3.3.8. Collecting tissues for molecular study

Molecular analyses can help to elucidate taxonomic problems and as such are complementary to morphological taxonomy.

Tissue must be removed immediately after euthanasia, never after fixation because formalin breaks DNA (although researchers already succeeded in DNA extraction from formalin-preserved samples). A small incision is made in the upper part of the abdomen and a small piece of liver is cut (Fig. 31A-B). In case you need several samples from the same individual, the whole liver can be extracted and divided into small pieces. A piece of thigh muscle is a suitable alternative. The slice of tissue is placed into a small screw-top plastic vial filled with 95% ethanol and a piece of waterproof paper on which you will write the number of the voucher specimen from which the tissue has been removed (Fig. 31C). Be very careful to write the number associated with the specimen during this process! Make sure that the ethanol completely covers the tissue sample. Do not screw the lid on too tightly. Vials are kept on plastic stands during the process, and packed in leakproof plastic bags for transport (Fig. 32). Keep the samples away from direct sunlight and try to store them in a cold place.

In order to avoid contamination between specimens, we use sterile disposable surgical blades (one blade per specimen) and sterilize the forceps in bleach. Before reusing the forceps, they are carefully rinsed with fresh water and dried with toilet tissue.

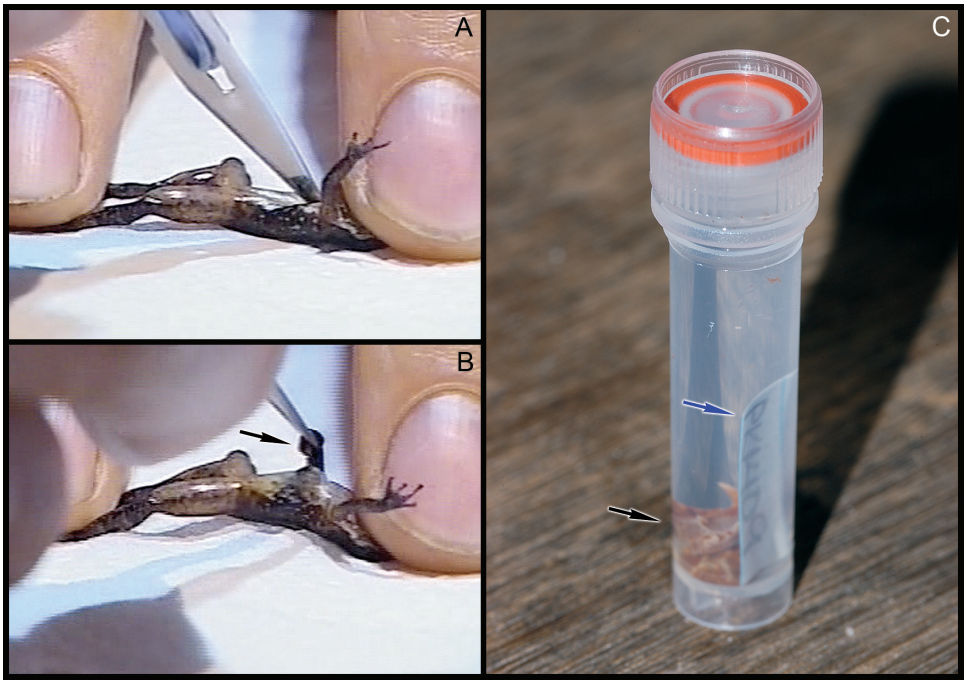

Fig. 31. Collecting tissues for molecular study. A. A small incision is made in the upper part of the abdomen; B. The liver or a piece of it is removed using small bleach-cleaned forceps (black arrow indicates liver); C. Tissue sample is placed in a vial together with a tag bearing the number of the voucher specimen (black arrow indicates liver, blue arrow indicates tag). (Photos A-B extracted from the documentary “Kaieteur” © Kanari Films, C by P. J. R. Kok).

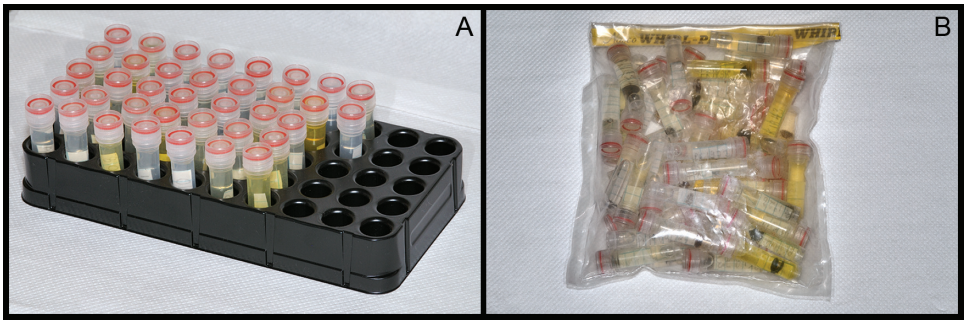

Fig. 32. Tissue samples. A. Vials with tissue for molecular analyses are kept in plastic stands during the process; B. They are packed in leakproof plastic bags for transport. (Photos by P. J. R. Kok).

➔ **Basic equipment needed for collecting tissues from voucher specimens:**

- Disposable surgical blades (expect 300 for a 3-weeks field trip).
- Scalpel(s).
- Small forceps.
- Syringes and needles to inject ethanol in vials.
- Bleach (*ca.* 250 ml).
- Small containers for bleach and rinsing water.
- Toilet paper to dry your forceps.
- Lidded vials for tissue samples (expect 300 for a 3-weeks field trip).
- 95% ethanol (expect *ca.* 1 litre for a 3-weeks field trip)
- Waterproof paper and pencil for labelling the tissue samples.
- Plastic stand for vials.
- Leakproof plastic bags to place your vials for transport.
- Latex gloves (optional).

3.4. Methods of collection

We mainly focus here on techniques used to collect voucher specimens within the framework of taxonomic studies.

The following descriptions of collecting techniques are mostly based on Heyer *et al.* (1994), a publication that should not be missed by investigators having an interest in collecting amphibians and measuring and monitoring amphibian diversity. Also see Simmons (2002) and Rödel & Ernst (2004).

3.4.1. Opportunistic collecting

This is probably the most traditional collecting technique in herpetological inventories. The principle of this productive technique is to slowly walk through adequate habitats, by day and by night, systematically searching for amphibians (visually and acoustically) in all possible microhabitats, turning over and breaking up logs, searching through the vegetation, in the leaf litter, turning over rocks, checking crevices and tree holes, and searching along the watercourses, checking both upper and undersides of leaves. Calling males are detected and collected. This technique does not involve any prescribed time period.

3.4.2. Visual encounter surveys

The visual encounter survey (VES) is a standard method for terrestrial herpetofauna inventories and monitoring. VES is conducted by walking through an area or habitat for a prescribed time period while systematically searching for animals that are visible to the researcher. Observers search surfaces, vegetation, turn over objects such as logs and rocks, and look in crevices in rocks and bark, replacing all surface objects after examining the ground.

The searching period is expressed as the number of person-hours searching in the sampled area. VES can be conducted day or night using flashlights. It is often

better to sample 10x100m transects than 1x1000m transect as it provides comparable data sets for analysis.

VES can be used to document the species richness of an area via a species checklist and to estimate the relative abundance of species within an assemblage. Often, VES is used in conjunction with other techniques such as transect sampling, mark-recapture, drift fences and pitfall traps, etc. VES is often best used to sample species that are unlikely to be caught using other techniques such as traps. The design for a VES will depend on the objectives of the research (*e.g.* is it a one-time inventory or long-term monitoring programme?), the information required *e.g.* species abundance, species composition or both, type and size of habitat, time frame *e.g.* diel or seasonal, species composition, and number of persons available to execute the VES. According to Heyer *et al*. (1994), there are three basic methodologies used for VES: randomized walks, quadrat and transect.

If only one methodology is used for sampling herpetofauna, VES is often the best to use due to its effectiveness across all habitat conditions and ease of implementation.

When sampling using VES, there are several assumptions to consider: every individual species has the same chance of being observed, each species will have the same probability of being detected regardless of seasonality, size, behaviour, activity, etc, an individual is recorded only once per survey, and results collected from the same area are not observer-related.

3.4.3. Quadrat sampling

Quadrat sampling (QS) entails exhaustively checking a series of small-defined (*e.g.* 10 m x 10 m) squares (quadrats), which are placed randomly in selected sites within the study area. The study area can be visualized as a series of numbered grids; a random number is then selected, indicating which square to sample. A preselected number of quadrats are chosen to be sampled *e.g.* Heyer *et al.* (1994) recommend 25 to 30 units be sampled in order to provide sufficient data for statistical analysis.

Quadrats can be sampled using either point sampling or broad sampling. Point sampling uses small squares to study single species with small, densely distributed individuals. Broad sampling uses large quadrats to sample species in which individuals are widely dispersed, large-bodied or both and to sample multispecies populations. In either case, all quadrats are of equal size in their respective study areas.

QS is often used to estimate the total number of species (whether species richness, abundance or densities) within the study area. Although QS is labour intensive, it is effective for sampling a variety of habitats and, for high-density species in forest litter, open-area habitats and aquatic environments. QS should only be used when animals do not leave the quadrat due to sampling disturbance before being counted, quadrats can be randomly but systematically placed, and quadrats yield independent data.

3.4.4. Transect sampling

Transects are predetermined length of straight lines that are established either permanently, or temporarily, depending on the objectives of the study, using a measuring tape. Data is collected by systematically walking the line and collecting/counting all herpetofauna seen on either side of the line. Randomized transects can be used to estimate species numbers, relative abundance and densities across habitat gradients. Transects are often effective for sampling along elevational gradients or lowland to upland habitat gradients.

The underlying theory behind the use of transects is that not all individuals will be detected as the probability of detecting species decreases as its distance from the line increases.

3.4.5. Patch sampling

Patch sampling (PS) entails randomly sampling microhabitats or patches where concentrations of herpetofaunal densities are the highest. As species composition and density changes dramatically from one type of microhabitat to another, PS is a very useful tool in sampling species confined to particular microhabitats within a larger study area.

PS is a sub-technique of quadrat sampling allowing to determine the number, relative abundance, and densities of species confined to particular microhabitats of an area of interest; QS indiscriminately samples all microhabitats while PS focuses on specific species that occupy specific microhabitats, ignoring all other species that occur between patches. As such, the patches that are sampled can be considered quadrats themselves. PS involves identifying all discrete patches in a particular area and systematically searching for amphibians in these specific microhabitats (*e.g.* leaf litter, bromeliads, etc.). As patches are discovered within the wider study area, a number is assigned to each patch in sequential order. The type and amount of patches will influence how they are sampled and how many are sampled respectively. Every individual of every species occurring in each patch must be detected and voucher specimens preserved.

The basic assumptions in PS are that each patch has a defined border, can be dimensionally defined *e.g.* 3m x 5m, can be observed and located within the wider study area, and individual species can be counted within the patches.

3.4.6. Drift fences and pitfall traps

Drift fences and pitfall traps are designed to collect animals that would not be found on opportunistic and other classical searches. This technique can be highly effective at surveying herpetofaunal communities and is particularly useful to collect fossorial and rare species. It can be used to encircle specialized habitats (breeding ponds for example).

Drift fences are barriers, usually 5-100 meters long, that redirect the travelling animal into traps placed at the ends, besides, or under the barriers. Drift fences can also be placed in arrays designed in Y or X. Traps can be pitfalls, funnel traps or a combination of the two, and made from either various sizes of plastic buckets or cans. Drift fences can be constructed from various materials, plastic

sheets being our preferred material because it is light and easy to transport.

We usually set 30 meters-long or 60 meters-long lines. Traps (plastic buckets of about 28 cm diameter at the top, 30 cm deep) are buried into the ground at *ca.* 3 m intervals under a drift fence of plastic sheet (approximately 50 cm in height) positioned to run across the open midline top of the buckets (Fig. 33A-B). Small holes are drilled into the bottom of the buckets for drainage. Traps are usually checked twice a day (in early morning and late afternoon).

Pitfall traps are more labour intensive and require significant personnel time and funding relative to vertebrate area searches and are often associated with high mortality rates for non-targeted taxa. However, they are effective in detecting a broad array of species, specifically the species richness of an area, the presence of rare species (if long-term monitoring is undertaken), relative abundance and habitat use of selected species. Drift fences with pitfall traps tend to capture terrestrial species more easily than other species (*e.g.* frogs that are strong jumpers or climbers).

A combination of three to four drift fences with pitfalls are better for sampling than a single drift fence with pitfalls. The length of the drift fences influences the number of animals captured, and this varies by habitat type. Shorter drift fences capture less amphibians than longer fences and larger traps tend to increase the number of specimens collected. Pitfall traps assembled in a matrix without fences can also be used to study the population ecology and habitat use of selected species. Population density can be estimated with this technique if used in conjunction with mark-recapture techniques.

Heyer *et al.* (1994) recommended that operating drift fences opportunistically, after rainfall to maximize capture of species. Other studies have indicated an operation of 30 days to 2 years, this depending on the available funds, personnel and time period for sampling.

➔ Basic equipment needed for setting a simple drift fence and pitfall traps

- Plastic sheet (*ca.* 50-100 cm in height, at least 100 m).
- Plastic buckets (*ca.* 35 buckets for a 100 meters-long drift fence). 20 litres buckets are efficient, but size of traps will mostly depend on what is locally available.
- Staple gun and staples.
- Stove.
- Machete.

Fig. 33. Some collecting techniques. A. Drift fence made of plastic sheet; B. Pitfall trap, here a plastic bucket of about 28 cm diameter at the top, 30 cm deep; C. Collecting tadpoles in a small puddle, using a “turkey baster”. (Photos A-B by P. J. R. Kok, C by I. Roopsind).

3.4.7. Canopy access

Accessing the canopy is very useful to collect arboreal species and/or record their advertisement call. We successfully used the single-rope technique (Fig. 34) both to climb in trees and to access bottom of caves in the forest. The technique was also used to reach the base of Kaieteur Falls in 2004.

Single-rope technique involves ascending a single length of rope through the use of a mechanical ascender. Climbing in the canopy using that technique is basically a two-step process: (1) the tree must be equipped with a climbing rope. To do so, a light line must be shot over a solid limb (we use very strong fish line, shot with a crossbow). The light line is used to haul a heavier line (usually 4 mm strong rope) that is then used to haul a climbing rope up and over the limb (usually 10.5 mm static rope). One end of the rope is tied off to a nearby tree trunk; (2) the investigator ascends into the canopy on the other end of the rope using specific equipment.

Please note that climbing techniques are life-threatening practices that require a lot of training. Never try to use single-rope technique or any other caving/climbing technique without having received proper professional instruction beforehand!

An alternative to the single-rope technique is the use of tree climbing spurs, but

this technique causes damage to the tree and should be used with caution.

For those interested in techniques to reach the canopy, do not miss Mitchell *et al*. (2002) and Merchant (2007).

Fig. 34. The senior author using single-rope technique to access bromeliads along the Kaieteur gorge. (Photo by H. Sambhu/P. J. R. Kok).

➔ **Basic equipment needed for single rope technique**

- 10.5 mm static rope (ideally 2 x 100 m).
- Harness (basic caving harnesses work great).
- Descender (we use *Petzl® Stop D09*).
- Ascender (we use *Petzl® Ascender B07*).
- Chest ascender (we use *Petzl® Croll B16*).
- Foot loop (we use *Petzl® Footape*).
- Shoulder strap for positioning the chest ascender (we use *Petzl Torse*).
- Asymmetrical Y-shaped lanyard used during rope manoeuvres (we use *Petzl® Spelegyca*).
- Gloves.
- Helmet.
- Maillons semi-circular.
- Maillons 7 mm inox.
- Webbings (various sizes).
- Carabiners (various sizes).

3.4.8. Sampling of amphibian larvae

Depending on the habitat, different techniques are used for sampling amphibian larvae such as seining, dipnetting, trapping and enclosure sampling. These techniques are quick, relatively thorough, with minimum personnel, material and time.

Seining is effective in shallow bodies of water with little vegetation; with an ideal length of 3-4 m long seine but length varies with the size of water body to be sampled. The seine is dragged from shore to shore, touching the bottom of the substrate and moved slowly along the aquatic habitat. Quantifying seine sampling can be done using square meter of bottom sampled (distance travelled x length of seine).

Dipnetting is the simplest method for sampling bodies of water clogged with vegetation, limited access stream habitat or specialized habitats such as tree holes. A standard small aquarium net (10 cm wide) is used to sweep under vegetation and in specialized structures. Sampling procedure can either cover approximately 20 to 50 sweeps in an hour or survey each aquatic habitat for an equal period. The rate of sweeps will either increase or decrease depending on the size of the aquatic environment. To collect tadpoles in bromeliads or small aquatic depressions, an aquatic pipette (turkey baster) is very effective (Fig. 33C).

Enclosure sampling (ES) includes box sampling, quadrat sampling and stovepipe sampling, and involves trapping animals inside an enclosure. ES is effective in shallow water habitats with relatively uniform substrates. ES can be objects such as PVC sewer pipes; 0.5 m^2 x 0.5 m deep metal box sampler or bottom net. The enclosure is dropped onto the substrate and pressing the sharp edge downwards, trapping the animal. The number of animals trapped within the closure is estimated.

Trapping is conducted using a funnel-trap principle and may be used to sample deep-water habitats or those with complex bottoms of stones, wood or rocks. Animals are encouraged to enter the funnel but cannot escape due to the small diameter and central location of the exit. Trapping is used specifically for estimating species richness and relative abundance.

Once collected, a number of tadpoles should be immediately euthanized and placed in small vials containing 10% formalin. Some tadpoles should be kept alive and reared in the field to obtain different developmental stages. Tadpoles can be reared in small containers or plastic bags. We usually use fish food to feed them.

Some tadpoles should be preserved in 95% ethanol for further molecular analyses.

➔ **Basic equipment needed for sampling amphibian larvae**

- Dipnets (various sizes).
- Aquatic pipette ("turkey baster").
- Containers or plastic bags for rearing tadpoles in the field.
- Fish food.

3.4.9. Sampling of caecilians

Due to their secretive habitat (most adult caecilians are terrestrial burrowers, some are aquatic), caecilians are difficult to collect and few sampling techniques have been established. In addition to drift fences and pitfall traps (with very variable success) and methods to sample amphibian larvae (see above), digging in suitable habitat (soft soil, under rotting plant materials, in fine gravel along streams for example) is required for terrestrial species. Aquatic species may be collected with a net, or by passive tracking by means of collapsible, nylon-meshed funnel traps using fresh fish bait (see Kupfer *et al.*, 2006a for details).

3.5. Collection management

As mentioned above, museum collections are extremely important, both to understand the past and to perform future research. Specimens must be preserved in a way that retains their original composition and be made available to the scientific community. As we saw before, adequate fixation is mandatory for long-term maintenance of your specimens. After all the efforts you have done to correctly fix the material you have the right to request that specimens' integrity will be preserved as long as possible. This means that loss of fluid preservative and protection from fluctuation in temperatures and humidity (two important threats to fluid preserved specimens) will be adequately supervised.

Adult specimens are best kept in 70% ethanol, while tadpoles are preserved in 10% formalin. Tissues should be preserved in a cold place, in 95% ethanol, or ideally cryopreserved (by freezing).

Describing herpetological collections management is beyond the scope of this manual and we encourage the reader to refer to Simmons (2002) for detailed guidelines and curatorial practices.

3.6. Deposition of specimens in Guyana

The Centre for the Study of Biological Diversity (CSBD) is the key institution in Guyana for the management of the national biodiversity collections and research information (Bernard *et al*., 2002). All floral and faunal specimens left in or returned to Guyana as a result of collecting expeditions are housed at the CSBD. These collections serve as a resource in the study of Guyanese flora and fauna and enable the identification of priority areas for conservation planning and resource management.

The CSBD, founded in 1992 and housed in the Department of Biology, University of Guyana (UG) on the Turkeyen Campus, has played an important role in the development of research as it relates to biodiversity conservation in Guyana.

The Museum houses approximately 668 specimens (58 species) of amphibians with approximately 119 species of amphibians known from Guyana (Señaris & MacCulloch, 2005).

The Collections are currently under the care of two Scientific Officers who are trying to reorganize, clean and database the specimens; some of which were damaged by a flooding in 2005. According to Bernard *et al*. (2002), and through the efforts of the staff of the Biology Department, UG, environmental NGOs in Guyana and foreign researchers, the collection and identification of plants and animals has progressed to the point that an estimated 70% of the plants, 90% of the mammals and birds, and 60-70% of the remaining vertebrate groups are known in Guyana.

Unfortunately specimens housed at the CSBD are currently of difficult (if not impossible) access to foreign researchers due to the lack of financial and human resources for sending material or for hosting investigators. Lack of resources could also affect the preservation of the specimens that demand storage in a cool place (which means functional air-conditioning) and regular checking of the amount and quality of the preservative.

If we agree that the deposition of voucher specimens at the CSBD is imperative to allow local students and researchers to examine museum material (it is also required by EPA), we also strongly suggest that part of the collections remains in larger institutions that have sufficient financial resources to ensure adequate conservation and accessibility to foreign researchers. This is especially true for type specimens.

4. Systematics

As we saw above (see Chapter 2), it may be very difficult to confidently assign an amphibian species to a family, notably because of convergence. Significant morphological diagnostic characters of families are often features of the internal anatomy and some families are even primarily defined by genetics. Readers should refer to Chapter 2 for basic descriptions of the amphibian families found in KNP.

Fortunately, several features of the external morphology are very informative to identify an amphibian to the generic or specific level, hence again the importance of well-preserved voucher specimens in which those morphological diagnostic characters are retained, and thus easily observed and studied. In the field, most of these characters can easily be observed without extensive handling of the animal, but some will require the usage of a magnifying glass. In the laboratory you will need a stereomicroscope to examine small characters.

Experienced taxonomists are usually able to easily assign a species to a genus, or identify the taxon without close examination; this could be much more difficult for the beginner. Below we list and illustrate the most important external

morphological characters that will help you to identify the amphibian genera and species occurring in Kaieteur National Park and in the Guiana Shield.

4.1. Caecilians (Order Gymnophiona)

Readers should refer to Chapter 2 for more information about the group.

4.1.1. Caecilians identification: key features

Identification of caecilians is mostly based on the following external morphological key features (many subtle characters are not discussed here):

Relative position and visibility of the eye

The eyes of caecilians may be plainly visible and functional or invisible and covered by a thin layer of skull bone or skin (Fig. 35). The distinctiveness and location of the eye are of taxonomic importance as well as its relative position to nostril, tentacle and mouth.

Location of the protrusible sensory tentacle in relation to the eye and external nostril

The tentacle is a protrusible, usually very small, sensory organ that is present in all caecilian species (Fig. 35). Its relative position to the nostril, eye, and mouth helps in species identification.

Presence or absence of tail and shape of terminal shield

The tail is absent in most caecilians, but is distinct in certain genera and species. The distinctiveness of the tail is very variable and it may be difficult to state if a tail is present or not. The tail may be considered as present if complete, discernible folds occur posterior to vent; but note that in some species these folds, although discernible, are incomplete. When the tail is not distinct, the terminal portion of the body is sometimes called the terminal shield, which may be conical, compressed or depressed.

Shape of cloacal opening

The cloacal opening (vent) may be longitudinal, circular, transverse, or V-shaped.

Number of primary, secondary and total folds

The number of folds (= annuli) is an important diagnostic character. The number of primary annuli reflects the number of vertebrae, but is never identical (usually there are slightly more vertebrae than primary folds). Secondary (= supernumerary) annuli develop on the primary annuli; they may be absent or very few in some species, while in others their number exceeds the number of primary annuli. Annuli may be complete or variously incomplete. Figure 35 shows how to distinguish primary annuli from secondary annuli.

Presence or absence of splenial teeth

Splenial teeth are located on the lower jaw, on the splenial bone (usually fused to the dentary bone) and their number is often lower than the number of dentary

teeth (= outer dental teeth located on the dentary bone) (see Fig. 36 for location of splenial teeth). Splenial teeth are absent in a number of genera.

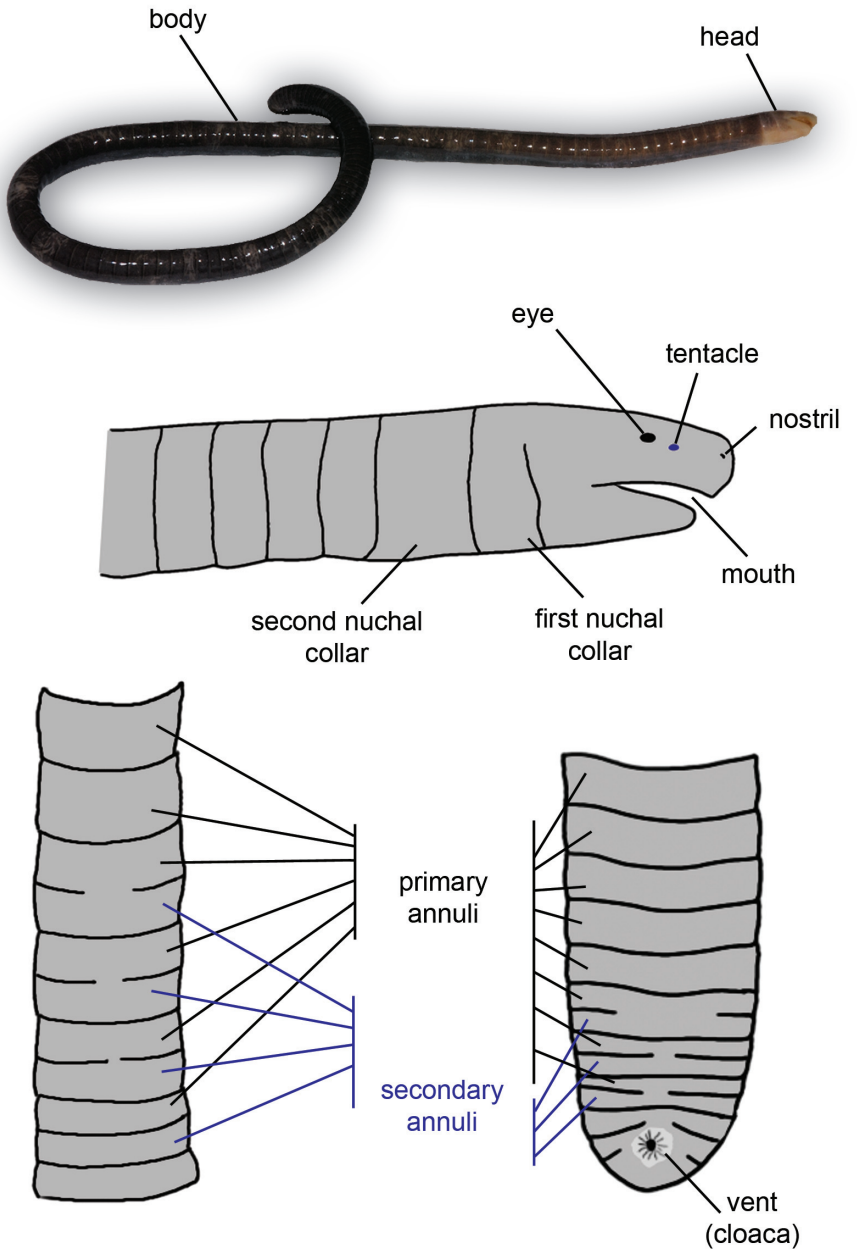

Fig. 35. Caecilian morphology and key morphological characters used in the identification of species. Modified from Taylor, 1968. (Photo by P. J. R. Kok).

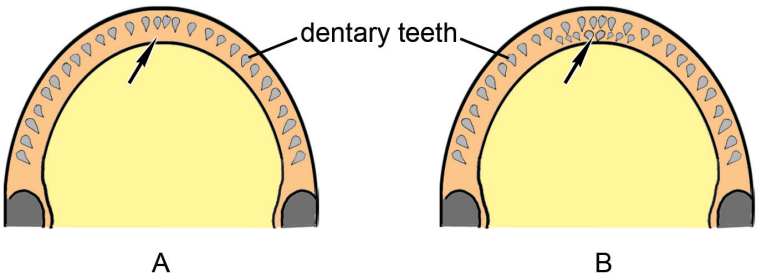

Fig. 36. Diagrammatic view of lower jaw and floor of mouth in caecilians. A. Splenial teeth absent; B. Splenial teeth present. Modified from Savage, 2002.

4.1.2. Field key to the caecilian genera of Kaieteur National Park

1. True tail present (complete folds discernible posterior to vent), yellow lateral band on body . . . . . ***Rhinatrema*** (p. 246)

1'. True tail absent (complete folds absent posterior to vent), no yellow lateral band on body . . . . . ***Microcaecilia*** (p. 244)

4.2. Frogs and toads (Order Anura)

Reader should refer to Chapter 2 for more information about the group.

4.2.1. Frogs and toads identification: key features

Identification of anurans is based on a very large number of external morphological characteristics.

Each genus generally has its own important diagnostic characters and it is impossible to list and detail all these characters of each anuran genus here. The following features are thus general and the reader should refer to specialized references to obtain more detailed information (some references are given in Chapter 5).

Figure 37 shows main general features (see “Morphometrics” below for additional terms and the manner in which various measurements are taken).

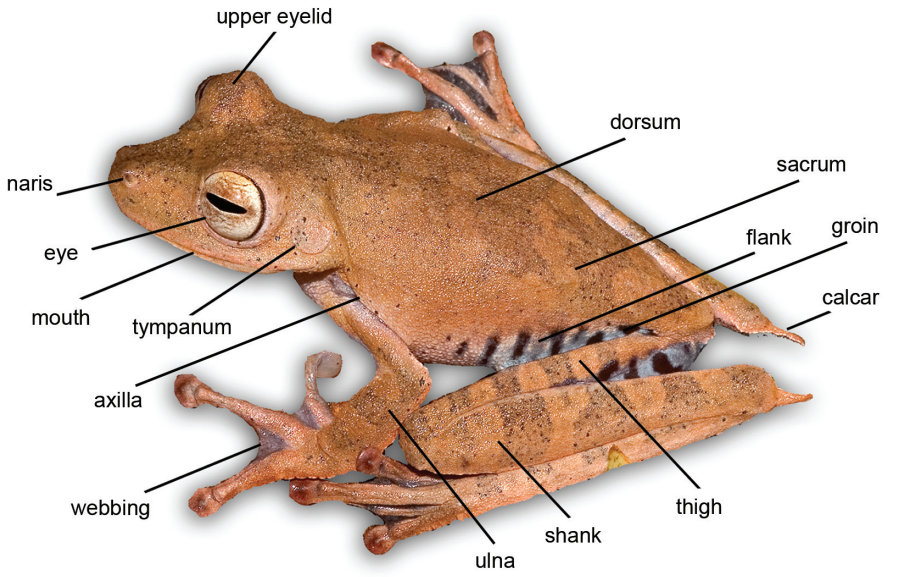

Fig. 37. An adult frog (*Hypsiboas calcaratus*, Hylidae) showing general morphology and features. (Photo by P. J. R. Kok).

The main and easiest observed key features are:

Size

Adult size is a useful distinguishing character in frogs and toads (Fig. 38). Size of anurans is measured from the tip of snout to the posterior margin of vent (see Fig. 55); it is usually abbreviated SVL (snout-vent length).

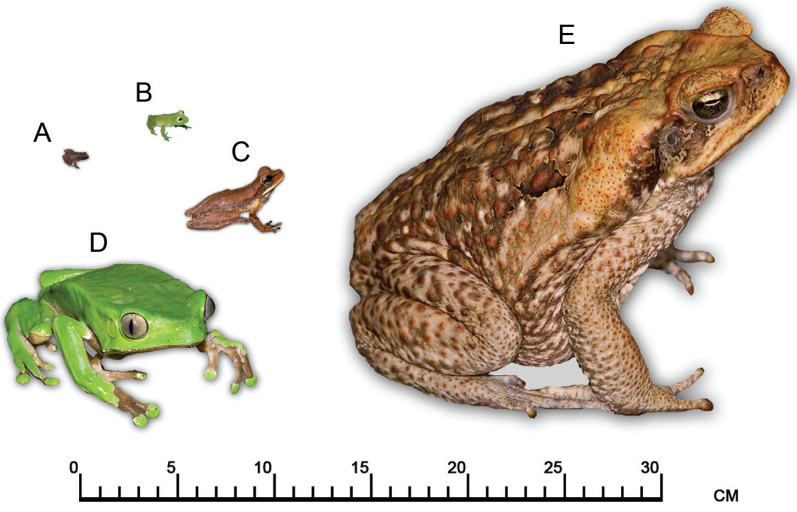

Fig. 38. Relative sizes of anurans in Kaieteur National Park. A. Very small/tiny (< 20 mm), *e.g. Adelophryne gutturosa*, Eleutherodactylidae; B. Small (20-30 mm), *e.g. Cochranella helenae*, Centrolenidae. C; Medium (30-60 mm), *e.g. Tepuihyla talbergae*, Hylidae; D. Large (60-200 mm), *e.g. Phyllomedusa bicolor*, Hylidae; E. Very large/giant (> 200 mm), *e.g. Rhinella marina*, Bufonidae. Photos by P. J. R. Kok.

Colour and pattern

Although colours and patterns have a large intraspecific variation and may change depending on light intensity, they are very important distinguishing features in anurans. Colours on flanks and anterior and posterior surfaces of thighs are highly diagnostic in some genera (*Scinax*, *Leptodactylus* for example).

In most anurans, the colouration depends on the arrangement of the following chromatophores (pigment-containing and light-reflecting cells found in the skin): xanthophores, erythrophores, iridophores, melanophores, and cyanophores.

Some species are uniform and cryptic, while others display vivid colours and complex patterns. Figure 39 shows principal colour patterns in frogs and toads, which are:

- **Spots**: small to medium, regular, roundish light or dark markings contrasting with the background colouration (Fig. 39A).
- **Blotches**: medium to large, irregular light or dark markings contrasting with the background colouration (Fig. 39B).
- **Ocelli**: medium to large light spots outlined by a darker border (Fig. 39C).
- **Flecks/speckles**: small or minute, more or less regular light or dark markings contrasting with the background colouration (Fig. 39D).
- **Anastomosis/reticulum**: dark or light network of lines contrasting with the background colouration (Fig. 39E).
- **Lines**: short to medium lineate dark or light markings (Fig. 39F).
- **Bands/stripes**: lines of various widths that may be transverse (bands) or longitudinal (stripes) (Fig. 39G).
- **Chevrons**: a dark or light V-shaped pattern contrasting with the background colouration (Fig. 39H).

Many species exhibit a combination of different patterns.

Do note that preserved specimens usually lose their bright colours, which commonly fade to white. Colour may also be drastically modified by the preservative [*e.g.* green may become lavender (in some glass frogs for example) or deep purple (in *Phyllomedusa* for example)]; patterns are usually retained but are lost in some species. Colour in preservative may thus be an additional useful distinguishing feature.

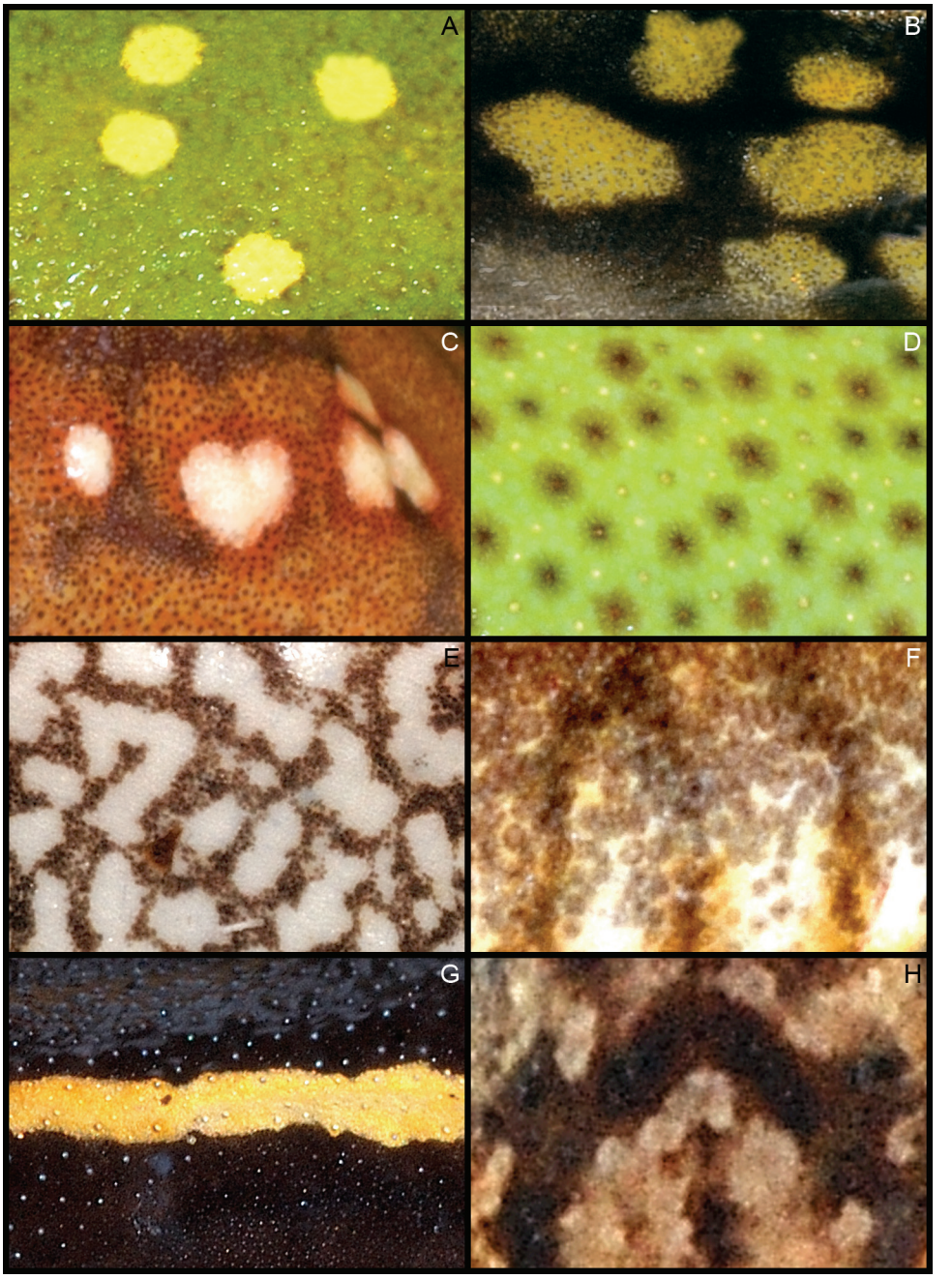

Fig. 39. Principal colour patterns in anurans. A. Spots; B. Blotches; C. Ocelli; D. Speckles; E. Anastomosis; F. Lines; G. Stripe; H. Chevron. (Photos by P. J. R. Kok).

Shape of head

Head shape is very variable in anurans and the dorsal outline of the snout and the snout profile are informative characters (Fig. 40). Note that there are subtle variations in dorsal outlines of snout, which are not illustrated here. We suggest the reader to refer to Heyer *et al.* (1990) for more information and original drawings.

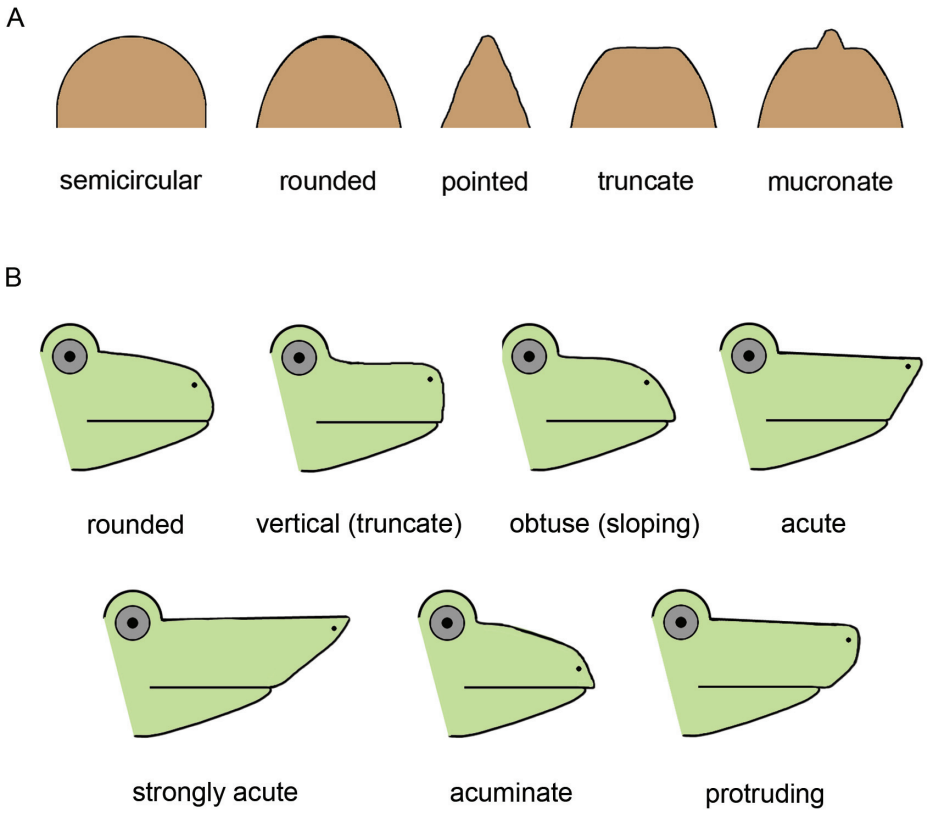

Fig. 40. Diagrammatic views of principal head shapes in anurans. A. Dorsal outline of snout; B. Snout profile. Modified from Heyer *et al.* (1990).

Absence or presence of cranial crests

Cranial crests are bony ridges on the skull that are found in many toads and in some frogs. The following cranial crests may occur: labial crest, suborbital crest, preorbital crest, canthal crest, supraorbital crest, postorbital crest, supratympanic crest, pretympanic crest, and parietal crest (Fig. 41). In some species these crests may be greatly expanded.

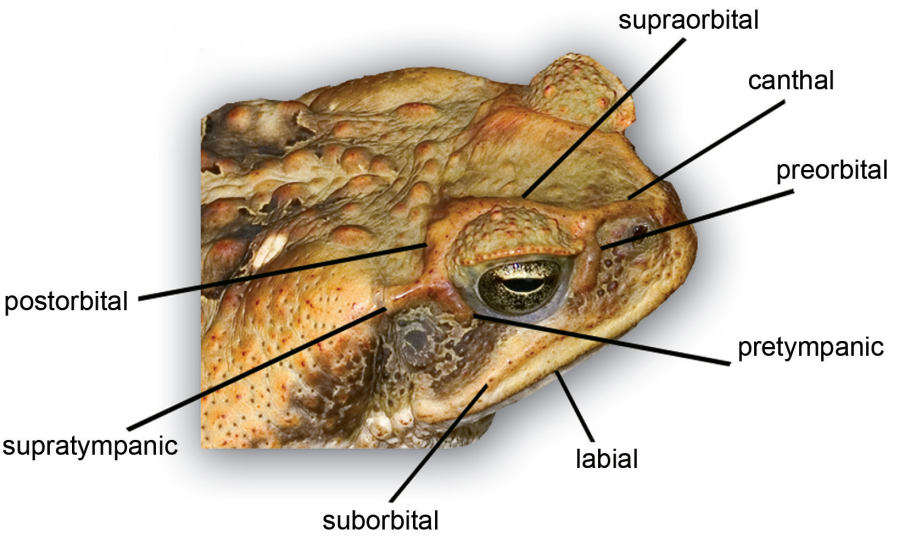

Fig. 41. Cranial crests in the toad *Rhinella marina* (Bufonidae). (Photo by P. J. R. Kok).

Shape of pupil and condition of palpebral membrane

In bright light, pupils of anurans may be horizontally elliptical (sometimes more or less heart-shaped), vertically elliptical (sometimes more or less triangular) or circular (Fig. 42). Note that this character is sometimes difficult to appreciate in preserved specimens.

The palpebral membrane (or nictitating membrane, the transparent lower eyelid) may be unpigmented or have a pigmented reticulation (Fig. 42D).