb Malformation in a Bangladesh Skittering Frog, *Euphlyctis kalasgramensis* Howlader, Nair, Gopalan, and Merila 2015 (Anura: Dicroglossidae).	*Reptiles & Amphibians*	2022, 29: 195–196	-	SCI	International Reptile Conservation Foumdation (IRCF): USA
16.	Fanai Malsawmdawngliana, Mathipi Vabeiryureilai, **Vanlal Siammawii,** Lal Muansanga, Gospel Zothanmawia Hmar, Ht. Decemson & Hmar Tlawmte Lalremsanga.	On the Occurrence of *Scorpiops longimanus* (Pocock, 1893)(Arachnida: Scorpiopidae) in Mizoram, Northeast india.	*Taprobonica*	2022, 11 (1): 4–11	2.13	SCI & SCOPUS	The Research Center for Climate Change (RCCC) at the University of Indonesia and the Faculty of Mathematics and Natural Sciences (FMIPA) at the same

							university: Indonesia
17.	**Siammawii, V**., Malsawmdawngliana, F. and Lalremsanga, H.T.	Color change in an Upland Treefrog (*Polypedates braueri*) from Mizoram, India.	*Reptiles & Amphibians*	2023, 30(1): e17126–e17126.	-	SCI	International Reptile Conservation Foumdation (IRCF): USA
18.	**Vanlal Siammawii,** Fanai Malsawmdawngliana, Lal Biakzuala, Lal Muansanga & Hmar Tlawmte Lalremsanga.	Natural History Notes. *Odorrana* mawphlangensis (Mawphlang Odorous Frog). Parasite**.**	*Herpetological Review*	2023, 54(1):103–104.	-	SCOPUS	Society for the Study of Amphibiana and Reptiles: USA
19.	**Vanlal Siammawii,** Fanai Malsawmdawngliana, Lal Muansanga, Mathipi Vabeiryureilai & Hmar Tlawmte Lalremsanga.	DNA barcoding reveals the first record of the Burmese Whipping Frog, *Polypedates mutus* (Anura: Rhacophoridae), from India.	*Reptiles & Amphibians*	2023, 30(1): e17036–e17036.	-	SCI	International Reptile Conservation Foumdation (IRCF): USA
20.	**Vanlal Siammawii**, Fanai	Observations on the	*Herpetology*	22024, 17*:*	0.679	SCOPUS	The publisher

	Malsawmdawngliana, Lal Muansanga & Hmar Tlawmte Lalremsanga.	breeding biology of the Upland Treefrog, *Polypedates braueri* (Vogt, 1911) from Mizoram, India.	*Notes*	25–33.			is the Societas Europaea Herpetologica (SEH): Germany
21.	**Vanlal Siammawii**, Hauzel Chinliansiama & Hmar Tlawmte Lalremsanga.	Direct development of the Reza Khan's bush frog *Raorchestes rezakhani* in north-east India.	*Herpetological Bulletin*	2024, 167: 25–29	-	SCI	The British Herpetological Society: UK
22.	**Vanlal Siammawii,** Fanai Malsawmdawngliana, Kholhring Lalchhandama,, P.B Lalthanpuii & Hmar Tlawmte Lalremsanga.	Tadpole description and oral morphology of Upland tree frog *Polypedates braueri* from Mizoram, Northeast India.	*Herpetological Journal*	2025, 35(2): 99–109	1.194	SCI & SCOPUS	The British Herpetological Society: UK

PAPER PRESENTED/ ATTENDED ON SEMINAR/CONFERENCE/SYMPOSIUM/WORKSHOP/TRAINING

Sl. No	Title of Presentation	Seminar/Conference/Symposium/Workshop/ Training	Type	Date
		Paper Presented		
1	Detailed Structure of Oral Apparatus of *Polypedates braueri.*	National Webinar on Herpetology Indian-Scenerio. Oganised by Natural Science Association, Department of Zoology, St. Joseph's College.	Oral	26–27 November, 2021.
2	Observations on the breeding biology of the Upland tree frog, *Polypedates braueri* (Vogt, 1911) from Mizoram, India.	Two day International Seminar on Climate Change: Impact & Resilience. Organised by Department of Zoology, Assam Don Bosca University in collaboration with Zoological Society of Assam held at Assam Don Bosca University, Tapesia Campus, Sonapur.	Oral	27–28 February, 2022
3	The First Comprehensive Study of the Normal Development and Tadpole Description of *Sylvirana lacrima* (Anura: Ranidae)	4th Mizoram science Congress. Organised by Mizoram Science, Technology and Innovation Council (MISTIC) in collaboration with Mizo Academy of Sciences (MAS), Mizoram Science Society (MSS), Science Teachers' Association, Mizoram (STAM), Geological	Oral	24–25 November, 2022.

		Society of Mizoram (GSM), Mizoram Mathematics Society (MMS), Biodiversity and Nature Conservation Network (BIOCONE), and Mizoram Information and Technology Society (MITS).		
4.	Studies in Oral morphology of *Polypedates braueri* with SEM	International Conference on Current Trends in Biological Sciences. Organised by School of Life Science, Mizoram University.	Poster	18–20 March, 2024
5.	Embryonic and Tadpole Development of *Polypedates braueri* from Mizoram, Northeast India	International Conference On Endocrinology, Metabolism and Reproduction: Exploring New Frontiers. Organised by Department of Zoology, University of Jammu, Jammu UT J&K, India, under the auspices of Indian Society for Comparative Endocrinology.	Poster	22–25 October, 2024

Participation

1	Forest Conservation & Watershed Management. Organised by MZUSC & Department of Geography and Resource Management, School of Earth Science, Mizoram University.	Attended	5 June, 2021
2	Webinar on AI Research, Applications on Computer Image & Biomedical Instrumentation Research. Organised by EduCare Taiwan with Yuan Ze University and National Chung Hsing University.	Attended	13 February, 2022
3	Webinar Training on QGIS Beginner Couse. Organised by TGIG, The Geographic Information System Lab, Ahmedabad, Gujarat.	Attended	14 April, 2022
4	Snake Awareness: Identification and Anatomy. Organised by The Nature's Eye powered by Wildlifearc	Attended	15 April, 2022
5	International Workshop on "Nanopore Sequencing and Data Analysis: Opportunities for Rapid Biodiversity and Biosurveillance programs and Local Capacity Building" at Mizoram University, Aizawl, Mizoram. Organised by Department of Zoology, Mizoram University in collaboration with Global Innitiative for Academic Networks and Ministry of Education, Goverment of India (Gian)	Attended	16–29. March, 2023

6	**National Workshop on “The Value and Interconnections of Human, Animal, Plant, and Microorganisms: Metataxonomics and Metagenomics Approach” at Mizoram University. Organised by Department of Zoology, Mizoram University.**	**Attended**	**18–25 March, 2023**
7.	International Workshop on “Computational Genomics with R: A hands-On Course on NGS Data Analysis” at Mizoram University, Aizawl, Mizoram. Organised by Department of Zoology, Mizoram University.	Attended	22–26 March, 2023

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NAME OF THE CANDIDATE	VANLALSIAMMAWII
DEGREE	DOCTOR OF PHILOSOPHY
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ABSTRACT

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STUDIES ON ECOLOGY, BREEDING BEHAVIOR AND DEVELOPMENT OF *Polypedates braueri* (VOGT, 1911) (AMPHIBIA: ANURA: RHACOPHORIDAE) FROM MIZORAM, INDIA.

BY

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ABSTRACT

Amphibians play keystone roles in forest ecosystems, influencing vital ecosystem processes and contributing to system resilienceresistance (=stability) (Davic & Welsh, 2004). Adult amphibians are important predators as well as prey and larval amphibians may be important herbivores (Blaustein et al., 1994). Therefore, a world-wide decline of amphibian populations could have a significant and detrimental impact on both natural ecosystems and human welfare (Gardner, 2001). According to the second Global Amphibian Assessment (GAA), amphibians are the most threatened vertebrate class (40.7% of species are globally threatened). While habitat loss and degradation remain the most common threat to amphibians, an increasing number of species are being pushed to the brink of extinction by disease and climate change effects. The urgency to act is becoming increasingly critical (IUCN, Luedke et al., 2023). Therefore, the present work is conducted for better understanding and contribution towards conservation of a poorly known Upland Treefrog, *Polypedates braueri*.

The following objectives were proposed for this study:

- To study the distribution and habitat of *Polypedates braueri* in Mizoram.
- To analyse the phylogenetic position and genetic divergence using partial 16S rDNA.
- To study the breeding biology with respect to environmental factors of *P. braueri*.
- To study the embryonic and larval development of *P. braueri*.
- To study oral morphology, food and feeding behavior of tadpoles and adults of *P.braueri.*

Amphibians, among the most ancient vertebrates, serve as crucial bioindicators due to their sensitivity to environmental changes. This study investigates the distribution, habitat preferences, and morphological characteristics of *Polypedates braueri* in Mizoram, a region within the Indo-Myanmar biodiversity hotspot in Northeast India. Amphibians of the family Rhacophoridae, particularly

arboreal frogs such as *Polypedates*, exhibit distinct morphological and ecological adaptations that facilitate their survival in forested environments. The genus *Polypedates* includes species with a wide distribution across Southeast Asia, with *P. braueri* reported in parts of China, Vietnam, Myanmar, and Northeast India, including Mizoram.

To assess the presence and ecological traits of *P. braueri*, extensive field surveys were conducted from 2021 to 2023 across 25 localities in nine districts of Mizoram, at elevations ranging from 80 to 1429 meters above sea level. Using Visual Encounter Survey (VES) and Acoustic Encounter Survey (AES) methods, 64 individuals were collected from 15 different localities across eight districts. The collection was documented through GPS and elevation records, and mapped using QGIS 3.10.8. Specimens were preserved in 70% ethanol and deposited at the Departmental Museum of Zoology, Mizoram University.

P. braueri exhibited a wide altitudinal distribution (312–1429 m asl) and was found in varied habitats, including tropical evergreen and sub-tropical mixed deciduous forests, semi-evergreen and montane sub-tropical forests. Habitats ranged from natural forested areas to disturbed environments with proximity to water bodies such as streams, ponds, and wetlands. Notably, this species demonstrated significant ecological plasticity, as individuals were observed on tree trunks, shrubs, grasses, and in leaf litter, highlighting both arboreal and terrestrial tendencies. Such flexibility suggests the species' resilience and capacity to adapt to diverse ecological niches.

Morphological assessments were performed on 64 individuals, including 37 males, 22 females, and 5 juveniles, using standard morphometric techniques. A total of 15 morphological characters were measured, including Snout-Vent Length (SVL), Head Width (HW), Head Length (HL), Forelimb Length (FLL), Tibia Length (TL), and Foot Length (FOL). Males and females exhibited sexual dimorphism, with females being significantly larger than males in all morphological traits. SVL ranged from 40.30 to 55.36 mm in males and from 51.10 to 72.60 mm in females. Morphological characters were analyzed using boxplot comparisons and Principal

Component Analysis (PCA). The PCA results showed distinct clustering of sexes based on size-related metrics, particularly in SVL, HL, HW, and limb proportions.

A key diagnostic trait of *P. braueri* is the presence of white or yellowish spots bordered by deep black on the posterior thigh surface, along with distinctive webbing patterns in the toes and fingers. Color variation was also observed, with individuals exhibiting dynamic changes from grayish brown to dark yellowish brown under disturbance, shifting to lighter shades under stable conditions. These color changes suggest behavioral plasticity potentially influenced by stress, microhabitat conditions, or reproductive cues. This phenomenon aligns with earlier studies reporting similar adaptive responses in congeners such as *P. maculatus*.

Compared with previous literature, this study provides the most comprehensive morphological dataset for *P. braueri* in Mizoram, identifying larger body sizes than previously recorded in Taiwan and China. The study confirms the species' presence in Mizoram, extending its known distribution and offering critical insights into its ecology. It also aligns with earlier research indicating broad altitudinal tolerance across its range, from lowland to montane forests, as observed in populations from Taiwan (0–2200 m) and Tibet (up to 1931 m).

This research highlights the importance of detailed ecological and morphological studies in documenting amphibian diversity. *P. braueri*'s adaptability to varied elevations and habitats, along with its distinct morphological features and sexual dimorphism, make it an ideal candidate for monitoring environmental changes in the region. The findings contribute valuable baseline data for conservation planning, especially in the face of habitat fragmentation, climate change, and increasing anthropogenic pressures. Continued fieldwork, coupled with genetic and ecological modeling, is recommended to understand population dynamics and inform strategies for preserving amphibian diversity in the Indo-Myanmar biodiversity hotspot.

Species identification remains a cornerstone of biodiversity research, yet it is complicated by cryptic taxa and morphological similarities. This study aimed to elucidate the phylogenetic position and genetic divergence of *Polypedates braueri* using partial mitochondrial 16S rRNA sequences. Liver tissue samples from 15 individuals collected in Mizoram, India, were analyzed through PCR amplification and Sanger sequencing. The generated sequences were aligned and analyzed alongside publicly available data using maximum likelihood phylogenetic methods. The resulting tree revealed that the Mizoram population forms a distinct and well-supported clade closely related to populations from Thailand, China, and Vietnam, suggesting recent divergence and possible range expansions. Pairwise genetic distance analysis showed minimal intraspecific variation within the Mizoram group (0.03%), while inter-population distances ranged from 0.21% to 0.44%. The presence of misidentified or closely related species within the dataset, including *P. himalayensis*, highlights the challenges of morphological identification. This study reinforces the utility of molecular techniques, particularly 16S rRNA analysis, in resolving taxonomic ambiguities and identifying cryptic species within the *Polypedates leucomystax* complex. The findings contribute to the broader understanding of amphibian diversity in Northeast India, a region with underexplored herpetofaunal taxonomy, and underscore the need for integrated taxonomic approaches in biodiversity assessments.

The present study comprehensively investigates the breeding biology of *P. braueri*, a rhacophorid frog species, in the montane subtropical forests of Mizoram, Northeast India. Field surveys were conducted over three consecutive breeding seasons (2021–2023) at two primary sites: Sailam and Reiek Community Reserved Forests. These habitats, characterized by seasonal rain-fed ponds and ephemeral pools, are critical for the reproductive activities of *P. braueri*.

Breeding activity was found to be highly seasonal, coinciding with the onset of the southwest monsoon from April to September, and was significantly influenced by environmental factors, notably rainfall and temperature. Kruskal-Wallis H tests demonstrated that rainfall and minimum temperature (Sailam) and both rainfall and air temperature (Reiek) had a statistically significant impact on breeding initiation ($p < 0.005$).

During the breeding season, males arrived at breeding sites earlier and began vocalizing to attract females. Mating behavior involved axillary amplexus, with amplexus lasting 55–140 minutes, followed by foam nest construction and egg deposition occurring over 30–55 minutes. The foam nests were pale whitish or yellowish, spherical in shape, and deposited either on the forest floor under vegetation or partially submerged leaf litter. The oviposition sites were located 0.5–3 meters from the water body, with plant species such as *Cyperus* sp., *Ageratina adenophora*, and *Commelina* sp. dominating the nesting microhabitat. Such plant structures may provide camouflage and protection from desiccation and predation, thus improving developmental success.

Acoustic analysis revealed that the advertisement calls of *P. braueri* consisted of 1–5 notes with 3–8 pulses per note. Call durations ranged from 43–598 ms, with dominant frequencies between 843.75–1406.25 Hz. These characteristics distinguish P. braueri acoustically from its congeners and reinforce the role of vocalization as a potential taxonomic tool. Unlike some related species, no female calls were detected during the study period.

Oviposition yielded foam nests containing clutches of 215–408 eggs (mean: 306 ± 17). A significant positive correlation ($r = 0.55$, $p = 0.05$) was observed between female snout-vent length (SVL) and clutch size, highlighting the influence of maternal body size on reproductive output. Males exhibited a smaller mean SVL (46.0 ± 2.2 mm) compared to females (54.5 ± 4.5 mm), indicating clear sexual size dimorphism.

Histological examination of gonads across breeding phases provided insights into the reproductive cycle. During the breeding phase, ovaries contained mature oocytes encased in vitelline membranes and abundant yolk, while testes exhibited all stages of spermatogenesis with prominent seminiferous tubules and abundant spermatids and spermatozoa. Post-breeding periods showed depleted ovaries and reduced testicular activity, suggesting the release of gametes and regression of reproductive organs. Environmental triggers like rainfall and temperature likely play a regulatory role in these gonadal changes, in accordance with patterns reported in other subtropical anurans.

Comparative analysis of breeding parameters with congeneric species revealed that *P. braueri* exhibits lower clutch sizes than *P. teraiensis*, *P. himalayensis*, and *P. megacephalus*, but higher than *P. otilophus*. Such variations may result from environmental adaptations or geographic variation in reproductive strategies. The study also recorded dipteran larval infestations in 60% of examined foam nests, indicating vulnerability to invertebrate predators.

The ecological specificity observed in the breeding sites and behaviors of *P. braueri* suggests a narrow reproductive niche, emphasizing the importance of habitat integrity for species survival. This study expands the limited existing knowledge on the reproductive biology of *P. braueri* and underscores the significance of understanding local environmental dynamics in developing effective conservation strategies. Furthermore, it highlights the need for continued ecological and taxonomic research on rhacophorid frogs in the biodiverse yet underexplored landscapes of Northeast India

Amphibian embryology and larval development are critical areas of study in taxonomy, phylogeny, ecology, and conservation biology. Among anurans, the developmental trajectories of larval stages exhibit significant diversity, both within and across taxa. This study presents a comprehensive description of the embryonic and larval development of *Polypedates braueri*, a rhacophorid frog species listed as Data Deficient on the IUCN Red List. Survey was conducted over three consecutive breeding seasons (2021–2023) in both natural habitats—Reiek and Sailam Community Reserved Forests of Mizoram—and under laboratory conditions. It documents all 46 developmental stages of *P. braueri*, aligned with Gosner’s (1960) standardized staging system, providing detailed morphological and morphometric observations, environmental parameters, and behavioral notes.

Fertilized eggs of *P. braueri* were found to be spherical with a mean diameter of 1.83 ± 0.01 mm. Embryonic cleavage began within hours of fertilization, progressing rapidly through the blastula, gastrula, and neurula stages. Tadpoles hatched at Gosner stage 20, approximately 2 days and 19 hours after fertilization, with a total length of 9.64 ± 0.13 mm. Development continued through a series of morphologically distinguishable stages encompassing tail elongation, operculum formation, hind limb and toe differentiation, and culminated in complete metamorphosis into froglets by day 75. The maximum body length was recorded at Gosner stage 41 (47.28 ± 0.38 mm), after which a progressive decrease in length was observed due to tail resorption during metamorphosis.

Environmental factors, particularly temperature and pH, significantly influenced developmental rates. Water temperatures in natural habitats ranged from 10.6°C to 30.3°C with pH levels between 6.0 and 7.4, while lab conditions maintained temperatures between 7.2°C and 30.2°C and pH between 5.78 and 8.88. Seasonal variations impacted development, with overwintering tadpoles observed during the cooler months of December to March. These tadpoles exhibited slowed growth, often completing metamorphosis only after temperatures increased in late February or March, although some failed to metamorphose entirely.

Morphometric data were systematically recorded for 10 individuals at each key developmental stage using standardized methods. Parameters included total length, body length, tail length, tail musculature height and width, body width, interorbital and internarial distances, and oral disc development. Significant positive correlation was noted between body length and tail length from stages 40 to 46 ($p < 0.001$), indicating synchronous growth patterns leading up to metamorphosis. Morphological descriptions at key stages highlighted changes in tail fin transparency, pigmentation, limb bud formation, and development of oral structures such as labial tooth rows and keratinized mouthparts.

The morphology of tadpoles at Gosner stage 34 was detailed from dorsal, ventral, and lateral views, providing diagnostic characteristics for taxonomic identification. These included dorsoventral body compression, dorso-lateral placement of eyes, a lateral sinistral spiracle, dextral vent tube, and lanceolate tail shape. Colouration in live specimens was light to dark brown with black mottling, while preserved specimens displayed faded pigmentation. A notable feature—a white patch at the tip of the snout—was observed from stage 24 to stage 40, a characteristic shared with certain congeners but absent in others.

Comparison with congeners such as *P. maculatus*, *P. leucomystax*, *P. megacephalus*, *P. teraiensis*, and *P. himalayensis* revealed interspecific differences in developmental timing, egg and tadpole size, and environmental conditions for metamorphosis. For instance, while *P. braueri* completed development in 75 days, other species such as *P. megacephalus* and *P. teraiensis* completed metamorphosis in 45–64 days under varying temperatures and environmental conditions.

The findings underscore the importance of ontogenic studies in rhacophorid frogs, contributing to the broader understanding of anuran development and aiding in taxonomic resolution. Given the ecological significance and conservation status of *P. braueri*, detailed documentation of its life cycle stages not only enhances taxonomic clarity but also supports conservation planning in its native habitats. Furthermore, the study highlights how larval morphological traits can be pivotal in resolving systematic questions and stresses the need for more integrative research combining morphological, molecular, and ecological data. As only 1.29% of known anuran species have documented normal developmental tables, this research fills a critical gap and establishes a baseline for future amphibian developmental and ecological studies.

Understanding the feeding ecology and oral morphology of anuran larvae is essential for assessing their ecological roles, evolutionary adaptations, and conservation needs.This study investigates the ontogeny of the oral apparatus and dietary preferences of *P. braueri* tadpoles across developmental stages, as well as the diet composition of the adult frogs. The research was conducted in northeast India, a region known for its rich but understudied amphibian diversity. Tadpoles were collected from temporary aquatic habitats such as rock pools and ephemeral ponds. Standard morphological staging was performed following Gosner's (1960) guidelines. The oral structures were studied using both light microscopy and scanning electron microscopy (SEM), while diet composition was determined through qualitative gut content analysis at various Gosner stages.

The oral disc of *P. braueri* is oriented anteroventrally and exhibits distinctive features including marginal and submarginal papillae, M-shaped upper jaw sheaths, V-shaped lower jaw sheaths, and well-developed keratinized tooth rows. Labial Tooth Row Formula (LTRF) varied significantly with developmental stage. At Gosner stage 25, LTRF was 3(2–3)/3; it changed to 4(2–4)/3 or 4(2–4)/3(1) between stages 26–34, and further to 5(2–5)/3 or 5(2–5)/3(1) between stages 35–41. These changes reflect morphological adaptation to different feeding intensities and nutritional demands during larval growth. SEM analysis revealed detailed structures of the keratinized teeth, including serrated edges and distinct head-neck-base segmentation, suitable for scraping and ingesting periphytic food sources.

Feeding activity began at stage 25 and continued until stage 46, with a noticeable decline in food diversity during metamorphic climax (stages 42–46), likely due to physiological remodeling. Gut content analysis across stages demonstrated that tadpoles primarily fed on planktonic and periphytic algae, with Bacillariophyceae being the most dominant group (65.95% of all dietary items). Among these, *Pinnulari*a and *Gomphonima* were the most frequently occurring species. Other food groups included members of Zygnematophyceae (16.21%), Trebouxiophyceae, Chlorophyceae, Euglenophyceae, and Tubulinea, as well as minor contributions from zooplankton, cyanobacteria, and fungi. Diversity indices such as the Shannon-Wiener Index and Berger-Parker Index were calculated to quantify species richness and evenness across developmental stages. The highest species diversity was recorded during stages 26–30, with values indicating moderate evenness (0.81–0.91), suggesting an uneven distribution of food items in the gut.

The study also examined the diet of adult *P. braueri* using the stomach-flushing technique. Adult frogs were found to be generalist carnivores, feeding on a wide range of arthropods including grasshoppers (Orthoptera), beetles (Coleoptera), ants (Hymenoptera), earwigs (Dermaptera), and insect larvae (Lepidoptera, Diptera). Other prey included soil mites (Oribatida), stick insects (Phasmida), snails

(Stylommatophora), and plant debris. These findings are consistent with those for congeners such as *P. himalayensis* and *P. leucomystax*, indicating a pattern of carnivorous non-selective feeding in adult *Polypedates* species. The wide variety of prey consumed is influenced by local prey availability and possibly constrained by morphological features like gape size, which determines the size range of ingestible prey.

Comparative analysis of oral structures with other *Polypedates* species from different geographical regions revealed considerable variability in LTRF, highlighting potential phylogenetic and ecological differentiation within the genus. Tadpoles of *P. braueri* are classified as nektonic exotrophs, adapted to feeding within the water column using their keratinized oral apparatus. Their ability to exploit diatoms and algae as primary food sources suggests that habitat quality—particularly in terms of algal diversity—plays a crucial role in larval development and survival.

This study contributes valuable data to the relatively sparse literature on the larval biology and diet of Indian anurans, especially in the northeastern region. The observations underscore the ecological importance of *P. braueri* tadpoles as primary consumers in freshwater ecosystems and their potential role as bioindicators of aquatic health. Furthermore, the shift from herbivory in the larval stage to carnivory in adulthood exemplifies the biphasic dietary adaptation in anurans. These findings emphasize the need for conserving ephemeral aquatic habitats that support critical life stages of amphibians. They also demonstrate the value of larval morphological studies in informing species identification, evolutionary relationships, and conservation strategies.

Therefore, this study fills critical gaps in the larval and reproductive biology of *P. braueri*, providing foundational data on distribution, habitat, behavior and normal table for embryonic and larval development for future taxonomic, ecological, and conservation efforts in a biologically rich yet under-studied region.