Cloudbridge Herpetofauna Study

Part 1. Amphibians

April 16 – May 10 2007 (to be continued Summer 2008)

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INTRODUCTION

Located in the neotropical zone in Central America, Costa Rica's $51,100 \text{ km}^2$ of land area – a mere 0.03% of the Earth's surface - supports a varied flora and fauna that ranks it globally among the top twenty countries in terms of biodiversity (NIB 2007). Washed on either coast by the Pacific and Atlantic Oceans, sandwiched between the North and South Americas, its varied topography, tropical climate, and location have all contributed to this rich variety.

The herpetofauna of Costa Rica is correspondingly diverse. From tiny frogs barely larger than a fingernail to the massive *Bufo marinus* weighing as much as a kilogram; cryptic fossorial salamanders to almost dinosaur-like *Ctenosaur* lizards; from massive, harmless pythons several metres in length to the ferocious *Fer de Lance* – this little country boasts over two hundred species of reptiles and approximately one-hundred fifty species of amphibians (Baker 2007). It is highly likely that there are many species yet to discover, as regions such as the southern Talamanca Range have yet to be researched or inventoried to any appreciable extent.

In light of this, the herpetofauna of the Rio Chirripó Pacificó and Cerro Chirripó drainage, where the Cloudbridge Reserve is located, is essentially unknown, as there have been no recorded studies in the area to date. Although a general species list can be inferred from existing research that has taken place in other areas of the country, at similar elevations and in bordering locales, at best this is a matter of educated guesswork. As such, a comprehensive, detailed inventory and study of the genera and species present in this area remains a necessity in order to adequately understand the composition of the herpetofauna found at Cloudbridge.

Additionally, while existing studies may assist in drawing a general picture of what genera and species we can expect to find within the reserve (given known elevations and habitats), scientists have observed that many organisms are expanding or altering their ranges towards higher elevations, possibly as a result of changes in climate due to global warming. Already at Cloudbridge this may have been observed within the herpetofauna, with two sightings of a *Ctenosaura* lizard thus far known only to occupy lowland habitats (Savage, p. 436).

OBJECTIVES

Due to the paucity of knowledge available specifically for the area, the initial purposes of herpetofauna study at Cloudbridge are twofold: first, to provide an idea of what genera (and where possible, what species) are found at the reserve; and second, to gain knowledge about variations in species composition, density and diversity in relation to altitudinal and habitat variations across it.

In order to accomplish these primary objectives, the greatest challenge lies in establishing effective methods of sampling. To this end, a variety of methods, both traditional (pitfall traps, manual surveys) and novel (bromeliad traps, PVC refugia) are currently being employed at Cloudbridge Reserve to sample as wide a range of the herpetofauna as possible. The ultimate goal is to be able to identify to genus and where possible, to species, the herpetofauna of Cloudbridge.

This project is being conducted in cooperation with Lindsay Dimitri, as part of a comprehensive inventory of the herpetofauna of the Cloudbridge Reserve. Because many of the same methods are effective for both reptiles (snakes, lizards) and for amphibians (frogs, salamanders, caecilians) we have chosen to combine sampling work to achieve greater efficacy and a potentially broader sampling range than either could achieve individually.

METHODOLOGY

Amphibian surveys took place through three primary methods:

- 1) Pitfall trapping transects in three distinct habitats (primary to secondary transition forest, primary forest, plantation)
- 2) Leaflitter and vegetation (up to 2 m above ground) manual surveys within 5 m x 5 m plots situated throughout the various habitats identified within the reserve (primary forest, secondary (or primary-secondary transition) forest, riparian, and plantation)
- 3) Night searches with headlamps and flashlights along existing trails, conducted by two or more persons. Anurans are located by sight and by tracking vocalizations.

Data collected on all specimens includes:

- weight
- sex, where possible to identify
- age (adult, juvenile, metamorph)
- identifying markings/characteristics (individual and to ID species)
 - tympanum and its size in relation to eye
 - distinctive colourations/markings
 - o toe/finger webbing
 - presence/absence of ventral disk
 - o orientation (horizontal/vertical) of pupil
 - \circ colour of iris
 - presence of distinctive tubercles
 - o if present, patterns of dorsal glandular ridges
- digital photos
- snout-vent length (SVL)
- location caught
- description of immediate location (i.e. under leaf litter, on tree branch at x height)
- date, time, weather and ambient temperature
- auditory remarks calls heard, approximate number (range), type of sound

Transects, traps and plots will also be surveyed for eggs and tadpoles. Eggs will be described with respect to morphology, location (general and specific, as outlined above) and approximate number. Tadpoles will be described with respect to morphology and identified to genus or species if possible; location (as above), and approximate density where found.

1) Pitfall Transects

Pitfall traps were constructed in five locations:

- a) Gavilan Primary-Secondary Transition Forest:
 - i. 5 each of 5 gal and 2.5 gal traps
 - ii. Transects placed in 2 parallel lines 25 m apart, perpendicular to slope
- b) Gavilan Lower Plantation:
 - i. 3 of 5 gal traps
- c) Gavilan Upper Plantation
 - i. 3 of 2.5 gal traps and 5 of 5 gal traps
 - ii. Transects minimum 25 m apart
- d) Smithsonian/Hectare Primary Forest
 - i. 5 each of 5 gal and 2.5 gal traps
 - ii. Transects separated by main trail (on either side of slope)
- e) Sendero Rio Riparian Primary 5 of 5 gal traps
- f) Sendero Rio Riparian Plantation 5 of 5 gal traps

2.5 gallon traps:

Each trap is a 2.5 gal green plastic bucket, dug into the ground to surface level with effort made to minimize disturbance of the surrounding terrain. The trap is covered with a 30 cm x 30 cm wire mesh square supported on four stakes, and subsequently covered with an equal-sized square of heavy duty black plastic. Loose leaf litter or vegetation of types surrounding the trap is placed on top of the cover for concealment.

5 gallon traps:

Each trap is a 5 gal plastic bucket (colours are white, black, yellow, or green), dug into the ground to surface level with effort made to minimize disturbance of the surrounding terrain. The trap is covered by a fitting plastic lid supported on four stakes, and covered with the sod cap removed from the hole. Loose leaf litter or vegetation of types surrounding the trap is placed on top of the cover for concealment.

All traps are placed a minimum of 10 m and no more than 12 m apart. Transects, where parallel or heterogenous, are placed a minimum of 25 m apart.

Traps are checked 5 days per week with 2 days per week designated as "rest days" to allow the site to recover somewhat from the disturbance of trap checking. Checking of traps south of ("above") Sendero Principal occurs between 6:30 and 10:00 am in the following order:

- a) Gavilan Lower Plantation
- b) Gavilan Upper Plantation
- c) Smithsonian/Hectare Primary Forest
- d) Gavilan Primary-Secondary Transition Forest
- e) Transects on the Sendero Rio are checked afterwards, in the early afternoon.





Step 1: Bucket is sunk into the ground to rim level. Three to four stakes placed around the rim will support the cover.

Step 2: Wire mesh cover is placed on top of the stakes and centred over the bucket.



Step 3: A square of heavy black plastic is placed on top of the mesh cover. This will both keep out rain and darken the inside of the trap.



Step 4: Leaflitter and small debris is used to camouflage the pitfall trap.

2) Plot Sampling

A sample plot location is selected for a general homogeneity of terrain and vegetation within a particular habitat. Plot size for two to four people working is 5 m x 5m (although larger sizes are feasible with more people); corners are flagged with flagging tape and GPS coordinates measured for each corner. Surveys are conducted with one person starting at a corner opposite their partner, then working inwards towards the centre. Leaflitter, dead logs, rocks, roots and bases of vegetation, and vegetation up to 2 m are searched carefully by hand (debris may be overturned using a snake stick or long pole). Any specimens found are captured and placed into appropriate transportation containers or bags, and the time of capture recorded in a field notebook. At the end of the survey (generally 40 minutes to 1 hour per plot) specimens are recorded, measured, and released at the original site of capture.

Plots are situated a minimum distance of 25 m from existing established trails, and a minimum distance of 50 m from pitfall transects or transects of other researchers. Each plot is described as thoroughly as possible with respect to vegetation and shade cover; level of disturbance; proximity to water, trails or other physical landmarks; incline; and other organisms found.

Plots are located randomly for an initial survey; additional plots will be added similar to the Adaptive Cluster Sampling methodology used by Noon, et al. 2006.

Owing to time constraints, only two leaflitter sample were completed during the duration of the project. The first (May 2) was a 10 m x 10 m plot near the Gavilan house, searched by six visiting university students; Lindsay, and myself. The second (May 9) was a 5 m x 5m plot in secondary forest west of the Gavilan trail, searched by Lindsay, Sarah Rathbone (see Lepidoptera studies), volunteer Amy Gode, and myself.

3) Night searches

Night searches are conducted approximately between the hours of 7 and 11 pm along established trails. Using headlamps and hand flashlights, vegetation, leaflitter, ground debris (logs, rocks), streams and standing water are manually searched for amphibians, reptiles, and their eggs and larvae. Three night searches were conducted during this study.

4) Additional Methods in Development

We are currently working on various trap designs for canopy exploration (specifically anurans), for ongoing amphibian monitoring and continuation of the herpetofauna inventory project. These include:

- Bromeliad traps fallen bromeliads (approximately 30 45 cm tall, 10 cm diameter base) will be collected. Each bromeliad is placed into a hole cut into a wooden platform. A small hole in each corner of the platform allows the attachment of ropes which will be used to move the platform in and out of the canopy for collection and monitoring of anurans.
- 2) PVC Refugia constructed from 60 cm lengths of dark gray or black PVC plumbing pipe, fitted with a T-junction at the top and a solid, removable cap at the bottom. A small hole drilled 10 cm from the bottom allows maintenance of a set water level to attract anurans for breeding and refuge. Refugia will be placed in expected anuran habitats, such as in the canopy (by suspension and placement with ropes), and at various heights above ground in trees where anurans are heard calling.

Equipment List

Gloves - heavy garden or leather gloves for fieldwork - disposable latex for handling Flagging tape (orange) Sharpie marker (or other permanent marker) GPS unit Thermometer Hand flashlight Plastic bags - open-ended - ziplock (1 quart) - ziplock (52 mm x 73 mm) Calipers Pesola spring scale or small electronic digital scale Wheel tape Small fine-mesh net (tadpole collection, manual capture) Elastic bands Magnifying glass Savage guide to herpetofauna of Costa Rica

DATA AND RESULTS

(An extensive and current data file containing details of all specimens captured during the course of this project is available on the Cloudbridge server, or on the main Cloudbridge computer in the casita. Photographs of each specimen are also available).

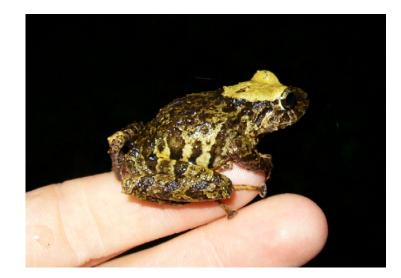


Through the methods described above, between April 17, 2007 and May 10, 2007, the majority of anuran specimens captured were of the genus *Eleutherodactylus*.

Additionally, one *Hyla pseudopuma* male (pictured at left) in breeding colouration was captured on April 18, 2007 during a night search.

Two specimens of *Eleutherodactylus podiciferus* were captured and identified in leaflitter habitats; three were captured and identified to genus as *Eleutherodactylus*, but identification to species level was not possible without examining internal features. Two more were observed but not captured. The physical characteristics, habitat and known specimens captured in the area allow us to logically conclude that these were also *Eleutherodactylus* species.

A single *Eleutherodactylus cruentus* adult female was captured during a night search, while several juvenile specimens are suspected to also be *E. cruentus* individuals, owing to similarity of unique colour patterns (primarily a distinct "cap" on the head, of a colour noticeably paler than the rest of the body). See picture at right.



One *Eleutherodactylus* specimen captured during a different night search is believed to be a distinct species from others captured; however, we have not yet been successful in finding a species description that adequately fits this specimen.

The first leaflitter plot (10 m x 10 m) sampled, on May 2, yielded four *Eleutherodactylus* leaflitter frogs that are, due to their size, believed to be juveniles (with one possible exception). It is likely

that these are either dark morphs of *Eleutherodactylus podiciferus* or *Eleutherodactylus cruentus*. Nothing was caught in the 5 m x 5m plot sampled in May 9. Additional surveys will be completed by Lindsay Dimitri (herpetofauna/reptiles) and associated volunteers in the future.

One salamander, an *Oedipina uniformis* adult, was captured in a pitfall trap at the Gavilan Primary-Secondary Transition Forest transect.

DISCUSSION

Due to the huge degree of variation – in habitat, biology and morphology – of species within the *Eleutherodactylus* genus, identifying all specimens to species level proved exceedingly difficult. Additionally, many characteristics differentiating one species from another are only observable through careful dissection, a process that we are at present reluctant to undertake given the fact that we know so little about the present herpetofauna at Cloudbridge. We are interested in exploring the possibilities of DNA analysis on tissue samples (for example, on those collected from routine mark-recapture toe clips) for accurate species identification in the future.

It is hoped that the use of novel and varied methods of sampling throughout the reserve will, over time, help to provide a wider picture of the species diversity. Methods found to be effective and efficient, in terms of both monetary costs (construction, implementation) and labour (time to construct, place, monitor and maintain) will be utilized during the May to September 2008 season, during which both Lindsay and I plan to return to Cloudbridge to continue the herpetofauna inventory.

PVC refugia will be constructed and placed at locations throughout the reserve during May 2007 in order to start monitoring amphibian usage in following months. We anticipate that these may become part of an ongoing project that can be operated by volunteers with basic instructions in specimen handling, data collection, and trap monitoring, so that data may be collected year-round. Because the traps may be exited and entered freely, they may be left unchecked for extended periods of time without detriment to the animals that may use them.

CONCLUSIONS

The anurans sampled were almost exclusively of the leaflitter genus *Eleutherodactylus*. Although one treefrog species (*Hyla pseudopuma*) was captured, this was likely to be an isolated event given the sampling methods used, which are heavily biased towards sampling ground-dwelling groups. Salamanders are extremely difficult to find in their habitats, due to their fossorial and subfossorial lifestyles and presumed scarcity (suggested by extremely low numbers of opportunistic sightings by area residents and Cloudbridge long-term volunteers and staff).

Further developments are currently underway to find methods of sampling canopy and treedwelling herpetofauna that are rarely captured through standard pitfall and ground-level manual survey sampling methods.

This project will be ongoing as effective and efficient trapping and sampling methods are established for the Cloudbridge Reserve; work will be continued through the summer of 2008 by both myself and Lindsay Dimitri. Ultimately we would like to establish a year-round herpetofauna monitoring program (particularly for anurans) that can be monitored by incoming volunteers, following some training and orientation.

LITERATURE CITED

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Appendix: Description of Adaptive Cluster Sampling Methodology:

Forest floor reptiles were sampled using adaptive cluster sampling, which gives better estimates of the density of animals that show patchy distribution12. The basic sampling unit was 5 m \times 5 m randomly laid quadrats. If an animal was sighted in one of these quadrats (called primary quadrats), additional quadrats (called secondary quadrats) of the same dimension were searched on four sides of the primary quadrat. There was a 1 m gap between the primary and secondary quadrat. If any of these quadrats had animals, further quadrats were laid around them until the quadrats with animals were bounded or surrounded by quadrats without animals. The quadrats with the animals then become a cluster. If the primary quadrat did not have any animals, the sampling was carried out in the next randomly selected primary quadrat. The search procedure in a quadrat followed Inger13. To minimize the chances of missing animals during search, two observers searched the quadrat from opposite sides towards the centre of the plot. The following parameters were estimated from these data:

The number of primary quadrats with animals: An indicator of the abundance of clusters.
Cluster size: The number of quadrats with animals in a cluster, an indicator of the area occupied by a cluster of animals.

3) Species richness in a cluster: An indicator of species assemblages in the area.

4) Density: This is the mean of the densities in clusters, including primary quadrats without animals (density of zero).

5) Community composition: The percentage of animals in a taxon out of the total number of animals recorded from quadrats.