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Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana

Philippe J. R. Kok
Michelle Kalamandeen

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Editors

Yves Samyn - Zoology (non African)

Belgian Focal Point to the Global Taxonomy Initiative
Royal Belgian Institute of Natural Sciences
Rue Vautier 29, B-1000 Brussels, Belgium
yves.samyn@naturalsciences.be

Didier VandenSpiegel - Zoology (African)

Department of African Zoology
Royal Museum for Central Africa
Chaussée de Louvain 13, B-3080 Tervuren, Belgium
dvdspiegel@africamuseum.be

Jérôme Degreef - Botany

Belgian Focal Point for the Global Strategy for Plant Conservation
National Botanic Garden of Belgium
Domaine de Bouchout, B-1860 Meise, Belgium
jerome.degreef@br.fgov.be

Instructions to authors

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Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana

by

Philippe J. R. Kok

Department of Vertebrates
Royal Belgian Institute of Natural Sciences
Rue Vautier 29, B-1000 Brussels, Belgium
Email: Philippe.Kok@naturalsciences.be

Michelle Kalamandeen

Centre for the Study of Biological Diversity, Department of Biology
University of Guyana
Turkeyen Campus, Georgetown, Demerara, Guyana
Email : michellek@bbgy.com

Cover illustrations: background photo, Kaieteur Falls; clockwise from top left, *Hypsiboas sibleszi* (Rivero), *Anomaloglossus beebei* (Noble), *Hypsiboas liliae* Kok, *Anomaloglossus kaiei* (Kok *et al.*). This page, *Stefania evansi* (Boulenger), a female carrying 30 juveniles. (Photos by P. J. R. Kok).

”To be a Naturalist is better than to be a King”
William Beebe, 1893, cited by Gould (2004)

Preface by His Excellency Patrick Gomes, Ambassador of Guyana in Brussels

The people of Guyana, our ancestors as well as the present generation, have always cherished the spectacular beauty of the Kaieteur Falls, known to most, however, mainly by the remarkable photography of that world-famous sight of a crystal clear sheet of 226 metres of water that sprays a mist of several million litres.

Accompanied by a thunderous roar, that is said to be enchanting and mysterious to the would-be visitor, Kaieteur Falls is truly the jewel and wondrous gift that our country shares with the world through its Kaieteur National Park, an area of more than 60,000 hectares, richly endowed by a biological diversity, little documented by scientists.

Now, this remarkable achievement of two young scientists, one Guyanese and the other Belgian, provides a seminal scientific account to serve as a manual with both theoretical and practical guidelines for other scientists, students and the reading public. All readers interested in learning more of the amphibians that make their habitat in the locale of the Kaieteur National Park within the wider region of the Guiana Shield, will benefit from the discussion and detailed descriptions provided by this Manual.

Beyond the readership and practitioners, whose knowledge and skills will be enriched by the study and use of this Manual, the publication will serve also as a significant step towards the designation by the United Nations Education and Scientific Organisation (UNESCO) of the Kaieteur National Park as a World Heritage Site.

Guyana is truly proud of the work of Philippe Kok and Michelle Kalamandeen.

Brussels, November 2008

Foreword by the authors

Original idea of writing this manual occurred in 2004 while working with amphibians in Kaieteur National Park in the context of a “training through research” program generously funded by the Directorate-General for Development Cooperation (DGDC) through the Belgian Focal Point to the Global Taxonomy Initiative.

We strongly believe that the science of taxonomy should be communicated to researchers, ecologists, and environmentalists - both beginners and experts - as it often shapes the survival of species in key ecosystems. Species are key in biodiversity conservation and estimation of biodiversity. Therefore it is important to properly identify the species in a given area. This is where taxonomy comes in.

There are numerous texts on amphibian taxonomy, but relatively few are dedicated to teaching the methods and techniques used in *performing* taxonomy. Concurrently, field guides dealing with amphibians of the Guiana Shield are surprisingly scarce. This manual will hopefully give extensive insight into the world of taxonomy of amphibians, using our knowledge from Kaieteur National Park.

We wrote the manual as a “frogs for dummies”, bearing in mind the kind of information that would have been most useful to us at the beginning of our own herpetological activities. Keeping it under the maximum number of pages allowed by the editors was quite challenging and sometimes frustrating. We expect this volume will stimulate the interest of Guyanese teachers, students and researchers that would like to specialize in amphibians, specifically given the increasing rate of disappearance of these vital bellwethers of the environment.

The manual is written to captivate undergraduate and graduate students with an interest in amphibian taxonomy, but can also be used to stimulate the interest of tourists and nature lovers. Professional herpetologists will enjoy the informative sections, which are easy to access and in a convenient format.

Studying and working with amphibians is not always glamorous, but it can be fulfilling and interesting working with these wonderful and complex animals. We trust we succeeded in synthesizing the most important information in this handy book.

So many questions are left unanswered and many things remain to be done!

Brussels, Belgium

Georgetown, Guyana

October 2008

Abstract

Kaieteur National Park is a protected area covering *ca.* 63,000 ha located at the eastern edge of the Pakaraima Mountains, in a largely unexplored region of west-central Guyana. Next to providing description of the area, its vegetation and climate, an overview of the equipment and appropriate techniques needed to study amphibian taxonomy, this manual also provides a brief summary of our current knowledge of the amphibian systematics in the region, key features useful to identify amphibians, and the very first field guide dealing with the amphibian fauna of Guyana, notably with the amphibians of Kaieteur National Park. A total of 48 species (46 anurans and 2 caecilians) are treated and illustrated in colour. Field keys, field identifications, brief information on natural history, calls, tadpoles and distribution within and outside the Park are also included. This work also reports the microhylid *Synapturanus salseri* Pyburn, 1975 for the first time from Guyana.

Keywords – advertisement calls; Allophrynidae, amphibian taxonomy; Aromobatidae; Bufonidae; Caeciliidae; Centrolenidae; collecting methods; descriptions; Eleutherodactylidae; field keys; Guiana Shield; Guyana; Hemiphractidae; Hylidae; Kaieteur National Park; Leptodactylidae; local communities; Microhylidae; Pipidae; preservation techniques; Rhinatrematidae; South America; Strabomantidae; tadpoles.

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1. Introduction to Kaieteur National Park, jewel of Guyana

Kaieteur National Park is located in west-central Guyana, South America, at the eastern edge of the Pakaraima Mountains (also known as Sierra Pacaraima).

The Cooperative Republic of Guyana (Guyana) is one of the six countries covering the geologically and biologically distinct unit called the Guiana Shield (Fig. 1), which contains one of the largest remaining tracts of untouched rainforest in the world and is well known for its high species richness and endemism.

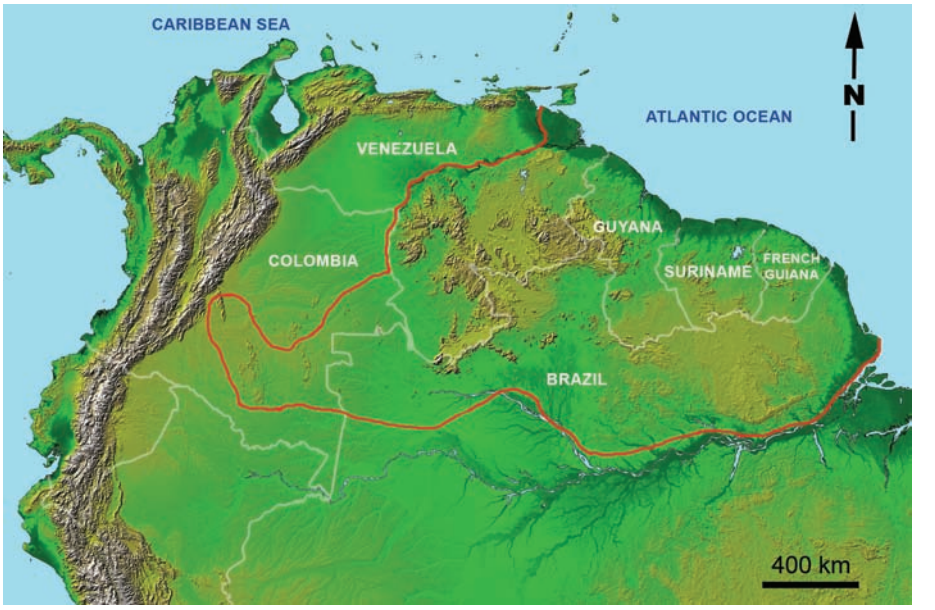

Fig. 1. Map of northern South America showing the boundaries of the Guiana Shield (red line). (Map elaborated by P. J. R. Kok after a radar image of South America by NASA/JPL/NIMA available at http://photojournal.jpl.nasa.gov/catalog/PIA03388 and the Guiana Shield map provided by Señaris & MacCulloch, 2005).

Guyana is bordered on the northwest by Venezuela, on the east by Suriname, on the south and southwest by Brazil, and on the north by the Atlantic Ocean, and is dissected by several major drainages (Fig. 2). More than 70% of the country is still covered with pristine tropical forest, making Guyana a biologically rich country, and an invaluable and attractive experience for scientists and any visitor captivated by nature.

Kaieteur National Park is probably one of the most neglected national parks in South America and its herpetofauna was hitherto never properly studied, although specimens were sporadically collected in the area since the beginning of the 20th century. The first and only published list of the reptile and amphibian species occurring in Kaieteur National Park is Kelloff's (2003) short compilation of 29 species, which unfortunately includes several obvious errors and dubious records.

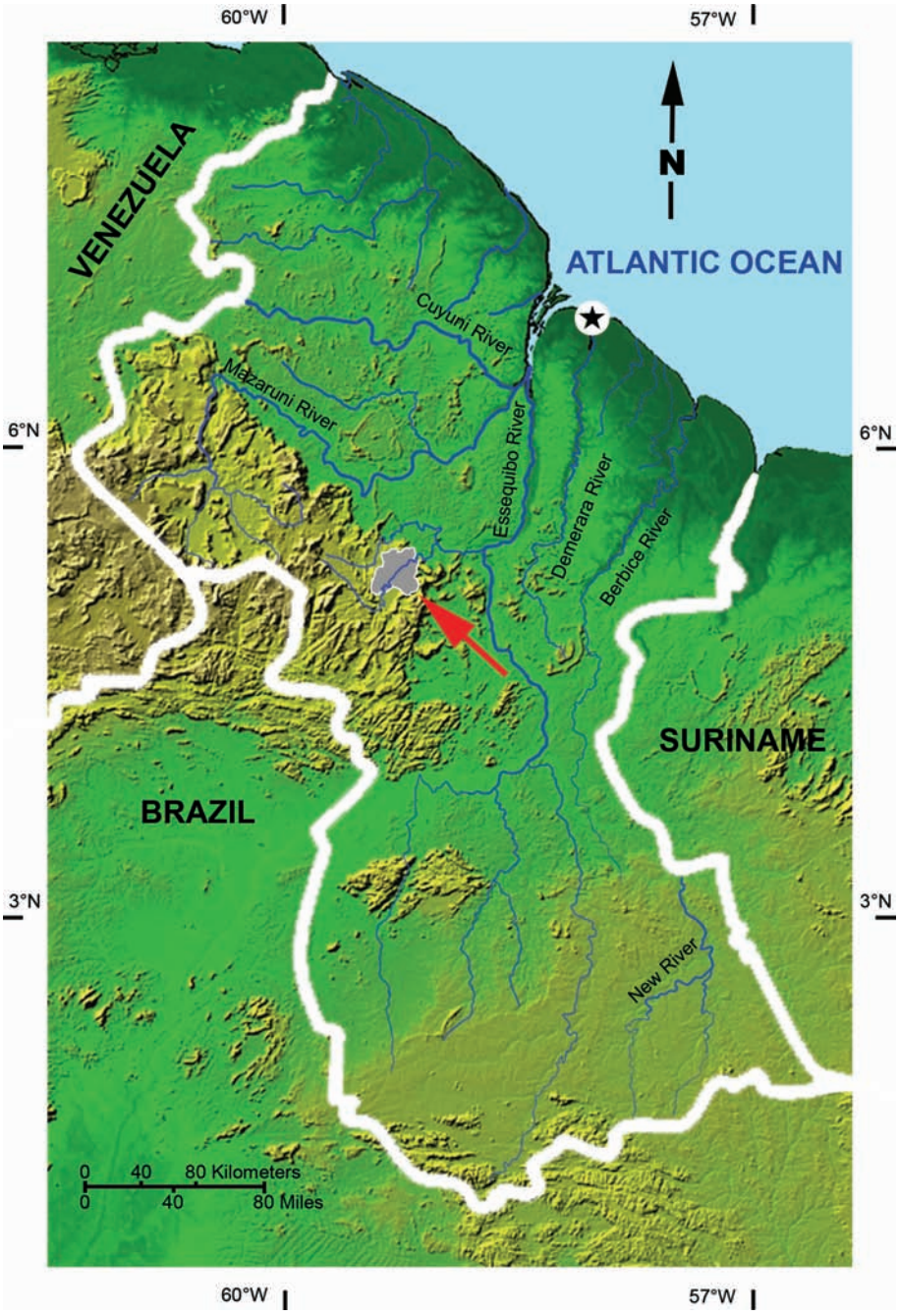

Fig. 2. Map of Guyana showing major drainages and the location of Kaieteur National Park (in grey, pointed by a red arrow); black star = Georgetown, capital city. (Map elaborated by P. J. R. Kok after a radar image of South America by NASA/JPL/NIMA available at http://photojournal.jpl.nasa.gov/catalog/PIA03388).

The British explorer and geologist Charles Barrington Brown was probably the first non-native to see the spectacular Kaieteur Falls in 1870.

Several decades later, in 1929, Kaieteur National Park (Fig. 3, located between *ca.* 5°08' to 5°19'N and *ca.* 59°22' to 59°38'W) was established by the British Commonwealth as one of the very first national parks in South America. Historically the boundaries of the original Park were drastically reduced from 11,400 ha to 1,940 ha in 1961, before being expanded in 1999 by President Cheddi Jagan (Kelloff, 2003).

At present the Park encompasses an area of 62,680 ha and lies in the Potaro-Siparuni District (formerly called Mazaruni-Potaro District).

1.1. Physiography and hydrography

Although Kaieteur National Park lacks the extensive mountainous topography and spectacular landscapes made from impressive plateaus (table-top mountains, locally called tepuis) that dominate the rest of the Pakaraima Mountains, its geological and biological diversity is significant.

Kaieteur National Park lies on Precambrian sandstone - one of the oldest exposed rock formations on earth - at the eastern edge of the Pakaraima Mountains, approximately 200 km airline SW of Georgetown, the capital city of Guyana. Formed about 300 millions years ago, the Pakaraima Mountains are located in the highlands of the Guiana Shield along the border between Venezuela, Brazil and Guyana, extending west to east for over 800 km. That region is also referred to as the phytogeographic province of Pantepui, which includes all upper slopes and summits of the Guiana Shield highlands. Mount Roraima (2,810 m above sea level) lies at the conjunction of the three countries and is the highest peak in the area.

This largely unexplored area is known for its relatively unspoiled habitat and highly endemic flora and fauna, however, as mentioned above, the herpetofauna of the region remains essentially undocumented. Elevation in the Park extends approximately 100–900 m (from the gorge to highest point on the plateau, see Fig. 3). The highest areas of the Park are located in the southwestern and southeastern parts, which remain largely unexplored. The centrepiece of the Park is the well-known 226 m high Kaieteur Falls situated where the Pakaraima Mountains give way to the interior lowlands (Figs 4, 5, 6A-B). This superlative phenomenon expels millions of litres of water as mist. The surrounding mist and prevailing winds partially influence the densities of some species in the vicinity of the fall. Many rivers and streams, including fast moving cascading streams with smaller waterfalls (Fig. 6C), are found throughout the Park. The largest river running through the Park is the Potaro River, which is 225km long, travelling approximately 32km through the deep Kaieteur gorge (Figs 6D, 7) and eventually into the Essequibo River, Guyana's largest waterway. The origin of the Potaro River is located in Mount Ayanganna (5°23'N, 59°59'W). Other major drainages running in the Park are Kurubia River, Aki River, Muri Muri River, Elinkwa River, Amamuri River, Amakwa River, and Chetu River (see Fig. 3).

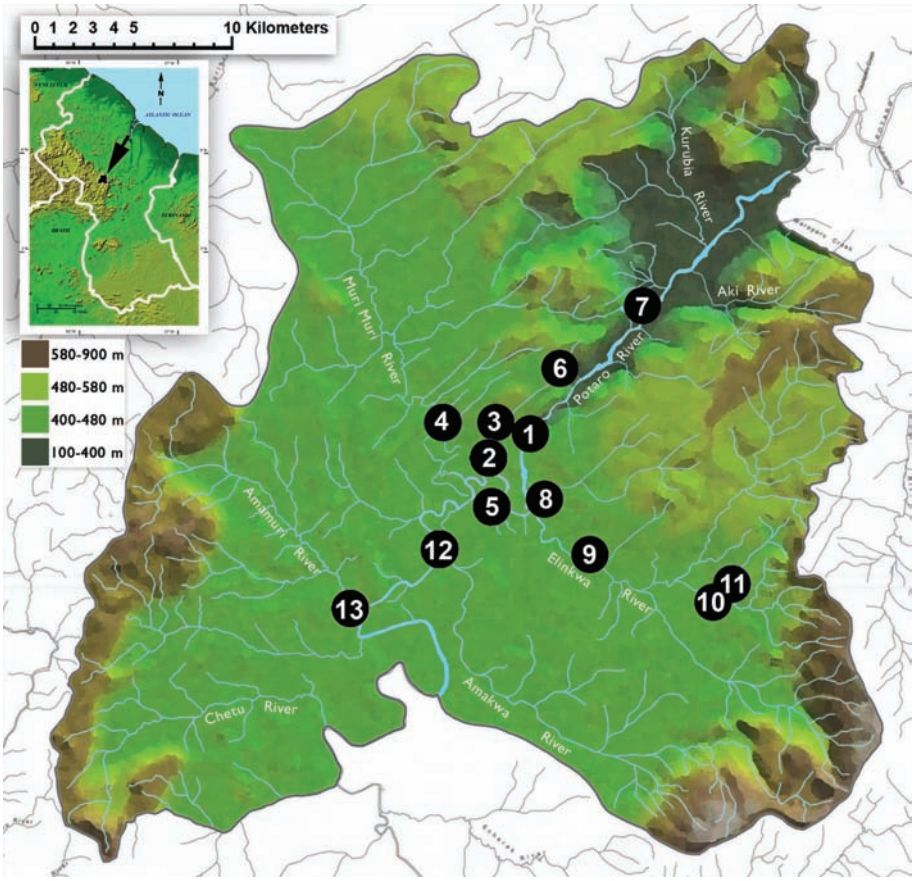

Fig. 3. Map of Kaieteur National Park with major drainages and main sampling localities (= localities where sampling efforts were concentrated): (1) Kaieteur Falls; (2) Menzies Landing trail; (3) Kaieteur airstrip; (4) Muri Muri trail; (5) Right bank Potaro River, opposite Menzies Landing; (6) Tukeit trail; (7) Tukeit Landing; (8) Elinkwa River mouth; (9) Elinkwa River; (10) Elinkwa camp #1; (11) Elinkwa camp #2; (12) Amakwa River mouth; (13) Amamuri River mouth. Insert map indicates the location of Kaieteur National Park in Guyana. (Maps elaborated by P. J. R. Kok after the Natural Resources Management Project, Topographic Map of Kaieteur National Park, Guyana and a radar image of South America by NASA/JPL/NIMA available at http://photojournal.jpl.nasa.gov/catalog/PIA03388).

Fig. 4. Scenic view of Kaieteur Falls from its base. (Photo by P. J. R. Kok).

Fig. 5. Kaieteur Falls splashing into the Kaieteur gorge. (Photo by P. J. R. Kok).

Fig. 6. Rivers and waterfalls. A. Kaieteur Falls flows at a rate of 660,000 litres per second during the wettest months; B. The physiognomy of Kaieteur Falls drastically changes during the driest months; C. Many smaller waterfalls like this one are found throughout the Park; D. The Potaro River running in the Kaieteur gorge. (Photos by P. J. R. Kok).

Fig. 7. Strong rapids on the Potaro River, below the Falls. (Photo by P. J. R. Kok).

1.2. Local communities

A small permanent settlement, called Menzies Landing (Fig. 8), is located inside the Park, less than 2 km SW by foot from the fall (Fig. 9). Menzies Landing is considered the gateway to the gold and diamond mining fields - some of them located within the Park - for “porkknockers” (local, low tech, freelance miners). These miners have built small wooden houses (Fig. 10) in which they live and rest when coming back from the “backdam” (mining field). In 2007, two small stores sold food and other basic supplies to miners and nearby Amerindian communities, but also bought diamonds and gold, which continue to be sent to Georgetown by plane from the Kaieteur airstrip. These human activities caused habitat destruction and pollution and are a serious threat to the biota of certain parts of the Park (Fig. 11).

The nearest community outside Kaieteur National Park is the Amerindian village of Chenapou (also spelled Chenapau or Chenapowu), located along the Potaro River, about 54 km SW of Kaieteur Falls by boat.

The Park encompasses ancestral lands and is an important traditional site for hunting and fishing for local indigenous communities. These local communities opposed the extension of the Park as it was made without their knowledge and without meaningful consultations. In the late nineties there were no regulations defining the rights of indigenous peoples to hunt, fish and conduct other traditional activities in the area, but by gazetting the 2006 Amerindian Act, traditional Amerindian practices are now officially allowed in the Park.

Consistent with the National Development Strategy (Anonymous, 2000), the community at Menzies Landing and all mining operations within the Park needed be closed down in order to rehabilitate and restore the area. Additionally, mining operations outside the Park were to be monitored so as to prevent damage to the Park's environment or, where this is not possible, terminated.

According to the National Parks Commission (NPC), the agency responsible for the management of Kaieteur National Park, effective monitoring and enforcement is currently unachievable due to the lack of financial and human resources. At present, four wardens control the Park, with two persons from the village of Chenapou currently involved in park ranger training by the joint Iwokrama-EPA-GFA programme. The NPC is considering hiring these trainees as full-time wardens to assist in the monitoring of the Park (N. Roopnarine, pers. comm. 2008).

Fig. 8. Menzies Landing along the Potaro River. (Photo by P. J. R. Kok).

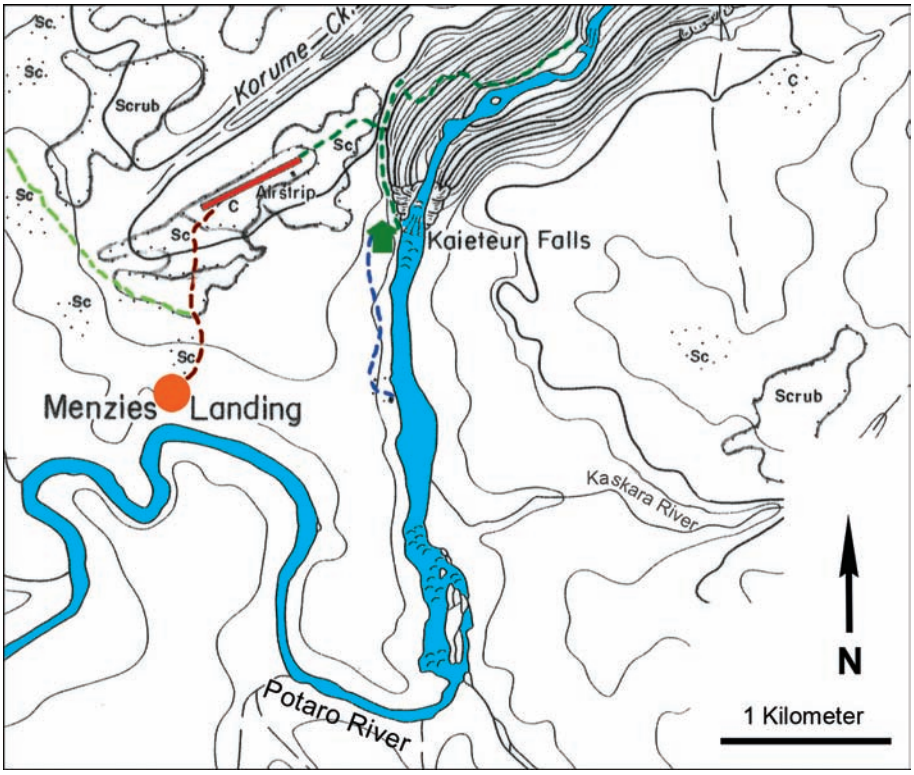

Fig. 9. Area map of Menzies Landing (orange dot) and surroundings. Brown dashed lines = Menzies trail; light green dashed lines = Muri Muri trail; dark green dashed lines = Tukeit trail;; blue dashed lines = Water gauge trail; green house = Kaieteur guesthouse. (Map elaborated by P. J. R. Kok after "Kaieteur Sheet 43 SW" published by the Survey Department of Guyana, 1972).

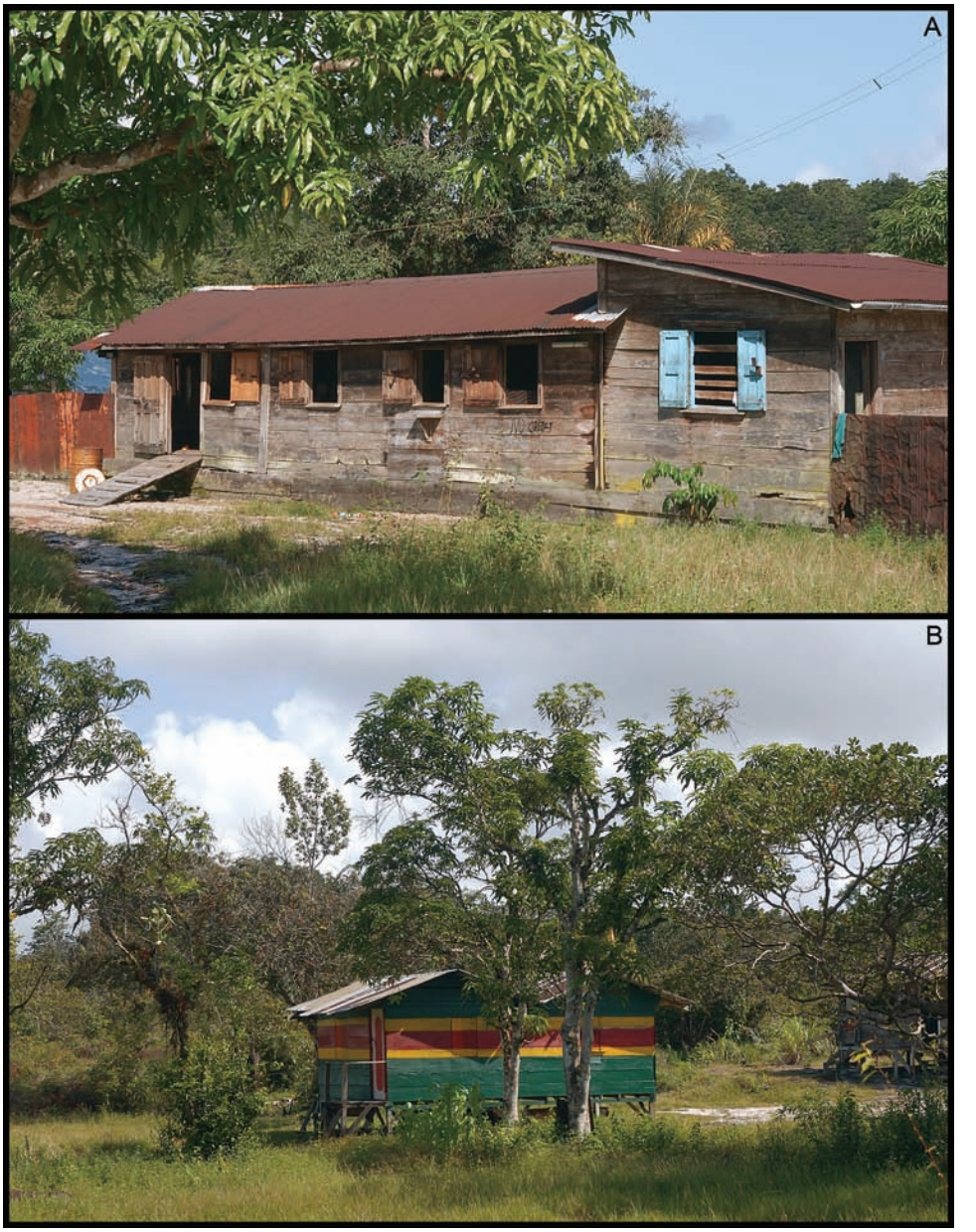

Fig. 10. A. Small store at Menzies Landing, centre of diamond and gold business; B. Typical wooden house at Menzies Landing. (Photos by P. J. R. Kok).

Fig. 11. Human activities and associated habitat destruction and pollution are a major threat to the fauna of certain parts of Kaieteur National Park. A. Illegal diamond-mining camp in the southeastern part of the Park; B. Illegal deforestation for farming around Menzies Landing; C. Burning of the savannah at the top of Kaieteur Falls in November 2004 - this kind of event could have extirpated several endemic species; D & E. Illegal mining (dredging) in the southeastern part of the Park. (Photos by P. J. R. Kok).

1.3. Climate

Guyana has a tropical climate, lying between 1-9°N and 56-62°W, with uniformly high temperatures, humidity and rainfall. Average annual rainfall ranges between 1778 mm and 2800 mm with a relative humidity of approximately 70%. Along the coast, temperature ranges from 20 to 38°C, while in the interior regions it ranges from 16 to 39°C (although temperatures on the summit of the highest tepuis may drop below 10°C).

There is a slight seasonal variation in temperature with two distinct wet seasons and two dry seasons. According to the Ministry of Agriculture, Hydrometeorological Service, Guyana (2008), seasonal rainfall variability is generally the dominant characteristic of climate in Guyana. The long wet season usually commences from mid-April to July, with major peak rainfall in June. The short wet season is from November to January with peak rainfall in December. The long dry season starts from August to November while the short dry season is from February-mid April.

At Kaieteur National Park, the yearly average relative humidity ranges between 80 and 87% with a dew point temperature averaging 21.6°C and an average mean temperature of 23.3ºC (Guyana Hydrometeorological Service, pers. comm. 2008). Figure 12 illustrates yearly mean temperature in the Park for the years 1997 and 2000-2007. The highest recorded month for rainfall is May (on average 728.3 mm) while the lowest is October (averaging 124.3 mm). The physiognomy of Kaieteur Falls is highly dependent on the seasons (see Fig. 6A-B). The data provided in Tables 1 & 2 are from the Meteorological Station located in the savannah at the top of Kaieteur Falls (Fig. 13). Due to the many different local environments (soils, elevations, exposures) occurring in the Park, average temperature and humidity may considerably vary in other locations. Some parts of the Park may experience intense downpours while a few kilometers away there is clear sky and strong sunlight.

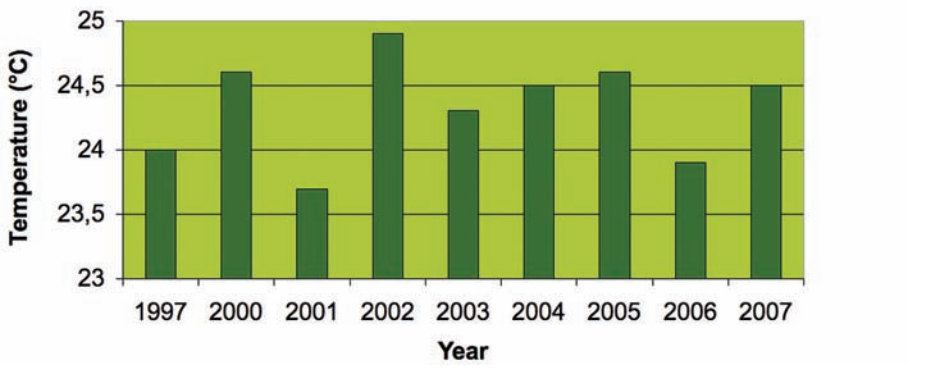

Fig. 12. Yearly mean temperature for Kaieteur National Park (data supplied by the Guyana Hydrometeorological Service, 2008).

Fig. 13. Kaieteur Hydrometeorological Station (indicated by black arrow in the upper left corner), along the Kaieteur airstrip at the top of Kaieteur Falls. (Photo by P. J. R. Kok).

Year	Dry Bulb (°C)	Wet Bulb (°C)	Relative Humidity (%)	Dew Point Temperature (°C)
1997	24.4	22.3	84	21.4
2000	24.1	22.4	87	21.7
2001	23.3	21.0	81	19.9
2003	25.5	23.2	83	22.3
2004	25.1	22.9	83	22.0
2005	25.9	23.3	80	22.1
2006	25.2	22.9	83	22.0
2007	24.2	22.5	86	21.7

Table 1: Yearly average relative humidity of Kaieteur National Park (data supplied by the Guyana Hydrometeorological Service, 2008).

Year/ Months	1997	2000	2001	2002	2003	2004	2005	2006	2007	Mean
1	326.9	523.8	168.1	DD	187.4	249.9	403.8	892.2	346.1	387.4
2	589.1	227.3	106.1	DD	138.8	107.5	311.0	211.9	136.2	228.5
3	DD	219.3	84.4	DD	139.9	201.1	146.8	265	296.1	193.2
4	DD	DD	258.0	DD	334.7	493.9	730.9	95.4	547.1	410.0
5	551.6	DD	633.4	DD	837.7	1089.7	849.7	407.6	DD	728.3
6	DD	DD	467.2	DD	596.2	721.6	318.4	778.9	626.5	584.8
7	483.9	DD	DD	DD	448.2	464.4	331.5	964.4	401.6	515.7
8	88.0	DD	DD	DD	318.6	276.2	460.5	197.7	304.2	274.2
9	DD	162.7	DD	105.6	88.7	252.9	36.2	101.9	194.1	134.6
10	213.6	111.5	DD	41.6	99.8	DD	60.3	141.1	202.3	124.3
11	DD	367.4	DD	395.7	294.4	163.8	138.1	292.4	DD	275.3
12	DD	585.9	DD	331.4	240.7	206.0	814.6	467.4	DD	441.0

Table 2: Monthly average rainfall (mm) for Kaieteur National Park for 1997, 2000 to 2007 (data supplied by the Guyana Hydrometeorological Service, 2008); DD = Data Deficient.

1.4. Vegetation

The vegetation at Kaieteur National Park is spectacular and supports a variety of different habitats. The Park harbours a mixture of the upland and lowland flora found on the Guiana Shield and supports a mosaic of forest, shrub and herbaceous formations.

According to Kelloff (2003), there are currently 22 endemic species of plants recorded for the early sixties' delineation of Kaieteur National Park (1,940ha), although some of these plants might prove to be more widespread both within and outside the Park. *Aechmea brassicoides* for instance, one of the 22 endemic species reported by Kelloff (2003) from the early sixties' delineation, was seen in other locations in the Park (P. Kok, pers. obs.). See Kelloff (2003) and Kelloff & Funk (2004) for more details on plants of Kaieteur.

Forest formations

Tall, mixed, evergreen, basimontane and submontane forests on white sand occur throughout the Park (Fig. 14A-B) and are mainly composed of tree species in the genera *Dicymbe, Dimorphandra, Eperua*, *Micrandra* and *Peltogyne*. Typical lower story trees belong to the families Annonaceae, Guttiferae, Lecythidaceae, Leguminosae, and Palmae, while members of the Araceae, Bromeliaceae, Marantaceae, Melastomataceae, and Rapateaceae noticeably dominate the vegetation of the forest floor.

Riparian forest consists of tree species such as wallaba (*Eperua*), brazilnut (*Lecythidaceae*), aromata (*Clathrotropis macrocarpa*), kakaralli (*Eschweilera spp.*), and coffee (*Rubiaceae*) families (Kelloff, 2003). The understory of this type of forest supports *Heliconia*, *Marantaceae*, and many species of *Melastomataceae* (Fig. 14C).

Patches of cloud forest are found in several parts of the Park, usually at elevations over 500-600m. One cloud forest habitat created by the cool mist rising from the gorge is found at the top of Kaieteur Falls (Fig. 14D). This habitat sustains numerous epiphytes, mosses, orchids, ferns and aroids.

Shrub and herbaceous formations

Patches of "savannah" (Fig. 15A-C) surround the top of Kaieteur Falls, but are also found elsewhere in the Park. These savannahs support a shrub-herb plant community with only few small trees. The pink sands mixed with bare rocks support scattered shrubs and a dense mat of small herbaceous plants (Kelloff, 2003). It must be noted that part of the savannah surrounding the top of Kaieteur Falls is anthropogenic (Fig. 15C).

During the rainy season, numerous species of lichens such as *Cladonia* spp. and *Cladina* spp., the small blue flowered herb *Burmannia bicolor*, two types of carnivorous plants, *Utricularia* spp. (bladderworts) and *Drosera kaieteurensis* (red sundew), appear from tiny cracks and on the surface of the bare, flat sandstone (Kelloff, 2003).

Usually the first plant to catch the eye in the vicinity of Kaieteur Falls is the tank bromeliad (*Brocchinia micrantha*), which can reach a height of 3.5 m as it takes

advantage of the humus caught in larger cracks and crevices. The water that collects in the phytotelm of this plant is an important habitat for the golden rocket frog, *Anomaloglossus beebei* (Fig. 15D), and the tiny bladderworts, *Utricularia humboldtii*, which uses its aquatic roots to capture insects that live in the stagnant waters.

Other notable bromeliads are the cabbage head, *Aechmea brassicoides*, and the carnivorous *Brocchinia reducta*, with tall, narrow, yellowish leaves, which often serves as a daytime refuge to the endemic frog *Tepuihyla talbergae*.

Kelloff (2003) highlighted that small trees such as *Andira grandistipula* and shrubs such as *Clusia* and *Erythroxylum* can develop into “bush islands” which support an entire community of plants and often differ from island to island.

Fig. 14. Forest formations found in Kaieteur National Park. A. Basimontane forest on white sand; B. Submontane forest; C. Riparian forest along the Potaro River; D. Cool mist rising from Kaieteur Falls creates a patch of cloud forest at the top of the fall. (Photos by P. J. R. Kok).

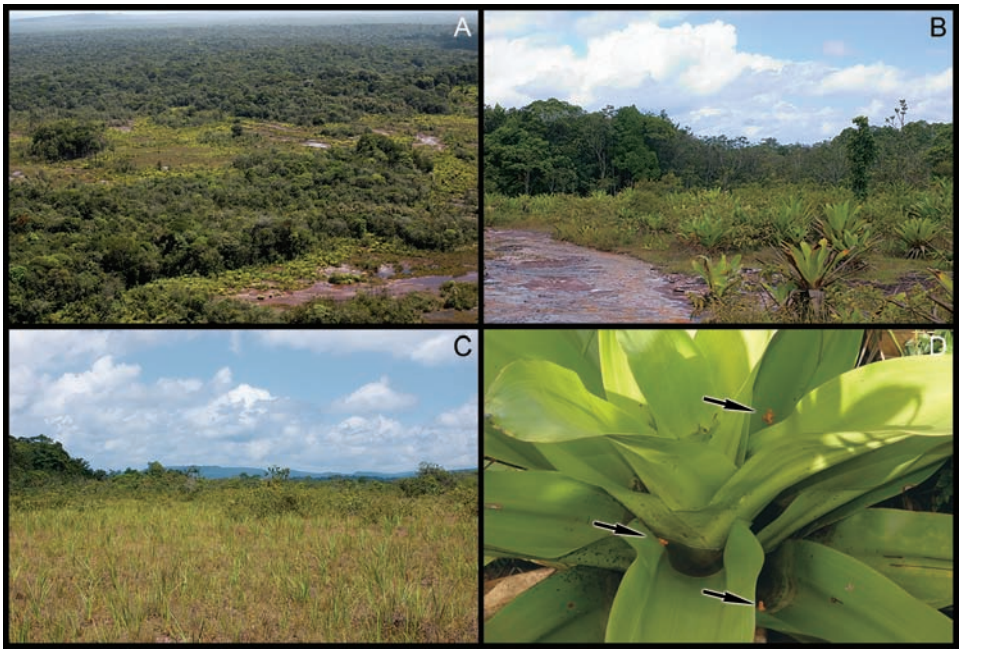

Fig. 15. Shrub and herbaceous formations found in Kaieteur National Park. A-B. Shrubland and forest at the top of Kaieteur Falls; C. Mostly anthropogenic herbaceous formation at the top of Kaieteur Falls; D. The terrestrial bromeliad *Brocchinia micrantha* is a major element of the savannah surrounding the top of Kaieteur Falls and is the exclusive habitat of *Anomaloglossus beebei* (three specimens are indicated by arrows). (Photos by P. J. R. Kok).

2. Class Amphibia Gray, 1825

Amphibian classification is undergoing major rearrangements. According to Frost *et al.* (2006), Amphibia is a monophyletic taxon composed of Gymnophiona ("caecilians") and Batrachia ("salamanders" + "frogs") (see Fig. 16).

Fig. 16. Basal structure of Frost *et al.*'s (2006) consensus tree with respect to outgroups and major amphibian taxa. Copied from Frost *et al.* (2006: 113), with permission of D. R. Frost.

The term Amphibia derives from the Greek *amphi* meaning both or double and *bios* meaning life; this is an allusion to the ability of amphibians to live both in

aquatic and terrestrial environments. Note that the term “Lissamphibia” is sometimes applied to the extant amphibian species. Amphibians are tetrapods (although limbs are reduced or secondarily lost in some groups) with a glandular skin that lacks epidermal scales, feathers, or hair. They are ectotherms, which means that they are dependent on external heat sources. Many internal and external morphological characters define the Class Amphibia. The purpose of this work is not to detail all of these features and we suggest the reader to refer to the numerous works extensively defining Amphibia (*e.g.* Duellman & Trueb, 1986; Trueb & Cloutier, 1991; Pough *et al*., 2004) for more exhaustive information. According to Trueb & Cloutier (1991) synapomorphies of Amphibia include the loss of the postparietal bones, the loss of the supratemporal bone, the loss of the tabular bone, the loss of the postorbital bone, the loss of the jugal bone, the loss of the interclavicle, the loss of the cleithrum, the presence of a specialized sensory area, the *papilla amphibiorum*, in the inner ear, the opercular element associated with the columella, the presence of fat bodies that originate from the germinal ridge associated with the gonads, and the presence of pedicellate and bicuspid teeth that are replaced mediolaterally (reversed in some taxa).

2.1. Order Gymnophiona Müller, 1832

Members of the order Gymnophiona, also called caecilians, are limbless amphibians that resemble earthworms or even snakes; the taxonomic name Gymnophiona derives from the Greek *gymnos* meaning naked and *ophis* meaning snake. Caecilians are found in most of the tropical regions, except Madagascar and Oceania.

The caecilian body is elongated and partly or completely segmented by annuli, which are separated by grooves. Limbs, rudiments of pectoral and pelvic girdles are lacking; frontal and parietal bones are distinct; palatoquadrate articulates with skull; atlas articulates with skull by atlantal cotyles. Only one currently known species is lungless [*Atretochoana eiselti* (Taylor, 1968)], all other known caecilians have lungs with the left one being usually rudimentary (similar adaptation is found in snakes). The tail is short or absent (it may sometimes be difficult to state if a tail is present or not). The cloaca is located at the end of the body. Variation in size is considerable ranging from *ca.* 100 mm to *ca.* 1500 mm. Eyes are small, often barely visible, covered by skin or by the bones of the skull. There is no tympanum, nor developed vocal structure (although sound production has been reported in a few species, see Duellman & Trueb, 1986), and all species have two small protrusible sensory tentacles on the head, each one usually located between the eye and the naris, sometimes below the naris. The skin is smooth; many species have numerous fish-like scales in pockets in the skin. Most species are drab in colour, although some are brightly coloured. Some caecilians produce skin toxins. All species have a dual-jaw closing mechanism and are equipped with several rows of sharp teeth used to capture small animals, mostly invertebrates. Larvae are very similar to adults, but are smaller and have gill slits, lateral line sensory organs and labial folds.

Unlike all other amphibians (with the exception of the leiopelmatid frog genus *Ascaphus,* and possibly the bufonid genus *Mertensophryne*), male caecilians

have a copulatory organ (phallus) and have internal fertilisation. The caecilian phallus (Fig. 17) is an eversible chamber (phallodeum) of the cloaca (Duellman & Trueb, 1986) and is a uniquely derived structure among vertebrates (Wake, 2006).

Fig. 17. Intromittent organ of the caecilian *Rhinatrema* cf. *bivittatum*. (Photo by Philippe J. R. Kok).

A number of species are viviparous, with epitheliophagous foetuses that, once the yolk mass is resorbed, feed on particular cells of the oviduct with specialized scraping teeth. These teeth are shed after birth. Foetal teeth are mainly specific to viviparous species, but at least two egg-laying species [*Boulengerula taitanus* Loveridge, 1935 and *Siphonops annulatus* (Mikan, 1820)] are known to feed their young - which are equipped with the same kind of teeth - by developing a special outer layer of skin that is peeled off by the young (Kupfer *et al.*, 2006b; Wilkinson *et al.*, 2008). Oviparous species lay gelatinous eggs that are guarded by the female (larvae may be terrestrial or aquatic).

Most caecilian species are soil-dwelling predators, but some are semiaquatic or aquatic (*i.e.* Typhlonectidae).

The caecilians are taxonomically challenging and several classifications have been suggested (see Wake & Campbell, 1983; Duellman & Trueb, 1986; Laurent, 1986; Lescure *et al.*, 1986; Nussbaum & Wilkinson, 1989; Frost *et al.*, 2006). The most recent classification was proposed by Wilkinson & Nussbaum (2006), who recognized the following six families: Rhinatrematidae, Ichthyophiidae, Uraeotyphlidae, Scolecomorphidae, Typhlonectidae and Caeciliidae. Only Rhinatrematidae, Typhlonectidae and Caeciliidae have representatives in South America.

Two families of caecilians are currently known to occur in Kaieteur National Park: Rhinatrematidae and Caeciliidae.

Rhinatrematidae Nussbaum, 1977

The main features characterizing this family are (Frost *et al.*, 2006; Wilkinson & Nussbaum, 2006): tail present; skin divided into annuli that are not congruent with segmetation of trunk musculature and with no distinction between primary and secondary annular grooves; scales numerous; nasals and premaxillae

present as separate bones; tentacle immediately anterior to or on the anterior edge of eye; eyes visible externally.

The family Rhinatrematidae contains two genera, one of which is present in Kaieteur National Park (*Rhinatrema*).

Caeciliidae Rafinesque, 1814

The main features characterizing this large family are (Frost *et al*., 2006; Wilkinson & Nussbaum, 2006): tail absent (although a pseudotail is present in Typhlonectidae); skin divided into primary annuli congruent with segmentation of trunk musculature, some of which may be divided posteriorly by secondary annular grooves; scales absent or present; nasal and premaxilla fused; septomaxilla reduced or absent; pterygoid absent; fused third and fourth ceratobranchials greatly expanded; vent circular or transverse; tentacle variously positioned; eyes visible or not.

The family Caeciliidae contains 21 genera, one of which is present in Kaieteur National Park (*Microcaecilia*).

2.2. Order Caudata Fischer von Waldheim, 1813

Members of the order Caudata, also called urodeles or simply “salamanders”, are characterized by the presence of a tail (*caudata* meaning tail in Latin) and two pairs of limbs (but see below). Most urodeles show a transition of aquatic life to a terrestrial mode of life. Urodeles are principally Holarctic and are found in Palearctic Eurasia, northwestern Africa and the Americas (Frost, 2008). Only one living family (Plethodontidae) extends into South America.

The salamander body is moderate or somewhat elongate, not annulated (although costal grooves may be present), with a long tail. Four limbs are present (except in the family Sirenidae, whose members lack pelvic limbs and girdle). Frontal and parietal bones are distinct; palatoquadrate fused by processes to cranium; atlas articulates with skull by atlantal cotyles and medio-ventral forward-directed process that meets the walls of foramen magnum on either side.

Since no urodeles are known to occur in the Guiana Shield (Señaris & MacCulloch, 2005) and *a fortiori* in Kaieteur National Park, we will not further discuss this order.

2.3. Order Anura Fischer von Waldheim, 1813

Members of the large and diverse order Anura, commonly called “frogs”, are easily distinguished from other amphibians by the absence of a tail (*anura* derives from the Greek *an* meaning without and *oura* meaning tail). Anurans are cosmopolitan, their diversity is greatest in tropical, subtropical and warm temperate regions and they are absent from high latitudes in the Arctic, Antarctica, most oceanic islands, and some xeric deserts (although they may be present in oases) (Duellman & Trueb, 1986; Frost, 2008).

The body of an anuran is short, relatively robust, not annulated, with elongated hindlimbs and feet. The mouth is usually large. Four limbs are present and adults lack a tail. Frontal and parietal bones are fused on each side (into a

frontoparietal); palatoquadrate fused by processes to cranium; atlas articulates with skull by atlantal cotyles. Eyes are functional and exposed. Most species have a functional tympanum, and well-developed vocal structures. Size varies from *ca.* 10 mm to more than 300 mm. Texture of the skin is highly variable, from smooth to warty.

While numerous anuran species are cryptic (which means that they cannot be easily detected), many species have bright colours that often serve as warning colourations (aposematism) associated with unpalatability and/or the presence of poisonous secretions. Many anurans exhibit defence behaviours when faced by a potential predator, some species feign death, other produce loud distress calls and some even bite (*e.g.* the hemiphractid *Stefania woodleyi* from Guyana, see Kok *et al.*, 2007a).

Most anurans are carnivorous and sit-and-wait predators. They feed on a great variety of invertebrates and sometimes on small vertebrates for the largest species. Preys are usually visually detected (olfactory and auditory detections are also reported) and captured with the tongue, on which they adhere due to the presence of a sticky secretion. The diet of the hylid *Xenohyla truncata* (from Brazil) includes fruits that are especially consumed during the dry season, when invertebrates are less abundant (da Silva & de Britto-Pereira, 2006).

Males almost invariably attract females with an advertisement call, although some species do not always produce sound and attract females using other strategies like “semaphoring” (arm waving, foot flagging). The latter behaviour is mainly observed in species living in noisy environments [*e.g.* the bufonid *Atelopus varius* from Costa Rica and Panama, see Hödl & Amézquita (2001) for more information]. Some species (*e.g.* the ranids *Huia cavitympanum* and *Odorrana tormota*) even produce ultrasonic calls, shifting the frequencies beyond the spectrum of the background noise (Feng *et al.*, 2006).

Mating typically takes place by the male grasping the female in a position that will allow him to externally fertilize eggs. Amplectic positions are variable and of phylogenetic significance. The male can grasp the female around the waist (inguinal amplexus, mostly in “primitive” frogs), behind the forelimbs (axillary amplexus, mostly in “advanced” frogs), or around the head (cephalic amplexus, mostly in “advanced” frogs). The male can also simply straddle the female, or be glued to the posterior part of the female by dermal secretions. Males of *Ascaphus* (see above) have an extension of the cloaca that is inserted into the cloaca of the female allowing internal insemination (internal insemination is also suspected in the bufonid *Mertensophryne*). In some cases amplexus is completely absent, like in species in the genus *Oophaga* (Dendrobatidae), which accomplish internal fertilization by cloacal apposition.

Reproductive strategies are amazingly diverse in anurans: 29 reproductive modes were recognized by Duellman & Trueb (1986), 16 years later Savage (2002) reported 35 reproductive patterns, and more recently Haddad & Prado (2005) recognized 39 reproductive modes in anurans. Since then, additional reproductive modes and strategies were identified (see for instance Gibson & Buley, 2004; Kok & Ernst, 2007). Eggs may be aquatic (*e.g.* simply deposited in water, laid in foam nests constructed in or over water, or even imbedded in

dorsum of the aquatic female), terrestrial (*e.g.* laid in burrows, on the ground, in excavated nests, in terrestrial foam nests), or arboreal (*e.g.* laid between leaves, above leaves, below leaves overhanging water, in leaf nests, in tree holes, etc.). Eggs can also be carried by one of the parents (on legs, on the dorsum, in a dorsal pouch, or even in the stomach), or be retained in the oviducts (ovoviviparous and viviparous species). Kok & Ernst (2007) recently described *Allobates spumaponens* (Aromobatidae) from Guyana that deposits tadpoles in foam nests of leptodactylid species, which is the first case of interspecific brood parasitism in amphibians. Some species provide extensive parental care (egg clutch attendance, larvae feeding, etc.).

Anuran larvae are nonreproductive and morphologically very distinct from adults. They have a short, usually globular, body and a long tail, which is resorbed during metamorphosis. Tadpole diversity is remarkable and McDiarmid & Altig (1999) provided no less than 15 ecomorphological guilds. Tadpoles may be endotroph (non-feeding tadpole) or exotroph (feeding tadpole) and present many adaptations to their environment (see McDiarmid & Altig, 1999 for further details). Tadpoles are vegetarian and/or carnivorous, some are cannibalistic.

The following 47 anuran families (*ca.* 5500 species) are currently recognized, even if some of them are still in debate among the herpetological community (families occurring in South America are in bold): **Allophrynidae**, Alytidae, **Aromobatidae**, Arthroleptidae, Bombinatoridae, **Brachycephalidae**, Brevicipitidae, **Bufonidae**, **Calyptocephalellidae**, **Centrolenidae**, Ceratobatrachidae, **Ceratophryidae**, **Craugastoridae**, **Cycloramphidae**, **Dendrobatidae**, Dicroglossidae, **Eleutherodactylidae**, Heleophrynidae, **Hemiphractidae**, Hemisotidae, **Hylidae**, **Hylodidae**, Hyperoliidae, Leiopelmatidae, **Leiuperidae**, **Leptodactylidae**, Limnodynastidae, Mantellidae, Megophryidae, Micrixalidae, **Microhylidae**, Myobatrachidae, Nyctibatrachidae, Pelobatidae, Pelodytidae, Petropedetidae, Phrynobatrachidae, **Pipidae**, Ptychadenidae, Pyxicephalidae, **Ranidae**, Ranixalidae, Rhacophoridae, Rhinophrynidae, Scaphiopodidae, Sooglossidae, and **Strabomantidae**.

Note that it may be difficult to confidently assign an anuran species to a family because many species closely resemble other species in unrelated families (due to convergent evolution); the most significant morphological diagnostic characters are often features of the internal anatomy (especially the skeleton). For some families none or very few external features allow identification, and in most cases only a combination of characteristics technically defines the family. In some cases, families are primarily defined by genetics.

Fig. 18. A few examples of the diversity of anurans in South America. A. *Lithobates palmipes* (Ranidae), a typical frog (note: this species is not recorded from KNP); B. *Rhinella marina* (Bufonidae), a typical toad; C. *Dendrobates tinctorius* (Dendrobatidae), a poisonous species that displays aposematic colouration (note: this species does not occur in KNP); D. The terrestrial and semi-fossorial *Otophryne steyermarki* (Microhylidae) (note: this species does not occur in KNP); E. The arboreal *Phyllomedusa bicolor* (Hylidae); F. The mainly aquatic *Pipa arrabali* (Pipidae). (Photos by Philippe J. R. Kok).

Eleven families of anurans are currently known to occur in Kaieteur National Park: Allophrynidae, Aromobatidae, Bufonidae, Centrolenidae, Eleutherodactylidae, Hemiphractidae, Hylidae, Leptodactylidae, Microhylidae, Pipidae, and Strabomantidae.

Allophrynidae

Although Frost *et al.* (2006) ranked the genus *Allophryne* in the subfamily Allophryninae of the family Centrolenidae, we maintain the use of Allophrynidae [see Guayasamin & Trueb (2007), and Guayasamin *et al.* (2008) for arguments].

The following main features are characteristic of the family (based on Zug *et al*., 2001): skull strongly ossified dorsally, with paired palatines and frontoparietals; vertebral column with eight holochordal, procoelous presacral vertebrae; ribs absent; maxillae toothless; sacrum with moderately dilated diapophyses and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle arciferal with distinct sternum; fibulare and tibiale fused at their proximal and distal ends; no intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges T-shaped; pupil horizontally elliptical. Amplexus axillary.

The family Allophrynidae currently contains only one genus, *Allophryne*, which is present in Kaieteur National Park.

Aromobatidae

Previously included in the Dendrobatidae, but removed after genetic analysis (Grant *et al*., 2006).

Similar to Dendrobatidae, but do not appear to have the ability to sequester alkaloids in their skin, and are usually not as brightly coloured. Members of this family are characterized by the following main features: skull with paired palatines (absent in *Allobates* and most *Aromobates*) and frontoparietals; vertebral column with eight holochordal, procoelous presacral vertebrae; ribs absent; upper jaw dentate; sacrum with cylindrical diapophyses (dilated in *Aromobates*) and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle firmisternal, with distinct bony sternum; fibulare and tibiale fused at their proximal and distal ends; no intercalary cartilage between terminal and penultimate phalanges of digits; supradigital scutes present; tips of terminal phalanges T-shaped; pupil horizontally elliptical. Amplexus cephalic or independent (absent).

The family Aromobatidae currently contains five genera, one of which is present in Kaieteur National Park (*Anomaloglossus*).

Bufonidae

The following main features are characteristic of the family [based on Zug *et al*. (2001), and Savage (2002)]: skull with paired palatines and frontoparietals; vertebral column with 5-8 holochordal, procoelous presacral vertebrae; ribs absent; upper jaw toothless; sacrum with moderately dilated diapophyses and bicondylar articulation with urostyle (except in some species); monocondylar or sacrum fused to vertebral column in taxa with reduced vertebral numbers; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle arciferal, rarely pseudofirmisternal, with distinct bony sternum; rudimentary ovary (Bidder's organ) retained in adult males (except in a few species); fibulare and tibiale fused at their proximal and distal ends; no intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges blunt to pointed; pupil horizontally elliptical. Amplexus axillary.

The family Bufonidae currently contains 45 genera, three of which are present in Kaieteur National Park (*Atelopus*, *Rhaebo*, *Rhinella*).

Centrolenidae

Members of this family are characterized by the following main features [based on Zug *et al*. (2001), and Savage (2002)]: ventral skin transparent, internal organs visible; skull with paired palatines and frontoparietals; vertebral column with eight holochordal, procoelous presacral vertebrae; ribs absent; teeth on upper jaw; sacrum with moderately dilated diapophyses and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle arciferal, with distinct cartilaginous sternum; fibulare and tibiale fused along entire lengths; short intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges T-shaped; pupil horizontally elliptical. Amplexus axillary.

The family Centrolenidae currently contains four genera, three of which are present in Kaieteur National Park (*Centrolene*, *Cochranella*, *Hyalinobatrachium*).

Eleutherodactylidae

The following main features are characteristic of the Eleutherodactylidae (Hedges *et al*., 2008; refer to that paper for extensive definition of the family): vertebral column with eight holochordal, procoelous presacral vertebrae; ribs absent; maxillary usually dentate; sacrum with rounded or barely dilated diapophyses and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle arciferal, rarely pseudofirmisternal, with distinct cartilaginous sternum; fibulare and tibiale fused at their proximal and distal ends; no intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges T-shaped; pupil usually horizontally elliptical. Amplexus axillary.

The family Eleutherodactylidae currently contains four genera, one of which is present in Kaieteur National Park (*Adelophryne*).

Hemiphractidae

Hemiphractidae is considered polyphyletic by Frost *et al*. (2006), who recognized Amphignathodontidae and Cryptobatrachidae as distinct from Hemiphractidae. Guayasamin *et al*. (2008) formally placed Amphignathodontidae and Cryptobatrachidae in synonymy with Hemiphractidae. Hemiphractidae (as Hemiphractinae) was formerly regarded as a subfamily of Hylidae, with which it is morphologically close.

Members of this family carry eggs and endotrophic embryos on the back or in a specialized dorsal pouch until hatching. The following main features are characteristic of the family [based on Hemiphractinae of Zug *et al*. (2001), and Savage (2002)]: skull with paired palatines and frontoparietals, strongly ossified, with or without dermis co-ossified to roofing bones; vertebral column with eight holochordal, procoelous presacral vertebrae; ribs absent; teeth on upper jaw; of the superficial mandibular musculature, the interhyoideus lies within the lower

jaw, and the intermandibular muscle has variable development of accessory lips; sacrum with rounded (cylindrical in some genera) slightly to moderately dilated diapophyses and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle arciferal, with distinct cartilaginous sternum; fibulare and tibiale fused at their proximal and distal ends; short intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges pointed or claw-shaped; pupil horizontally elliptical. Amplexus axillary.

The family Hemiphractidae currently contains five genera, one of which is present in Kaieteur National Park (*Stefania*).

Hylidae

The following main features are characteristic of the family [based on Hylinae, Pelodryadinae, and Phyllomedusinae of Zug *et al*. (2001), and Savage (2002)]: skull with paired palatines and frontoparietals, ossification variable, dermis usually not fused to roofing bones; vertebral column with eight holochordal, procoelous presacral vertebrae; ribs absent; teeth on upper jaw; of the superficial mandibular musculature, the interhyoideus extends posteriorly beyond the lower jaw, and the intermandibular muscle is undifferentiated, has lateral accessory slips or a separate apical element; sacrum with rounded (cylindrical in some genera) slightly to moderately dilated diapophyses and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle arciferal, with distinct cartilaginous sternum; fibulare and tibiale fused at their proximal and distal ends; short intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges pointed or claw-shaped; pupil horizontally elliptical (vertically elliptical in Phyllomedusinae). Amplexus axillary.

The family Hylidae currently contains 45 genera, seven of which are present in Kaieteur National Park (*Dendropsophus*, *Hypsiboas*, *Osteocephalus*, *Phyllomedusa*, *Scinax*, *Tepuihyla*, *Trachycephalus*), but see taxonomic comments about *Hypsiboas liliae* on page 172.

Leptodactylidae

The following main features are characteristic of the family [mostly based on Leptodactylinae of Zug *et al*. (2001), and Savage (2002)]: no webbing on hand; skull with paired palatines and frontoparietals; vertebral column with eight holochordal, procoelous presacral vertebrae; ribs absent; maxillary dentate; sacrum with rounded diapophyses and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle arciferal, with distinct cartilaginous sternum; fibulare and tibiale fused at their proximal and distal ends; no intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges variable; pupil horizontally elliptical. Amplexus axillary.

The family Leptodactylidae currently contains four genera, one of which is present in Kaieteur National Park (*Leptodactylus*).

Microhylidae

This family is characterized by the following main features [mostly based on Zug *et al*. (2001), and Savage (2002)]: 1-3 transverse dermal folds running across palate anterior to pharynx (except in two taxa); skull with paired palatines and frontoparietals; vertebral column with eight holochordal, procoelous presacral vertebrae, or eighth presacral vertebra biconcave and sacrum biconvex; ribs absent; maxillary toothless (except in Dyscophinae and some Cophylinae); sacrum with cylindrical to broadly dilated diapophyses and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle firmisternal, with distinct cartilaginous sternum; fibulare and tibiale fused at their proximal and distal ends; no intercalary cartilage between terminal and penultimate phalanges of digits (except in one genus); tips of terminal phalanges variable; pupil horizontal or round. Amplexus usually axillary, but in some robust taxa males adherent to posterior part of female.

The family Microhylidae currently contains 52 genera, one of which is present in Kaieteur National Park (*Synapturanus*).

Pipidae

This family is characterized by the following main features [based on Duellman & Trueb (1986), and Zug *et al*. (2001)]: body dorsoventrally depressed; hindlimbs large and muscular; feet extensively webbed; tongue absent; presence of a lateral-line organ; skull lacking palatines, with a single frontoparietal; vertebral column with 6-8 epichordal, opisthocoelous presacral vertebrae; ribs present; maxillary usually toothless, but dentate in some species; sacrum with broadly expanded diapophyses, fused with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle pseudofirmisternal, with distinct sternum; fibulare and tibiale fused at their proximal and distal ends; no intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges pointed; pupil round. Amplexus inguinal.

The family Pipidae currently contains five genera, one of which is present in Kaieteur National Park (*Pipa*).

Strabomantidae

Characteristics of this family are mostly similar to those of the family Eleutherodactylidae, from which it is mainly distinguished on the basis of molecular data (Hedges *et al*., 2008). The following main features are characteristic of Strabomantidae (refer to Hedges *et al*., 2008 for extensive definition of the family): vertebral column with eight holochordal, procoelous presacral vertebrae; ribs absent; maxillary usually dentate; sacrum with rounded or barely dilated diapophyses and bicondylar articulation with urostyle; facial nerve exits through anterior acoustic foramen in auditory capsule; trigeminal and facial nerve ganglia fuse to form a prootic ganglion; pectoral girdle arciferal,

rarely pseudofirmisternal, with distinct cartilaginous sternum; fibulare and tibiale fused at their proximal and distal ends; no intercalary cartilage between terminal and penultimate phalanges of digits; tips of terminal phalanges T-shaped, knobbed, or bearing hook-like lateral process; pupil usually horizontally elliptical. Amplexus axillary or inguinal.

The family Strabomantidae currently contains 16 genera, one of which is present in Kaieteur National Park (*Pristimantis*).

3. Taxonomic study of amphibians

Unless you plan to work on already collected material (*i.e.* museum collections), taxonomic study of any group of animals implies the development of various techniques and protocols to build a reference collection. This also means that you must spend significant time in the field to gather specimens and detailed, accurate and associated data.

The collected or "voucher" specimens are specimens that are sacrificed to serve as a basis of study and reference. These specimens must be deposited in a recognized natural history collection, which will ensure long-term care and maintenance, accessibility to other researchers, and independent verification of results.

Voucher specimens are an extremely important part of scientific research. Their main purposes are: (1) to allow correct identification of the species under study, (2) to allow resolution of species limits [*e.g.* in a complex of closely related species] and understand intraspecific variation, (3) to allow confirmation and a verification of the occurrence of a species at a certain place at a certain time.

It must be emphasized that the collection of voucher specimens is essential in almost any biological research project, including systematics, ecological or behavioural research, environmental assessment, etc. Correct identification of the animals under study is always crucial to the outcome of the research, and the quality of your sample will play a major role. Identifying specimens that were poorly prepared or lack accurate data is very frustrating and these specimens are of little or no scientific use. Additionally it poses ethical problems to collect specimens that will prove to be useless. High quality of preparation will also ensure proper future studies of important morphological traits that could disappear in ill-prepared specimens, and is also a token of respect for the killed animal.

In case new species are discovered among the collected material, some individuals will be selected as "type specimens" (= permanent and objective standards of reference to the scientific name given to the new species), Other kind of samples (*e.g.* photographs, drawings, call recordings, etc.) can complement the type series.

It is thus essential to master collection techniques, fixation protocols, and collection management. The protocols to succeed in these tasks are explained below.

3.1. Permits

The first step of any biological fieldwork is to obtain appropriate permits to conduct research, including permits to capture, handle and euthanize a number of specimens. Permits to export those specimens from their country of origin will also be required if you plan to take them away or send the material to foreign specialists. This can be a time-consuming and frustrating task, since it is not unusual that the official authorities in charge to grant permits have poor or inadequate knowledge of the biota and/or the ways fieldwork must be completed. Each country has developed its own set of requirements for granting collection and exportation permits. It is essential to comply with local laws and regulations, even when the required documentation seems unreasonable. If the latter is the case good reasoning and communication will usually resolve many problems and might even help to simplify the bureaucracy for future researchers.

Do note that if you are collecting species protected by the Convention on International Trade in Endangered Species (CITES), additional export permits will be required (and usually supplementary fees will must be paid).

In case you plan to work in indigenous land, additional permits might be required to allow you to conduct field research among indigenous communities.

In Guyana the following agencies must be consulted before any biological research is conducted:

The Environmental Protection Agency (EPA), Lot 7 Broad and Charles Street, Charlestown, Georgetown, Guyana.
The Ministry of Amerindian Affairs (MoAA), 251-252 Quamina & Thomas Sts. South Cummingsburgh, Georgetown, Guyana.

3.2. Living in the field

As mentioned above, building a reference collection usually implies spending a long period in the field. This is certainly a very enjoyable part of the research, if you are well prepared. Ill-prepared fieldtrips will usually not yield good results and may sometimes become a true nightmare. Over the many months spent in the field, we have tested a large number of different equipment and we would like to share parts of our experience and preference here.

Here are a few basic tips and tricks that, we hope, will facilitate your fieldwork:

Carrying food and equipment

The amount of material needed during biological field research may be pretty large: a total weight of 250 kg (including food) is not uncommon for a 3-week field trip in remote areas (based on three main investigators total). Most of the time you will rely on the assistance of local inhabitants to help you carry food and equipment. Sometimes you will have to hire boats to reach your final destination.

Solid, waterproof bags that can be easily carried on the back should be used to carry food and most of the material. We have a preference for the *Ortlieb® X-Tremer* dry bags, which are valuable alternative to rigid boxes. They are waterproof, and have shoulder straps that support up to 500 kg (!). When empty,

they are easy to fold flat into a small package. They can be filled with air to ensure protection of fragile equipment. Some of our indigenous counterparts even used them as sleeping bags during very cold nights.

Try to put each specific equipment in a specific bag/container.

For cameras, laptop and other delicate precision devices we use watertight, crushproof and dust proof *Peli*™ cases. These cases even float if your boat flips over. When carrying cameras and DAT recorder out of the base camp we use the waterproof *Dryzone 200* backpack from *LowePro*®.

Comfortable base camps for sweet jungle nights

An important consideration in establishing a base camp is the amount of time you plan to spend in it. You may intend only to spend one or two nights, but you also may plan to stay more than two weeks at the same location. Hammocks are by far the most comfortable way to sleep in the jungle; in addition they avoid contact with the ground and its numerous small inhabitants (see “Hygiene” below). In case of overnight camps or short-time stays, a simple hammock tied between two trees is generally sufficient (Fig. 19A); during rain specimens will be processed on the ground, just below the hammock’s tarp. We recommend *Hennessy Hammock*™, which are light, solid, all-in-one hammocks that include a mosquito net and a tarp. In cases of long-term stays, a larger “solid” camp should be built. We usually build two separate “rooms”; one will house hammocks and people, the other will serve as a “field lab” where specimens will be photographed and processed (Fig. 19B-C).

The location of the base camp is important and the following points should always be considered: (1) proximity of water (for drinking water, washing, etc.), but keep in mind that the area you chose could be flooded in case of heavy downpours; (2) proximity of large dead trees or very high trees with many bromeliads that could fall on your camp in case of a storm or heavy rain. Falling branches and trees are a real hazard in tropical rainforests. If you travel with local companions, always rely on their judgment; they know the place better than you.

In case of camping in savannah or on the summit of tepuis (table-top mountains) where trees and other supports are absent or too small to attach a hammock, light tents are an excellent alternative (Fig. 19D). Note that expeditions on tepuis require robust equipment due to cold temperatures, heavy rains and harsh winds. Products made for extreme conditions are expensive but are the only ones that will ensure more or less comfortable nights.

➔ **Basic equipment needed to set up your base camp:**

- Hammock with mosquito nest (or robust lightweight tent if necessary).
- Large heavy-duty tarps (size will depend on the number of researchers staying in the camp).
- Plenty of solid rope to attach your hammock and fix the tarps.
- Heavy-duty tape to repair potential tears in the tarp.
- Light sleeping bag (nights may be surprisingly cold in the forest).
- Light pillow.
- Machete + file.
- Light foldable seat (an optional luxury).

Fig. 19. Base camps. A. Basic base camp for short-time stays; B-C. Solid base camps for longer stays, note the separated "field lab" on photograph C (front); D. Tents on the summit of a tepui, note solar panels and 12 volts battery to provide electric power. (Photos by P. J. R. Kok).

Energy solutions

We use rechargeable batteries, which are charged through a 12 volts solar battery alimented by two solar panels (Fig. 20). All of our electronic and electric equipment (laptop, satellite phone, recorder, headlamps, etc.) runs thanks to solar power. Rechargeable LR6 (AA) NiMH batteries (used in headlamps, DAT recorder, etc.) are charged with a 15 minutes charger. If you plan to run a laptop, solar panel(s) with a minimum power output of 52 watts is recommended. We chose this solution mainly for ecological reasons and for minimizing our impact

on the environment (no dead batteries, no fuel to run a polluting generating unit). Note that this technology evolves very quickly and many new excellent products can be found on the market. Modern solar panels are very light, foldable, and charge even in cloudy conditions. Some new batteries include an inverter, are very small and lightweight. We have a preference for *Brunton*® products and use two *Solaris*® *26* foldable solar panels.

➔ **Basic equipment needed to provide electric power:**

- Foldable solar panel(s), minimum power output of 52 watts recommended.
- Inverter: necessary to operate your electronic/electric devices.
- Solar controller: prevents overcharging the 12 volts battery and safely permits the battery to remain in constant charging.
- 12 volts solar battery, preferably dry cell.

Fig. 20. Electric power provided by solar panels, here charging the emergency satellite phone. See text for details. (Photo by P. J. R. Kok).

Food and cooking

Adequate food is essential during field research. Do not forget that you will probably walk long distances, sleep little and work hard. We believe that food should always be bought in the country where the research is done, avoiding expensive excess baggage costs, contribute to the local economy instead!

You will need to find a clever compromise between weight and calorific value. We usually take cereal bars, oatmeal instant packs (there are different flavours), cereals, raisins, coffee/tea, sugar cane and dehydrated milk for breakfast; Chinese noodles instant packs, rice, cassava farine, dehydrated soya (called "chunk" in Guyana), onions, garlic, and hot sauce for other meals. When in the field, we usually only eat twice a day, once in early morning and once in late afternoon.

Lighting a wood fire can be somewhat tricky in some wet places and it may be risky to rely on it to cook. Furthermore cooking on wood fire is often time-consuming and maintaining the fire requires time and attention. We prefer to use a liquid-fuel stove and we have a fondness for the *Dragonfly* from *MSR*®, which is lightweight and burns many different fuels (white gas, kerosene, unleaded auto fuel, diesel, and jet fuel). Only a few minutes are needed to boil water and the stove is very fuel-efficient.

➔ **Basic equipment needed for cooking and eating:**

- Multifuel stove and accessories.
- Set of lightweight cookware.
- Robust cups, knifes, and spoons.
- Weatherproof lighter(s).
- Small fuel container.

Water

In tropical rainforests water is everywhere and is usually not a problem. It is recommended that all water be sterilized, especially near local communities, as there might not be a distinction between washing and drinking water. We usually do not disinfect water when travelling in remote areas, but it is always better to do it (gastric problems can ruin your expedition). You can obtain safe water by adding water purification tablets (*e.g. Micropur*). However, we prefer to use Ultraviolet disinfection (*SteriPEN*®) because it is much faster (about 50 seconds for 0.5 litres) and does not give a bad taste to water. You also can collect rainwater using a tarp and adequate containers. We always take an inflatable water container to stock water at the base camp.

Hygiene and clothing

Good hygiene is important in the field, especially to avoid skin problems. Avoid walking barefoot near some local communities, especially those in sandy areas as you have a high chance of collecting sand fleas (*Tunga penetrans*, also called "chiggers"). These parasites may be very painful and must be carefully extracted. Regularly check your body and remove ticks and other parasites as soon as possible as some carry diseases.

Use lightweight clothes that dry quickly, wear long rubber boots in the field (do not forget good socks), and slippers in the camp. Always try to keep a set of dry clothes and use small waterproof bags to pack them. Do not forget a robust rain cap.

Pharmacy and safety equipment

During field research you will probably be out of reach of immediate medical aid, as such some basic safety equipment and drugs from a good pharmacy, and some common sense precautions are thus necessary.

Covering all health hazards is beyond the scope of this manual; you should always carefully check with your doctor for the recommended vaccination and the appropriate medication to carry with you in the field. When possible, use orodispersible tablets as you can take them while walking or if clean water is not immediately available.

Malaria is rampant in many tropical countries and, if contracted, oftentimes kills both residents from local communities and researchers. An adequate malaria prophylaxy is mandatory. Note that antimalarial tablets are often much less expensive in tropical countries.

Leaving your pharmacy to local communities once the field trip is completed is an excellent idea, but this is only valuable if you explain drugs indication and dosage!

➔ **Basic field first-aid kit:**

- Plenty insect repellents.
- Malaria prophylaxy.
- Thermometer.
- Skin suture set.
- Syringes and needles.
- Topical anaesthetics.
- Disposable scalpels.
- Sterile skin closure strips, several sizes (*Steri-Strip*™).
- Tourniquet.
- Sterile compresses.
- Sterile plasters, including blister plasters (*Compeed*®).
- Bactericide aqueous disinfectant.
- Pain tablets (avoid aspirin if there is a malaria risk).
- Allergy relief/allergy symptoms medicine.
- Epinephrine.
- Broad-spectrum antibiotics.
- Antibiotic cream for skin/eyes.
- Antifungal cream.
- Flu medicine.
- Anti-inflammatory cream.
- Elastic bandages.
- Anti-diarrhoea medication/adsorbing preparations.
- Intestinal amoebiasis treatment.
- Non-steroidal anti-inflammatory (also exist in very useful patches).
- Sun blocker.
- Sleeping tablets.
- Tweezers.

➔ **Basic safety equipment:**

- Venom suction pump.
- Survival blanket.
- Satellite phone (optional).

3.3. Specimens and data collection

For further detailed information, we strongly suggest the reader to refer to Heyer *et al.* (1994), which is the most important reference available for researchers interested in measuring and monitoring biological diversity in amphibians. Also see Simmons (2002).

3.3.1. Basic collecting equipment

In order to collect specimens and data you will need some basic equipment (additional specific equipment is provided below, under specific sections).

As most amphibians are nocturnal, a good headlamp is probably one of the most important devices you will need in the field: at night frogs and toads may be spotted by their bright red eyeshine, which is the reflective effect of the *tapetum lucidum*, a reflecting layer found behind the retina that improves vision in low light conditions. We use the *Duo Led 5* from *Petzl*® (with rechargeable batteries, see "Energy solutions" above), which is waterproof down to -5 meters and allows two kinds of lighting: halogen for focused lighting (up to 100m) and LED's for wide, proximity lighting. The *Petzl® Myo XP* is lighter and is an excellent alternative.

Most of the time, amphibians are captured by hand or with a small aquarium net, quickly slamming the net over them.

It sometimes happens that a member of the collecting team detects a frog at a certain distance (by eyeshine detection for instance) that other field investigators cannot locate. While walking through dense vegetation it may be difficult to stay focused on the animal and the specimen may be lost. To circumvent that problem, a member of the team can use a small laser pointer to indicate the frog's position while another investigator goes to collect it.

To avoid getting lost take some colour flagging tape to mark the trails, which will help you find your way back.

For note taking at nights in the camp, we use a small dynamo headlamp (rarely candles) in order to save batteries. If you have a well-charged 12 volts battery, an economic bulb in the camp is helpful.

After capture, medium to large specimens are placed in plastic bags (ziplock bags are quite effective), and small or tiny specimens are transferred in screw-top small containers (urine sample containers are ideal). If possible the field tag should be immediately placed with the associated specimen (see "Field notes and labels" below).

A global positioning system (GPS) is a must that will allow you to record the exact geographic coordinates of your base camps and collecting localities. We use the *60CSx* model from *Garmin*®, which also records altitude.

You will also need some measuring devices to record environmental and specimen data. A thermometer and a hygrometer are the basics, a pHmeter will be useful to record acidity of water in which tadpoles are found. We use callipers for measuring small specimens and measuring tape for larger individuals. Measuring tape is also used to record, for example, distances between animals and water, or distance between the animal and the ground. Spring scales are used to weigh specimens.

➔ **Basic equipment needed for collecting specimens and data:**

- Headlamp, batteries and spare bulbs.
- Dynamo headlamp, candles, or economic bulb to take notes at night.
- Small aquarium net.
- Airtight plastic bags and small containers.
- Shoulder bag to carry the collected specimens.
- Global Positioning System (GPS).
- Colour flagging tape for trail marking.
- Thermometer/Hygrometer, pHmeter (optional).
- Callipers.
- Measuring tape.
- Spring scales (10, 100, and 500 grams are sufficient for amphibians).
- Hand lens.
- Binoculars.
- Machete and knife + file.

3.3.2. Number of voucher specimens required

The number of specimens required to establish identification is variable from one species to another and it is impossible to generalize. Whenever possible, we recommend the minimum of 10 adult voucher specimens from each site; ideally 25 adult specimens should be collected. It is recommended to collect both sexes, juveniles, and larvae. The minimum number of collected larvae should be 20, preferably including different stages of development (see “Sampling of amphibian larvae” for further details).

The number of specimens collected will, of course, depend on the rarity of the species and/or the difficulty to collect species representatives (*e.g.* in case of highly arboreal species or fossorial species). It is usual that even during an extensive survey you will not encounter more than one specimen of a peculiar taxon.

Preparing specimens is time-consuming and you should never collect more specimens than you will be able to handle properly. Fewer well-prepared specimens associated with accurate data are always better than many specimens in poor state of preservation and lacking pertinent information.

Note that collection of rare species may endanger the population: in case of known endangered species or very rare taxa, fewer vouchers should be collected, but at least a single representative should be retained.
It should be mentioned that some species considered as common are curiously poorly represented in museum collections, which for example precludes exhaustive study of intraspecific variation. Thus do not refrain to collect good samples of so-called “common species”.

3.3.3. Field notes and labels

It is crucial that each collected specimen be associated with detailed relevant data. In order to do so, a numbered label (tag) is securely attached to each specimen. Tags should be made of solid paper instead of any hard material (like metal or plastic) that could damage the specimen during transport. Indelible ink or tags with perforated numbers (Fig. 21A) should be used. The use of coloured tags or coloured inks must be avoided as they might discolour the specimens.

In frogs the tag should be attached around the knee (Fig. 21C), or around the waist in very small specimens (Fig. 21D). In caecilians, the tag is attached around the neck or around midbody. The best knot to attach the tag, avoiding that it unties during transport, is probably the surgical double knot (Fig. 21B); make two to be sure. Our numbered tags always include initials of the main investigator (Fig. 21A).

Series of tadpoles, or other very small samples collected together, are preserved separately in screw-top vials (see below) and are kept in a small leakproof plastic bag (we use *Whirlpak*® from *Nasco*), in which the tag is inserted (Fig. 22).

The tag number will be retranscribed in the field book and associated with temporary field identification and detailed data about the collected specimen.

Minimum data associated with the collected specimens are: precise locality (if possible geographic coordinates, which are referenced to map datum WGS84), elevation, date and time of collection, collector’s name, sampling/detection method, general habitat, microhabitat, type of activity before capture and basic weather data (see Fig. 23). It might be difficult to take extensive notes while collecting in the field, especially at night or during heavy rains. An interesting alternative is to use a mini voice recorder to record the data on a tape (digital ones are very small and record on a hard drive) and subsequently report the data in your field book. These recordings are also great back-up solutions.

Field books should be made of solid, all-weather, waterproof material, and waterproof inks must be used (we recommend *Rite in the Rain*® products, Fig. 23). We always use paper with metric grid, which is useful when taking photographs (see below). Pencils are suitable and cheaper alternative to all-weather pens. All data should be saved as soon as possible in electronic format (*e.g.* on a CD or external hard drive) in case you lose or damage your field book. Keep in mind that your field book itself is as valuable as your voucher specimens.

Fig. 21. Tagging voucher specimens. A. Two different types of tags that can be used in the field: printed tag with indelible black ink (upper), and tag with perforated number (lower); B. How to tie a surgical double knot; C. In frogs, tag should be attached around the knee; D. In very small specimens tag should be attached around the waist. (Photos by P. J. R. Kok).

Fig. 22. Series of tadpoles (like illustrated here) and other small samples are packed in small leakproof plastic bags in which the field tag is inserted. (Photo by P. J. R. Kok).

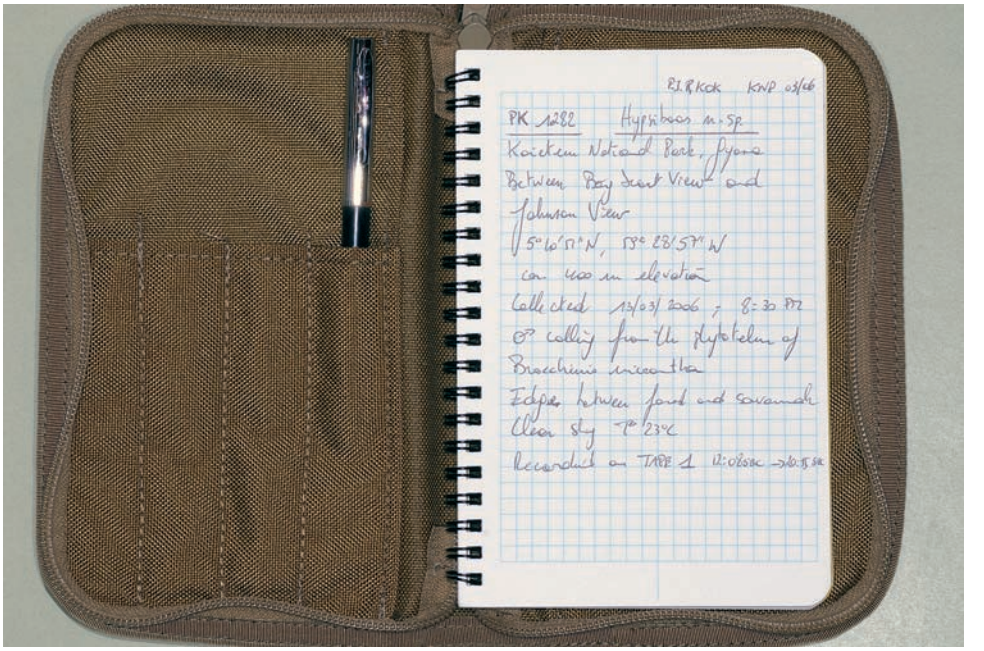

Fig. 23. *Rite in the Rain*® field book and basic notes about a voucher specimen. (Photo by P. J. R. Kok).

➔ Basic equipment needed for labelling your specimens and recording your data:

- Field tags (to be sure, take *ca.* 1000 tags for a three weeks long fieldtrip).
- String to attach field tags to specimens.
- Small scissors to cut strings and tags.
- Small forceps.
- All-weather notebook(s).
- All-weather pen(s) and/or pencil(s).
- Digital mini voice recorder (optional).
- Laptop and external hard drive to back-up your data (optional).

3.3.4. Photography of voucher specimens and habitats

Most amphibians quickly lose their colour in preservative. Sometimes colours may drastically change (*e.g.* the bright green *Phyllomedusa bicolor* becomes purple in preservative, the green *Hypsiboas cinerascens* fades to white). Good photographs of preserved specimens in life are invaluable. They will facilitate the description of colours and patterns, and zooming in the digital picture will help you to distinguish some features that often disappear in preservative (folds, texture of skin, etc.). You will sometimes be surprised to see some details that were completely overlooked in the field.

Photographs should show features used for identification. We suggest taking at least a dorsolateral and a ventral view of each specimen (ventral view on paper with metric grid if possible). We usually take much more photographs of each individual, from different angles, including details of peculiar patterns and/or morphological characters.

Photographs of tadpoles are also very valuable. We usually take photographs of larvae in a Petri dish deposited on a paper with metric grid (the same paper used in our field book, Fig. 24).

Fig. 24. Taking photographs of tadpoles is invaluable, notably to record their colour in life. (Photo by P. J. R. Kok).

When possible, photographs should be taken *in situ*, but this is rarely achievable. We recommend the use of a small tent with a large front opening (Fig. 25A) in which you will reconstitute the microhabitat of the animal. In case the animal tries to escape (which will happen many times!), it cannot disappear in the surrounding vegetation and can easily be secured with a net or a small container.

A good tip to photographing “nervous” specimens is to place an opaque container over them and wait a few minutes. Usually the specimen will stay quiet for some time when you remove the container.

To avoid any confusion between specimens and photographs, we always photograph the tag associated with the specimens before taking photographs of the next specimen. Many digital cameras allow you to assign a peculiar number to each photograph, but we found that method slower and more restricting.

Most recent digital cameras are robust and can be used in the field on condition that it avoids contact with water. Be sure to keep them in waterproof bags or suitcases when you are not using them (see “Carrying food and equipment” above). Always place desiccant in your bags/cases; we use reusable silicagel

placed in small transparent containers that have small holes in the lid. This type of silicagel is blue when dry and becomes pink while wet, so you can easily detect changing conditions and when necessary, the time to replace it. The silicagel will become blue again once exposed to high temperature (on the stove for example), allowing for water evaporation.

A digital reflex body camera with a macro lens is a must to photograph amphibians. A wide-angle lens should be used for habitats and microhabitats. The senior author uses a remote macro flash system that gives excellent results (Fig. 25C). We also carry a small shockproof and waterproof compact digital camera that record short video sequences, which may be useful when observing a peculiar behaviour.

Taking good photographs requires some skills and is time-consuming, but efforts are worthwhile!

➔ **Basic equipment needed for taking photographs of your specimens:**

- Camera body (preferably digital).
- Macro lens.
- Wide-angle lens (a 18-70 mm zoom is ideal).
- Flashes, remote macro flash system is ideal.
- Several memory cards (or film rolls if you run a non-digital camera).
- Memory card reader.
- Batteries.
- Battery charger.
- Compact digital camera allowing recording video sequences (optional).
- Small tent with large front opening.
- Net to secure the animal if it tries to escape.
- Laptop and external hard drive or other media storage device to backup your photos and empty your memory cards (optional if you have plenty memory cards).

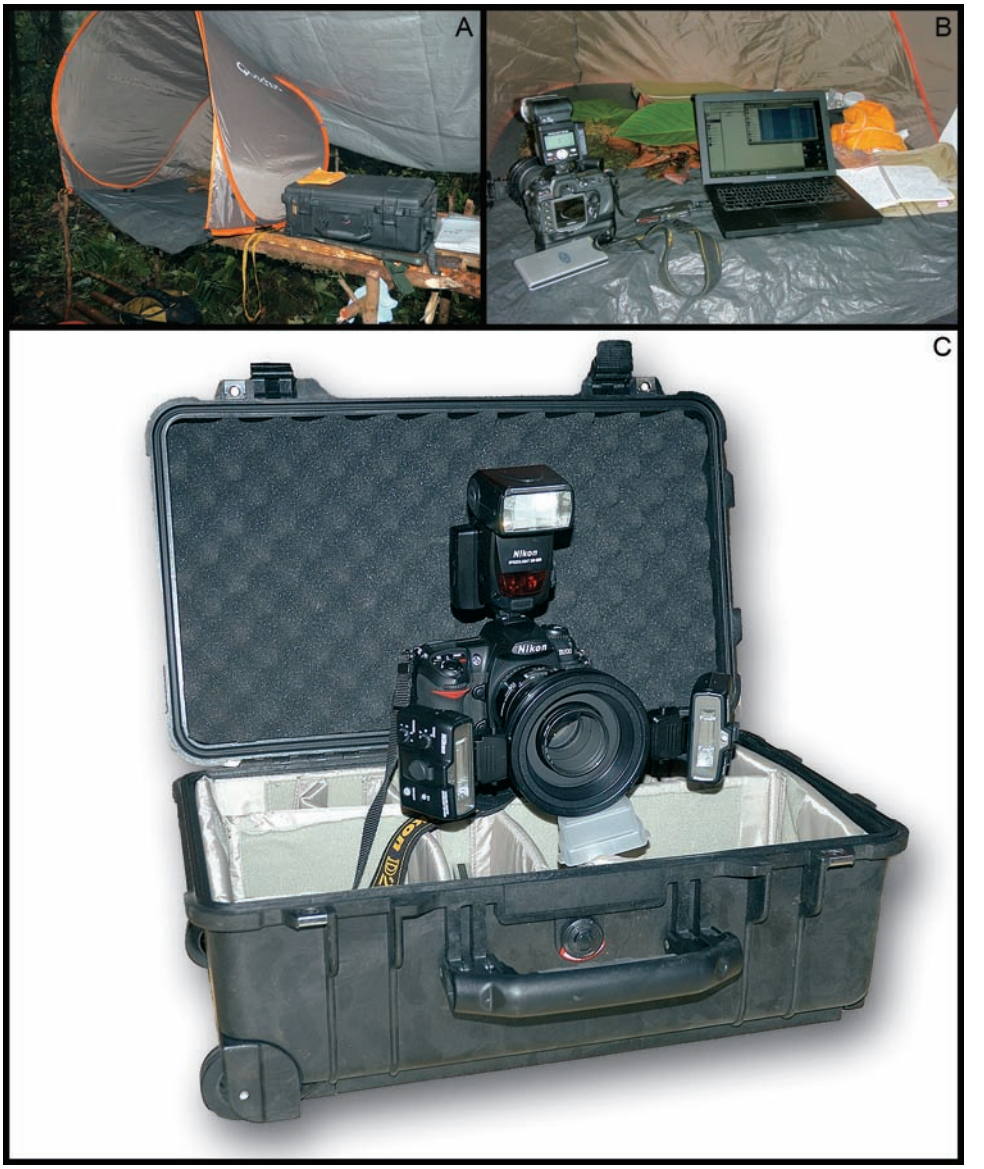

Fig. 25. Taking photographs of specimens. A. Small tent with large opening used as a "field studio"; B. Digital images are downloaded on a laptop right in the field and saved on an external hard drive; C. Digital reflex with macro lens and remote macro flash system as used in the field by the senior author, note the protective *Peli*™ case. (Photos by P. J. R. Kok).

3.3.5. Recording of advertisement calls

Male anuran advertisement calls are species-specific, and bioacoustics analyses of frog vocalizations are invaluable in the discovery of new taxa, assessment of taxonomic rank, and species identification (see “Call analysis” below). Frog recordings can even detect species otherwise thought to have been absent in a specific area. In some studies tape recordings may be used as voucher material.

It can be surprisingly challenging to locate a calling frog or toad. Some species call from beneath leaves or under the ground, and in many cases their calls are so ventriloquial that the position of the calling male is very difficult to estimate.

You should always collect the specimen recorded as a voucher and take associated data as recommended in “Field notes and labels” above; do not forget to include tape identification (name of the person making the recording + tape number) and temperature during recording (see below). One voucher specimen per species per calling site is a minimum.

To acquire quality recordings that will allow you to perform reliable analyses you will need a good recording system that offers very ultra-low distortion levels and is immune to speed errors, tape noise, and non-linear frequency anomalies. Dominant frequency of the advertisement call must be accurately captured and a recorder with a flat frequency response from *ca.* 20-15000 Hz is appropriate. A recording level-meter is mandatory to avoid distorted signals due to too high-level recording. Avoid devices that utilize an audio compression algorithm (like Digital MiniDisc recorders). We recommend DAT recorder, Hi-MD recorder, digital hard drive or solid-state recorders. Using an expensive recorder with a low quality microphone is not recommended. Choose a directional, omnidirectional or a shotgun microphone with no noticeable distortion in the 20-10000 Hz range. Preferably use 30 minutes tapes. Headphones will allow you to evaluate the quality of your recordings in the field and should offer some degree of isolation from ambient noises. We use a *Sony* DAT *TCD-D100* recorder with a *Sony ECM-MS907* microphone with very good results.

Note that temperature variation affects call parameters and a critical step in recording frogs is obtaining temperatures during recordings. If a frog is calling in water, water temperature should be recorded. Do not forget to always carry a waterproof thermometer along with your recording equipment.

Single calling individuals or choruses may be recorded. For the purpose of taxonomic research, recording of calling individuals is required. Frogs should be recorded at distances from 0.5 to 1.5 meters using an appropriate gain level (test gain level before recording). Record at least 5-10 calls from each individual and do not forget to keep recording between calls, this will allow you to know the intercall interval (see “Call analysis” below). Before each recording you should add a voice label giving basic information like the name of the person recording, locality, time, temperature and field identification. Some species are very shy and stop calling if they are only slightly disturbed (by your voice for example), you might thus prefer to report these data in your notebook referring only to the number of the recording. Saying “stop” at the end of each recording will help you to locate different recordings from different individuals. DAT recorders allow to

automatically stamp the current date and time on the tape and allow quick access to the starting points of your recordings thanks to an indexing system. The caveat of digital audio recorders is that they are sensitive to high humidity level and might stop working in very humid environments. We always protect the device in a ziplock bag during recordings and place the recorder in a protective case with desiccant (see above) when not in use.

Some species call only during heavy rain, which creates a lot of background noise. Rain falling on your microphone or on the ground next to the frog may be a problem. We use a small umbrella to avoid that trouble.

➔ **Basic equipment needed for recording frog advertisement calls:**

- Recorder.
- Microphone.
- Headphones.
- Batteries.
- Tapes.
- Ziplock bag to protect the recorder from rain.
- Waterproof thermometer.
- Small light umbrella.

3.3.6. Euthanasia of voucher specimens

Once voucher specimens have been photographed, they must quickly be killed using a humane method of euthanasia. This for evident ethical reasons and practical motivations: specimens humanely killed will be relaxed and much easier to fix in the proper position. Do not expose your specimens to inappropriate handling, temperature extremes or any other undue suffering. Never place living specimens in formalin without prior euthanasia, their agony will be long and painful and specimens could be contracted making further examination problematical.

Many investigators use a chlorobutanol solution in which the animal is immersed. We prefer to use local anaesthetics like lidocaine or similar drugs that have the advantage of not being controlled substances. Also they usually are easily available in pharmacies in most countries, and are available in a wide range of presentations (injection, spray, gel).

Specimens are immersed for a few minutes in the solution, which must be regularly replaced. Note that amphibians are species-specific in their response to anaesthetic chemicals and that some large specimens (*e.g.* large *Rhinella* or *Leptodactylus* species) may require intracardiac or intraperitoneal injection of the solution.

➔ **Basic equipment needed for voucher specimens euthanasia:**

- Syringes and needles.
- Containers for euthanasia.
- Lidocaine or similar drug.

3.3.7. Preservation of voucher specimens

As stated above, good preservation of the voucher specimens will simplify their identification and the description of possible new species; it will also guarantee long-term preservation. Preserving specimens is basically a two-step process: (1) the specimen is fixed in preservative; (2) the specimen is transferred to 70% ethanol for permanent storage. For step 1 we typically use 10% formalin. Pure formalin can be bought in pharmacies or drugstores in many countries and you just will need to dilute it: one part of 100% formalin in nine parts of water will give you a 10% formalin solution. Be careful with formalin because it is irritating, carcinogenic, and very harmful to the environment. Always wear gloves and be careful not to receive projection of the solution in the eyes. This can happen when you inject specimens or simply if a bottle falls on the ground. Formalin in the eyes is a very unpleasant experience and eyes must be immediately washed with water for several minutes. Never abandon formalin in the field.

Ideally the 10% formalin solution should be buffered with magnesium carbonate to avoid acidification or alkalinisation of your fixation solution (use 1/2 teaspoon of magnesium carbonate per litre of 10% formalin). Acidification or alkalinisation will cause excessive discolouration, clearing and/or decalcification of your specimens.

70% ethanol is an alternative fixing solution if formalin is not available.

Step 2 will usually only happen once you are back from the field (see “Collection management” below).

Once the formalin solution is ready you can prepare your fixative trays. We typically use lidded plastic containers of *ca.* 40x25x9 cm (*Really Useful Boxes*®). The bottom of the tray is covered with white tissue saturated with 10% formalin (we use strong cellulose paper or cheesecloth; avoid coloured tissues that could discolour your specimens, see Fig. 26).

Once you are sure that the specimens are killed and completely relaxed – for frog specimens a stimulus on the frog’s eye is a good indicator: if the eye retracts, the frog is still living – you must dispose them in a way that will facilitate measurements and further examination of important morphological characters (webbing for instance, see Figs 26, 27A-B). In case of large specimens, you will need to gently inject them with 10% formalin to be sure that they will not partly rotten. Figure 28 shows multiple injection points, and figure 27C positioning of amphibians for final fixation. We usually attach a tag before fixing the specimen to avoid tags and specimens mixing. However, this is not always feasible, especially in small specimens that will not fix in the right position with the tag. In this case, the tag is deposited on the back of the specimen and will be attached

immediately after fixation. Once your specimens are correctly positioned, cover them with another piece of saturated tissue, gently add a little more of fixing solution and cover the tray.

Fig. 26. Fixative tray. (Photo by P. J. R. Kok).

Fig. 27. Fixation of specimens. A. Hand of a properly fixed frog, note that webbing is easily examined and that measurements will be taken without difficulty (length of Finger III for example); B. Hand of an incorrectly fixed frog, note that measurements could be approximate and examination of webbing difficult; C. Ideal position of a frog in the fixative solution, which will facilitate measurements and further examination. (Photos by P. J. R. Kok).

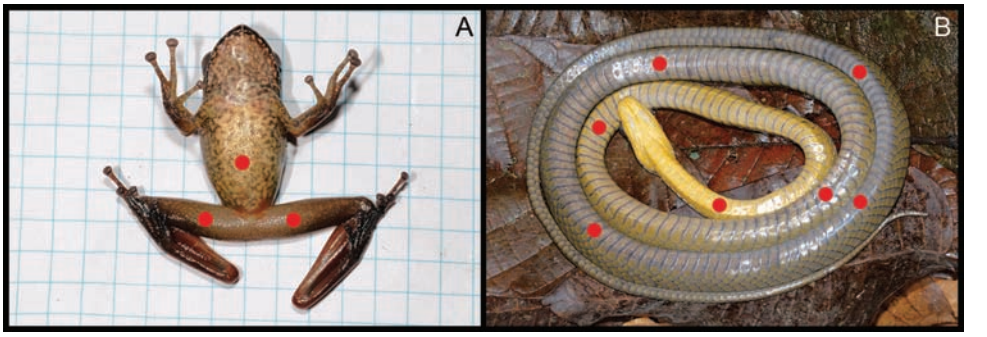

Fig. 28. Formalin multiple injection points (red dots). A. In frog; B. In caecilians (here shown on a snake, in which the same method is applied). (Photos by P. J. R. Kok).

After a few hours or a full day, depending on the size of the animal, specimens are hard enough to be transferred to a container filled with 10% formalin (Fig. 29). Check specimens often to judge when the transfer may occur, but do not be afraid to leave them too long in the trays. Specimens will remain in 10% formalin-filled containers until the end of the field trip. We use different sizes of wide-mouth jars and try to keep together specimens having approximately the same size. Avoid mixing tiny specimens with large ones and be sure to not overcrowd your jars, but do not leave too much space because if you transport the specimens – which will be the case if you move from one location to another with all your equipment – they might be damaged by friction with others. To avoid that, we usually fill the container with soaked tissue, or wrap most fragile specimens with cotton tulle. Fragile specimens can also be kept in separate small vials. Jars and containers must absolutely be kept out of direct sunlight because this will accelerate discolouration, could interfere on the fixation process of the specimens, and could modify the pH of your solution (which will affect your specimens, see above).

Fig. 29. When they are hard enough, specimens are transferred to a container filled with 10% formalin in which they will remain until the end of the field trip. (Photo by P. J. R. Kok).

If you are not a local resident, at the very end of the field trip you will probably need to have your specimens checked by local colleagues before exporting them. This is the perfect occasion to pack them for transport. Good packing of specimens is almost as important as fixation because if you are careless you might have disagreeable surprises (specimens desiccated, distorted, etc.).

The best procedure to pack specimens is the following: use large pieces of formalin-saturated cotton tulle to wrap 1-10 specimens together (again do not mix small specimens with large ones). Once the specimens are wrapped, be sure that the packet is wet enough and transfer it in a leakproof plastic bag (Fig. 30). Close the plastic bag tightly. We usually pack specimens by species and by size to facilitate our work in the laboratory. Avoid overcrowding your plastic bags and be careful that toes and fingers of specimens will not be stressed. We usually slightly inflate the plastic bags for shock protection. Insert the bag in a second plastic bag for security, put all the plastic bags in solid waterproof jars – the same you used in the field for your fixative solution – and add a notice for customs with the following text: “This package contains dead, preserved animals for scientific studies that have no commercial value. If this shipment is inspected, it is absolutely imperative that animals wrapped in wet tissue be returned to and sealed inside the plastic bag. If not, the material will dry rapidly and become useless. We thank you very much for taking good care of this invaluable resource”.

Specimens are now ready to be shipped to the laboratory.

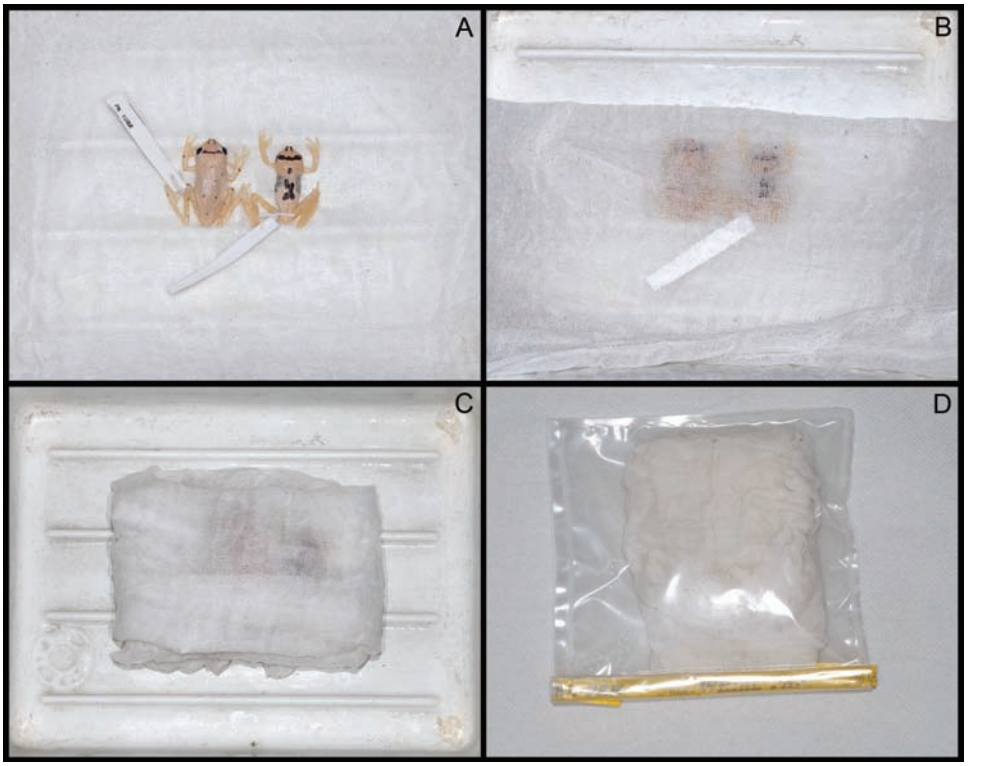

Fig. 30. Packing of specimens for transport. A-C. Specimens are wrapped in formalin-saturated cotton tulle; D. The packet is well soaked and transferred in a leakproof plastic bag. (Photos by P. J. R. Kok).

➔ **Basic equipment needed for preserving and packing voucher specimens:**

- Full-strength formalin (ideal), 70% ethanol (alternative).
- Buffer for formalin (magnesium carbonate).
- Plastic teaspoon.
- Forceps (long and small).
- Dissecting scissors.
- Syringes and needles (various sizes).
- Preserving trays with lids.
- Tissue (strong cellulose paper or cheesecloth).
- Cotton tulle.
- Leakproof plastic bags.
- Nitril gloves.
- Wide-mouth air/watertight bottles for fixation solution.
- Wide-mouth air/watertight jars for fixation solution, storage of large specimens and shipping.

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.

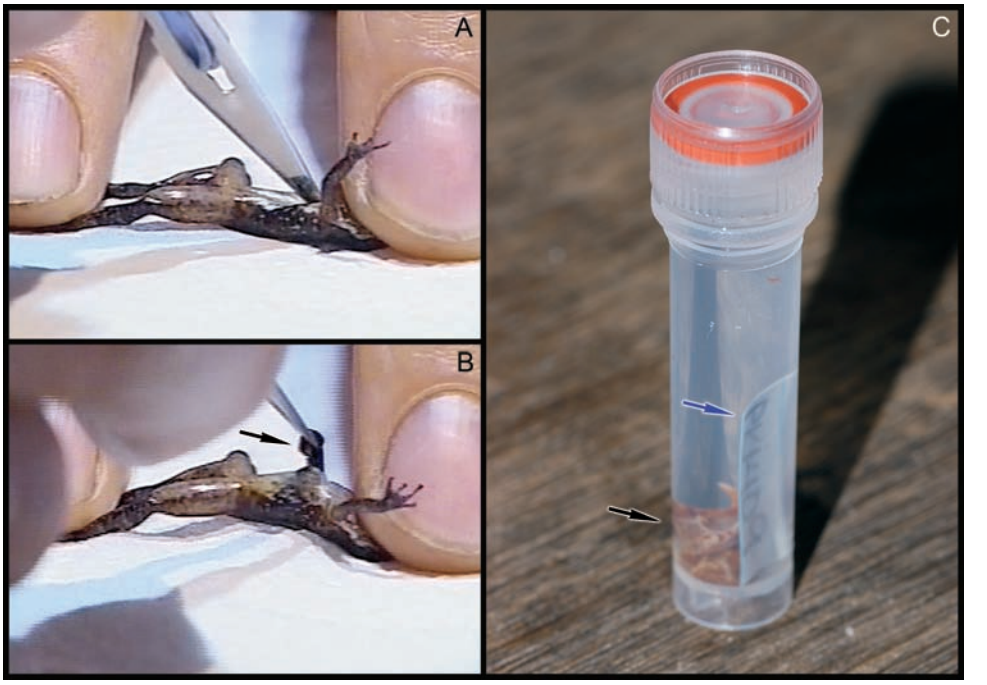

Fig. 31. Collecting tissues for molecular study. A. A small incision is made in the upper part of the abdomen; B. The liver or a piece of it is removed using small bleach-cleaned forceps (black arrow indicates liver); C. Tissue sample is placed in a vial together with a tag bearing the number of the 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).

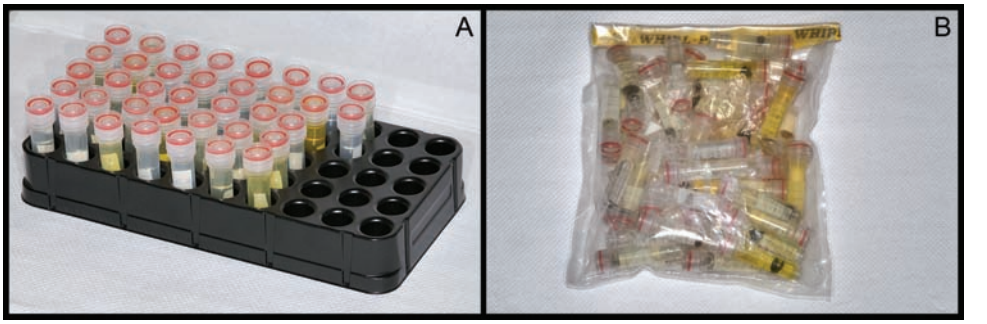

Fig. 32. Tissue samples. A. Vials with tissue for molecular analyses are kept in plastic stands during the process; B. They are packed in leakproof plastic bags for transport. (Photos by P. J. R. Kok).

➔ **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.

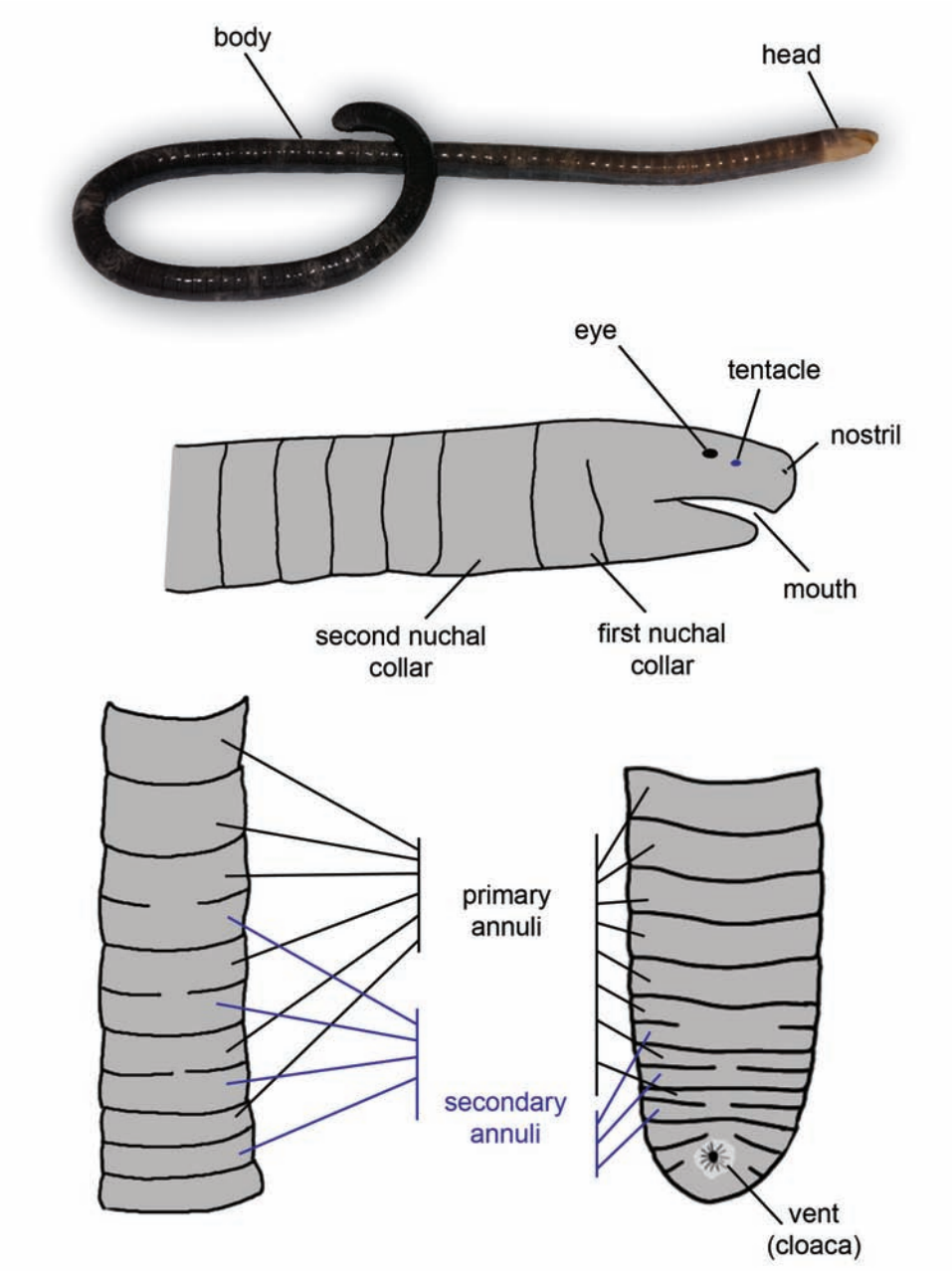

Fig. 35. Caecilian morphology and key morphological characters used in the identification of species. Modified from Taylor, 1968. (Photo by P. J. R. Kok).

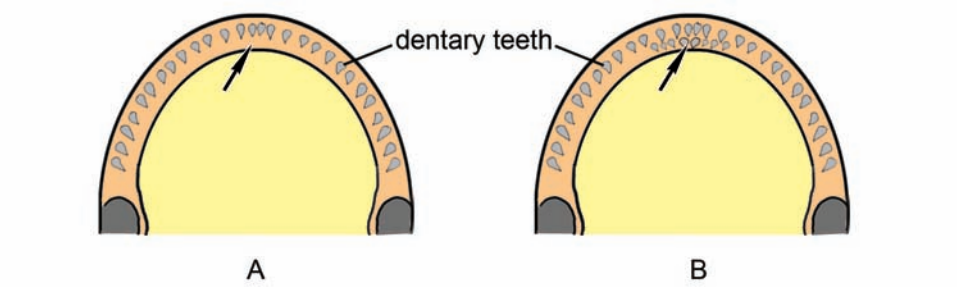

Fig. 36. Diagrammatic view of lower jaw and floor of mouth in caecilians. A. Splenial teeth absent; B. Splenial teeth present. Modified from Savage, 2002.

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).

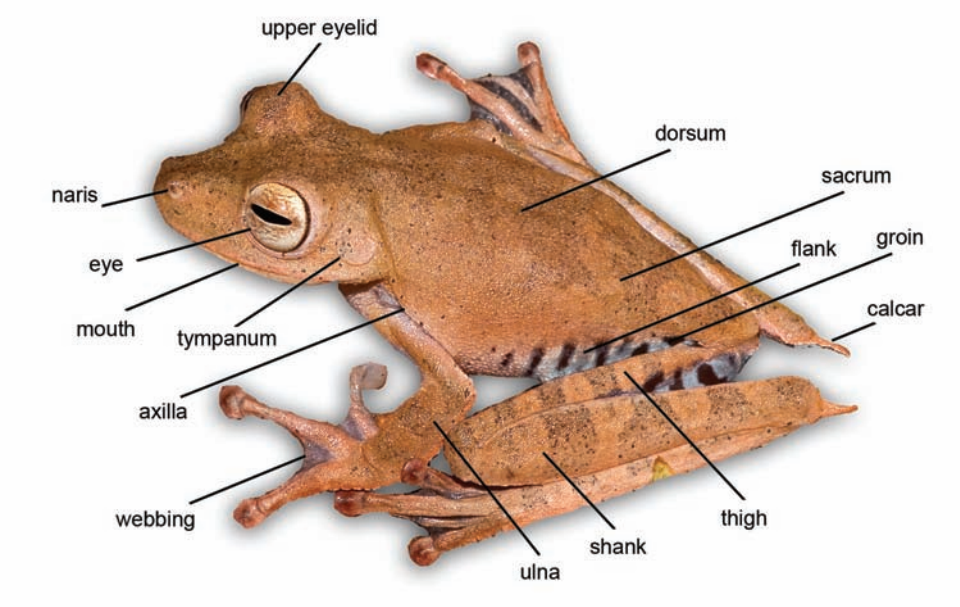

Fig. 37. An adult frog (*Hypsiboas calcaratus*, Hylidae) showing general morphology and features. (Photo by P. J. R. Kok).

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).

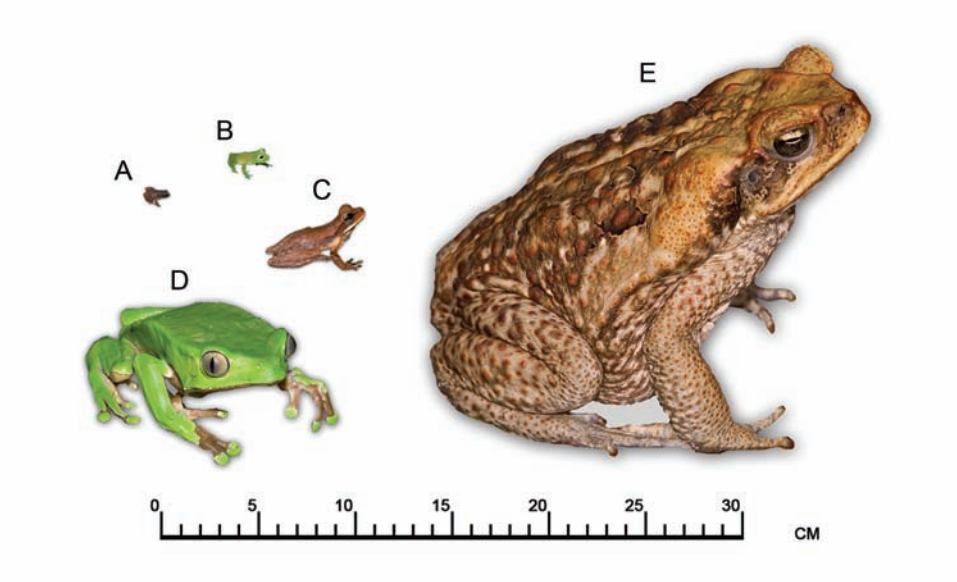

Fig. 38. Relative sizes of anurans in Kaieteur National Park. A. Very small/tiny (< 20 mm), *e.g. Adelophryne gutturosa*, Eleutherodactylidae; B. Small (20-30 mm), *e.g. Cochranella helenae*, Centrolenidae. C; Medium (30-60 mm), *e.g. Tepuihyla talbergae*, Hylidae; D. Large (60-200 mm), *e.g. Phyllomedusa 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.

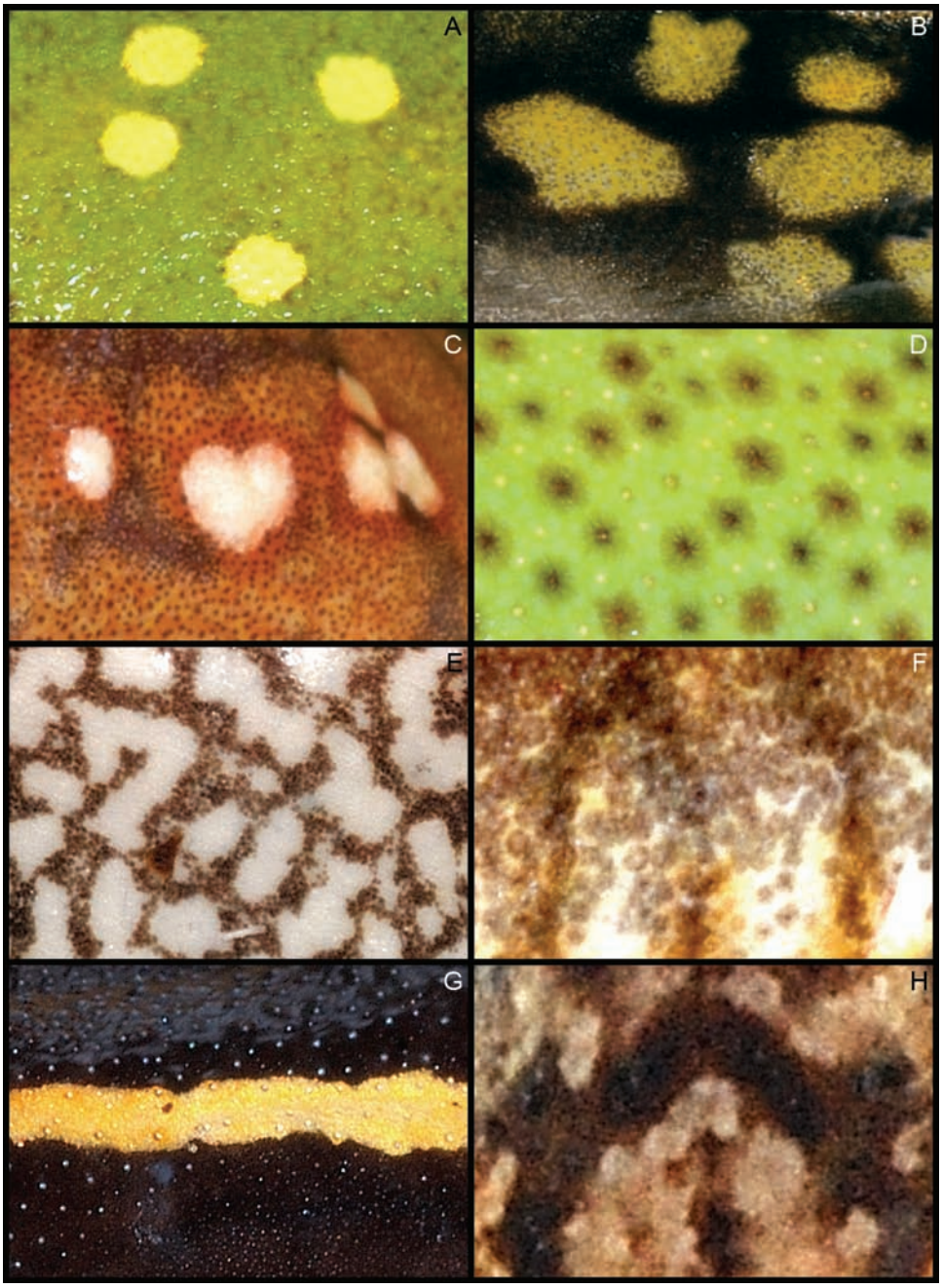

Fig. 39. Principal colour patterns in anurans. A. Spots; B. Blotches; C. Ocelli; D. Speckles; E. Anastomosis; F. Lines; G. Stripe; H. Chevron. (Photos by P. J. R. Kok).

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.

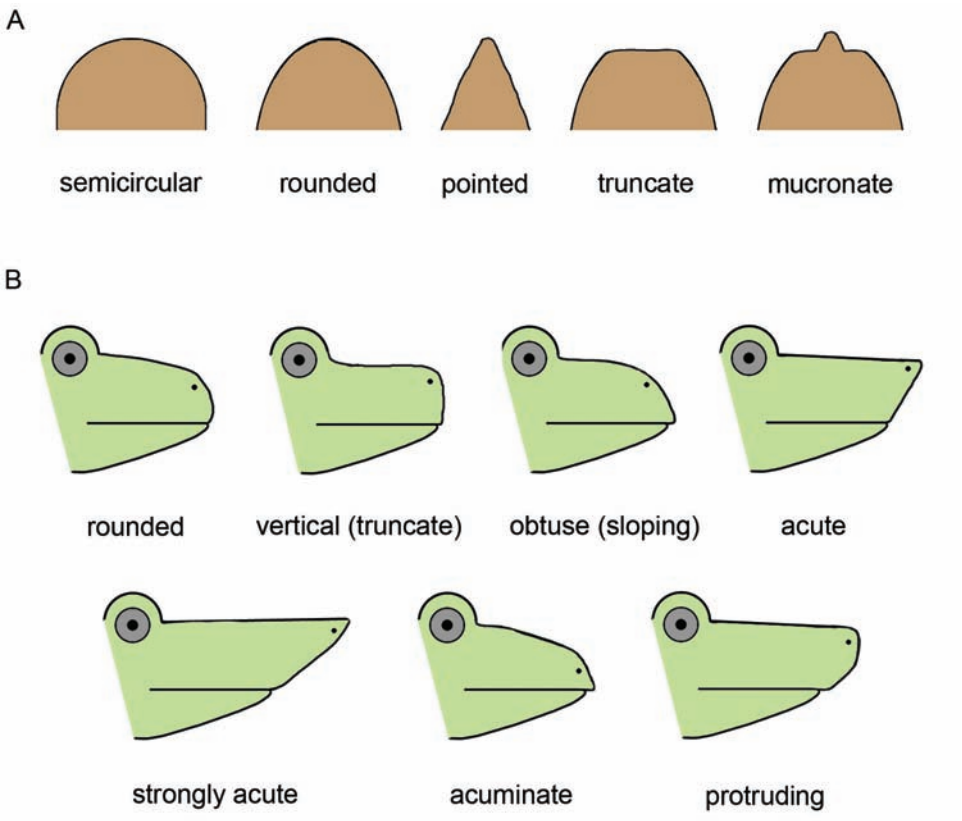

Fig. 40. Diagrammatic views of principal head shapes in anurans. A. Dorsal outline of snout; B. Snout profile. Modified from Heyer *et al.* (1990).

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.

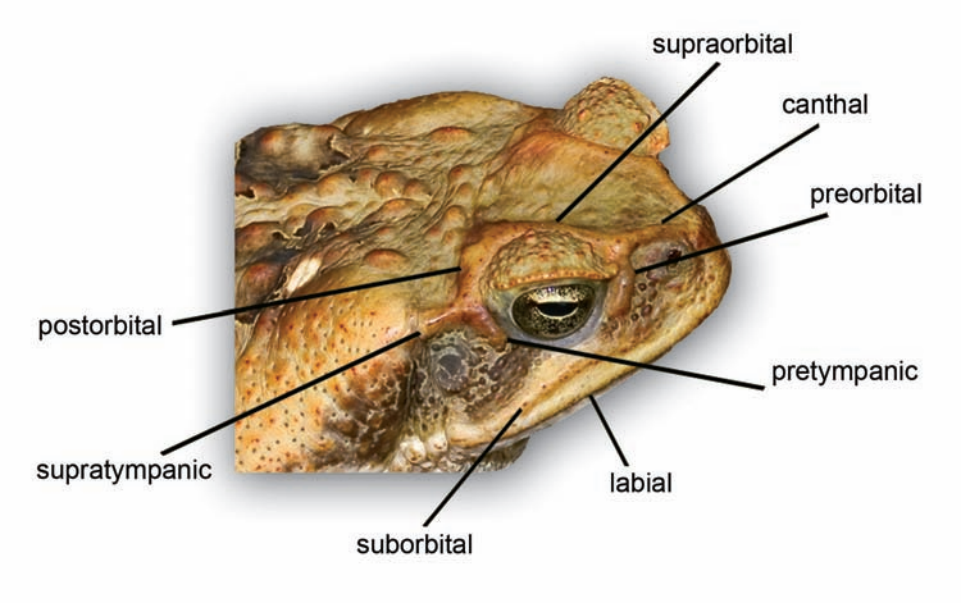

Fig. 41. Cranial crests in the toad *Rhinella marina* (Bufonidae). (Photo by P. J. R. Kok).

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).

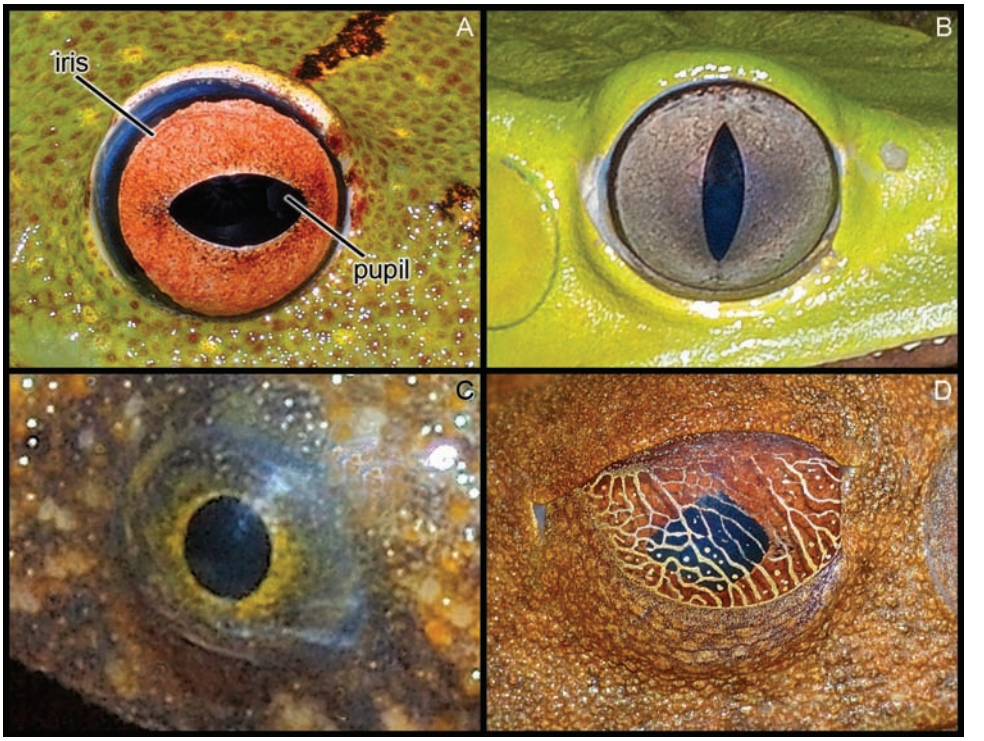

Fig. 42. Shape of pupils and palpebral membrane in anurans. A. Pupil horizontally elliptical (*Hypsiboas cinerascens*, Hylidae); B. Pupil vertically elliptical (*Phyllomedusa bicolor*, Hylidae); C. Pupil circular (*Pipa arrabali*, Pipidae); D. Palpebral membrane with pigmented reticulation (*Hypsiboas geographicus*, Hylidae). (Photos by P. J. R. Kok).

Condition of tympanum

Tympanum may be externally distinct or not, and tympanum condition is sometimes described as: prominent (very distinct with tympanic annulus prominently ringing the well visible tympanum), distinct (tympanum well visible, but tympanic annulus less visible), indistinct (tympanic annulus not visible, upper tympanum barely visible), very indistinct (tympanic annulus not visible, most tympanum barely visible) or absent (no tympanic annulus and tympanum visible). Most of the time the tympanum is described as distinct (Fig. 43A), indistinct (Fig. 43B) or absent (Fig. 43C). Note that this character is prone to post-mortem and preservation artefact.

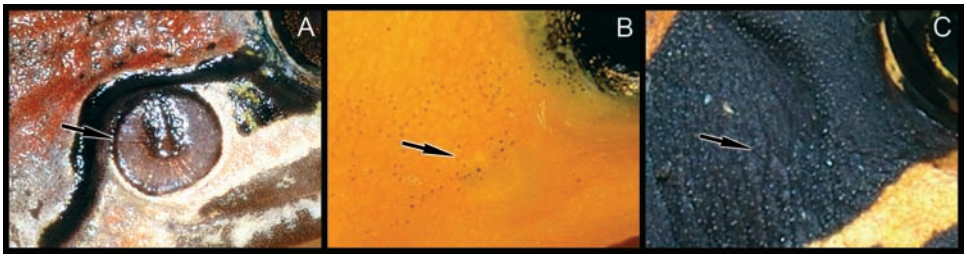

Fig. 43. Condition of tympanum in anurans (eye is in the right upper corner). A. Distinct (*Leptodactylus longirostris*, Leptodactylidae); B. Indistinct (*Anomaloglossus beebei*, Aromobatidae); C. Absent (*Atelopus hoogmoedi*, Bufonidae). (Photos by P. J. R. Kok).

Texture of skin

Texture of dorsal skin is of considerable taxonomic importance. Skin texture is very variable in anurans and can mostly be described as:

- **Smooth**: free from projections (Fig. 44A).
- **Shagreened**: rough to the touch, covered with numerous very small close-set tubercles (Fig. 44B).
- **Granular**: bearing small, rounded, relatively flat grains of approximate equal size (granules) (Fig. 44C).
- **Tuberculate**: bearing rounded bumps of various sizes (tubercles) with no keratinized tip (Fig. 44D).
- **Spiculate**: bearing small pointed tubercles, often with keratinized tip (Fig. 44E).
- **Warty**: bearing protuberances of various sizes, often with keratinized tip (Fig. 44F).
- **Areolate**: skin covered with circular, closely-set, barely elevated protuberances (Fig. 44G); a condition most often found on the flanks or the venter.

There is some variation among these textures, and adverbs like weakly, finely, coarsely, thickly, etc. are often used to refine the description of the skin.

Some species exhibit a combination of skin textures (the dorsum may be shagreened anteriorly and granular posteriorly like in some *Anomaloglossus* for example, Fig. 44H).

Note that skin texture is prone to post-mortem and preservation artefact and may be difficult to appreciate on preserved specimens.

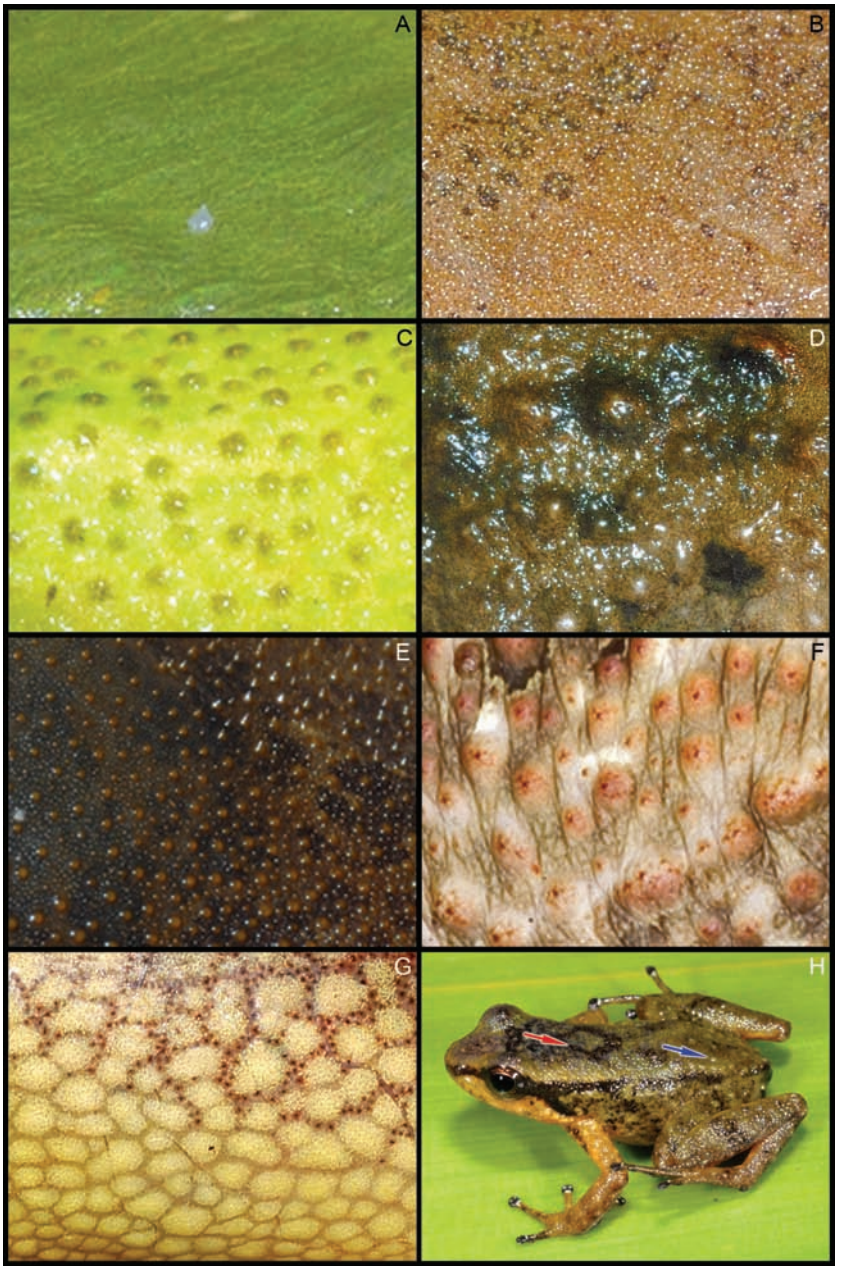

Fig. 44. Principal skin textures in anurans. A. Smooth (*Phyllomedusa bicolor*, Hylidae); B. Shagreened (*Hypsiboas calcaratus*, Hylidae); C. Granular (*Hypsiboas liliae*, Hylidae); D. Tubercular (*Leptodactylus petersii*, Leptodactylidae); E. Spiculate (*Pipa arrabali*, Pipidae); F. Warty (*Rhinella marina*, Bufonidae); G. Areolate (flanks of *Osteocephalus leprieurii*, Hylidae); H. Combination of skin textures in *Anomaloglossus* cf. *roraima* (Aromobatidae), a species that does not occur in KNP (red arrow shows shagreened skin on anterior dorsum, blue arrow shows granular skin on posterior dorsum). (Photos by P. J. R. Kok).

Presence or absence of an axillary membrane

The axillary membrane is a skin membrane that may occur at the posterior insertion of the upper arm (= axilla or armpit) (Fig. 45). It is characteristics of some species and may be more or less developed.

Fig. 45. Axillary membrane in anurans. A. Absent (*Osteocephalus leprieurii*, Hylidae); B. Present (*Dendropsophus marmoratus*, Hylidae). (Photos by P. J. R. Kok).

Presence or absence of dermal folds and fringes

A number of variously visible folds in the skin may occur on the anuran body and limbs: dorsolateral fold, middorsal fold, lateral fold, supratympanic fold, ulnar fold, tarsal fringe, etc. (see Fig. 46 for location of the principal fringes and folds). Folds may be interrupted or not and more or less elevated. A relatively developed ventral discoidal disc (thickening of ventral integument) may be visible in some species.

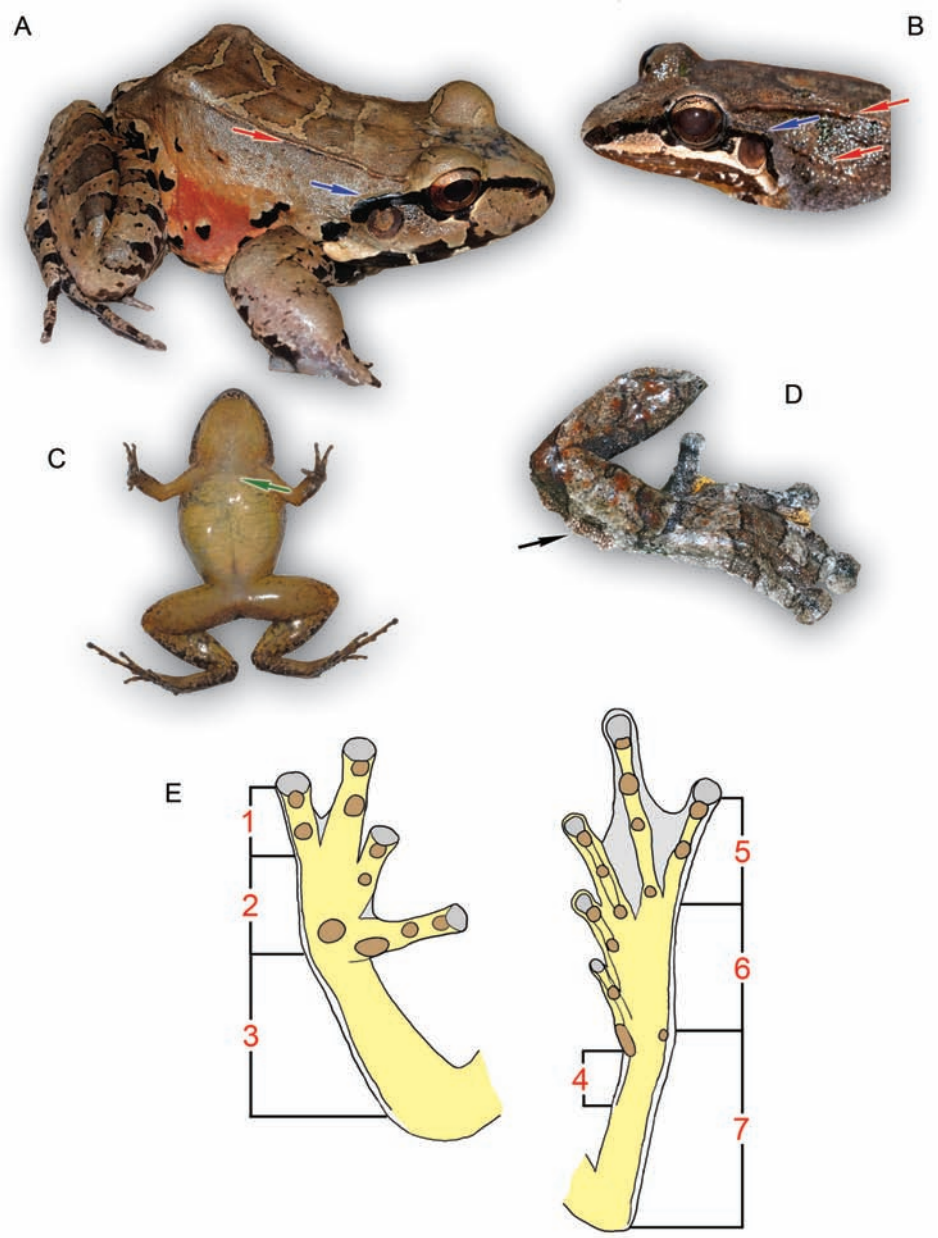

Fig. 46. Principal fringes and folds in anurans. A. Dorsolateral fold (red arrow) and supratympanic fold (blue arrow) in *Leptodactylus knudseni*, Leptodactylidae; B. Supratympanic fold (blue arrow) and dorsolateral and lateral folds (red arrows) in *Leptodactylus longirostris*, Leptodactylidae; C. Pectoral (= thoracic) fold (green arrow) in *Leptodactylus lutzi*, Leptodactylidae; D. Ulnar fold (black arrow) in *Dendropsophus marmoratus*, Hylidae; E. Fringes and folds on arm and leg: (1) fringe on postaxial edge of Finger IV, (2) metacarpal fold, (3) ulnar fold, (4) tarsal fringe, (5) fringe on postaxial edge of Toe V, (6) metatarsal fold, (7) tarsal fold. (Photos A-D by P. J. R. Kok; E modified from Kok & Castroviejo-Fisher, 2008).

Presence or absence of glands

Parotoid glands and other small glands may be visible on the skin (Fig. 47); some of them produce toxins (*e.g.* parotoid glands), others are used in defensive postures (*e.g.* inguinal glands).

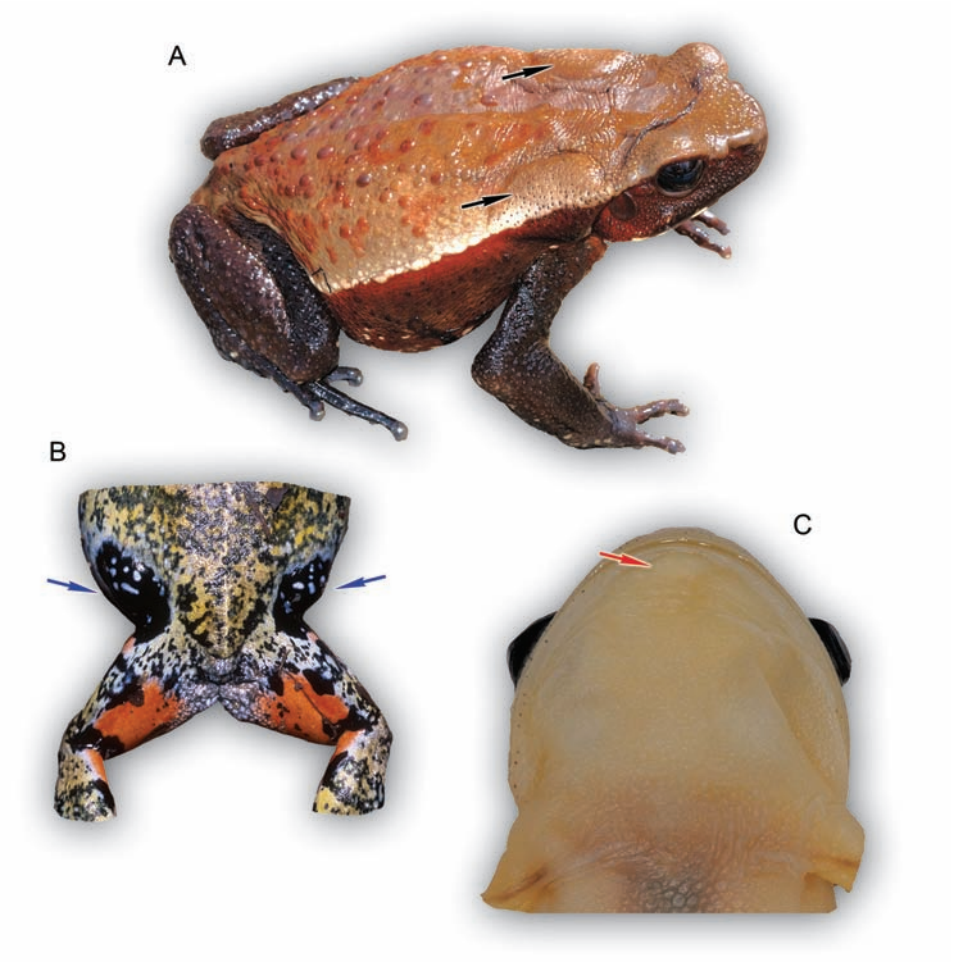

Fig. 47. Some glands found in anurans. A. Parotoid glands (black arrows) in *Rhaebo guttatus*, Bufonidae; B. Inguinal glands (blue arrows) in *Pleurodema brachyops*, Leiuperidae (note: this species does not occur in KNP); C. Mental gland (red arrow) in *Hypsiboas cinerascens*, Hylidae. (Photos by P. J. R. Kok).

Palmar structures

Figure 48 shows main palmar structures, which involve various tubercles, fringes, folds (see also Fig. 46), and the presence or absence of a visible prepollical spine. See Fabrezi (2001) for prepollex and prehallux variation in anuran limbs.

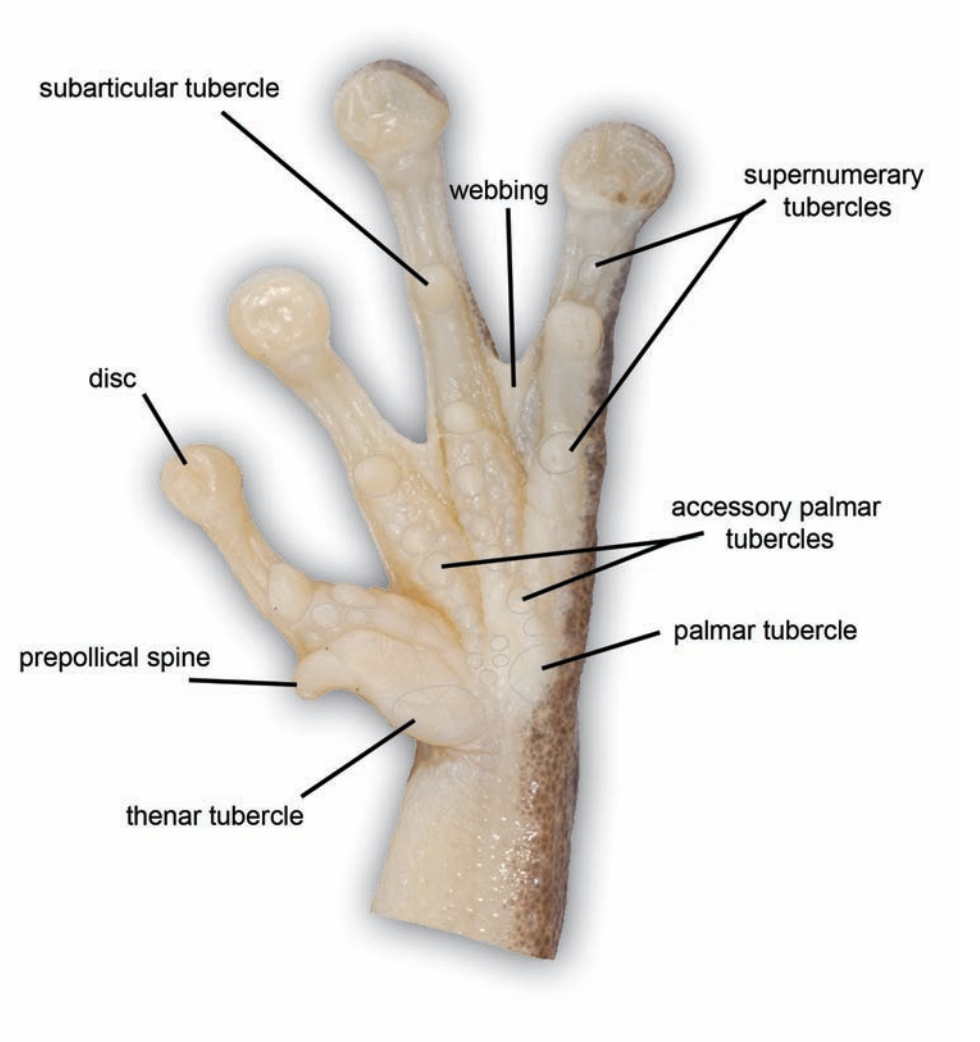

Fig. 48. Palmar structures in anurans. (Photo by P. J. R. Kok).

Degrees of webbing on hand and foot

Similar species may be distinguished by the amount of finger and/or toe webbing they possess. Although some authors (see Edwards, 1974; La Marca, 1997) proposed different terminologies, the most widely used system for webbing formula follows Savage & Heyer (1967), with modifications proposed by Myers & Duellman (1982) and Savage & Heyer (1997). Recently, Guayasamin *et al*. (2006) slightly refined the system for centrolenid frogs.

The degree of webbing is described in enumerating phalanges (including metacarpals and metatarsals) that are free of webbing. Each finger and toe is represented by a Roman numeral and the number of phalanges completely or partially free of webbing by an Arabic numeral (Fig. 49). A notation of "0" indicates that the web extends to the disc, while "1" indicates that the web extends to the intercalary tubercle (distal, just below the disc). A "+" indicates that the web reaches the proximal margin of the structure (tubercle or disc), a "-" indicates that the web reaches the distal margin of the structure, and no superscript means that the web reaches the middle of the structure. Fractions are used when the web does not reach a structure, but only a point between two structures: for example "1/2" when half of the phalanx is free of webbing, "1/3" when the distal one-third of the phalanx is free of webbing, "2/3" when the distal two-thirds of the phalanx are free of webbing, etc.

Note that webbing may be somewhat variable intraspecifically and that females may have slightly more webbing than males.

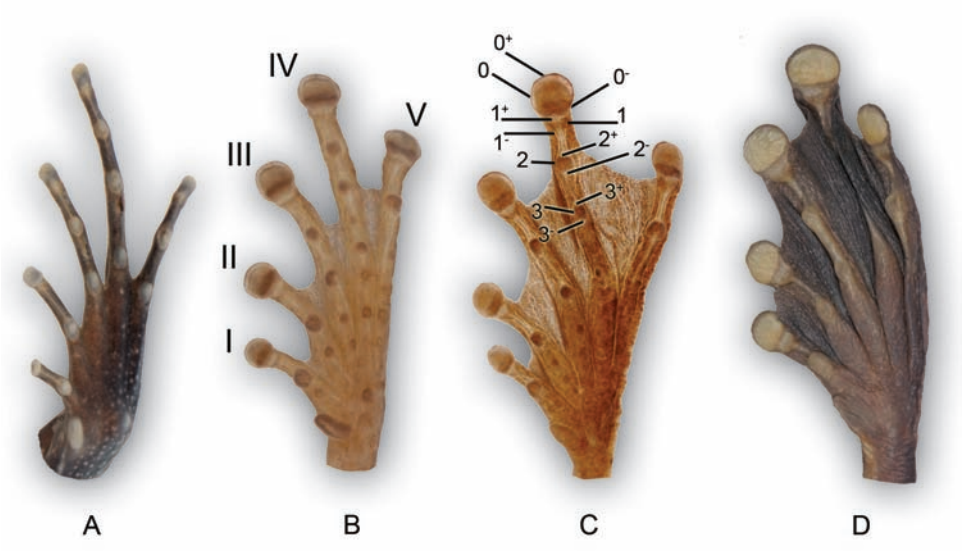

Fig. 49. Degrees of webbing in anurans. A. Unwebbed; B. Basally webbed; C. Half-webbed; D. Fully webbed. (Photos by P. J. R. Kok).

Plantar structures

Figure 50 shows main plantar structures, which involve various tubercles, fringes, and folds (see also Fig. 46).

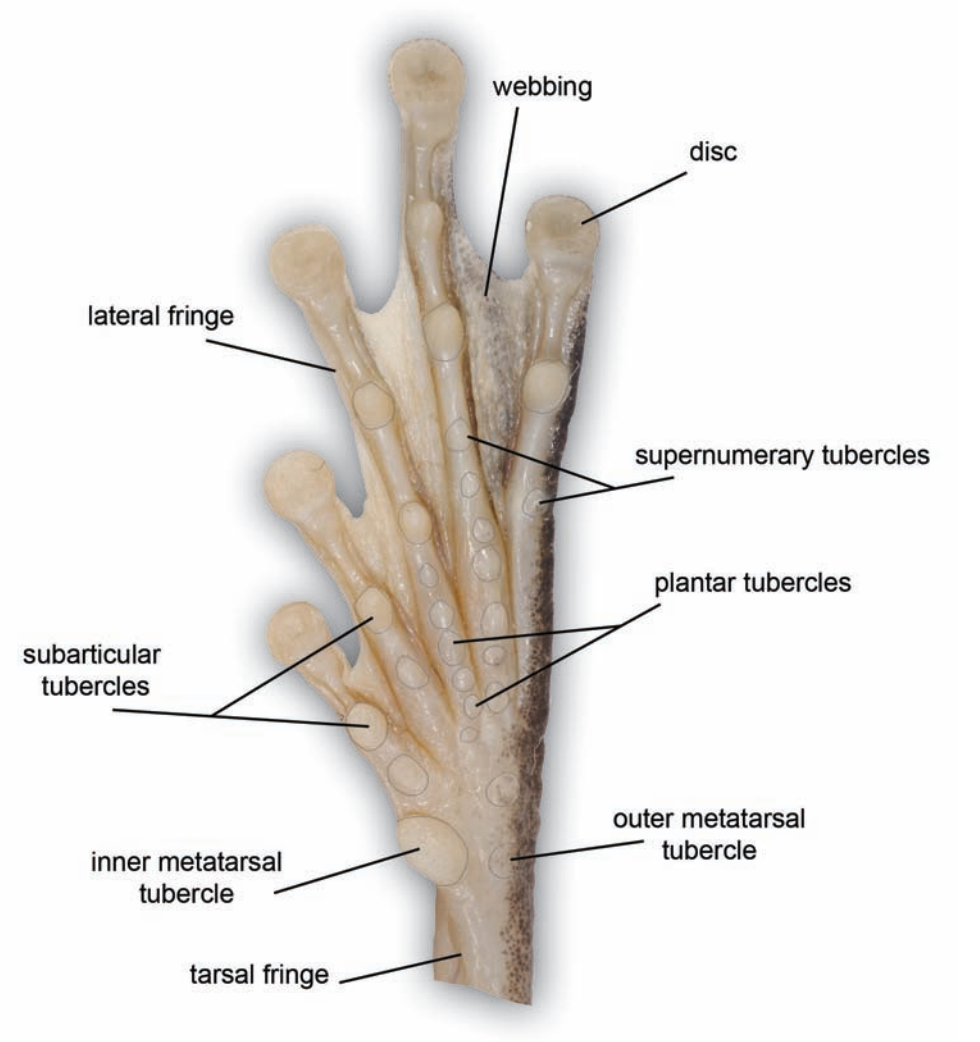

Fig. 50. Plantar structures in anurans. (Photo by P. J. R. Kok).

Structure of digital discs and subarticular tubercles

Variation in external digital features is of taxonomic importance. Digital disc structure is very variable (and not related to the shape of the distal phalanx); figure 51 shows some common shapes (see Savage, 1987 for additional digital disc character states).

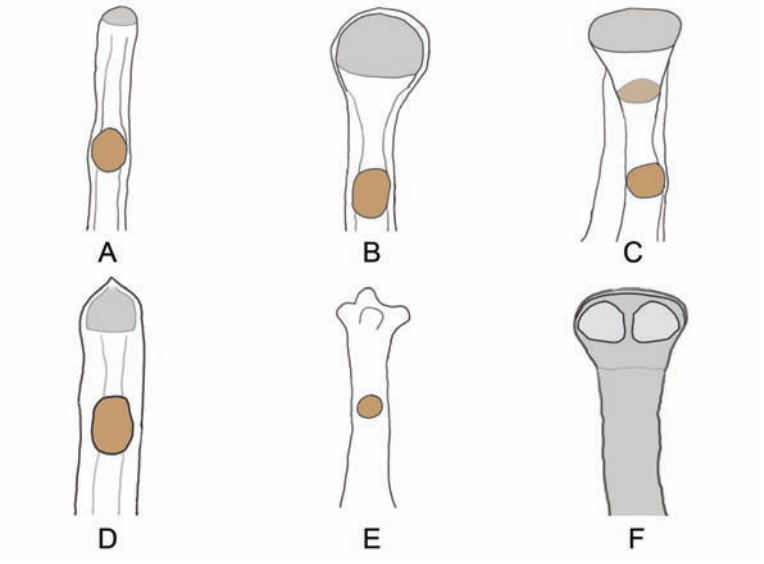

Fig. 51. Diagrammatic views of main structures of digital disc and tip of digit in anurans. A. Disc unexpanded *(e.g.* in *Leptodactylus petersii*, Leptodactylidae); B. Disc expanded, broadened (*e.g.* in *Hypsiboas liliae*, Hylidae); C. Disc expanded, truncate (*e.g.* in *Allophryne* and some glass frogs); D. Disc not, or slightly, expanded with pointed tip (*e.g.* in *Adelophryne gutturosa*, Eleutherodactylidae); E. No terminal disc, but four minutes lobes (*e.g.* in *Pipa arrabali*, Pipidae); F. Dorsal surface of finger disc with two scutelike flaps (*e.g.* in *Anomaloglossus*, Aromobatidae).

Presence/absence and structure of the distal subarticular tubercle on the fourth finger is also variable and helpful for identification (Fig. 52).

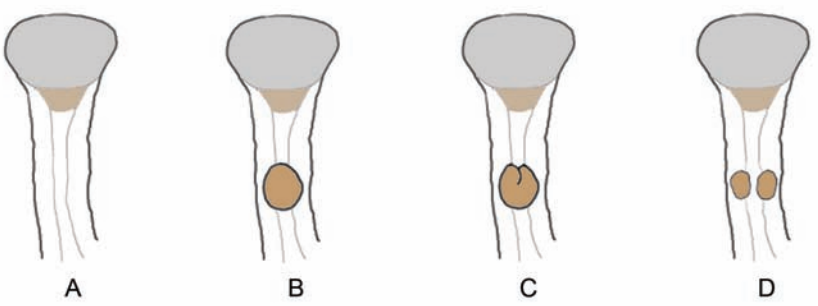

Fig. 52. Diagrammatic views of structures of distal subarticular tubercle on Finger IV. A. Absent; B. Single; C. Bifid; D. Divided. Modified from Duellman, 1970.

Buccal structures: condition of odontophores, and shape of tongue

Maxillary teeth (= teeth that are on the maxilla) may be absent (*e.g.* in Allophrynidae and Bufonidae) or present, in which case they may have various shapes that are characteristic and helpful for identification.

The absence or presence of odontophores (= the portion of the vomer bearing the vomerine teeth) and their shape and position is also of taxonomic importance (Fig. 53). The number of vomerine teeth is usually related to the age of the frog and juveniles may lack vomerine teeth or have only a few while adults of the same taxon may have very distinct odontophores bearing numerous teeth.

Shape of choanae (singular choana) and interchoanal distance is also considered of taxonomic importance in some genera, but this character may be intraspecifically variable.

Shape of vocal slits is variable with taxa and may also help for identification (see below “Condition of vocal sacs”).

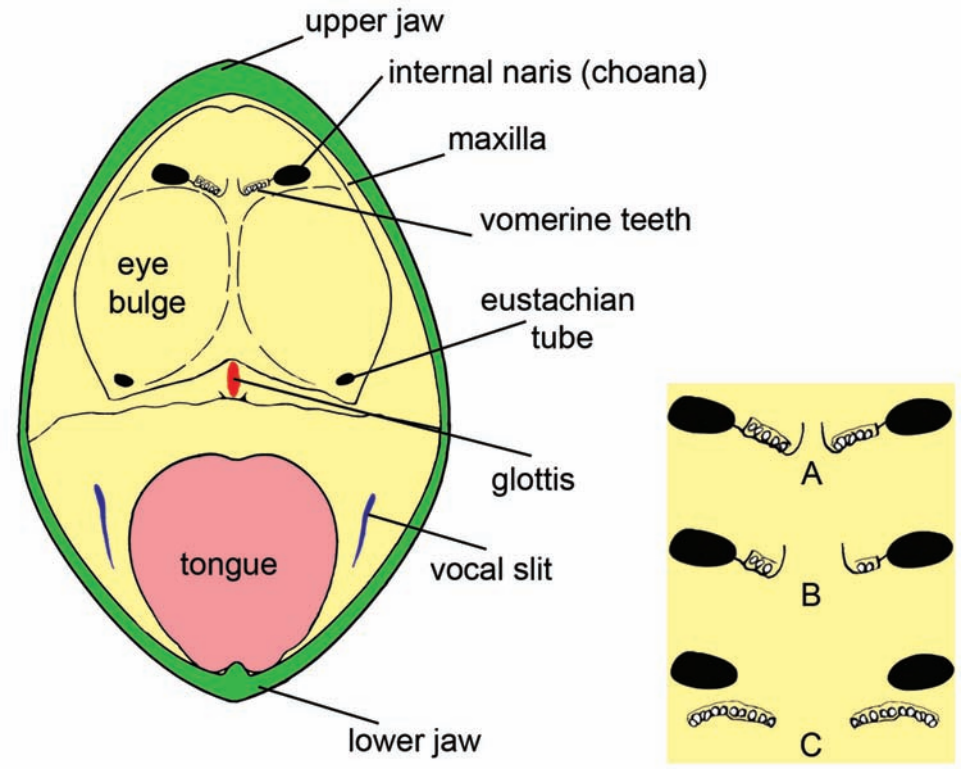

Fig. 53. Generalized diagrammatic view of anuran buccal cavity showing principal structures and some conditions of odontophores. A. Odontophores oblique and barely separated, between choanae. B. Odontophores oblique and widely separated, between choanae. C. Odontophores arched and widely separated, below choanae. Modified from Duellman & Trueb, 1986.

Shape of tongue is also variable with taxa and is of some taxonomic importance. Figure 54 shows the principal shapes of tongue in anurans. Note that this character is prone to post-mortem and preservation artefact.

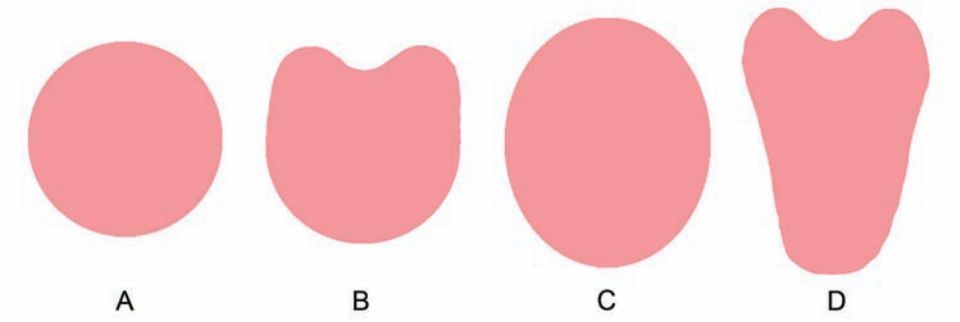

Fig. 54. Diagrammatic views of principal shapes of tongue in anurans. A. Round; B. Cordiform; C. Ovoid; D. Lanceolate. Modified from Duellman, 1970.

Morphometrics

Morphometric comparisons, including comparison of relative length of fingers (*e.g.* relative length of Finger I versus Finger II, or relative length of Toe III versus Toe V), and relative position of various structures (*e.g.* the relative position between the tip of Finger II and the distal subarticular of Finger III when Finger II and III are adpressed together, or the relative position between the tibiotarsal articulation and the tip of snout when hindlimb is adpressed along the body) are helpful to distinguish similar species.

The use of statistics and comparison of measurement ratios are also invaluable in many cases.

It is thus mandatory to take a number of measurements in order to compare species' morphometry. Principal landmarks are indicated in figure 55 and are defined below:

- **Snout-vent length** (SVL): from the tip of the snout to the posterior margin of the vent.
- **Head length**: from the posterior edge of the jaw (sometimes from the posterior edge of the tympanum) to the tip of the snout.
- **Head width**: the greatest width of the head, usually at the level of the anterior edges of the tympani, sometimes at the level of the angle of jaws.
- **Eye-naris distance**: from the posterior edge of the naris to the anterior edge of the eye.
- **Eye length** (= diameter): the greatest length of the orbit from the anterior margin to the posterior margin of the eye.
- **Tympanum length** (= diameter): the greatest length of the tympanum from the anterior margin to the posterior margin of the tympanum.

- **Eyelid width**: the greatest transverse width of the upper eyelid.
- **Interorbital distance** (IOD): the distance between the median margins of the orbits.
- **Internarial distance** (IND): the distance between the median margins of the nares.
- **Snout length**: from the anterior margin of the eye to the tip of the snout.
- **Hand length**: from the proximal edge of the palmar tubercle to the tip of Finger III.
- **Upper arm length**: from the margin of the body insertion to the tip of the elbow.
- **Forearm length**: from the tip of the elbow to the proximal edge of the palmar tubercle.
- **Thigh length**: from the vent to the outer edge of the flexed knee.
- **Shank length**: from the outer edge of the flexed knee to the tip of the heel.
- **Tarsus length**: from the heel to the proximal edge of the inner metatarsal tubercle.
- **Foot length**: from the proximal edge of the inner metatarsal tubercle to the tip of Toe IV.
- **Width of disc** (usually on Finger III and Toe IV): the greatest width of the disc.

Remark: taking precise, comparable, measurements in amphibians is almost impossible due to the soft and flexible nature of preserved amphibians (see Hayek *et al*., 2001). The value of the measurements used in morphometric studies is also closely related to the quality of the preservation of the specimens and the training level of the observer. Hayek *et al*. (2001) pointed out that intra- and interobserver differences in measuring specimens are recurrent and can lead to statistically significant differences in the variables, which may result in different biological interpretations. They suggested several recommendations to use in frog morphometric studies (*e.g.* remeasure at least one individual 20 times for estimation of measurement error) and we encourage the reader to refer to that publication.

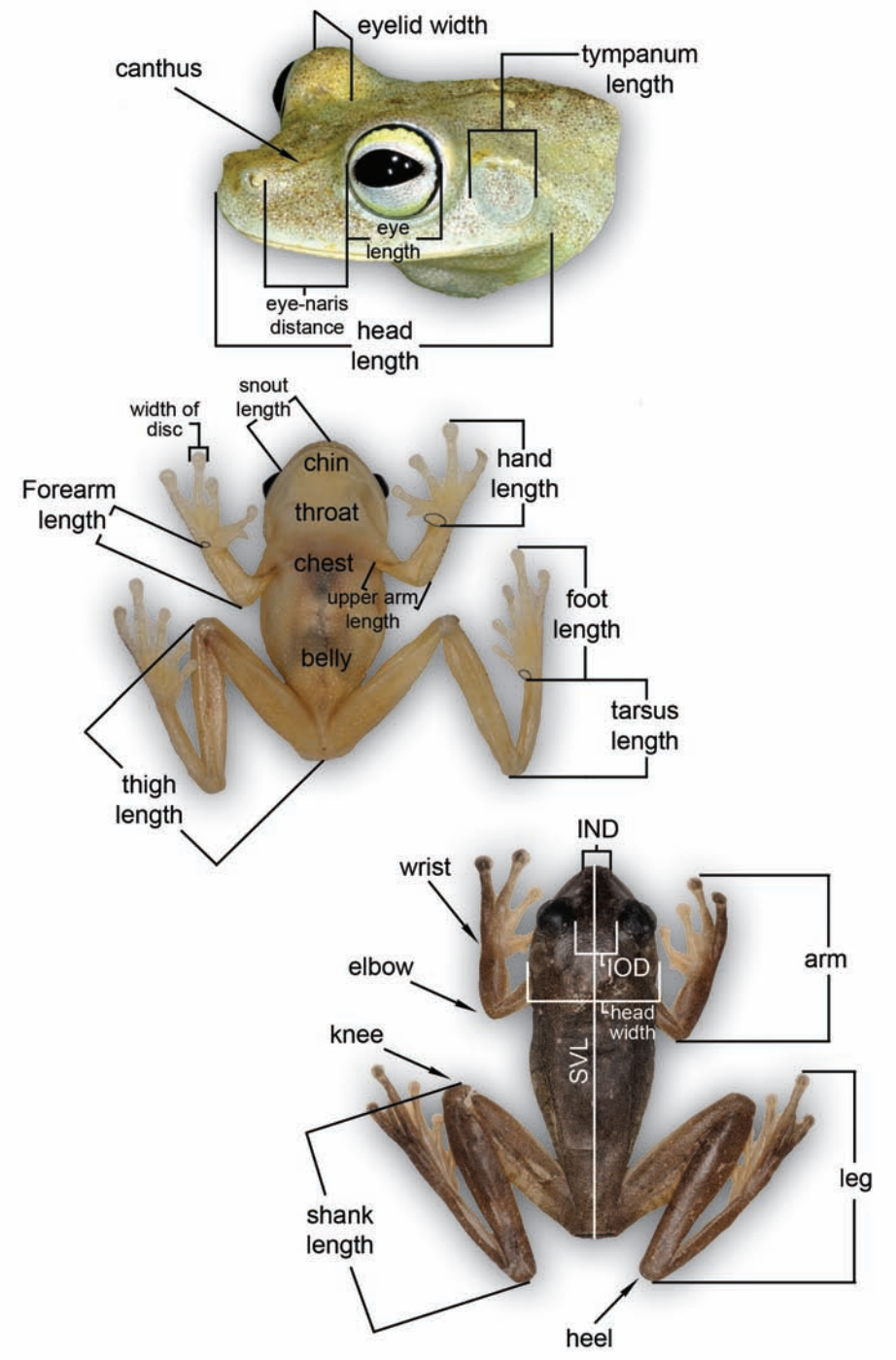

Fig. 55. Main terms and landmarks in anurans. Abbreviations are explained in the text. (Photos by P. J. R. Kok).

The following external morphological diagnostic features are secondary sexual characters found only in males:

Condition of vocal sacs

The vocal sac(s) communicates with the buccal cavity via two small apertures called the vocal slits, which may be round or slitlike and variously elongated (see Fig. 53). The skin covering the external vocal sac is usually modified and it is possible to discern some dermal lobes or folds. In some species males lack an external vocal sac, in this case the skin covering is totally unmodified. Some species completely lack vocal sac and vocal slits (*e.g. Stefania* spp., Hemiphractidae). Vocal sacs may be subgular (single, bilobate, or paired) or lateral (paired) (Fig. 56).

The pulsating sac may increase the attractiveness of advertisement calls in some species (see Rosenthal *et al.*, 2004).

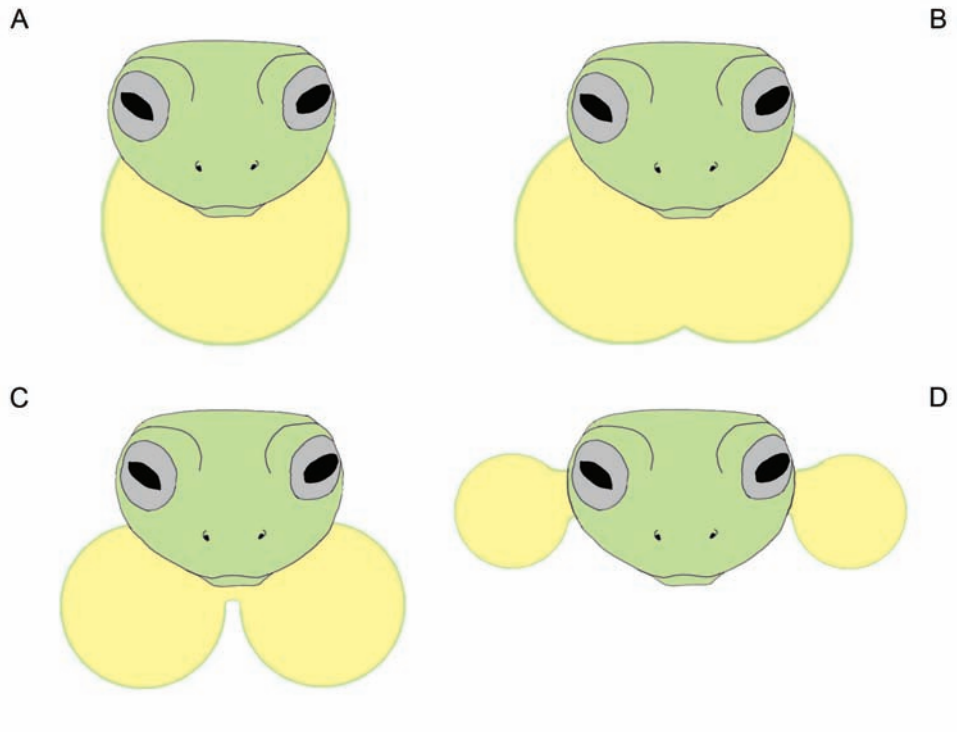

Fig. 56. Diagrammatic views of main types of vocal sacs in anurans. A. Single, median, subgular; B. Bilobate subgular; C. Paired subgular; D. Paired, lateral. Modified from Duellman, 1970.