

# Larval taxonomy of Indian Anurans

## Abstract

Keys for tadpoles of 38 species of anurans from India belonging to various genera collected from various aquatic systems are herein described. For the current study, we used a set of external, buccopharyngeal and internal anatomical characters to build a robust taxonomic key system that would facilitate in proper taxonomic identification of anuran larvae from the Indian subcontinent.

## Introduction

Most amphibians especially anurans, have a larval stage that is morphologically different from their adult forms. In general, anuran larvae live in different ecological setups than the adult, have different physiological and dietary requirements, and have different predators while having the same set of selection pressure as of the adults. During this larval phase, the tiny larva (tadpole) develops from an aquatic gill-breathing organism with a tail to a lung breathing four-legged metamorph. In addition, many species have highly specialized larval morphologies, with various morphological characters essential for their survival that are modified or lost upon metamorphosis. Traditionally, anuran taxonomy was mainly focused on variation and characteristics of adults with a few studies dealing with larval morphology. The larval stages of anurans are termed as "tadpoles" which was derived from medieval english term "taddepol" (tadde- toad and pol-head). Physically the tadpoles resemble fishes with a large body and an elongated caudal tail. Research on tadpole morphology has grown rapidly in recent years, but many larval anurans remain unknown. Unfortunately, difficulties in tadpole identification had been a major obstacle in

anuran systematics and conservation programs.

## Tadpole work in India

Indian subcontinent is home to more than 350 species of anurans of which of which ~ 60 species have direct development and the rest have free-living larval forms (tadpoles). Of these species having free-living tadpoles, larval (tadpole) identities of ~100 species

## Key words:

*Tadpole; External morphology; Buccopharyngeal; Chondrocranium; Morphological key.*

Stream living tadpoles show special characters for adaptation in torrent habitat.  
Photo Credit: Abhijit Das

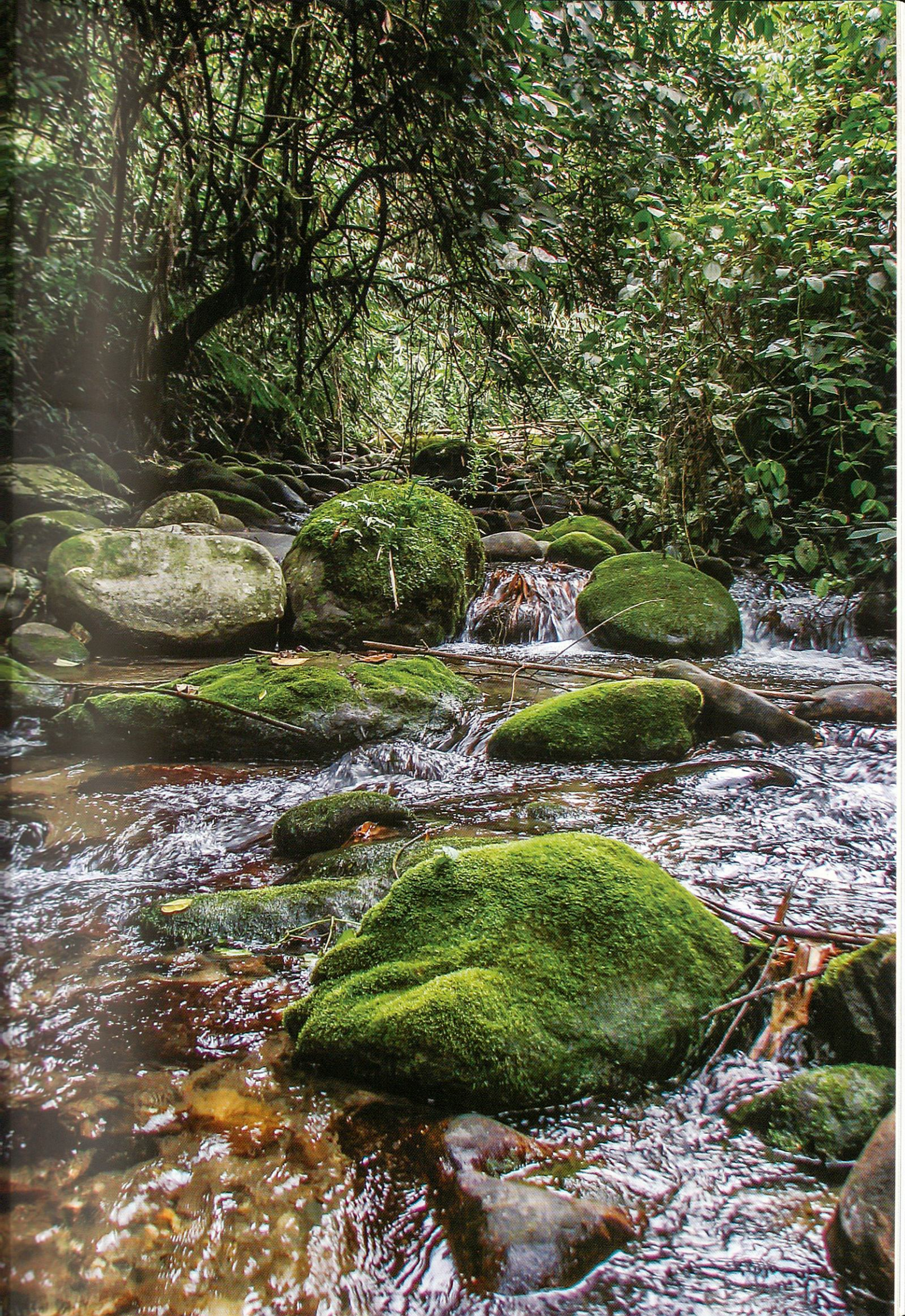
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are known. In comparison to work on adult anurans from India, little detailed systematic work has been carried on the larval stages. By early 20th century, biologists started describing larval forms for many anurans from the Indian subcontinent and provided characters for their identification. Annandale and Rao during this period has taken the lead and made extensive morphological descriptions for larvae mostly on peninsular Indian anurans and a few from the Himalayas. Work by Annandale (1905; 1906; 1908; 1912; 1913; 1917; 1918; 1919), Annandale & Hora (1922), Annandale & Rao (1917; 1918) and Rao (1914; 1915; 1918; 1922; 1937; 1938) are the most comprehensive that has appeared so far. Later during the middle of last century larval descriptions for a few species were made by Bhaduri (1935; 1944a; 1944b), Bhaduri & Daniel (1956), Bhaduri & Kripalani (1954), Kripalani (1953a; 1953b). This was followed by Agarwal & Niazi (1977; 1980), Bhatia (1969), Bordoloi et al. (2001), Chanda & Talukdar (1973), Chari (1962), Daniel (1962; 1963a; 1963b; 1975), Das (1996; 1998), Dubious (1974; 1976), Dutta (1997), Dutta et al. (1993; 1994; 2001; 2004), Dutta & Mohanty-Hejmadi (1976; 1984), Hiragond et al. (2001), Inger (1985), Khan (1965; 1969; 1982a; 1997; 2002; 2003a; 2003b; 2003c; 2004), Khan et al. (1979), Khare & Sahu (1984), Kuramoto & Joshy (2002), Leong (2002; 2004; 2005), Mallick et al. (1979), Mehta (1983), Mohanty et al. (1996), Mohanty-Hejmadi & Dutta (1979),

Pillai (1978), Sahu (1994), Sahu & Khare (1980; 1983), Sekar (1990a; 1990b; 1992) in the later part of last century and early this century. However, many of these works on tadpole external morphology were inadequately characterized. On the anatomical studies, pioneering work has been carried by Ramaswami (1932; 1933; 1934; 1936; 1938; 1940; 1943; 1944) on musculature and chondrocranial morphology of many larval anurans from India which still remains to be the only complete comparative anatomical studies on tadpoles of India till date. This was followed with work by Chaco (1965) and Khan (2003d); however, after this no subsequent work was carried on materiel from India. Work on buccopharyngeal anatomy of tadpoles of a few anuran species from India was taken up by Das (1994), Grosjean (2003; 2004) and Hora (1922). Buccopharyngeal studies of few species with extra-limital distribution were taken up by Khan (1996a; 1996b; 2000), Khan & Malik (1987), Khan & Mufti (1994; 1995). Few keys to anuran larval faunas from India are available. However, subsequent to the general larval keys for few anurans from India and Pakistan by Annandale (1918), Annandale & Rao (1917; 1918), Khan (1982) and Sahu (1994) there have been no attempts to provide comprehensive keys to Indian tadpoles. In the past few decades, there had been major systematic rearrangements of many anuran species and several new genera were described

Metamorph of *Hyla annectans*, Nagaland.  
Photo Credit: Abhijit Das



with considerable amount of new information. Tadpoles of species restricted to known hotspots, such as the Western Ghats and the Eastern Himalayas in India, remain poorly known, especially since spectacular discoveries have been made in the recent past from these regions.

### **Taxonomy of anuran larvae**

Tadpoles of most species tend to have similar body forms and which makes them notoriously difficult to identify for a layman. Morphologically tadpoles of most species show homoplasy and distantly related species have very similar appearing tadpoles. There are few keys available for identifying tadpoles to species. Tadpoles are generally identified using a set of characters. The most important character is the denticle row formulae (LTRF- Labial Teeth Tow Formula) (Fig 1). However, going by LTRF alone would lead to ambiguity. One needs to identify them using a set oral characters and a set of external morphological characters. In many groups, larval morphology is conserved and tadpoles of many species of the same genus tend to look alike. Tadpoles due to various ecological factors have different anatomies and show considerable variation during the ontogeny in terms of their morphology. However, they do have certain morphological traits that can be used to distinguish among species. Recent advances in molecular studies had made identification of tadpoles easier and one can easily ascertain the taxonomic identity of the tadpole.

### **Methods**

#### **External morphology**

There is a lot of confusion on the usage of terminology on anuran tadpole morphology. For external morphological and oral disc terminology, it is advised to follow Altig & Johnston (1989), and Altig & Mc Diarmid (1999). In recent years, Altig (2007) and Haas (2011) summarized much of the known information. For a layman, taxonomic identity of anuran larvae can be done using some simple external (Fig 2) and oral morphological keys. Externally tadpoles can be differentiated based on the position of the oral disc, position of the eye, opening of

the vent tube, shape of the tail, etc. With in oral disc, one needs to look into the shape of the jaw sheath, pigmentation of the jaw sheath, nature of the oral disc, distribution of marginal papillae, pigmentation on the jaw sheaths, presence/absence of denticle rows, indentations of the oral disc, etc. Tadpoles of anurans during their ontogeny are referred by various developmental stages usually defined by various morphological attributes. One of the most widely used staging tables was the one developed by Gosner (1960) primarily based on hind limb development. The developmental stages of anurans can be broadly grouped into two phases: (i) the initial growth phase, which is characterized by an aquatic free-swimming tadpole (Gosner stage 25-40) and; (ii) the metamorphosis phase (Gosner stage 40-46), during which the tadpole loses the free-swimming neonatal characters and starts to resemble the adult. Morphometric measurements of various morphological variables are of great taxonomic value and can be useful in taxonomic identification of tadpoles.

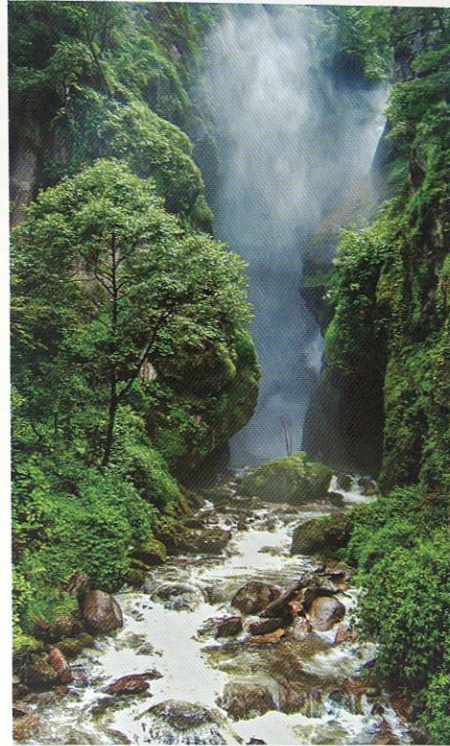
#### **Buccopharyngeal study**

The internal buccal and the pharyngeal anatomy of tadpoles vary across species. The internal surface of buccopharyngeal region is dotted with a number of chemoreceptory pustules and papillae (Fig. 3). The number and nature of these structures is species specific and can be of great taxonomic importance. These internal structures can be studied either by using photographs taken by Scanning Electron Microscope or by simple staining of internal structures using Methylene Blue and observing under stereo zoom microscope (SEM). Careful dissection of the tadpole by making insertions through the lateral corners of the jaw sheath would divide the buccal region into dorsal (buccal roof) and ventral (ventral floor) regions. For standard terminology and methods for studying buccopharyngeal structures of tadpoles, one can refer to published literature by Wassersug (1976, 1980) and Inger (1985).

#### **Anatomical characters**

Anatomical characters are more conserved

and can be useful in ascertaining phylogenetic relationships. Chondrocranium along with the ceratohyal forms the major structural composition of the tadpole anatomy (fig. 4). Despite this, so far there few good studies on larval anatomy and little is known about the function of the chondrocranium and the concerned musculature or how developmental factors contribute to inter-specific morphological variation. One of the easiest ways of studying larval anatomy is through staining. Anuran larval anatomy during the tadpole stage constitutes only cartilage and gets replaced with bone during metamorphosis. Alcian blue is used to stain cartilage and Alzarin red is used for staining bone.



A typical stream habitat showing various microhabitats.

## Results

### Larval keys

Based on this morphological characterization, taxonomic key for tadpoles of 38 anuran species are being produced.....	1
1 A. Without nares; without jaw sheath, spiracle ventral;.....	2
B. With nares; with jaw sheaths, spiracle sinistral;.....	4
2 A. Oral disc with a fold on the lower labium with no denticle and papillae; one medial vent and a medial spiracle; large lateral eyes with a transparent body .....	3
B. Oral disc with a fold on the lower labium with no denticle and papillae; dorsal side speckled with reddish brown melanophores; small lateral eyes with a translucent body diffused with many melanophores. ....	<i>Uperodon systoma.</i>
3 A. Dorsum with a diamond marking due to congregation at the centre of the abdomen; Caudal fin with melanophores spread uniformly .....	<i>Microhyla berdmorei.</i>
B. Dorsum with uniformly spread tiny melanophores; Caudal fin with melanophores concentrated at the distal end of the fin .....	<i>Microhyla ornata.</i>
4 A. Oral disc with multi lobed oral disc and without labial tooth rows, dorsal fin origin posterior to body tail junction .....	5
B. Oral disc with emarginated or non-emarginated oral disc with labial tooth rows .....	6
5 A. Small tadpoles, with body length less than 11 mm (stage 35-40), tadpoles with pale body and sparsely dotted with few brown melanophores .....	<i>Nyctibatrachus cf. minor.</i>
B. Large tadpoles, with body length more than 11 mm (stage 35-40), tadpoles with dirty brown body colouration .....	<i>Nyctibatrachus cf. major.</i>
6 A. Eyes lateral/ near lateral; mouth anteroventral; origin of the ventral fin anterior to body tail junction .....	7
B. Eyes dorsal/ dorsolateral; mouth ventral/ anetero-ventral; origin of the tail at body tail junction/posterior to body tail junction .....	11

- 7 A. Eyes near lateral; vent tube medial; both fins of equal height; marginal papillae on the oral disc distributed with wide dorsal gap; single row of marginal papillae; jaw sheath was broadly rounded with a long lateral process (trapezoidal) ..... *Hyla annectans*.
- B. Eyes lateral; vent tube dextral; ventral fin taller; marginal papillae on the oral disc distributed with wide dorsal gap and narrow ventral gap; marginal papillae were single row on the anterior labium and double row on the posterior labium; jaw sheath arch shaped 8 A. Opening of the nostrils circular/oval with the rim elevated or depressed; pre narial arena with an arched medial ridge and many secondary lateral papillae or a simple arched medial ridge; Infralabial papillae pointed with rugose margin; lingual papillae smooth ..... 9
- B. Opening of the nostrils reniform with rim elevated; pre narial arena with arched medial ridge and many secondary lateral papillae; Infralabial papillae dilated; lingual papillae postulated ..... 10
- 
- 9 A. Nostril opening depressed; pre narial arena of the buccal roof was simple arched medial ridge; four lingual papillae; A5 (4)/P3 (1) ..... *Polypedates cf. pseudocruciger*.
- B. Nostril opening elevated; pre narial arena of the buccal roof was arched medial ridge with many secondary lateral papillae; two lingual papillae; A4 (3)/P3 (1) ..... *Polypedates maculatus*.
- 
- 10 A. two lingual papillae; A5 (4) (1)/P3 (1) ..... *Polypedates cf. himalayanus*.
- B. Four lingual papillae; A4 (3)/P3 ..... *Polypedates teraiensis*.
- 
- 11 A. Eyes dorsal placed back on the dorsum; oral disc large and ventral; tail muscle wide in dorsal view; origin of the tail posterior to body tail junction ..... 12
- B. Eyes dorsolateral placed near the anterior end of the body on the dorsum; oral disc small to moderate and positioned either ventral/anteroventral; tail muscle wide/narrow in dorsal view; origin of the tail at body tail junction/ posterior to body tail junction ..... 13
- 
- 12 A. Absence of cutaneous granular glands on the body; absence of suction organ formed by gastrozymus muscle; vent tube medial; labial tooth row formula was A 2/P3 (1) ..... *Nasikabatrachus sahyadrensis*.
- B. Presence of cutaneous granular glands on the body; presence of suction organ formed by gastrozymus muscle; vent tube dextral; labial tooth row formula was A8 (5)/P3 (1) ..... *Amolops assamensis*.
- 
- 13 A. Presence of cutaneous granular glands on the body ..... 14
- B. Absence of cutaneous granular glands on the body ..... 17
- 
- 14 A. Large tadpole; presence of parotid gland, posteroventral gland and supracaudal gland ..... 15
- B. Moderate sized tadpole; presence of parotid gland, posteroventral gland and absence of supracaudal gland; labial tooth row formula is A2 (1)/P3 (1) ..... 16
- 
- 15 A. presence of ocellus on the caudal musculature; labial tooth row formula is A6 (4)/P7 (1) ..... *Clinotarsus alticola*.
- B. Absence of ocellus on the caudal musculature; labial tooth row formula is A8 (6)/P7 (1) ..... *Clinotarsus curtipes*.
- 
- 16 A. Nostrils located midway between the eye and snout; internal nares narrowly separated; Buccal Roof Arena (BRA) papillae were abundant; straw coloured body with few melanophores ..... *Indosylvibana indica*.
- B. Nostrils located midway between the eye and snout; internal nares moderately separated; BRA papillae were fewer; Body straw coloured but with many melanophores ..... *Indosylvibana indica*.

C.	Nostrils closer to eye; internal nares narrowly separated; BRA papillae were fewer; Body dirty yellow with numerous tiny brown melanophores .....	<i>Indosylvibana indica</i> .
17 A.	Vent tube opening medial .....	18
B.	Vent tube opening dextral .....	23
18 A.	Jaw sheath broadly rounded with long lateral process (trapezoidal); caudal musculature strong with low dorsal and ventral fin .....	19
B.	Jaw sheath with elongated thick middle portion and thin lateral processes; caudal musculature strong with low dorsal and ventral fin .....	22
19 A.	Centripetal wall of the spiracle totally fused to the body wall with distal end free; broadly rounded tail .....	20
B.	Centripetal wall of the spiracle partly formed; rounded tail .....	21
20 A.	Shape of the narial opening was oval/rounded; both caudal fins of equal height; jaw sheath keratinization moderate; median ridge bifid; BRA pustules few .....	<i>Duttaphrynus himalayanus</i> .
B.	Shape of the narial opening was reniform; Dorsal fin taller; jaw sheath keratinization weak; median ridge trifid; BRA pistules absent .....	<i>Bufo latastii</i> .
21 A.	Body colour black olive; ethmoid plate of the chondrocranium elongated; coupla 1(Basihyal) present; Gap below the hypobranchial plate was wide .....	<i>Duttaphrynus melanostictus</i> .
B.	Body colour black olive with golden speckles; ethmoid plate of the chondrocranium intermediate; coupla 1(Basihyal) absent; gap below the hypobranchial plate was intermediate .....	<i>Duttaphrynus cf. melanostictus</i> .
22 A.	Semi terrestrial tadpoles; large dorsolateral eyes; triangular body; oral disc positioned anterolateral; Spiracle with no centripetal wall and tube reduced/dumitive .....	<i>Indirana cf. beddomii</i> .
23 A.	Presence of external naso-lacrimel groove .....	24
B.	Absence of external naso-lacrimel groove .....	26
24 A.	Narial opening elevated; nostrils closer to the eye; oral disc with a wide dorsal gap and narrow ventral gap; Fewer BRA and Buccal Floor Arena (BFA) pustules; labial tooth row formula was A5 (4)/A3 (1) .....	<i>Rhacophorus maximus</i> .
B.	Nostrils placed midway; oral disc with wide dorsal gap; abundant BRA and numerous BFA pustules .....	25
25 A.	nostril opening shape oval/rounded; dorsal fin taller; median ridge triangular; labial tooth row formula was A6 (4)/A3 (1) .....	<i>Rhacophorus lateralis</i> .
B.	Nostril opening shape oblong; both fins are of equal height; median ridge triangular; labial tooth row formula was A7 (5)/3(1) .....	<i>Rhacophorus malabaricus</i> .
C.	Nostril opening shape oblong; dorsal fin taller; median ridge bifid; labial tooth row formula was A6 (4)/3(1) .....	<i>Rhacophorus pseudomalabaricus</i> .
26 A.	Oral disc located ventrally .....	27
B.	Oral disc located antero ventrally .....	29
27 A.	Shape of the nostril oval/round; both fins of equal height; marginal papillae distributed on the oral disc with narrow dorsal and ventral gaps; submarginal papillae present on both labia; shape of upper jaw sheath Inverted v-shaped with a medial protrusion; no pre narial medial ridge; labial tooth row formula is A5 (4)/P6 (6) .....	<i>Leptobrachium smithi</i> .
B.	Shape of the nostril opening reniform; marginal papillae distribution on the oral disc with a wide gap on the upper labium; submarginal papillae present at the lateral corners and lower labium of the oral disc; Jaw sheath keratinization strong; upper jaw sheath arch shaped .....	28

28. A. Snout broadly rounded; widest part of the body middle of the abdomen; broadly rounded tail tip; simple arched pre medial ridge; labial tooth row formula was A6 (4)/P3 (1) ..... *Nanorana vicina*.
- B. Snout rounded; widest part of the body at the back of the abdomen; rounded tail tip; single papilla pre medial ridge; labial tooth row formula was 5(4)/3(1); ..... *Nanorana minica*.
- 
29. A. Opening of the nostrils depressed; marginal papillae were uniseriate on upper labium and biseriate on the lower labium; shape of the upper jaw sheath Inverted v-shaped with a medial protrusion ..... 30
- B. Opening of the nostrils elevated ..... 31
- 
30. A. Infra-labial papillae shape dilated ..... *Chiromantis dudwaensis*.
- B. Infra-labial papillae shape pointed ..... *Chiromantis simus*.
- 
31. A. Infra-labial papillae shape dilated ..... 32
- B. Infra-labial papillae shape bifurcate/ compressed ..... 33
- 
32. A. nostril located closer to the eye; tail tip pointed; submarginal papillae present on the lateral corners of the oral disc; Jaw sheath keratinization weak; upper jaw sheath arch shaped with medial protrusion; internal nares widely separated; BRA pustules few; two lingual papillae; labial tooth row formula is A2 (1)/P3 ..... *Fejervarya cf. keralensis*.
- B. Nostril located midway between eye and snout; tail tip pointed; submarginal papillae present on the lateral corners of the oral disc; Jaw sheath keratinization weak; upper jaw sheath broadly rounded with a short lateral process and a medial protrusion; internal nares narrowly separated; BRA pustules abundant; two lingual papillae; labial tooth row formula is A2 (1)/P3 (1). ..... *Fejervarya cf. orissaensis*.
- C. Nostril located closer to the eye; tail tip rounded; submarginal papillae present on the lateral corners of the oral disc; Jaw sheath keratinization moderate; upper jaw sheath arch shaped with medial protrusion; internal nares moderately separated; BRA pustules abundant; two lingual papillae; labial tooth row formula is A3 (2)/P3 ..... *Fejervarya cf. pierrie*.
- D. Nostril located closer to the eye; tail tip rounded; submarginal papillae present on the lateral corners of the oral disc; Jaw sheath keratinization moderate; upper jaw sheath arch shaped with medial protrusion; internal nares moderately separated; BRA pustules abundant; four lingual papillae; labial tooth row formula is A2 (1)/P3 ..... *Fejervarya cf. limnocharis*.
- E. Nostril located midway to the eye; tail tip pointed; submarginal papillae absent Jaw sheath keratinization moderate; upper jaw sheath arch shaped with medial protrusion; internal nares moderately separated; BRA pustules few; four lingual papillae; labial tooth row formula is A2 (1)/P3 (1) ..... *Fejervarya cf. nilagarica*.
- 
33. A. Widest part of the body was at the back of the abdomen; submarginal papillae absent; jaw sheath keratinization strong; Jaw sheath arch shaped with medial protrusion; labial tooth row formula is A1/P2 ..... *Euphlyctis cyanophlyctis*.
- B. Widest part of the body was at the middle of the abdomen; submarginal papillae at the lateral corners of the oral disc; jaw sheath keratinization weak; Jaw sheath arch shaped with medial protrusion; labial tooth row formula is A2 (1)/P3 ..... *Sphaerotherca breviceps*



Tadpole of *Indirana* sp. are semi-aquatic.

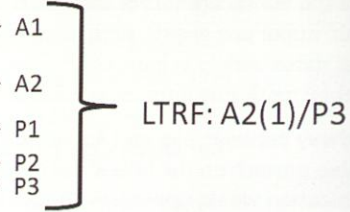
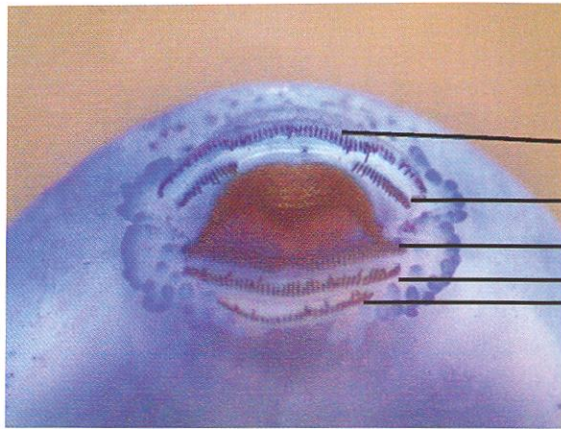


Figure 1: Labial Tooth Row Formula (LTRF) of *Fejervarya* cf. *nilagirica*

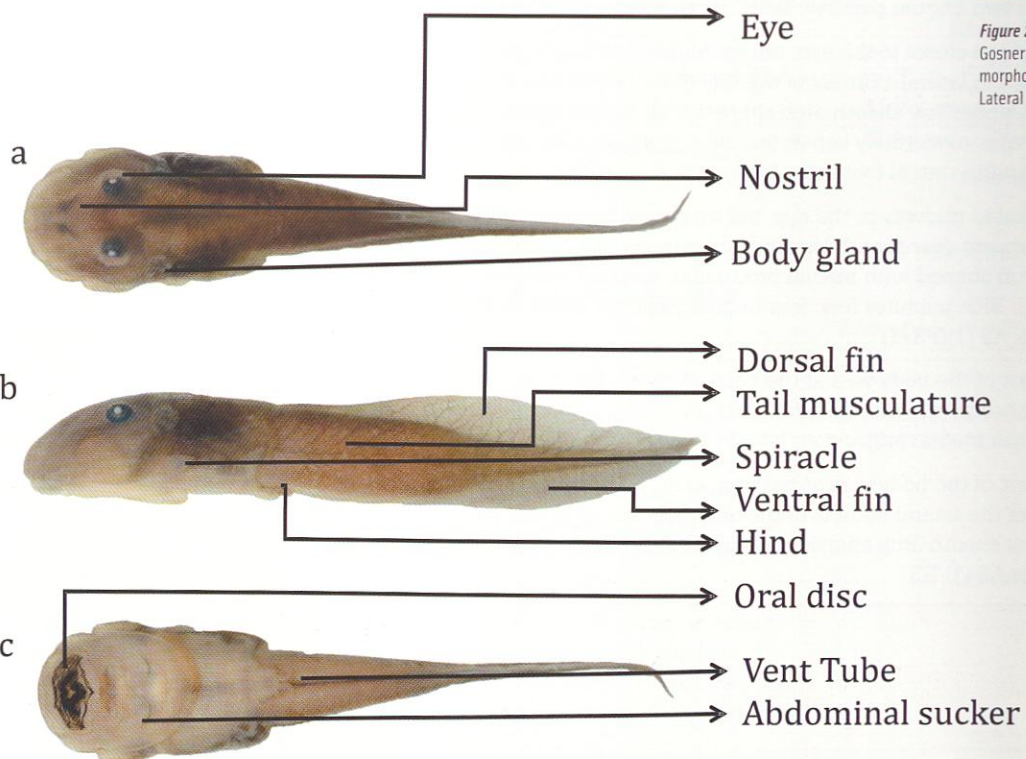
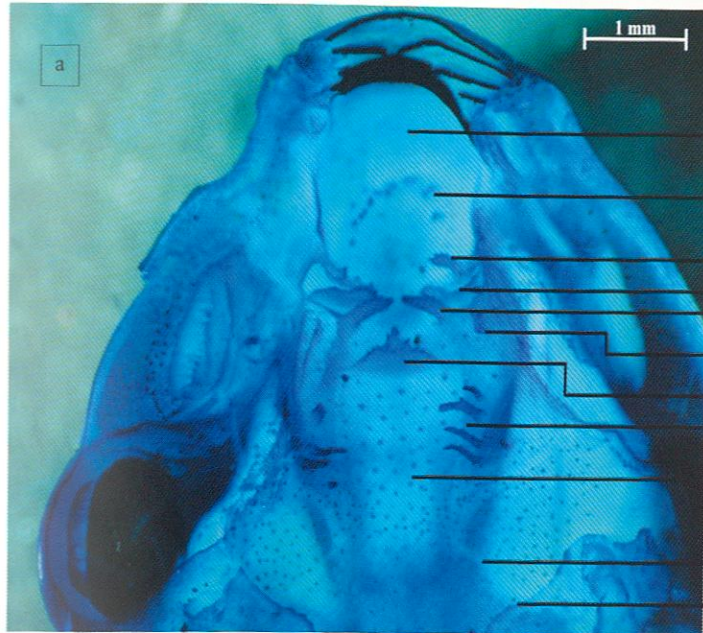


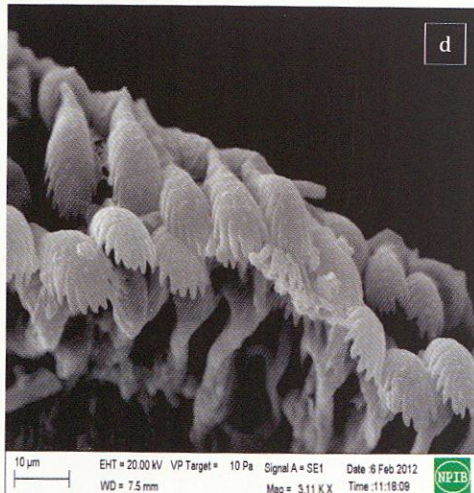
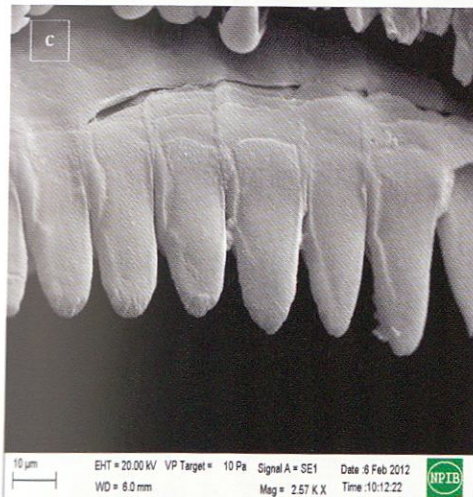
Figure 2: *Amolops assamensis*, Gosner stage 37. External morphology (a) Dorsal view; (b) Lateral view; (c) Ventral view.



- Pre-narial arena
- Pre-narial ridge
- Anterior narial wall papilla
- Posterior narial wall
- Posterior narial papilla
- Lateral ridge papilla
- Median ridge papilla
- Buccal roof arena papilla
- Buccal roof arena pustules
- Glandular zone
- Dorsal velum



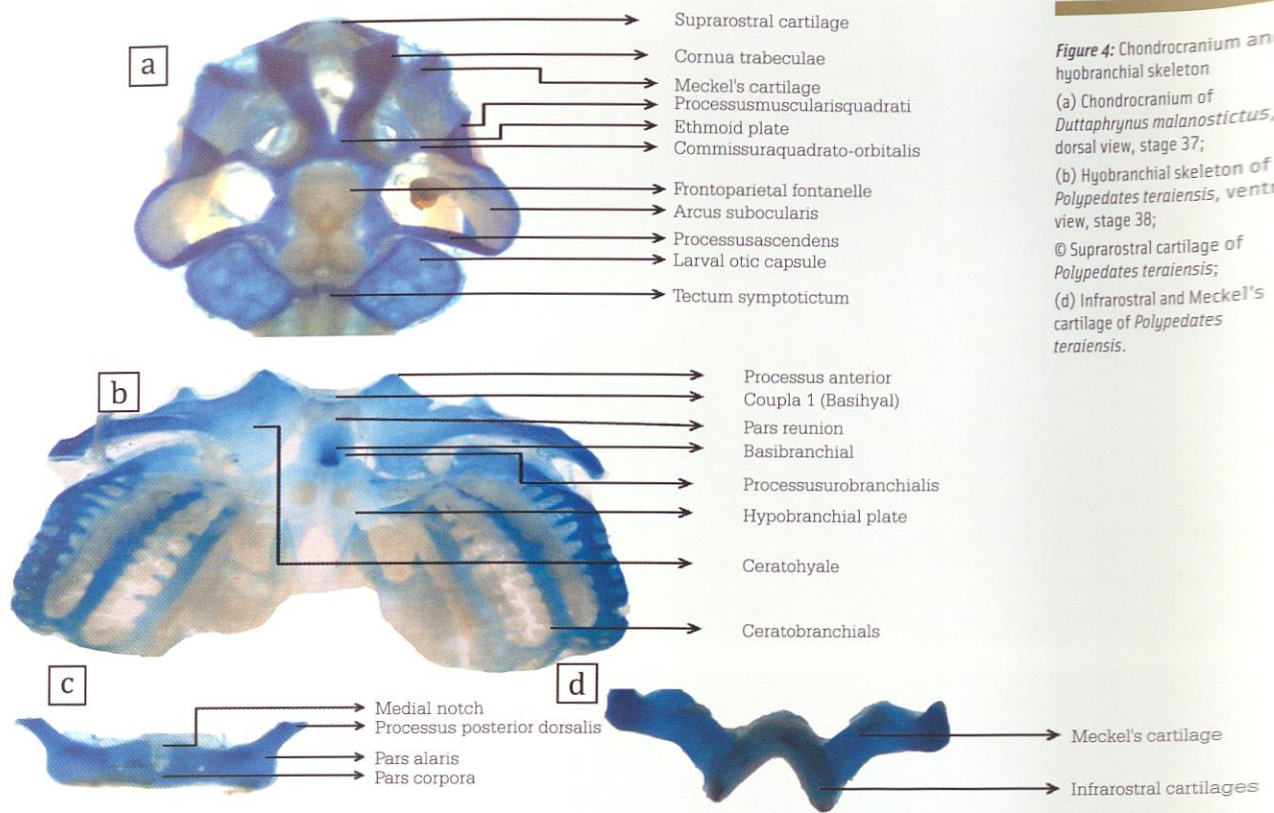
- Pre-lingual arena
- Pre-lingual papillae
- Tongue anlage
- Lingual papillae
- Buccal floor arena
- Buccal floor arena papillae
- Buccal pockets
- Ventral velum
- Gill clefts



**Figure 3:** *Polypedates cf. occidentalis*, stage 37. Buccopharyngeal and denticles (a) Buccal roof, Methylene Blue stained photograph, ventral view, scale = 1 mm; (b) Buccal floor, Methylene Blue stained photograph, ventral view, scale = 1 mm; (c) Individual denticles, scale = .1 mm; (d) Jaw sheath serrations = .1 mm.

10 µm EHT = 20.00 kV VP Target = 10 Pa Signal A = SE1 Date : 6 Feb 2012  
WD = 6.0 mm Mag = 2.57 K X NPTB

10 µm EHT = 20.00 kV VP Target = 10 Pa Signal A = SE1 Date : 6 Feb 2012  
WD = 7.5 mm Mag = 3.11 K X NPTB



**Figure 4:** Chondrocranium and hyobranchial skeleton  
 (a) Chondrocranium of *Duttaphrynus melanostictus*, dorsal view, stage 37;  
 (b) Hyobranchial skeleton of *Polypedates teraiensis*, ventral view, stage 38;  
 (c) Suprarostrals cartilage of *Polypedates teraiensis*;  
 (d) Infracrural and Meckel's cartilage of *Polypedates teraiensis*.

## Glossary

Anterior: front of the body

Basihyal cartilage (Coupla 1): cartilage that chondrifies between the anterior tips of the ceratohyal cartilage

Bucco- Pharyngeal: the region starting from the buccal to the pharynx or nasal passage, or (b) the most ventral element of a gill arch

BRA: Buccal roof arena

Branchial arches: The successive bulges in the lateral walls of the pharynx found between successive branchial groove and branchial pouches or both

Choanae: Internal narial opening

Chondrocranium: The anterior part of the axial skeleton that encases the special sense organs and contributes to the skeletal elements encasing the brain

Caudal: Toward the tail end, inferior

Dextral: Right side

Ethmoid plate: bone that separates the nasal cavity from the brain

Fontanelles: Temporary openings between bony plates of the skull, formed during the formation and fusion of the cranial bones

Infracrural cartilage: Cartilage which acts as a precursor to the lower jaw

Jaw sheath: A chondrofied structure present inside the oral disc used for engulfing food

Lateral: towards the sides

Melanophore: Pigment cell that contains melanin

Neurocranium: Portion of the cranium that houses the brain

Papillae: Small, nipple like projections

Pistules: Tiny projections

Posterior: Nearer the back of the body, dorsal

Spiracle: A tube through which water is released from the body

Sinistral: Left side

Suprarostrals cartilage: Cartilage found attached at the anterior end of the chondrocranium and supports the jaw sheath

Vent tube: A tube at the posterior end of the body for discarding excreta

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