4.2.3. Tadpoles identification: key features

Characteristics of anuran larvae (= tadpoles) are often used in taxonomic descriptions. In some taxa adults may be problematic to identify even though their tadpoles are very distinctive. Reversely (and surprisingly) some fairly different species may have extremely similar larvae.

Tadpoles show a great diversity in morphological types and are perfectly adapted to the many different environments in which they are found (from ponds and streams to bromeliads and tree holes); their morphology also reflects phylogenetic relationships.

Gosner’s (1960) staging system subdivides tadpole development in 46 stages, those below 25 being of little use for identification. Ideally tadpoles should be in stages 26 to 38 to be accurately identified. Hence it is important to rear some larvae in the field and preserve tadpoles at different developmental stages.

The Gosner (1960) staging system is recommended for use with exotroph tadpoles. Figure 61 illustrates Gosner stages from 23 to 41, which are briefly explained below [see Gosner (1960) and McDiarmid & Altig (1999)]. Before stage 23 larvae are non-feeding and mostly immobile.

Stage 23: oral disc distinct, external gills very distinct on both sides.
Stage 24: oral disc distinct, external gills atrophied, operculum closes on right.
Stage 25: oral disc obvious, external gills absent, spiracle forms on left.
Stage 26: hind limb development begins, length of hind limb bud inferior to 50% of its height.
Stage 27: length of hind limb bud superior or equal to 50% of its height.
Stage 28: length of hind limb bud superior or equal to its height.
Stage 29: length of hind limb bud inferior or equal to 150% of its height.
Stage 30: length of hind limb bud equal to 200% of its height.
Stage 31: foot paddle visible.
Stage 32: indentation between the fourth and the fifth toes visible.
Stage 33: indentation between the third and the fourth toes visible.
Stage 34: indentation between the second and the third toes visible.
Stage 35: indentation between the first and the second toes visible.
Stage 36: Toes III-V separated.
Stage 37: all toes separated.
Stage 38: inner metatarsal tubercle appears.
Stage 39: subarticular patches visible.
Stage 40: outer metatarsal tubercle and foot subarticular tubercles visible, vent tube still present.
**Stage 41:** forelimbs bud visible, vent tube absent.

From stage 41 the larval mouthparts disappear and are replaced by adult jaws, tail is resorbed and limbs become functional. Stage 46 corresponds to complete metamorphosis.

*Fig. 61.* Gosner (1960) developmental staging system, from stage 23 to stage 41. Modified from McDiarmid & Altig (1999).
The principal structural features of anuran larvae are illustrated below (Fig. 62).

![Diagram of anuran larva with labeled landmarks]

**Fig. 62.** Principal structural features of anuran larvae (here the arboreal tadpole of *Anomaloglossus beebei*, Aromobatidae). (Photo by P. J. R. Kok).

Shape and position of several structural characters are of taxonomic importance in tadpoles. The spiracle, for example, may be single and sinistral with a short spiracular tube (as illustrated in Fig. 62); single and sinistral with a long spiracle tube; dual and lateral; dual and lateroventral; single and posterior ventral; single and midventral. As for the eyes, they may be lateral or dorsal; the tail tip may be pointed or ending by a filament; the body may be adpressed or not; the tail fin may be extensive or not (compare Figs 62 & 63); the vent tube may be dextral or medial; etc. The development of the lateral line system is also variable. See McDiarmid & Altig, 1999 and Altig, 2007 for extensive descriptions of these structural characters.

Colour and pattern are also helpful for identification and are usually not very variable intraspecifically (although they may change during development, hence the importance to compare larvae of the same developmental stage).

As in adults, comparisons of morphometrics and measurement ratios are helpful to distinguish similar species. Grosjean (2005) recommends tadpoles between stages 32-40 for best morphological intra- and interspecific comparisons.

Principal landmarks are indicated in figure 63 and are explained below:

- **Total length** (TL): from the tip of the snout to the tip of the tail.
- **Body length** (BL): from the tip of the snout to the junction of the posterior body and the tail musculature.
- **Tail length** (TAL): from the junction of the posterior body and the tail musculature to the tip of the tail.
- **Body width** (BW): the highest width of the body.
• **Body height** (BH): the highest height of the body.
• **Head width at level of eyes** (HW): self-explanatory.
• **Tail muscle height at base of tail** (TMH): self-explanatory.
• **Upper tail fin height** (UTF): the highest height of the upper fin, from the upper margin of the tail musculature to the upper margin of the upper fin.
• **Lower tail fin height** (LTF): the highest height of the lower fin, from the lower margin of the lower fin to the lower margin of the tail musculature.
• **Tail muscle width at base of tail** (TMW): self-explanatory.
• **Maximum height of tail** (MTH): the highest height of the tail.
• **Eye-naris distance** (END): from the anterior corner of the eye to the posterior margin of the naris (nostril).
• **Naris-snout distance** (NSD): from the anterior margin of the naris to the tip of the snout.
• **Snout-spiracle distance** (SSD): from the tip of the snout to the posterior margin of the spiracle.
• **Internarial distance** (IND): the distance between the median margins of the nares.
• **Interorbital distance** (IOD): the distance between the median margins of the orbits.
• **Eye diameter** (ED): the greatest length of the orbit from the anterior margin to the posterior margin of the eye.

Note that measurements are accurately compared only when they involve the same landmarks and tadpoles of the same developmental stages!

Some authors suggest that measurements between structures should be taken from the centre of these structures (e.g. internarial distance measured between the centre of the nares). As the centre of a structure is not a fixed point, we find this method too subjective and prefer taking measurements between anterior or posterior margins of structures.

See also aforementioned remark in "Morphometrics".
Shape and location of the oral disc are also very characteristic (see McDiarmid & Altig, 1999 for further details) and are usually related to the feeding habit of the larva (which may feed on detritus, dead invertebrates, other tadpoles, conspecific or heterospecific eggs, etc.). Figure 64 shows principal terminologies used in oral disc description, which are briefly explained below (see McDiarmid & Altig, 1999 and Altig, 2007 for extensive details):

- **A-1, A-2**, etc.: anterior tooth rows (= rows of labial teeth), which are numbered from the anterior margin of the upper labium toward the mouth.

- **Dorsal gap in marginal papillae and A-2 gap**: the term “gap” is used to indicate that there is a space (usually medially) that is free of papillae or labial teeth. There is often a medial gap in marginal papillae on the upper labium and sometimes a gap in the second anterior tooth row. Medial gaps may also occur elsewhere (in P-1 for example). They should not be confused with “artificial” gaps due to the loss of labial teeth or papillae. Number and location of gaps are of taxonomic importance; the size of the gap may vary with developmental stage.
• **P-1, P-2, P-3,** etc.: posterior tooth rows, which are numbered from the mouth toward the posterior margin of the lower labium.

• **Marginal papillae:** they are found on the edges of the oral disc. They may completely encircle the disc, or be interrupted by gaps. Marginal papillae may be laterally indented (= emarginated). The number of papillae rows and the length and shape of papillae vary among taxa and are helpful for identification.

• **Upper and lower jaw sheath:** they form what is sometimes called the tadpole “beak”. Shape of jaw sheath is of some taxonomic importance.

• **Jaw sheath serration:** they are the keratinized projections of various sizes and shapes occurring on the cutting edge of the jaw sheaths.

The LTRF (abbreviation of Labial Tooth Rows Formula) is very useful for comparison. It is expressed as a fractional notation in which the numerator equals the number of anterior tooth rows and the denominator equals the number of posterior tooth rows. Natural gaps are noted between parentheses [e.g. LTRF of the tadpole illustrated in Fig. 64 (Anomaloglossus kaiei, Aromobatidae) is 2(2)/3]. Numbers in bracket indicate variation in the presence of a medial gap.

![Diagram of oral disc](image)

**Fig. 64.** Oral disc of an anuran larvae (Anomaloglossus kaiei, Aromobatidae) showing principal terminologies. Abbreviations are explained in the text. (Scanning electron micrograph by J. Cillis & P. J. R. Kok).
4.2.4. Preparation of tadpole oral disc for electronic microscopy

Observation of the tadpole oral disc using scanning electron microscopy (SEM) is very effective to distinguish very small features that are of taxonomic importance (variation in labial teeth for example).

The oral disc must first be carefully dissected under a stereomicroscope and transferred to 100% ethanol. The sample will then be “critical-point dried”. Critical point drying is a technique of drying soft, naturally hydrated, tissues without deforming their structure. This technique is mostly used for examination under high vacuum conditions, as in the case of a scanning electron microscope. Allowing the oral disc to dry under high vacuum conditions would damage it due to the surface tension that occurs when changing from the liquid to the gaseous phase.

Within the critical-point drier apparatus, the ethanol (called the intermediate fluid) is exchanged for the transition fluid (CO₂) and the “critical point” at which the density of the liquid and the gas is the same is achieved by controlling pressure and temperature within the instrument. Once the CO₂ is fully converted to gas the specimen is dry.

Because freshly dried specimens are highly hygroscopic (which means they readily absorb water), they must be quickly coated with a thin layer of conductive metal (usually gold).

After gold coating the oral disc is carefully positioned on a small stand with a sticky surface and is ready to be examined.

4.2.5. Call analysis

Although sound emission is reported in some caecilians (see Duellman & Trueb, 1986), only anurans produce sounds to attract conspecific females, defend their territory and communicate stress. Call analysis is a valuable tool in species identification: the advertisement call is an important mate recognition character and anuran advertisement calls are species-specific.

Vocal communication in anurans and call analysis are rather complex matters and we only provide here a brief introduction to the very basics of frog call analysis, i.e. the principal terms used in call analysis, their meaning, and the way to obtain essential information from your recordings through the sound analysis software.

The sound analysis software

There are several software programs available on the market; some of them may be downloaded for free from the Internet. We use Raven Pro (version 1.3) from the Cornell Lab of Ornithology, thus the appearance of the oscillograms and spectrograms provided below (and the calculation methods) may change according to the software you will use.

Acquiring input

This depends on the software you use. Connect your recorder to the audio input device on your computer and follow the software user guide.
Types of calls and terminology

There are four types of calls in anurans:

- **The advertisement call**: is produced by males and has two principal functions: attracting conspecific females, and announcing to other males (both conspecific and heterospecific) that the territory is occupied.

- **The reciprocation (or response) call**: is emitted by receptive females in response to conspecific males advertisement call (currently known only in a very few species).

- **The release call**: is produced by males and unreceptive females in response to a tentative amplexus. Often accompanied by body vibrations.

- **The distress call**: is emitted by several species in response to severe disturbance. Usually explosive and very loud (Fig. 65).

![Fig. 65. Distress call of Rhaebo guttatus, Bufonidae. Compare with advertisement call in figure 86.](image)

We focus here on the advertisement call, the most commonly heard call that is widely used in species identification.

The advertisement call is the assemblage of one or more acoustic signals (called the notes) produced in a given time sequence. The notes are nothing else than sound waves transmitted through the air (most of the time) or through water (in some species, *e.g.* *Pipa aspera*, Pipidae).

The characteristics of a sound wave (Fig. 66) are:

- **The amplitude**: usually measured in decibels (dB), the amplitude is the loudness of the sound. Variation of amplitude is visible on an oscillogram (also called the waveform).
• **The frequency**: measured in Hertz (Hz) or kilohertz (kHz), the frequency is the pitch of the sound, which depends on the number of vibrations imposed on the air per second. Variation of frequency is visible on a spectrogram (= audiospectrogram).

Acoustic components of the call are well visible on a spectrogram (Fig. 66) and are:

• **The note**: the smallest unit of the call. The advertisement call may be a single note (Fig. 67), or a series of similar or different notes (Figs 68-70).

  The notes may be unpulsed, meaning that there is no extreme change in the amplitude over time (Fig. 71A), or pulsed, meaning that there is severe change(s) in amplitude over time (Fig. 71B). This phenomenon is called the amplitude modulation. Notes may contain one or several pulses of various intensities.

  The frequency (= pitch) of the note may be unmodulated, meaning that there is no variation in the pitch over time (Fig. 72A), or distinctly modulated, meaning that there are conspicuous changes in frequency over time (Fig. 72B). This phenomenon is called the frequency modulation. Frequency modulation may have different patterns (*e.g.* upwards, downwards, up-down, etc.).

• **The fundamental frequency**: the lowest (= first) frequency harmonic.

• **The dominant frequency**: the frequency harmonic within which the greatest amount of sound energy is concentrated; also called the main harmonic. In some cases, the dominant frequency is the fundamental frequency (see Fig. 68 for example).

• **The harmonics**: the separated, evenly spaced frequencies that are multiples of the fundamental frequency.

![Oscillogram and spectrogram](image.png)

**Fig. 66.** Oscillogram and spectrogram of the call of *Scinax boesemani* (Hylidae) showing acoustic components.
Fig. 67. Oscillogram and spectrogram of the call of *Leptodactylus lutzi* (Leptodactylidae) illustrating a call composed of a single note.

Fig. 68. Oscillogram and spectrogram of the call of *Adelophryne gutturosa* (Eleutherodactylidae) illustrating a call composed of a series of notes (here 13 notes).
Fig. 69. Oscillogram and spectrogram of the call of *Scinax boesemani* (Hylidae) illustrating a call composed of a series of identical notes (here 18 notes) produced in a very short period of time. This kind of call is named a trill. In this case the entire call is given in less than half a second; compare with figure 68 in which the call is given in about 2s.
Fig. 70. Oscillogram and spectrogram of the call of *Osteocephalus leprieurii* (Hylidae) illustrating a complex call composed of a series of very different notes.
Fig. 71. Oscillograms and spectrograms illustrating amplitude modulation. A. An unpulsed note [*Allobates spumaponens* (Aromobatidae)]; B. A pulsed note [*Allobates granti* (Aromobatidae)]. None of these species occur in KNP.

Fig. 72. Spectrograms illustrating frequency modulation. A. Dominant frequency of the call of *Centrolene gorzulae* (Centrolenidae), mostly unmodulated; B. Dominant frequency of the call of *Leptodactylus longirostris* (Leptodactylidae), distinctly modulated (upwards). Red arrows highlight the change of frequency between the beginning of the note (on the left) and the end of the note (on the right).

The following principal temporal variables and parameters are usually considered in the call analysis and allow comparisons between calls:

- **The call duration**: measured in seconds (s), from the beginning of the first to the end of the last note.

- **The note duration**: measured in seconds (s), from the beginning of the note to the end of the note.

- **The inter-call interval**: measured in seconds (s), from the beginning of one call to the beginning of the next.

- **The number of notes**: the number of notes within the call.
• **The inter-note interval**: measured in seconds (s), from the end of one note to the beginning of the next.

• **The note period**: measured in seconds (s), from the beginning of one note to the beginning of the next.

• **The call rate**: the rate at which entire calls are produced, expressed in calls/min.

• **The note repetition rate**: the rate at which notes are produced, expressed in notes/s. The note repetition rate is obtained by measuring the time between the beginning of the first note and the beginning of the last note, and dividing the number of notes included within this period by the time in seconds. Equivalent to the call rate when the call is composed of a single note.

• **The dominant frequency**: generally measured from a spectral slice taken through the portion of the note with the highest amplitude, expressed in Hertz (Hz).

**Acquisition of the data**

Open your sound using the sound analysis software. Calls and notes are usually not well visible (Fig. 73A) and you will need to zoom in the recording to see the calls and the notes closer (Fig. 73B-C). Play with the contrast if needed and use the software tools to calculate the data you need.

Figure 74 illustrates how to calculate the call duration using the selection borders in *Raven Pro*. The same method is applied for other temporal variables.

Figure 75 shows calculation of the dominant frequency from a spectral slice in *Raven Pro*. Placing the cursor at the top of the first peak will provide the frequency at the bottom of the display.

Remarks:

• Descriptive data are always obtained from multiple measurements of different calls from an individual (ideally from as many individuals as possible).

• As we saw above (in “Recording of advertisement calls”), temperature notably influences some attributes of the acoustic signals (*e.g.* frequency, note repetition and note repetition rate) and comparisons between calls recorded at different temperatures may lead to misinterpretations.
Fig. 73. Zoom in the sound recording to identify calls and notes (here the call of *Anomalousus beebei*, Aromobatidae). A. Calls are difficult to detect and details are not discernible; we zoom in the area highlighted yellow; B. After zooming, five calls are well visible and the number of notes is discernible; we zoom again in the area highlighted in yellow; C. After zooming, one call composed of three notes is isolated.
Fig. 74. Example of calculation of a temporal variable: estimation of the call duration of *Anomaloglossus beebei*, Aromobatidae. The call is pinpointed between the selection borders. The black arrow indicates the beginning of the call (= time at the beginning - in seconds, which is encircled in black in the left column); the blue arrow indicates the end of the call (= time at the end - in seconds, which is encircled in blue in the left column). The difference (delta) between these two times (which is encircled in red in the left column and shown by a red curly bracket on the oscillogram) is the call duration (0.291 s in this case).

Fig. 75. Spectral slice of the first note of the first call illustrated in figure 74 (*Anomaloglossus beebei*, Aromobatidae). The first harmonic is the fundamental harmonic. In this case, the dominant frequency is the second harmonic. The black arrow indicates the peak at which the dominant frequency is measured.
5. Identification guide, and how to use it

The main goal of this identification guide is to allow for a quick and easy identification of the species of amphibians currently known from Kaieteur National Park.

Most of the time, collection and handling of the animal will be necessary for examining discrete structures and hidden colour patterns. Always handle amphibians with wet hands, as their skin may be fragile. Additionally, you should never handle an amphibian if you have insect repellent on your hands, as it would kill it. No “poison frogs” (family Dendrobatidae) are currently recorded from the Park, but some species secrete large amounts of toxins (e.g. large *Leptodactylus*, *Rhaebo* and *Rhinella*) that can irritate your skin and mucous membranes, or even kill other amphibian species if they are in contact with the secretions. Always rinse your hands thoroughly after handling an amphibian.

Genera and species are treated alphabetically within each family. Each species is illustrated by at least a dorsolateral view in life and a ventral view (in life or in preservative). Whenever possible the colour variation is illustrated. Illustration of hand and foot, peculiar morphological characters that may help the identification, and an oscillogram and spectrogram of the call (if known and when relevant) are provided as well. Whenever possible, oscillograms and spectrograms were generated from recordings made in Kaieteur National Park. When adequate recordings were not available we prepared audiospectrograms from recordings made outside the Park. This was the case for the following species: *Allophryne ruthveni*, *Rhinella marina*, *Hypsiboas calcaratus*, *H. boans*, *H. geographicus*, *Osteocephalus leprieurii*, *O. taurinus*, *Phyllobates bicolor*, *Trachycephalus coriaceus* (species recorded in French Guiana, calls courtesy of C. Marty and P. Gaucher), *Atelopus hoogmoedi*, *Rhaebo guttatus*, *Trachycephalus resinifictrix*, *Leptodactylus mystaceus*, *L. rhodomystax*, *Pristimantis cf. marmoratus* (species recorded at Mabura Hill Forest Reserve, Guyana, calls courtesy of R. Ernst), *Dendropsophus marmoratus* (species recorded in Ecuador, Napo, Jatun Sacha, call courtesy of K. H. Jungfer), *Phyllobates vaillantii* (species recorded in Peru, Panguana, call courtesy of A. Schlüter), and *Leptodactylus lineatus* (species recorded in Peru, Tambopata, call courtesy of A. Schlüter).

You can use the field keys provided on p. 64 (caecilians) and p. 87 (anurans) to identify the genus, and then use the field keys provided under each generic account to identify your specimen up to the species. You also may wish to first consult the colour figures that illustrate the species and check the diagnostic characters given in the accounts. Both methods should allow for fast identification. If you experience problems in identifying a specimen you can contact one of the authors (see the beginning of the manual for contact information), as it is possible that you have found a species not previously reported from the Park or even an undescribed taxon.

In addition to the field key for species, each generic account provides basic information on the genus, some external morphological characters that may be useful for identification, and, when necessary, briefly mentions species of possible occurrence in the Park that were not collected during our surveys. Do
note that morphological characters provided for each genus are not always
discriminant because no morphological synapomorphies have currently been
detected in some genera (e.g. *Dendropsophus*, *Hypsiboas*).

The taxonomy of a few specimens collected in the Park remains too unclear and
so these possible new taxa were voluntarily excluded from this guide. The
elucidation of the taxonomy of these specimens will be dealt with later, once
more material becomes available.

Cryptic species are distinct taxa that are not, or hardly distinguishable on a
morphological basis (see for example *Hypsiboas cinerascens* and *Hypsiboas* sp.
on p. 168 and 176, respectively). Some widespread species might in fact be
complexes of cryptic taxa (Fouquet et al., 2007) and many of those species
probably remain to be described. This suggests that current estimates of
amphibian species richness are too low, but also that the taxonomic status
and/or the distribution of some species treated in this manual could be re-
evaluated in the future.

Each species account is provided as follows:

- **Scientific name of the species** (Genus and species) followed by authorship
  and date of publication.
  Year and page of the original description + references to original illustrations
  (when relevant) are also given.

- **Pictograms** illustrate the size of the animal and its nycthemeral activity
  pattern (Fig. 76). This may be useful for quick identification and comparison
  without reading the text.

Diurnal species are active during the day (whatever the meteorological
conditions). Nocturnal species are primarily active by night, or near dawn or
dusk (or both), but some may be found during the day when cloudy and/or
heavily raining.

![Fig. 76. Pictograms illustrating the nycthemeral activity patterns. A. Diurnal. B. Nocturnal.
C. Used for nocturnal species that may be found during cloudy days and/or heavy rains.](image)

- **English name**: the most commonly used English name(s). We usually
  propose an English name if none is currently available.

- **Local name**: the name of the species in Patamona dialect, when known.
• **Type locality**: the geographical location where the holotype (the original preserved specimen designated for naming and describing the species) or the lectotype (a specimen serving the function of a holotype when no holotype was designated in the original description) was collected.

• **Selected references**: a maximum of three important references that should be consulted by the reader.

• **Field identification**: this section provides the maximum theoretical size (SVL) in males and females (*i.e.* the maximum size reported in the literature, not the maximum size reported from KNP). If examination of specimens collected in Kaieteur National Park resulted in increases in the known maximum size for a species, this is indicated by an asterisk (*). Eight to nine characters that are easily observable in the field, even for people having little knowledge in amphibian taxonomy, are emphasized. The reader should refer to the previous chapters of this manual for more details about diagnostic characters. We tried to deal with the same characters for each species of a same genus in order to facilitate comparisons. Colour arrows refer to these characters in the identification section and pinpoint them in the corresponding figures.

• **Life history**: this section provides basic information on the biology of the species.

• **Call**: this section provides reference to the first description of the advertisement call (when relevant), and a brief description.

• **Tadpole**: this section provides reference to the first description of the tadpole (when relevant), a brief description as well as its ecomorphological guild (see McDiarmid & Altig, 1999 for details).

• **Abundance and distribution in KNP**: this section provides a subjective estimation of the abundance of the species in the Park, which is expressed as very common (occurs in considerable numbers and easily observed every day), common (commonly seen, easily observed every week), rare (not usually observed more than once every few month), or very rare (seen very occasionally, sometimes known from a single specimen). Note that a species may be rare in some parts of the Park, but locally abundant due to adequate habitat, environmental conditions, etc. (this is especially true for species like *Anomaloglossus beebei* and *Leptodactylus lineatus*). Some species may be locally abundant only during a very short period of time (*e.g.* explosive breeders) and otherwise be seen only very occasionally. Main sampling localities where the species was recorded in KNP are provided as well (refer to Fig. 3 to locate sampling localities on a map).

• **Geographic range**: the general distribution of the species.

• **Taxonomic comments**: when necessary, this section provides some important remarks on the taxonomy of the species.

• **Remark**: when necessary, this section mentions if some photos used to illustrate the species have been taken outside the Park.
Anura | Allophrynidae | Allophryne Gaige, 1926

Allophryne Gaige, 1926

“TUKEIT HILL FROGS”

Fig. 77. Allophryne ruthveni, the only described species in the genus. (Photo by P. J. R. Kok).

- Small size
- Head small, triangular
- Snout short
- Maxillary teeth absent
- Pupil horizontally elliptical (Fig. 42A)
- Skin on dorsum smooth with unevenly distributed spicules (Fig. 44A, E)
- Vocal sac single, subgular (Fig. 56A)
- Fingers basally webbed
- Finger I < II when fingers adpressed
- Toes half-webbed
- Finger discs truncate, wider than digits (Fig. 51C)