

Spatial Distribution Patterns of an Amphibian Community in a Threatened West Africa Rainforest (Ivory Coast)

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Abstract: *The amphibian communities in Banco National Park (BNP) and two forest fragments were studied. The surveys were realised on 24 transects from July 2003 to July 2006. These surveys permit to study amphibian spatial distribution patterns. The standardized transects technique, based on the acoustic and visual surveys, was used. A total of 7933 anurans were sampled. We identified 28 species, 13 genera and 8 families in BNP. In Filtisac forest, we sampled 13 species, 8 genera, 6 families and in University forest 9 species, 7 genera and 6 families. Hyperoliidae (32.14 %) were the most diversified group. Dicroglossidae and Pipidae were represented by one species (3.57 %). Four habitats types were distinguished based on species assemblage. In BNP swampy areas, Phrynobatrachus ghanensis, P. phyllophilus and Aubria subsigillata were abundant. The closed and sparse dry habitats are characterized by the abundance of Arthroleptis spp. and Phrynobatrachus tokba. In the open areas, Phrynobatrachus latifrons, Hyperolius fusciventris lamtoensis, Afrixalus dorsalis, Ptychadena pumilio, two Amietophrynus species were encountered. Canopy, humidity, and water bodies were the main environmental factors which influenced the spatial distribution. These results showed that BNP was well preserved so far, except the central clearing. The two forest fragments were altered by human activities.*

Keywords: Anura, Diversity, West Africa, Rainforest, Conservation

1. Introduction

The Upper Guinea Forest Block, which ranges from Senegal to Togo, is home to a large diversity of endemic plant and animal species [1,2]. However, since the 1980s more than 100.000 km² of forest in this African area are destroyed every year. The main causes of forest destruction are linked to agricultural activities and urbanization [3]. In Ivory Coast, the policy to address these threats has led to the creation of several protected areas. The oldest one is the Banco National Park (BNP). This park, located in the center of the city of Abidjan, is subject to strong anthropogenic pressure.

Several authors have demonstrated a close relationship between the composition of animal communities and habitat diversity. Indeed, it was shown that forest fragmentation and habitats degradation affect the dynamic and structure of biological communities and may e.g. cause local extinction of amphibians [4].

Amphibians are among vertebrates the most endangered group in the world, especially in tropical forests [5,6]. Several factors are responsible for this threat. However, the loss and alteration of habitat are probably the most serious causes of the decline, that affects more than 32% of amphibian species [7]. Indeed, these animals represent a taxonomic group which is very sensitive to changes in environmental conditions [8]. Their species composition may thus reflect the degree of disturbance or habitat alteration [9-11]. Amphibians are closely related to certain microhabitats and/or micro-climates [12-13]. Thus,

according [14] and [15], amphibians are excellent indicators of the biotic integrity of forest ecosystems. Despite their importance, the ecology of amphibians in Africa is little known [16,17]. In Ivory Coast, particularly, the majority of studies on amphibians concerned inventories and systematics. This work has enumerated 93 species in 9 protected areas [9, 10,18-22]. Very few studies have focused on the ecology of amphibians [e.g. 23-26]. More specifically in the Banco National Park, studies on amphibians are few. Indeed, the available publications are those which led to the description of the species *Kassina arboricola* [27], revising *Aubria subsigillata* [28], anuran fauna [29], prey composition of two syntopic *Phrynobatrachus* species [30], anuran community calling activities [31], systematic of a new tree-frog genus and species [32] and dietary strategies of *Hoplobatrachus occipitalis* [33]. Thus, anuran community spatial distribution patterns of BNP is not sufficiently known.

The present study aims to provide a better understanding of the diversity and ecology of amphibians of BNP to define the health state of the forest ecosystem for future conservation and sustainable management measures. It will be more specifically (1) assess the diversity of amphibians of Banco forest, (2) analyze the spatio-temporal dynamics of the amphibian community, (3) identify environmental factors determining their dynamic and (4) compare the anurans community of BNP to those of two forest fragments located near the Filtisac company and within the University of Nangui Abrogoua (ex-University of Abobo-Adjamé).

2. Materials and Methods

2.1 Site

This study focused on the Banco National Park and two nearby forests fragments, i.e. one near the Filtisac company and one within the area of the University Nangui Abrogoua (Figure 1). BNP is located in Abidjan between 5° 21' to 5° 25' N and 4° 01' to 4° 05' W at an altitude between 0 and 113 ma.s.l. [34]. Its area is estimated at 3474 ha [35]. This park, created by the decree of October 31, 1953 is the oldest of the 11 national parks and natural reserves of Ivory Coast. It is the remnant of an evergreen forest that once extended over an average width of 20 km. The BNP takes its name from a river "Banco" which originates on the northern edge of the forest, extends through the park and flows south into the lagoon Ebrié. This river is fed by a network of small streams. Its main bed is 9 km long, with an average width of 3 m. Inside the park, a forest school (founded in 1938), an accommodation (camp) and a fish farm, occupy an area of about 5 ha.

Two relics of the former forest bloc are located at the north-eastern outskirts of the park. The first, called in this study "Filtisac Forest", is part of the administrative boundaries of the park. But the extension of Abidjan city isolated this forest from the protected area of the park on an average distance of about 500 m. Its area is estimated at about fifty hectares [36]. The second forest fragment is smaller (about 6

ha) and located within the University area. The distance between this small forest and BNP is about 2 km.

The equatorial-type climate include [37,38] two rainy seasons (long season: April to July; short season: October and November) and two dry seasons (long season: December to March; short season: August and September). The average annual rainfall in the study area varies between 1650 to 2000 mm [34,39].

Banco forest and the two forest fragments are part of the Guinean rainforest zonen [40], characterized by evergreen forest types. The vegetation is dominated by *Turraeanthus africanus* (Meliaceae) and *Heisteria parvifolia* (Olacaceae) trees.

2.2 Data Collection

The sampling sites were selected taking into account all present types of habitats [41]. These include primary and secondary forests, open or closed canopy areas, or conserved and disturbed habitats. Wetlands which are generally breeding site of amphibians have also guided the choice of sampling sites. Habitat parameters and amphibian data were collected in a standardized way along rectangular (200 m/100 m) transects (for exact transect and sampling design see [22]). The survey sampling was realized on 20 transects from BNP from July 2003 to February 2005 and from April to July 2006 on 4 transects in the forest fragments (Filtisac and University forests).

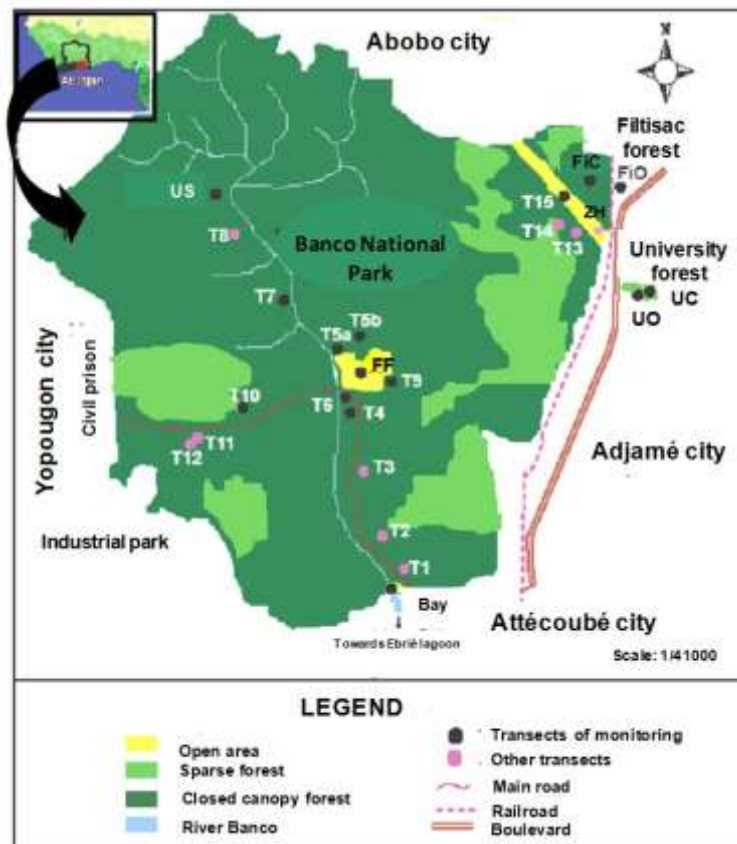


Figure 1: Location of transects in the Banco National Park and Filtisac forest and the University Nangui Abrogoua forest (source: CNTIG 2006; modified satellite image of Google Maps 2009).

2.2.1 Environmental variables

Measurements of air temperature and relative humidity in each transect were conducted at day and night of each survey event. They were taken in different segments of each transect during the sampling. The proposed methodology by [22], [42] and [43] was used to estimate canopy cover, density of trees and shrubs, as well as the leaf litter cover and density. Additional parameters were taken into account. This was the density of the grass cover (expressed as % of ground cover), and the presence of water (ponds, streams, river) in different habitats. The canopy was estimated by the same observer using the following scale: 0% for open sites, 25 and 50 % for sparsely vegetated sites, 75 and 100 % for closed canopy. The leaf litter thickness was evaluated by measuring the height thereof at different points within a radius of 0.25 m. The density of trees and shrubs has been evaluated on an area of 4 m² on each transect. All these parameters were considered invariant over the sampling period and this measured only once.

2.2.2 Sampling

Amphibians were sampled opportunistically during visual surveys in different habitats. Sampling was carried out day and night according to standard techniques of [22] and [41]. These study techniques included visual observations (day and night) and investigation of potential shelters (i.e., rocks, dead wood or leaf litter). Amphibians were also sampled using acoustic monitoring in all different habitat types. The collection of data on each transect always started in the southeast corner to ensure identical geographical orientation between the different sample events. During the transect walks, frogs were captured, determined, sexed and measured, before releasing them at the same point. Measures were taken with dial-callipers (precision: ± 1 mm). Representatives of each species were collected, anesthetized and killed in chlorobutanol solution and thereafter preserved in 70% ethanol. Specimen of all species were deposited in the collection of the Laboratoire d'Environnement et de Biologie Aquatique at the University Nangui Abrogoua (Ivory Coast). Vouchers are further housed in the Staatliches Museum für Naturkunde Stuttgart (SMNS) and the Museum für Naturkunde Berlin (ZMB). Tissue samples (toe tips) of recorded species were preserved in 95% ethanol. These samples are stored at the Museum für Naturkunde, Berlin (ZMB), Germany. Identification and description of new species was realized in separate papers. These new taxa concern a tree frog of the family Hyperoliidae, namely *Morerella cyanophthalma* (see [44]) and an arthroleptid frog: *Astylosternus laticephalus* (see [45]).

2.3 Data Analysis

2.3.1 Species richness and species diversity

To evaluate the effectiveness of sampling techniques, two nonparametric estimates of species richness statistical methods were used. Based on the assumption that the relative sampling effort was the same in each transect (always three people involved in searching), the theoretical number of amphibian species was calculated using the Jackknife 1 [46-48] and Chao 2 estimators [49], based on presence / absence data. The estimation of species richness was performed with EstimateS (version 7) [50].

Species richness, diversity and evenness are descriptive indices used to compare different populations or different states (changes over time) in a community [51]. The [52] species diversity index is used to highlight the overall diversity. Given that two different communities may have the same diversity, evenness [53] was also calculated. It describes the relative abundances of species within a community.

2.3.2 Self Organizing Maps (SOM)

To investigate the transect data in relation to environmental variables and species assemblages, we used the algorithm of "Self Organizing Maps (SOM)" of Kohonen [54-56]. This method of nonlinear classification has already proven its effectiveness in ecological analysis [57]. It has also the advantage of visually representing profiles simplified from complex databases by identifying similar groups [56-58].

SOM usually consists of two layers of neurons. The input layer consists of the data matrix (species and environmental variables), and is directly connected to the output of two-dimensional layer Kohonen map, composed of artificial neurons or output nodes. The number of nodes is selected as a result of a calculation of the error which is a topographical indicator of how the card is preserved [59]. This topographic error must be less than 1 [58]. At the end of the learning process, an output map is obtained. In each hexagon of the map a virtual object is located for which the values of descriptors are calculated. These virtual objects represent the distribution patterns of real objects (species, sampling site and environmental variables).

The SOM Toolbox (version 6.1) interface for Matlab used in this study was developed by the Helsinki University of Technology and available on the website: <http://www.cis.hut.fi/projects/somtoolbox>

The discriminate factor analysis (DFA) was used to test the degree of discrimination of parameters used to distinguish the habitat groups determined by the SOM map. Nonparametric tests of Kruskal-Wallis ranks and Mann-Whitney test for independent samples were used for small samples. For large samples, the Student-t test was used instead. These tests were conducted to test significant differences of environmental variables and community indices. DFA, Kruskal-Wallis and Mann-Whitney test were performed with the STATISTICA 7.1 program [60].

3. Results

3.1 Community assemblage

3.1.1 Species richness

A total of 28 species of anurans distributed in 13 genera and 8 families were identified in the different transects of the Banco National Park, the Filtisac forest and the University Nangui Abrogoua forest. The theoretical species richness obtained by Jackknife and Chao indices was between 34 and 38 species. Thus we recorded 73.7 or 82.4 % of the estimated species richness, respectively. In the Filtisac forest, 13 species have been collected against 11 species in the University forest.

Closed canopy habitats (except Filtisac forest) had a species richness varying between 7 and 21 species. These habitats are represented by Banco transects US, T4, T5a, T5b, T6, T7, T8, T9. *Astylosternus laticephalus* were encountered in this type of habitat. In addition, apart from *Phrynobatrachus latifrons*, all other species of the family Phrynobatrachidae were present in closed canopy habitats. In contrast, sparse habitats of BNP (transects T1, T2, T3, T10, T11, T12, T13, T14) and the University forest, had the lowest species richness (2 to 6 species). In this type of habitat, the Arthroleptidae and Phrynobatrachidae were represented by only one species (*Leptopelis spiritusnoctis* and *Phrynobatrachus tokba*, respectively).

In open canopy areas of BNP (T15, ZH, FP, Bay), the Filtisac forest (FiO) and the University forest (UO), 6 to 17 anurans species were sampled. Almost all species of tree frogs (except *Leptopelis macrotis*) have been identified in these habitats.

In total, 7933 anuran were captured during the survey. Of this, 94.7% were recorded from the BNP, 3.4% from the Filtisac forest and 1.9 % from the University forest. Phrynobatrachidae was the most abundant family (41.9%). It was followed by the Hyperoliidae (24.9%). The least abundant families were Bufonidae (3.3%) and Pipidae (0.01 %).

The amphibian community of the three forests was dominated by *Phrynobatrachus latifrons* (23.8%). The less abundant taxa in these forests were *Ptychadena bibroni*, *Ptychadena aequiplicata*, *Leptopelis macrotis* and *Xenopus tropicalis*. They represented 0.01% to 0.1% of the total abundance.

3.1.2 Species diversity

The Shannon species diversity index calculated for the different transects varied from 0.17 to 3.31 (Figure 2). As for the evenness index, it was quite high especially in the open areas (0.52 to 0.83) regardless of season. The fish farm was the site where these indices were very high ($H' = 2.72$ to 3.03, $E = 0.72$ to 0.78).

In closed and sparse canopy areas, evenness index varied between 0.29 and 0.99. Higher species diversity was observed in closed wet habitats of transects US and T6 during rainy seasons (3.23 and 3.31 respectively). In contrast, sparse canopy areas of transects T9, T10, FiC and UC had the lowest species diversity (0.30 to 1.34).

3.2 Species Distribution Patterns

The distribution of the amphibian community was analyzed using the SOM (Self Organizing Maps). This modeling process was based on abundance and occurrence data. Based on the quantization and topographic errors, 30 cells (6 rows \times 5 columns) were selected for both the environmental data and species assemblages mapping.

The trained SOM permitted to classify all samples on these 30 units (Figure 3A). On output SOM map, four groups (I-IV) were characterized (Figure 3B). Each group represented

on the SOM map with the same pattern, contains samples with similar species compositions.

Group I contained predominantly samples of transects T4, T9, T10, FiC and UC (represent 74.2% closed and sparse canopy dry habitats). In group II, it gathered transects US, T5a, T5b, T6 and T7 (100% wet closed canopy habitats). Group III was composed of 85.7% of the samples of the fish farm. Finally, Group IV included samples of the transect Bay, T15, FiO and UO (100% open forest areas).

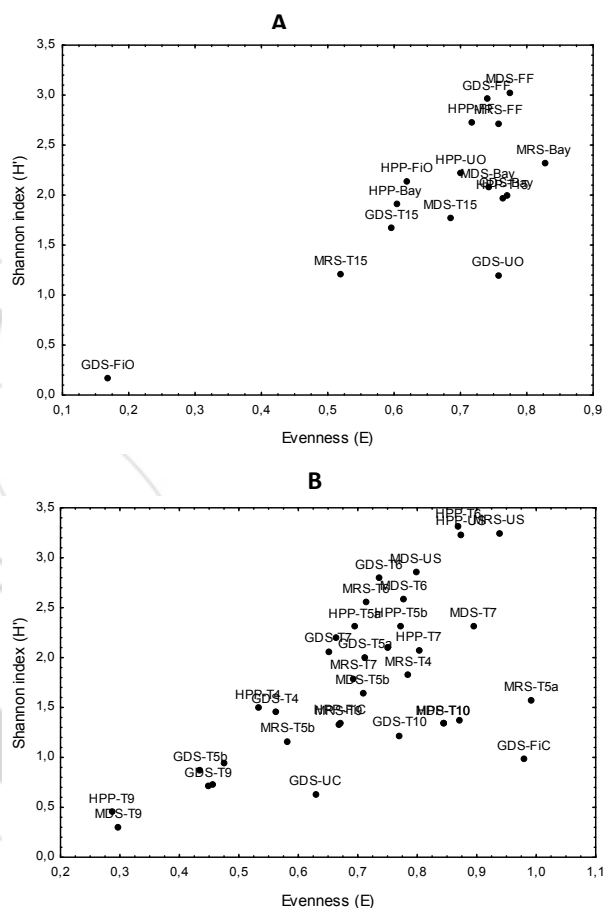


Figure 2: Spatio-temporal variations (transect per season) of open (A) and closed (B) canopy amphibian diversity made on the basis of Shannon index (H') and evenness (E); HPP = highest precipitation period, GDS = great dry season, MRS = minor rainy season, MDS = minor dry season.

Species assemblages in each of these groups are summarized in figure 4. Group I (closed and sparse canopy dry habitats) contained the taxa *Phrynobatrachus tokba*, *Arthroleptis* sp.1 and *Arthroleptis* sp.2. The only specimen of *Leptopelis macrotis* captured belongs to this group as well. In addition, *Phrynobatrachus tokba*, *Arthroleptis* sp.1 and *Arthroleptis* sp.2 are present in group II (closed canopy wet habitats) with relatively high abundance. In this group, the characteristic taxa are *Phrynobatrachus ghanensis*, *P. phyllophilus*, *P. liberiensis*, *Ptychadena aequiplicata*, *P. longirostris*, *Aubria subsigillata* and *Astylosternus laticephalus*. Concerning groups III and IV (open habitats), they are determined by tree frogs (except *Leptopelis macrotis*) and *Phrynobatrachus latifrons*, *Hoplobatrachus*

values were significantly higher (Student t test, $p < 0.05$) in groups I and II (84.3 to 85.6 %) than in groups III and IV (69.6 to 72.0 %).

Regarding the canopy, the thickness of leaf litter and density of shrubs, values observed in the closed and sparse canopy habitat groups (I and II) were similar (Student t test, $p > 0.05$). The density of trees was significantly larger (Student t test, $p < 0.05$) in wet closed canopy habitats (group II) (6.7 trees / m²) than closed and sparse canopy dry habitats (group I) (5.2 trees / m²). In groups III and IV, habitats were characterized by grassland (open areas), no trees and shrubs are present.

Discriminate analysis was performed on four groups established by the SOM and the main environmental variables to identify the factors determining the distribution patterns of diversity. The discriminate analysis indicates that for variables included in the model, only the relative humidity contributed significantly ($p < 0.05$) to discriminate the groups. The contribution of the temperature in the groups was not significant ($p > 0.05$).

Table 1: Summary of environmental variables in the different habitat groups, as defined by the SOM map (see Figs. 3 & 4), the median values assigned the same letter (a or b) did not differ significantly (Student t test, $p > 0.05$) from each other, different letter indicate significant differences, n = sample size, SD = standard deviation.

		Groupe I n = 58	Groupe II n = 47	Groupe III n = 12	Groupe IV n = 31
Temperature (°C)	Mean	26.9 ^a	27.1 ^a	31.8 ^b	30.6 ^b
	SD	2.1	2.0	5.5	3.8
Relative humidity (%)	Mean	84.3 ^a	85.6 ^a	69.6 ^b	72.0 ^b
	SD	6.8	7.4	15.8	9.6
Canopy (%)	Mean	53.9 ^a	59.0 ^a	0.0	0.0
	SD	15.0	12.1	0.0	0.0
Leaf litter thickness (cm)	Mean	5.6 ^a	5.4 ^a	0.0	0.0
	SD	2.5	2.8	0.0	0.0
Shrubs density (ind./m ²)	Mean	13.8 ^a	15.4 ^a	0.0	0.0
	SD	5.6	7.7	0.0	0.0
Trees density (ind./m ²)	Mean	5.2 ^a	6.7 ^b	0.0	0.0
	SD	2.2	3.8	0.0	0.0

Discriminate analysis provided a matrix (Table 2) indicating that the typology made on the basis of the SOM allowed 64.9% correct classifications. However, the best classification was obtained with the group IV (93.6%), followed by group I (72.4%). This analysis indicated 9 samples of group III (25.0%) that should be included in group IV. In group II (46.8%), 25 samples would be grouped in I.

Table 2: Classification matrix of four habitat groups formed by the SOM map based on discriminate analysis of the main environmental variables; rows: observed classifications, columns: classification provided, P = weight of the point clouds.

Groupe	Best classification (%)	Groupe I P=0.39	Groupe II P=0.32	Groupe III P=0.08	Groupe IV P=0.21
I	72.4	42	15	0	1
II	46.8	25	22	0	0
III	25.0	0	0	3	9
IV	93.6	0	0	2	29
Total	64.9	67	37	5	39

4. Discussion

Our qualitative and quantitative analyzes of the Banco National Park, Filtisac forest and the University forest amphibian communities showed that wetland habitats (closed or open canopy) contained the largest species diversity. In contrast, dry habitats (closed, sparse or open forest area) had the lowest taxonomic diversity. Also, it appeared clear that the spatial variation in species richness and abundance of amphibians were related to habitat diversity. Indeed, the presence of the river, streams and ponds in moist habitats of BNP created favorable conditions for the development of amphibians. The low species richness observed in Filtisac and University forests was due to the absence of permanent water in these sites. Our observations are confirming those of [24], [61], and [62] who argue that the permanent presence of water points in a habitat is essential for the reproduction of amphibians and determine their spatial distribution.

In moist habitats, species richness in closed canopy was relatively close to that of open areas. However, species composition in these two areas was different. Closed wet habitats were dominated by forest species and leaf litter species, while wet open habitats were characterized by the strong presence of grass land species. In addition, the species *Aubria subsigillata*, *Ptychadena aequiplicata*, *P. bibroni*, *Phrynobatrachus ghanensis*, *P. phyllophilus*, *P. liberiensis*, *Astylosternus laticephalus*, *Phrynobatrachus tokba*, *Arthroleptis* sp.1 and *Arthroleptis* sp.2 were constantly present in woodland habitats (closed and sparse canopy) characterized by a wet microclimate (low temperature, high relative humidity). In contrast, taxa associated with open habitats determined by grasslands are *Afraxalus dorsalis*, *A. fulvovittatus*, *Hyperolius concolor*, *H. fusciventris*, *H. guttulatus*, *H. picturatus*, *Hoplobatrachus occipitalis*, *Ptychadena pumilio*, *P. mascareniensis*, *Amietophrynus maculatus*, *A. regularis* and *Phrynobatrachus latifrons*. Thus, the composition of the amphibians population was influenced by vegetation type and cover as well as humidity. This conclusion is consistent with [63] reporting that the canopy cover influences the choice of breeding site for amphibians and thus determines their movements. As for [12] and [63], they state that the irregular distribution of amphibians is often due to local environmental conditions, including special microclimates. [62] showed that the change of plant assemblages, allied to soil moisture and type of water body, was the most

important environmental factor acting on the structure of the anuran community. Finally, [64] and [65] show that the dense leaf litter is an ideal environment with abundant prey.

Nevertheless, there were some species that occurred in several habitat types. This was *Hylarana albolabris*, *Hyperolius fusciventris*, *Leptopelis spiritusnoctis* being present in relatively open and closed canopy habitats. These is consistent with observations of [9] in the classified forest of Haute Dodo and Cavally and [21] in the National Park of Mount Sangbé who met these frogs in open and closed forests.

Phrynobatrachus tokba, *Arthroleptis* sp.1, *Arthroleptis* sp.2, were abundant in both wetlands and dry habitats (closed or sparse canopy). Water does not seem to be a limiting factor for the distribution of these taxa. This may be related to their reproductive strategies. Indeed, *P. tokba* and species of the genus *Arthroleptis* are independent of running water or ponds for spawning [66,67], as they have no aquatic larval phase. *P. tokba* has endotrophic larvae whose development take place within the original clutches on moist leaves and *Arthroleptis* undergoes direct development in moist soil.

In BNP, the presence of the river Banco and larger surface of forest ascertains the persistence of wet microclimates favorable to the development of a high anuran species diversity, e.g. *Xenopus tropicalis* (aquatic), *Aubria subsigillata* (swampy habitats species), *Hylarana albolabris* (frequent on the edges of streams), *Ptychadena aequiplicata* (wet primary forest species) and *Morerella cyanophthalma* (constant in wetlands). It also meets the characteristics of primary forest species such as *Phrynobatrachus ghanensis*, *P. phyllophilus*, *Astylosternus laticephalus* and secondary forest species, e.g. *P. liberiensis*, *P. tokba*. These latter 10 species were absent from Filtisac and University forests with significantly smaller areas, presence of water bodies (ponds) being only temporary during the rainy seasons. In addition, there we likewise note the absence of the species found, but rare in BNP: *Ptychadena bibroni*, *P. aequiplicata* and *Leptopelis macrotis*. This could be a result of forest fragmentation, in particular the reduction or loss of certain microhabitats and / or microclimates indispensable to the survival and maintenance of special amphibian species with specialized requirements [4,68]. According to [69], species showing small populations are the ones most likely to disappear from small fragments. They are often very sensitive to changes in environmental factors such as temperature and humidity [70,71].

Half of the amphibian species identified in the BNP, have a wide distribution across Africa. The other half is endemic to West Africa, with 35.7% being endemic to the Upper Guinea forest block (from Sierra Leone to Togo). Finally, five taxa are even restricted to the eastern part of the Upper Guinea forest block and concerning conservation, are the most important anurans of BNP. These are *Phrynobatrachus ghanensis*, *Arthroleptis* sp.1, *Arthroleptis* sp.2, *Astylosternus laticephalus* and *Morerella cyanophthalma*. In addition, the aquatic *Xenopus tropicalis* and the arboreal *Leptopelis macrotis* seem to be extremely rare in BNP. Only one specimen of these species was captured during the 20 months of sampling. The rate of endemism of the Banco

forest is low compared to that of forests in other parts of West Africa and Upper Guinea. In the forests of western Ghana, about 45 % of the anuran species are endemic to Upper Guinea and two thirds are endemic to West Africa [72]. Similar values (42-53 % of species) were observed in the south-eastern Guinea by [19]. However, [73] showed that, generally, the richness of amphibian declines through the forest block of Upper Guinea from west to east (specifically from Sierra Leone to Togo). In contrast [72] noted that there is still high species richness (with a high rate of endemism) in forests remnants on the southern border of Ghana and Ivory Coast. Thus, the low taxonomic richness of anuran fauna of BNP could be due to its small size [12,74] and / or the impact of the anthropogenic environmental deterioration due to its located in Abidjan (economical capital of Ivory Coast).

5. Conclusion

The species composition encountered in BNP indicated that this forest still possesses a high potential in the conservation of forest amphibians. However, the presence of many disturbance indicators and the absence of many forest species is a pressing hint to enhance and improve conservation measures for this most valuable forest.

Located in midst of the megalopolis Abidjan, Banco National Park is a precious remnant forest which represents a huge educational potential for the conservation of biodiversity. To do this, an appropriate policy for the sustainable management of this ecosystem should be conducted.

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