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Engineering School of Agronomy and Food Process

Internship report
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Frog survey during the wet season in Primary and Secondary Forests

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Foreword

Before beginning this report, I would like to thank all those who contributed to the development of this project namely Genevieve Giddy (Founder of the reserve) for welcoming me, Thomas Gode and Frank Spooner (co-manager) who were my tutors, helped me in the realization of the project and answered my questions. I would also like to thank Matt Smokoska (referring biologist for the reserve) for his help in the identification of frogs (sometimes complicated) and the entire group of researchers and volunteers at any time of my internship helped me. I also thank Ms. Gibot-Leclerc (Lecturer in Agronomy) which was my tutor and advised me well and Ms. Fant (Statistic teacher) for her help in the statistical analysis of data.

And finally I thank my parents who have been behind me for this adventure in Costa Rica but also all the Ticos for their wonderful welcome.

This document is the culmination of the collective work.

Abstract

Costa Rica is a country rich in **biodiversity** and works to its **preservation and protection** through the work of many parks and reserves ; Cloudbrige one of them. Private Reserve, it started a **reforestation project** in 2002 ; today , it is time for questioning. Many studies have been conducted in this reserve on the fauna and flora. The **Frogs** are dependent animals in their environment : if there is deforestation, they have no more middle life and their **reproduction is threatened**. This work is an a**nalysis of the various studies** conducted on their population and a conclusion on the **effectiveness of reforestation**. By this report, reforestation efforts are proved, it has a real **positive impact** on frog species : their number has been growing and a **new species** is present.

131 words

Introduction

Fauna and Flora diversities are a richess it is important to preserve. There are numerous projects for this and some countries fight for this like Costa Rica. This little country of 51.100 km² is situated in Central America and surrounded by two oceans : its typography ans localisation fact that it is a country possessing a rich biodiversity : currently Costa Rica has 6% of the world biodiversity. It aims to preserve this with lot of national parks, potected areas and private natural reserves.

Cloudbridge is one of these reserves, founded in 2002, it is the link between National Park of Chirripo and Talamanca Reserve : it permits migratory flow between the both. It divided between two forests : the Primary forest never attained by Humans effects and the Secondary one composed of reforested areas and natural regrouth ones. The second forest permited rehabilitation of numerous species of animals or plants like frogs which their reproduction were threatened because of the destruction of their habitats (tree, buissons and litter).

We can wonder “how can the forest type impact on the frog biodiversity during the wet season ?” In order to answer, we can focus firstly on the subect gaol, secondly and material and methods used for it, thirdly on the results and finally on a descusion of these and some recommandations.

1. Project and goal

1.1. Context of the project

One of the Reserve goal is to protect the biodiversity ; some studies have been made since 2007 in order to survey frogs diversity and to take an inventory of the found species. This project is done during the “wet season” and it's the continuity of an other project realized in the dry season by Jasper van Kessel from February to April 2015. This study is devided in different parts : the first one (from mid-March to May) is for the setting up of th material and methods and the second one is for the field experiences during the wet season which begins in May.

1.2. Problematic and tiebreakers

1.2.1. Problematic and goal

This study is based on the fact that the reserve want to know the different species it is possible to see in the forests through the question : “ How did the reforestation effected frogs diversity ?”. In reality, the first goal of this study is to make an inventory of the found species during this season.

1.2.2. Tiebreakers and expectations

How did the frogs biodiversity envolved from the first researchs (in 2007) to today ? For this question we can compare the past researchs and the present research and we can expect on an increase of the number of the species found thanks to the reforestation project.

Is there a biodiversity difference between the two seasons ? For this we can focus ourselves on just two studies : Jasper one (Van Kassel J., 2015) and mine ; in fact we can compare firstly the differents species and secondly the study areas.

Is the reforestation project really effective ? Finally for this tiebreaker we can study the results of this research and look at the differences and the likness observed.

2. Material and methods

2.1. Subject studied : Frogs

2.1.1. General taxonomy

Frogs take part of the *Eukaryota* Domain caracterised by the presence of mitochondrions and nucleus inside cells of mono- pluricellulaire organisms. *Animalia* Reign is a taxon from this Domain ; the *Chordata* Phylum resulting is recognizable : with few exceptions, chordates are active animals with bilaterally symmetric bodies that are longitudinally differentiated into head, trunk and tail. Frogs take part of *Craniata* Subphylum which means that there is the presence of a cranium ; besides they are Amphibians, in other words, thought of as cold-blooded, these tetrapod vertrebates are ectotherms, meaning they are unable to regulate their own body temperature independently of the temperature of their surroundings. The *Amphibia* Class is divided in three Orders : *Anura* (Frogs and Toads), *Caudata* (Salamanders) and *Gymnophiona* (Caecilians).

This *Anura* Order is characterized by :

- body short, relatvely robust, no tail in adults, postsacral vertebrae fused to form rodlike coccyx that supports the pelvic girdle
- pectoral and pelvic limbs and girdle and sternal elements present
- pelvic limbs composed of four segments : femur, tibia and fibula, elongate tibiale and fibulare, and foot
- eyes present, exposed, and functional
- usually a well-developed tympanum, middle ear, and Eustachian tube
- larvae lacking true teeth, although keratinized beaks and denticles are usually present ; gills covored by an operculum (except in very early stages), as are the forelimbs until just before metamorphosis ; opercular chambrs open to outside through one or two spiracles
- body not annulate or with costal grooves ; no specialized cephalic chemosensory tentacles ; no phallodeum
- palatoquadrate fused by processes to cranium
- atlas arrticulates to skull by atlantal cotyles
- no teeth on lower jaw (exceptin the hylid Gastrotheca guentheri) ; upper jaw and vomerine teeth variably present
- frontal and parietal bones on each side fused into a single element (a frontoparietal)

The *Anura* Order is divided in 50 Families (cf. Annexe 1) ; frogs we can observed take part of the *Neobatrachia* taxon which has 41 Families.

2.1.2. Expected species

In Costa Rica, there is 6% of world biodiversity ; for frogs, there are 141 species in all of the country. At Cloudbridge, we expect to find almost 37 species.

Family	Number of species in Costa Rica	Number of species potentially present	Name	Elevation where find them
Bufonidae	18	6	Atelopus hiriquiensis Atelopus chirripoensis Atelopus senex Atelopus varius Crepidophryne epiotica Incilius (Bufo) fastidiosus	Multiple Multiple Low Low Low Low
Eleutherodactylidae	6	3	Diasporus diastema Diasporus hylaeformis Diasporus ventrimaculatus	Low Multiple Multiple
Craugastoridae	28	9	Craugastor crassidigitus Craugastor fleischmanni Craugastor gulosus Craugastor melanosticus Craugastor obesus (punctariolus) Craugastor phasma Craugastor podiciferus Craugasor rayo Craugastor rhyacobatrachus	Low Low Low Multiple Low Low Multiple Low Low
Stabomantidae	9	1	Pristimantis cruentus	Low
Leptodactylidae	5	0		
Leiuperidae	1	0		
Hemiphractidae	1	0		
Hylidae	43	13	Agalychnis lemur Duellmanohyla rufioculis Duellmanohyla uranochroa Ecnomiohyla fimbrimembra Isthmohyla angustilineata Isthmohyla debilis Isthmohyla picadoi Isthmohyla pictipes Isthmohyla pseudopuma Isthmohyla rivularis Isthmohyla tica Isthmohyla zeteki Ptychohyla legleri	Low Low Low Low Low Low High High Multiple Low Low Low Low
Centrolenidae	13	2	Espadarana prosoblepon Hyalinobatrachium feischmanni	Low Low
Dendrobatidae	7	0		
Microhylidae	3	0		
Ranidae	7	3	Lithobates taylori Lithobates. vibicarius Lithobates warszewitschii	Low Multiple Low

2.2. Field experiences

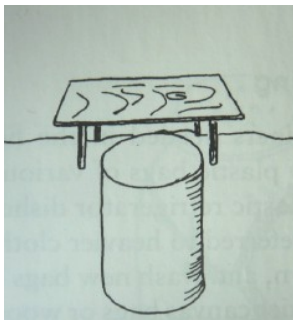
2.2.1. Explanations

There are some methods in order to catch frogs ; in this study we use two of these methods. The first one is the use of pitfall traps put on the different parts of the reserve and the second one is the fact to do some night search because most of frogs are nocturnal.

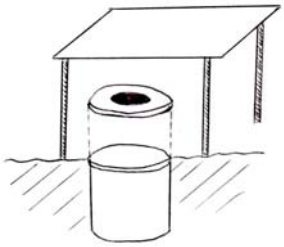
2.2.2. Pitfall traps

Some pitfall traps were put on the reserve before my arrival by Jasper, the previous frogs researcher and a map was made (cf. Annexe jasper). After Jasper report analysis, I found some mistakes on the installation. In order to be rigorous and have coherent results it is important to have th same number of pitfall traps in each part of the two forests : we also choose to have in each part two observation areas.

The building of pitfall traps was realized like J. M. Savage explained (Savage J. M., 2002) : a bucket is put in the ground and a raincover is installed to protect it. It's important that the top of the bucket is on the ground level and not above. If the bucket is too little (when a frog can jump out), we can enhance the trap with a tapped lid which prevents frogs escape. Then it is well if the bucket is drill : it allows the water evacuation and the frogs catched couldn't drown.



Picture 1 : Initial trap
(from Savage J. M., 2002)



Picture 2 : Trap with tapped lid
(from me)

In order to increase frogs capture, two type of traps have been installed for a forest part. The first installation represents two pitfall traps separated by 5 meters, the second one is the same thing with a wall between the traps. The “wall” is a hessian fixed on sticks and with 5 cm put underground (it prevents frog to go under the wall).

The traps control will be done every two days at the beginning and if there is some frogs inside, we will check every days. There is a particular method to check the “wall trap” : We have to begin by one side of the wall, walk on the other side checking the bottom of the wall and

the litter around it with a stick.

2.2.3. Night searchs

Night research is a good way to find frogs. For this, we need a flash light, and some plastic bags with zip lock to catch frogs. In order to make a good night survey, there are some rules :

- Make a selection of each area with almost the same caracteristics : it is better to have the caracteristics for the future analysis.
- One observation area per each forest part : it permits to focus ourselves in on special place and not in all the forest (cf. Annexe 2).
- Spend almost one hour per area and begin the research after 6 am. : a night research is better when it is completely dark and the period allows to be meticulous.
- Take a stick : a stick permits to remove the litter in order to find frogs living inside.
- Be very focused : Glassfrogs have the same colour as a leaf so be carefull !

3. Results

Results presented are only about night researchs because the traps wee always empty ; we wonder in the next part the reasons and the solutions we can apply.

3.1. Found species

3.1.1. Found species through past studies

Since 2007 to today, six studies have been made in order to study herpetofauna and more especially frogs. The differents species which are grouped in the table below.

Found species	Study 1 04/2007 – 05/2007	Study 2 2007	Study 3 07/2007	Study 4 04/2011 – 03/2012	Study 5 02/2015 – 04/2015	Study 6 03/2015 – 08/2015
Craugastor bransfordii		X				
Craugastor crassidigitus	X	X	X			
Craugastor fitzingeri		X	X	X	X	X
Craugastor gollmeri		X				X
Craugastor melanosticus		X				
Craugastor podiciferus	X	X	X		X	X
Craugastor ranoides						X
Craugastor stejnegarianus		X				
Diasporus diasthema			X	X		
Diasporus hylaeformis			X			
Espadarana prosoblepon					X	X
Hyalinobatrachium colymbiphyllum						X
Isthmohyla pseudopuma	X				X	
Pristimantis cruentus	X	X	X	X	X	X
Pristimantis ridens		X		X	X	

3.1.2. Found species for this study

For this study, our observation in each part of the reserve were needed to have these informations.

Species	Primary forest	Secondary forest		
		Planted regrouth	Natural regrouth	
			less than 30 years	more than 30 years
Craugastor fitzingeri	X	X	X	X
Craugastor gollmeri				X
Craugastor podiciferus	X		X	
Craugastor ranoides	X		X	X
Espadarana prosoblepon		X	X	
Hyalinobatrachium colymbiphyllum		X		
Pristimantis cruentus	X	X	X	X

3.2. Tiebreakers answers

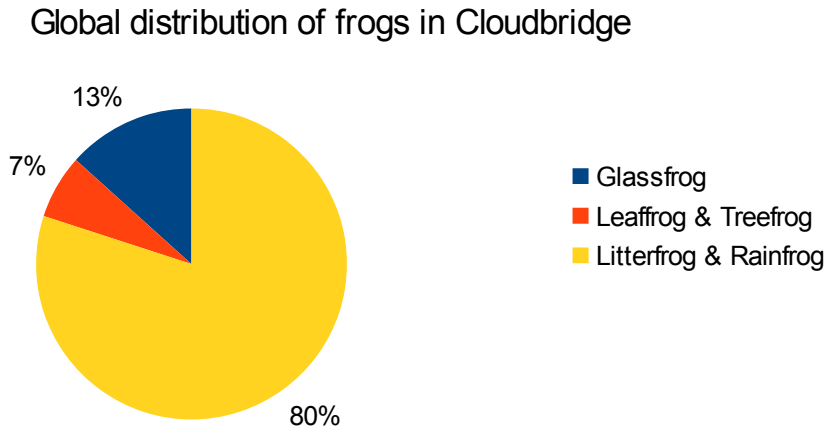
3.2.1. How did the frogs biodiversity envolved from 2007 to today ?

Since 2007 to today, fifteen species were found. After analyse, the most comon frogs (frogs found frequently) are *P. cruentus*, *C. fitzingeri* and *C. podiciderus*. Some species are found only in one search : *C. melanosticus*, *C. ranoides*, *C. stejnegarianus*, *D. hylaeformis*, *H. colymbiphyllum*.

When we classify the different species according to the type of frogs, the Glassfrogs, the Litterfrogs & Rainfrogs and the Leaffrogs & Treefrogs are represented, but there is more types in Costa Rica.

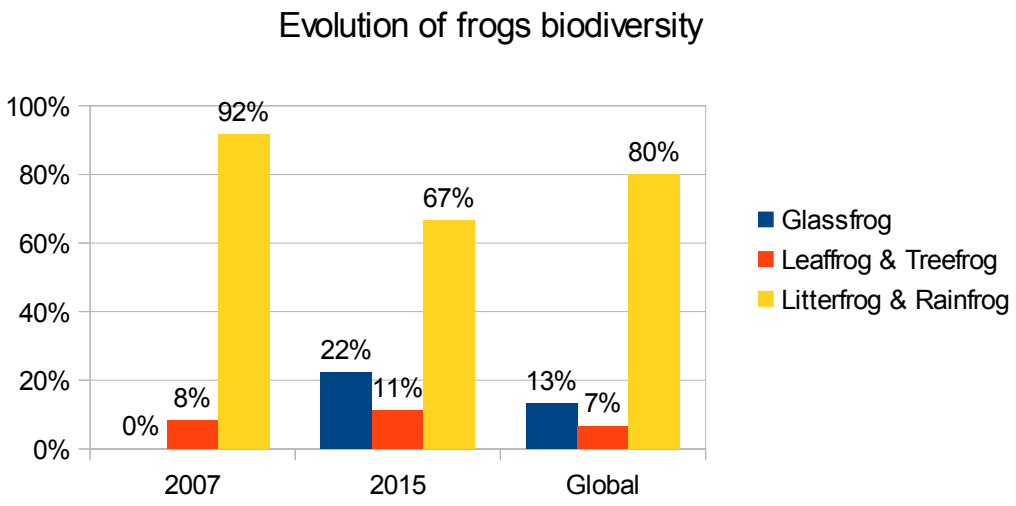
Type	Genus	Specie
Glassfrog	Espadarana	*E. prosoblepon*
	Hyalinobatrachium	*H. colymbiphyllum*
Leaffrog & Treefrog	Isthmohyla	*I. pseudopuma*
Litterfrog & Rainfrog	Craugastor	*C. bransfordii* *C. crassidigitus* *C. fitzingeri* *C. gollmeri* *C. melanosticus* *C. podiciferus* *C. ranoides* *C. stejnegarianus*
	Diasporus	*D. diasthema* *D. hylaeformis*
	Pristmantis	*P. cruentus* *P. ridens*

We can see that Litterfrogs & Rainfrogs have been more seen thant the two other types with thirteen species against two for the first type and only one for the second one. In Cloudbridge, the third type is also the richest. According all the studies, Cloudbridge frogs take part at 80% for Litterfrogs & Rainfrogs, 13% for Glassfrogs and only 7% for Leaffrog & Treefrogs.



When we campare frogs biodiversity between 2007 and 2015, we cans see that

at the begeening of the researchs (year 2007) there were 12 species with only two types : Leaffrog & Treefrog and Litterfrog & Rainfrog. For this year, Litterfrogs & Rainfrogs proportion was of 92%, but in 2015 it passed at 67% because of the discovery of three news species.



Finally we can say that the frogs biodiversity increased between 2007 and today with the presence of Glassfrogs. A phylogenetic tree was created in order to see relationship between the species (cf .Annexe 3)

3.2.2. Is there a biodiversity difference between the two seasons for one year ?

Forest or season effect

To answer, we use two-way ANOVA test with informations put in the below table in order to notify if there is an affect of the season or of a part of the reserve.

Season	Forest	Nb species
DS	1F	5
DS	2FN	4
DS	2FR	2
WS	1FN	4
WS	2FN	6
WS	2FR	4

For realize this test, we say that there is no effect from the forest and the season, this hypothesis es called H_0.

Source	SS	df	MS	F	f
Total	8,83	5			
Season	1,50	1	1,50	1,00	18,50
Forest	4,33	2	2,17	1,44	19,00
Error	3,00	2	1,50		

NB : f is the Table Fisher value.

For season or forest factor, $F < f$; we can say that we accept H_0 and also there is no effect form the forest or the season.

Comparaison entre les espèces recensées

We can focus now on “biodiversity” factor.

Found species	Dry Season			Wet Season		
	1F	2FN	2FR	1F	2FN	2FR
Craugastor fitzingeri	X	X	X	X	X	X
Craugastor gollmeri					X	
Craugastor podiciferus	X			X	X	
Craugastor ranoides				X	X	
Espadarana prosoblepon	X	X			X	X
Isthmohyla pseudopuma		X				
Hyalinobatrachium colymbiphyllum						X
Pristimantis cruentus	X	X	X	X	X	X
Pristimantis ridens	X					

Two species were found in each part of the reserve for the two seasons (in red on the table) and for species were found just one time for these two studies (in blue). We kno now that there is no effect from the season and the part of the forest, but we can forget the “researcher” effect which can be a bias and the “observation area” because for the two studies we analyse the are different (it is a second bias).
que ces dernières n'étaient pas les mêmes pour chaque étude.

3.2.3. Is the reforestation project really effective ?

During the present study, seven species were observed .

Species	Primary forest	Secondary forest		
		Planted regrouth	Natural regrouth	
			less than 30 years	more than 30 years
Craugastor fitzingeri	X	X	X	X
Craugastor gollmeri				X
Craugastor podiciferus	X		X	
Craugastor ranoides	X		X	X
Espadarana prosoblepon		X	X	
Hyalinobatrachium collymbiphyllum		X		
Pristimantis cruentus	X	X	X	X

In order to verify the effectiveness of reforestation work realized since 10years, we will be interested only in secondary forests : planted regrouth and natural regrouth less than 30 years. Indeed, these two forest types are almost similar in the faunal diversity and are located at similar altitudes.

After analysis, we see that these forests have 50% of species in common with a newly observed (*Espadarana prosoblepon*). In addition, the planted regrouth forest has the same species as those found in the natural regrouth forest, then we can say that the work of reforestation is effective. In addition, the plnted regrouth forest presents a new species of frogs (*Hyalinobatrachium collymbiphyllum*) observed only in this part. It would be interesting to continue the research to see if this species is not found in other part of the reserve.

4. Discussion and perspectives

4.1. Discussion

4.1.1. Discussion on the project

It is worth remembering that firstly the study was to focus on the tree frogs who soon find themselves in danger because of the destruction of their natural habitat and breeding area. This study could not be achieved from a practical point of view ; indeed, the tree frogs live several meters high in the trees and therefore by the measures implemented in the reserve impossible to capture. The subject also envolved on the frogs in general. Thus the list of frogs in the park is exhaustive because the tree frogs have not been observed and one can question to the presence of Dendrobates.

In addition, studies conducted since 2007 are not made according to the same criteria (observation area, observation period and amount of observation). It was asked to make a summary and comparison of all these studies to make a point about the diversity of frog species. It would have been desirable to have all the necessary

information as the period or observation zones and select the studies that will be used. For an inventory job, this is sufficient.

4.1.2. Project limits

Pitfall traps

Traps installed at the reserve did not work although they have proven themselves in our place in Costa Rica and Cloudbridge in previous studies. Then one can assume that this is because the settlements were not suitable for this, as these places there was not necessarily frogs.

Moreover, as specified during installation, you had to restore the litter in order not te create an environmental difference and also disturbing wildlife. If this restoration was not done well, this can explain the fact that traps have remained empty.

Moreover, it was hypothetically have two viewing areas in every part of the reserve, easily accessible areas and with the same characteristics. However, this could not be feasible: different forests of the reserve are not located at the same altitude, there is no place with the same characteristics for each.

Night searchs

It is possible to observe several limiting factors

- Time : if there is no rain during the day, areas are low humidity and frogs will have less tendency to get out. Heavy rainfalls can damage traps : during my study a tree collapsed on a "wall"
- The observer : if a search is made in a downpour, concentration is difficult to maintain and observations are of lesser quality ; too many observers may also impacted on the quality of work
- The maintenance of the park : we must be ensured before any research night there was not a maintenance work during the day or the previous two days , this work can disturb wildlife and it is possible to have any subjects of observation

Frogs

We must not forget that frogs are living beings capable of moving ; this is a limiting factor in this study. Indeed , although owe choose an area where it is known to observe frogs, it is possible that at the evening they is nothing to observe.

The appearance of a new species within the planted regrouth forest is not necessarily characteristic of the latter, it is necessary to do a thorough study on this subject ; it is possible that there is trade between the different forest types that are the source.

4.2. Perspectives

There is no reason that traps do not work for this year at Cloudbridge. With more time, we should consider a field study to select suitable areas for observation with the same or approaching characteristics (humidity, presence of a water point, specific flora ...) and adjust the location of the traps for the selected project. It will still remain difficult to compare primary and secondary forest if you want to have the same characteristics, but we can adapt the study conditions.

Thanks to my knowledge and to those people surrounding me during my internship, we have made changes to the traps but it was not enough. It will be interesting to have a person on the site who have already achieved these traps and having got a result, but also able to explain why they have not worked. This person arrived shortly after my departure.

Also it would be interesting to set up traps to catch tree frogs. This could be a new topic of study.

Conclusion

During the eight years that have been carried out various studies on frogs, we have observed a growing number of species. Currently it is possible to say that the season (dry or wet) and forest type (primary, secondary planted regrouth forest, secondary natural regrouth more than 30 years and under 30 years forests) have no influence on these species.

In addition, the reforestation work is efficient in terms of the diversity of frog species : the species present in the young natural regrouth forest are found in the planted regrouth forest. It is even possible to observe only one species in this forest type : *Hyalinobatrachium collymbiphyllum*. These statements however, are to qualify without thorough research .

Eventually we can say that reforestation impacts on biodiversity at different levels : positive development in the number of found species and discovery of a new one.

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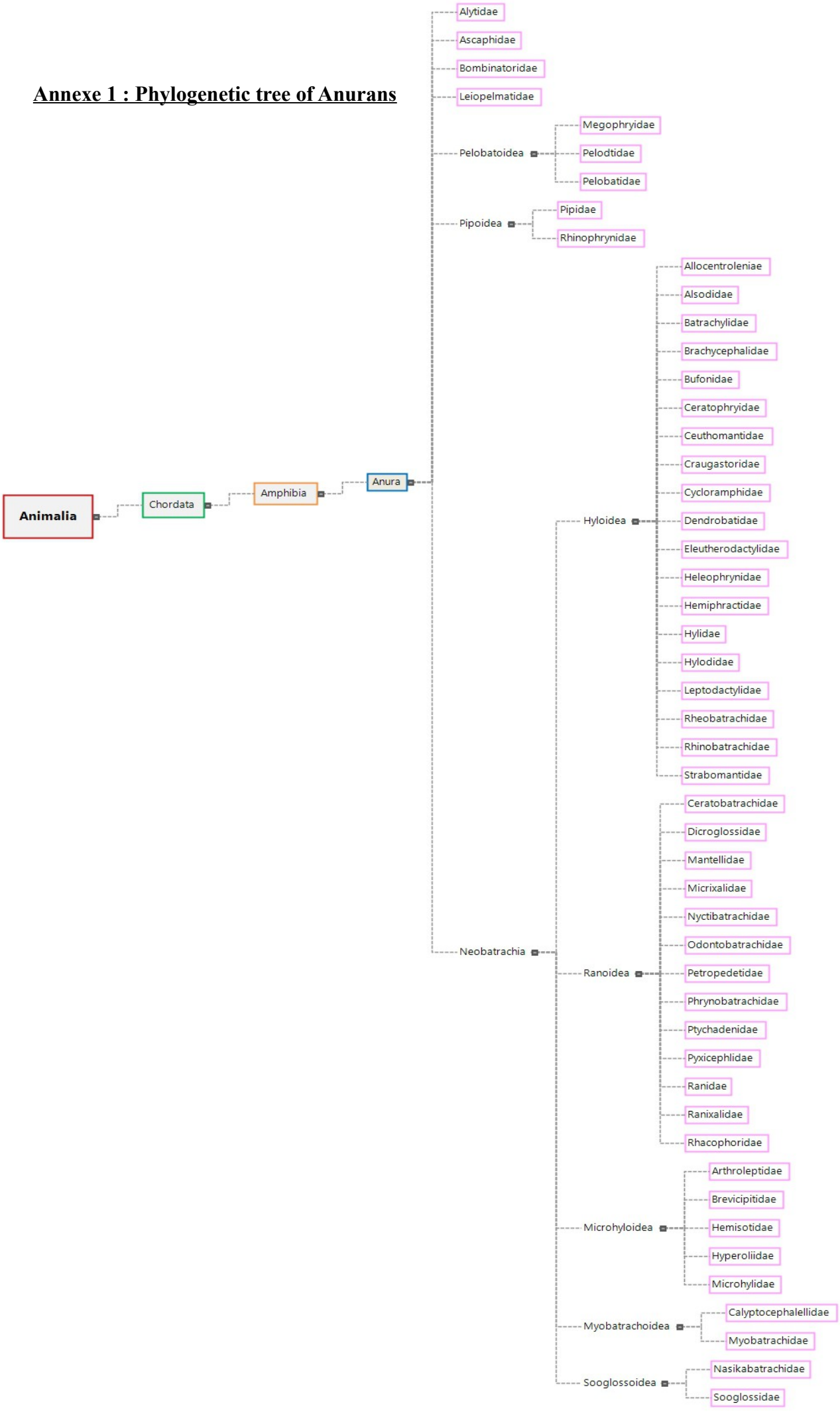
Table of Annexes

Annexe 1 : Phylogenetic tree of Anurans

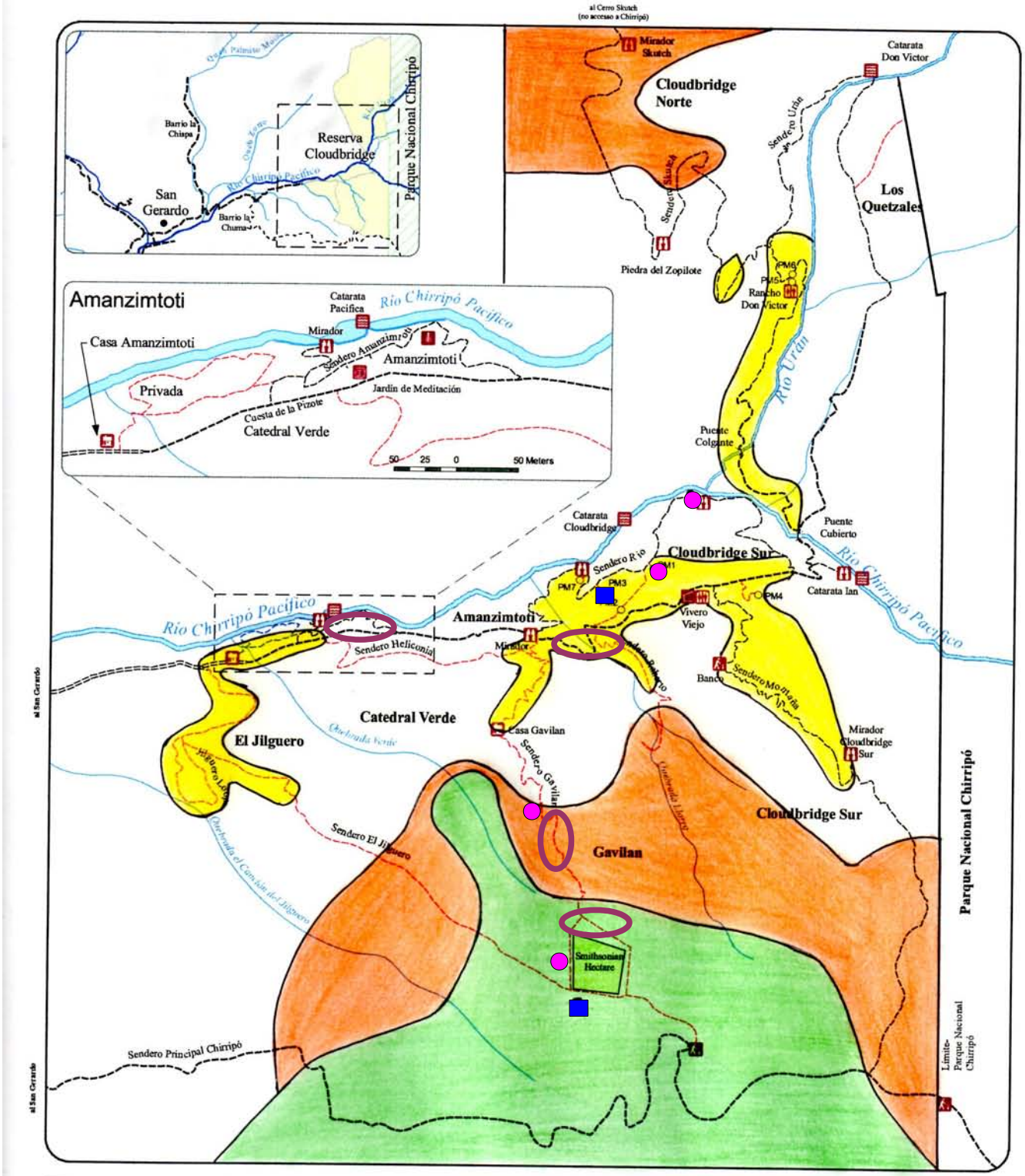
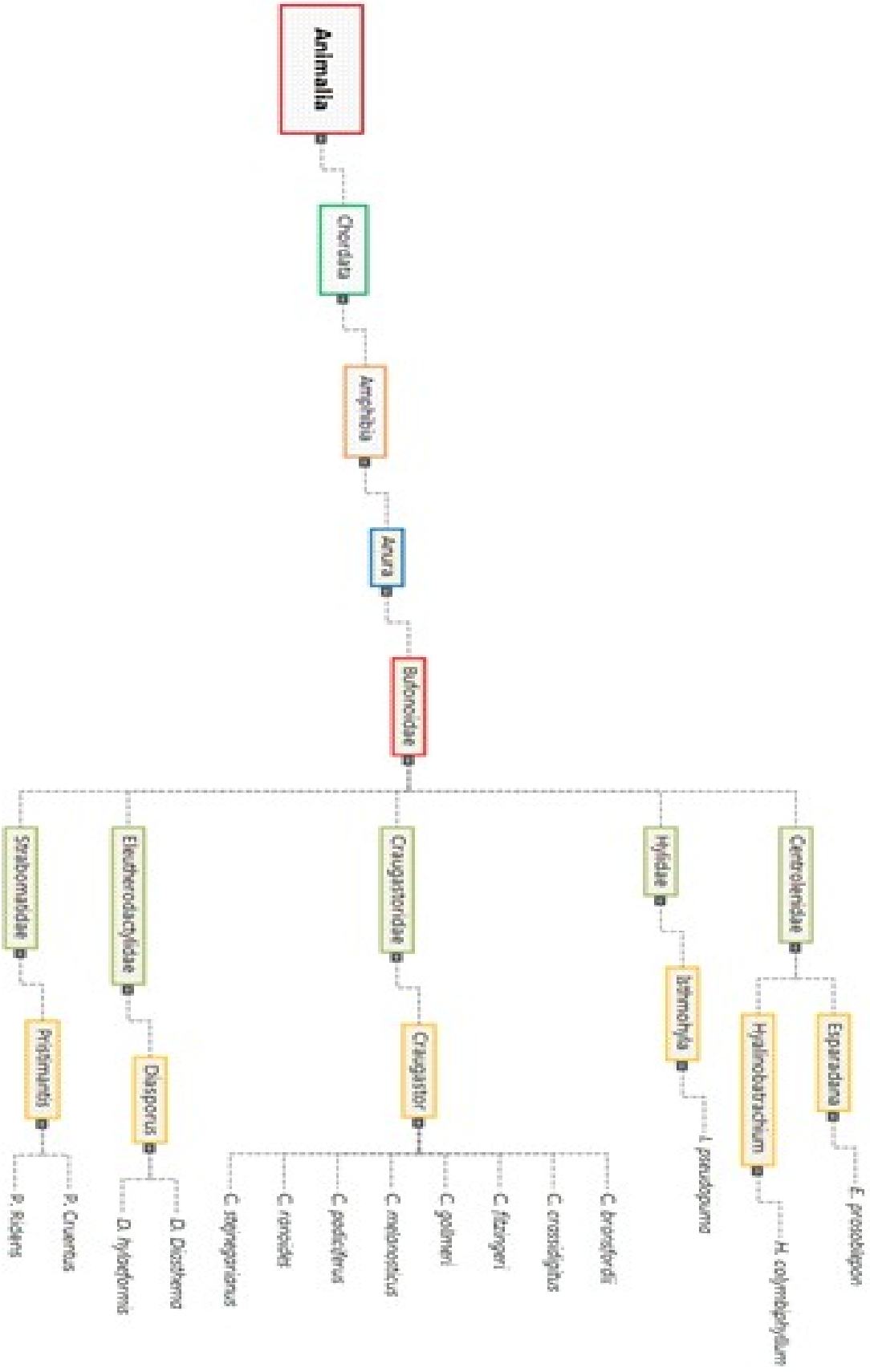
Annexe 2 : Cloudbridge map

Annexe 3 : Phylognetic tree of frogs species in Cloudbrige

Annexe 1 : Phylogenetic tree of Anurans



Annexe 2 : Cloudbridge map



Annexe 3 : Phylognetic tree of frogs species in Cloudbrige