





Diversity of glass frogs (Centrolenidae: Anura) in tropical rain forest areas in the center of the department of Choco, Colombia

Diversidad de ranas de cristal (Centrolenidae: Anura) en zonas de bosque pluvial tropical en el centro del departamento del Chocó, Colombia

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How to cite: Palacios-Rodríguez, L.J.; Arango-Córdoba, A.M.; Rengifo-Mosquera, J.T. 2019. Diversity of glass frogs (Centrolenidae: Anura) in tropical rain forest areas in the center of the department of Choco, Colombia. Rev. U.D.C.A Act. & Div. Cient. 22(2):e1108. <http://doi.org/10.31910/rudca.v22.n2.2019.1108>

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Received: November 26, 2018

Accepted: June 13, 2019

Edited by: Ingeborg Zenner de Polanía

ABSTRACT

The central area of the department of Choco (Colombia), is composed of a tropical rain forest. Has a large of variety of amphibians that provides an ecosystem service, being the Family Centrolenidae listed as an excellent indicator of the forest condition. The focus of this study was to determine the richness and distribution in the habitats of the family Centrolenidae in two vegetation coverings in a tropical rain forest of the department of Choco, using the Visual Encounter Survey (VES) method with an effort of 240 per hour. There were 53 individuals registered, distributed in five genera and six species, being the *Hyalinobatrachium* genus the best represented. The most abundant species was *Teratohyla spinosa*, being the most dominant for the secondary forest and *Hyalinobatrachium collymbiphyllum* for the primary forest. *Espadarana callistomma* and *Teratohyla spinosa* were the most plastic species in

terms of habitat since they were recorded in both coverages. The centrolenids registered showed a preference for high (73.5%) and medium (26.4%) perches, in terms of the substrate leaf (83%) and the branch (17%). Regarding the state of conservation, the species are in the category of LC, DD, and NT; however, it is important to conserve their habitats since some centrolenids are tolerant to intervened ecosystems that have good vegetation and bodies of water with a permanent flow for their development.

Keywords: Anurans; primary forest; secondary forest; centrolenids; Choco frogs.

RESUMEN

La zona centro del departamento del Chocó (Colombia) está compuesta por bosque pluvial tropical, alberga una significativa

riqueza de anfibios, que brinda servicios ecosistémicos, siendo la familia Centrolenidae, catalogada como una excelente indicadora de las condiciones del bosque. El objetivo de este estudio fue conocer la estructura y el uso de hábitat de especies de la familia Centrolenidae en dos coberturas vegetales, en un bosque pluvial tropical, del departamento del Chocó, empleando el método Relevamiento por encuentros visuales (VES), con un esfuerzo de 240h/per. Se registraron 53 individuos, distribuidos en cinco géneros y seis especies, siendo el género *Hyalinobatrachium*, el mejor representado. La especie más abundante fue *Teratohyla spinosa*, siendo la más dominante para el bosque secundario e *Hyalinobatrachium colymbiphyllum*, para el bosque primario. *Espadarana callistomma* y *Teratohyla spinosa* fueron las especies más plásticas, en términos de hábitat, ya que se registraron en ambas coberturas. Los centrolénidos registrados mostraron preferencia por las perchas alta (73,5%) y media (26,4%); en términos del sustrato indicaron mayor preferencia por las hojas y ramas, con 83 y 17%, respectivamente. Referente al estado de conservación, las especies se encuentran en la categoría de NT, DD y LC; sin embargo, es importante conservar sus hábitats, puesto que la transformación de los hábitats naturales genera el declive en las poblaciones.

Palabras clave: Anuros; bosque primario; bosque secundario; centrolénidos, Chocó.

INTRODUCTION

Amphibians are an important component of the biota present in many ecosystems, and in some cases are considered the largest fraction of vertebrate biomass, thus contributing to the trophic dynamics of the communities to which they belong (Valencia-Aguilar *et al.* 2013). However, the loss, fragmentation and transformation of natural habitats as a consequence of human activities (Knutson *et al.* 1999) in the last 50 years has generated a considerable and irreversible loss of diversity in ecosystems (Andrade-C., 2011). It is believed that there are approximately 277 species of anurans nationwide in some category of threat (Acosta Galvis, 2019).

The family Centrolenidae groups the commonly known glass frogs due to its translucent skin, distributed in 10 genera and 79 species approximately for Colombia (Acosta Galvis, 2019). This group of amphibians is well represented in the humid forests of the Pacific (Bustamante *et al.* 2007; Rengifo & Lynch, 2010). The central area of the department of Choco has a registry of six genera and approximately 13 species.

The ecological contribution of amphibians to ecosystems, together with the global disappearance of a large number of species and the decline of 43% of their populations worldwide (Stuart *et al.* 2004; Wake & Vredenburg, 2008), reflects the urgent need to carry out immediate actions that deepen in the knowledge of the specific causes of decline, in the study of their biology, ecology and in the design of conservation strategies.

The Centrolenidae family presents a variety of characteristics and behaviors that make it possessor of a high species richness, for this

reason, it is important to understand the ecology and distribution in the forests of the department of Choco, where some studies by Hayes & Starrett (1980), Lynch & Suárez-Mayorga (2004) and Rengifo & Lynch (2010) have shown that the central area of the department of Choco tropical rainforest presents a high potential to house a high richness of species of centrolénidos, however, the change of land use and the transformation of habitats at the hand of man (Gibbs *et al.* 2010; Carr, 2004) could be affecting the diversity of organisms due to the reduction of plant cover.

In the department of Choco, the diversity of amphibians is very important, however, many species lack the assessment of their conservation status, particularly in areas where diverse anthropogenic actions exert a negative pressure, which could lead to the reduction of their diversity (Myers *et al.* 2000), which is a cause for concern because many of the species of the Centrolenidae family are not very tolerant of the transformation of their habitats and require bodies of water with favorable conditions for their development. The present study aims to generate information about the structure and habitat use of species of the family Centrolenidae in an area currently impacted by different anthropic activities, which will allow the development of future management and conservation plans with the communities that live in the areas of Tropical rainforest.

MATERIALS AND METHODS

Study area. The study area was located between the geographic coordinates of 5°00'-6°45'N and 76°30'-77°15'O (Table 1). The precipitation regime is bimodal-tetra-stational with a period of higher rainfall between April and October and a period of lower concentration from November to March (Poveda *et al.* 2004). The annual rainfall is on average 8558mm with a monthly average of 395.5mm (Rangel-Ch. & Lowy, 1993). The general climatic limits are an average temperature higher than 24°C (Table 1).

The five sampling zones, Pan-American Union, Certegui, Lloró, Atrato, and Quibdó, are located in the alluvial plains, low hills and foothills in the central area of the Choco department of tropical rainforests (bp-T), where the largest concentration pluviosity of the Pacific platform is concentrated (Chocó Biogeográfico). Although the forests of the Chocó Biogeográfico are homogeneous, these zones present a great variety of ecosystems, which means the presence of suitable conditions to show a wealth of remarkable fauna and flora, however, the region is being affected by some actions anthropic, such as mining, selective extraction of fauna and flora and plantation of agricultural crops (Palacios Rodríguez *et al.* 2018; Rengifo M. *et al.* 2014; Rengifo M. *et al.* 2019).

Fieldwork. The present study was conducted in periods between March and June 2016, with a duration of six days in each vegetation cover. Two types of vegetation cover (primary and secondary forest) were selected and replications were made at the five sampling points. It is considered the primary forest, such as that which has existed without significant human disturbance and other disturbances during periods exceeding the normal life span of mature trees of 60 to 80 years according to FAO (Anón, 1982). The secondary

Table 1. Location and biophysical characteristics of the five sampling areas in a tropical rain forest in the central area of the department of Choco. Temperature (Temp °C), Relative humidity (%), Average annual precipitation (PP mm), Tropical rain forest (bpt). Data were taken from Rengifo *et al.* (2014).

Municipality- Sample points	Coordinates	Temp °C	Humidity %	Altitude	PP mm	Life zone
Unión Panamericana- Salero	5°22' N, 76°36' W	28	90	100	7.600	bp-T
Certegüi-Recta Larga	5°41'41" N, 76°39'40" W	28	95	43	7.000	bp-T
Lloró-CMUTCH	5°30'37" N, 76°33'15" W	28	85	48	10.000	bp-T
Atrato-Samurindó	5°35'15" N, 76°39'15" W	28	91,8	30	8.000	bp-T
Quibdó-Urbana y Suburbana	5°43'13" N, 76°37'40" W	26,5	87	47	12.000	bp-T

forest, corresponds to a stage of the forest cover that arises after a total anthropogenic intervention (of more than 90%) of the primary vegetation, creating regeneration conditions, which lead to a structure different from the original forest, with another composition of arboreal species and another dynamic, without having yet reached its original state again, that is, it is clearly differentiated from the state of the original forest (ECO, 2000).

In the sampling sites, the aforementioned plant coverings were qualitatively identified. For the primary forest, observations were made around trees, shrubs bordering the current water currents of waters, rocks, soil. For the secondary forest, places with little vegetation, bodies of water with apparently low quality or with some type of contamination (transformed habitats) were observed.

The samplings were carried out at night times from 6-10pm. through free and unrestricted travels through surveys by Visual Encounter Survey (VES) (Crump & Scott, 1994), using a sampling effort of 240 hours/person (120 hours Primary Forest and 120 hours Secondary Forest).

The identification of the registered species was made based on specialized guides (Renjifo & Lundberg, 1999; Páez *et al.* 2002; Ron *et al.* 2018) and scientific articles (Guayasamín *et al.* 2009; Ruiz-Carranza & Lynch, 1991a; b; Ruiz-Carranza & Lynch, 1998). Additionally, the determinations were corroborated with reference material from the Herpetology Collection of the Universidad Tecnológica del Chocó. Additionally, a bibliographic review of the centrolénidos species registered for the area under study was carried out.

During the samplings, records of some ecological data suggested by Heyer *et al.* (1994) as the vertical position or height of the perch (from the ground or water) and classified in the following ranges: soil (0cm), low (1 - 49cm), medium (50 - 149cm), high (> 149cm). The type of substrate or perch site was classified as branches, stems, leaves, litter, rock. The information on the state of conservation and endemism was documented according to the IUCN (2018).

Data analysis. To determine if the sampling effort in the study areas was sufficient to know the species richness, non-parametric estimators such as CHAO2 and ICE were used, which relates the accumulated species according to the sampling effort, using presence and absence data of registered species using the EstimateS Version 6.0 program. The alpha diversity was evaluated by determining the number of individuals in each cover and the relative abundance was determined by dividing the number of individuals per species in each cover, over the total of captured individuals, and also the capture success was calculated to determine the representativeness of the applied sampling effort.

In addition, the dominance index (Simpson) and equity (Shannon-Wiener & Pielou) were used (Baev & Penev, 1995). Beta diversity, defined as the similarity of species among the sampling areas (primary forest and secondary forest), was analyzed using the Jaccard similarity coefficient (Magurran, 1988).

Nonparametric methods were used to compare the coverage and determine if there are significant differences in the composition and use of habitat through the PAST program. Version 1.15 (Hammer & Harper, 2003). X² chi-square was performed to evaluate habitat use.

RESULTS AND DISCUSSION

Composition of Species. In total, 53 individuals belonging to six species and four genera were recorded (Table 2, Figure 1), in a sampling effort of 240 hours/person, obtaining a capture success of 0.22 individual/hour.person. The bibliographical review carried out in this geographical area reports the presence of five species of centrolénidos that were not registered in this study (*Cochranella ramirezi*, *Espadarana prosoblepon*, *Hyalinobatrachium chirripoi*, *Sacbatamia albomaculata* and *Sacbatamia ilex*) event that could be related to the different anthropic interventions that currently facing these ecosystems due to habitat removal due to mining at different scales, the indiscriminate felling of trees, and contamination of water bodies by contamination with chemicals, which are the main habitats of this faunal group, possibly generating the decrease in the populations of this group of amphibians.

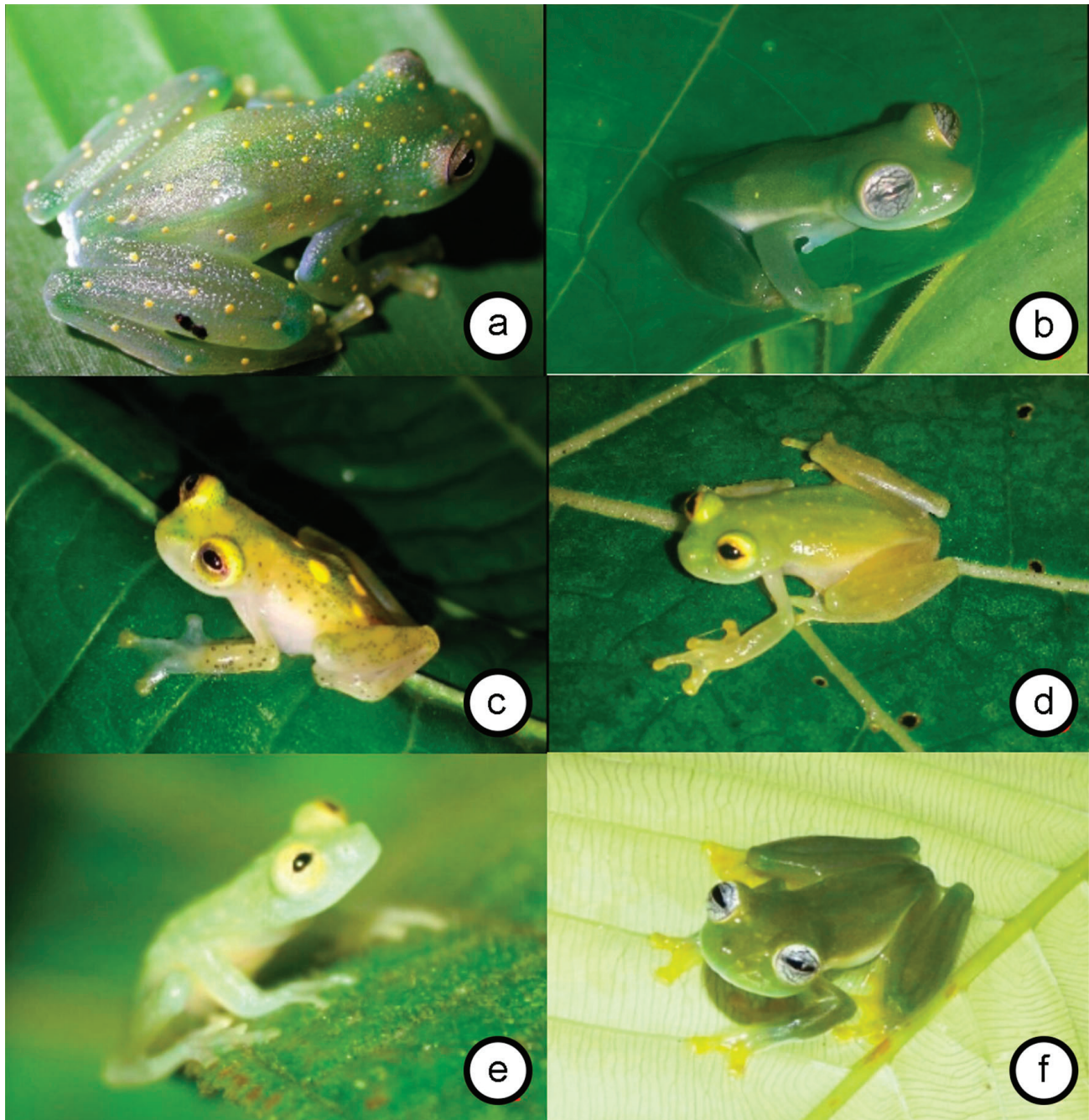


Figure 1. Species of centrolenids present in areas of tropical rain forest in the center of the department of Choco. a. *Cochranella eucknemus*; b. *Espadarana callistomma*; c. *Hyalinobatrachium aureoguttatum*; d. *Hyalinobatrachium colymbiphylum*; e. *Hyalinobatrachium fleischmanni*; f. *Teratohyla spinosa*.

Table 2. Taxonomic composition, total (n) and relative abundance (%) of centrolénidos in the vegetal coverings sampled in the department of Choco. Primary forest (BP), secondary forest (BS), number of individuals (N), relative abundance (%).

Genera	Species	Vegetables cover		Abundance		IUCN	Reference
		BP	BS	(N)	(%)		
<i>Cochranella</i>	<i>Cochranella euknemus</i>	1	0	1	1,89	LC	1, 2, 3, 4
	<i>Cochranella ramirezi</i>	-	-	-	-	DD	2
<i>Espadarana</i>	<i>Espadarana prosoblepon</i>	-	-	-	-	LC	2, 3
	<i>Espadarana callistomma</i>	6	7	13	24,53	DD	4
<i>Hyalinobatrachium</i>	<i>Hyalinobatrachium aureoguttatum</i>	2	0	2	3,77	NT	2, 3,4
	<i>Hyalinobatrachium chirripoi</i>	-	-	-	-	LC	1, 2
	<i>Hyalinobatrachium colymbiophyllum</i>	10	0	10	18,87	LC	2, 3, 4
	<i>Hyalinobatrachium fleischmanni</i>	0	1	1	1,89	LC	1, 3, 4
<i>Sachatamia</i>	<i>Sachatamia albomaculata</i>	-	-	-	-	LC	1, 2
	<i>Sachatamia ilex</i>	-	-	-	-	LC	1, 2
<i>Teratohyla</i>	<i>Teratohyla spinosa</i>	2	24	26	49,06	LC	3,4
Total		21	32	53	100		

LC: Least Concern; DD: Deficient Data

Hayes & Starrett, 1980; 2) Lynch & Suárez-Mayorga, 2004; 3) Rengifo & Lynch, 2010; 4) Registered in the present study.

The non-parametric wealth estimators in the two vegetation cover sampled showed different behaviors; for the primary forest, the Chao2 estimator indicates that the registered species equals 83.3% while for ICE 76.1% of the estimated species (Figure 2a). In the secondary forest area, the registered species are equivalent to 75% of the species estimated by ICE, while according to Chao2, the registered species correspond to the totality of the species with the effort of inverted sampling (Figure 2b). When estimating the richness of centrolénidos for the primary forest and to graph the curve of accumulation of species, this did not reach its asymptote, indicating that some additional species could be registered when intensifying the effort of sampling. For the secondary forest, on the other hand, the species accumulation curve reaches its stability, showing that the sampling effort applied to this area was significant since the species estimated for this coverage were recorded. The behavior of the curve of the primary forest is normal for the tropical ecosystems since the prolonged work and in different periods of the year are those that achieve the stabilization of the curves (Vargas & Bolaños, 1999; Rengifo *et al.* 2015). that it is necessary to carry out samplings with more time to register more species that could possibly be found in these sites and be able to evaluate the status and size of their populations, the possible threats they may be facing and factors that could be having a negative impact.

Of the four registered genera, *Hyalinobatrachium* was the best represented with three species, while the rest of genera were represented by a single species, data that coincide with a study conducted by Vargas & Bolaños (1999) in an inventory of frogs in the

bass Anchicayá, Valle del Cauca, finding the genus *Hyalinobatrachium* as the best represented within the family. Of the total of recorded species *T. spinosa* was the most abundant species with 49% (n = 26) and the rest of the species were represented together with a percentage of 50.9% (Table 2). In this study we recorded the species *T. spinosa*, *H. fleischmanni* and *E. callistomma* in secondary forest areas, the fact that these species are found in this type of habitat could indicate that some species of centrolénidos present resilience or tolerance to modifications in their habitats causing these species to persist in these ecosystems, the secondary forests without direct and obvious intervention in the surrounding vegetation of the streams, could present the conditions that allow the reproduction and development of some species since the presence of some species of centrolénidos it is determined by the males when selecting the sites with the ideal conditions for their reproductive processes (Rojas-Morales & Escobar-Lasso, 2013), Guayasamín *et al.* (2009), affirm that centrolénidos species require streams with permanent flow and the presence of suspended vegetation to adhere their mass of eggs. As has been demonstrated in some investigations with glass frogs (Ramírez J. *et al.* 2009; Basto-Riascos *et al.* 2017), however, it is important to use conservation strategies since some species of centrolénidos do not tolerate changes or transformations in their habitats, which evidences an imminent risk for these species.

According to the analysis of nonparametric estimators, no significant difference was found between the coverages evaluated with respect to the composition and use of habitat (P = 0.6759), which may be related to the closeness and forestry continuity of both coverages,

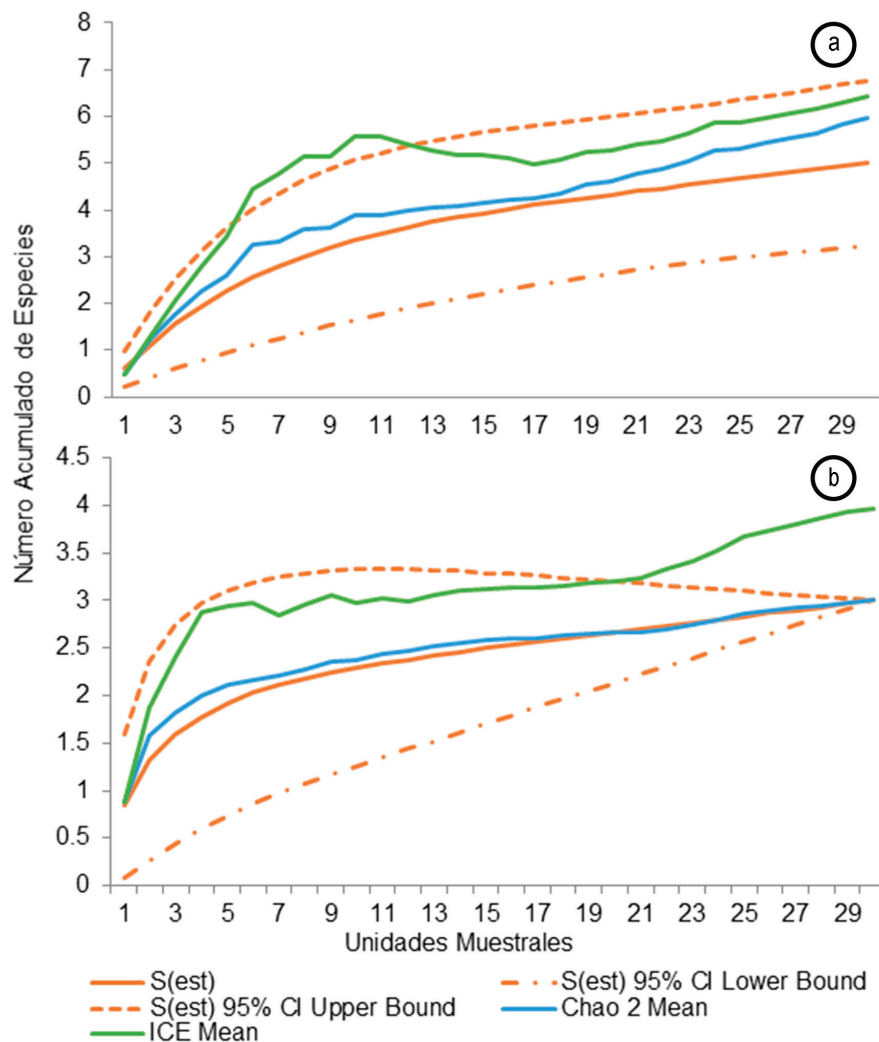


Figure 2. Curve of accumulation of species. a. Primary Forest; b. Secondary Forest.

making both forest types (primary and secondary) are similar, which shows that the support vegetation in both forests is significant, which allows the presence of these species in these sites. The Jaccard similarity coefficient reveals that the primary forest zone shares 33.3% of the species with the secondary forest, among which are *E. callistomma* and *T. spinosa*. The dominance-diversity index indicates that *H. colymbiphyllum* was the most abundant species for the primary forest and *T. spinosa* for the secondary forest. Some species vocalize from the high perches, mostly from the beam or the back of the leaves to attract the females for the amplexus and they register in this type of heights to avoid being easily preyed by crawling animals, due to greater availability of food and favorable conditions. for the development of their populations (Cardozo-Urdaneta & Señaris, 2012) (Table 3).

Regarding the registered hanger height for each of the species, there was no significant statistical difference ($X^2 = 7.12$, $P = 0.21$) within the evaluated heights, but when analyzing the percentage values a greater number of records on high hangers, with 73.5%, followed by middle positions with 26.4%. *Cochranella euknemos* is

the species recorded at the highest level of vegetation structure in the primary forest, followed by the species *T. spinosa* (Figure 1f). For the secondary forest, *H. fleischmanni* was the highest recorded species with only one individual, followed by the *E. callistomma* species (Table 4). The species *T. spinosa* presented a higher number of individuals in high and medium positions, followed by the *E. callistomma* species in both coverages while *H. colymbiphyllum* was recorded in this type of height only in the primary forest, the results agree with (Rada *et al.* 2007; Cardozo-Urdaneta & Señaris, 2012), where they refer that centrolénidos species were registered in high and medium positions.

All the individuals of centrolénidos were registered to heights greater than 100cm of the ground, as they refer different studies carried out (Ortega-Andrade *et al.* 2013; Rojas-Morales & Escobar-Lasso, 2013; Vargas-S. & Castro-H., 1999), show that the species *H. aureoguttatum* was registered in terms of its vertical location or height in medium and high position, data that agree with the results of our investigation. Although few species were recorded in the secondary forest area, all were above 2m, this may be because this

Table 3. Wealth and diversity values of Cristal frogs for the sampled coverages (primary forest and secondary forest). BP = Primary Forest, BS = Secondary Forest.

Indices	BP	BS
Richness	5	3
Abundance	21	32
Shannon Wiener (H)	1,304	0,6565
Dominance (λ)	0,3288	0,6113
Richness (Margalef)	1,314	0,5771
Pielou equitability (J)	0,8103	0,5976
Simpson Dominance (λ)	0,6712	0,3887

Table 4. Vertical position and substrate of the glass frogs in the study area. (N = number of individuals). Number of individuals (N), relative abundance (%).

Species	Vertical position				Substrates			
	half		upper		leaf		branch	
	N	(%)	N	(%)	N	(%)	N	(%)
<i>Teratohyla spinosa</i>	8	57.1	18	46.2	19	43.2	7	77.8
<i>Espadarana callistomma</i>	5	35.7	9	23.1	11	25.0	2	22.2
<i>Hyalinobatrachium colymbiophyllum</i>	1	7.1	8	20.5	10	22.7	0	0.0
<i>Hyalinobatrachium aureoguttatum</i>	0	0.0	2	5.1	2	4.5	0	0.0
<i>Hyalinobatrachium fleischmanni</i>	0	0.0	1	2.6	1	2.3	0	0.0
<i>Cochranella euknemos</i>	0	0.0	1	2.6	1	2.3	0	0.0
Total	14	100.0	39	100.0	44	100.0	9	100.0

coverage presents traces of the natural forest, which makes it contain trees, shrubs, and good vegetation, allowing to find these species in this type of coverage and also, some species prefer the perches to ensure the reproductive success of their eggs and to avoid crawling predators or being trodden by other animals that use these sites as forages (Rueda-A., 1994).

There was no significant statistical difference ($X^2 = 4.71, P = 0.45$) in terms of substrates or perch sites (leaves and branches) where the family species Centrolenidae were recorded (Table 4), but by percentage comparison, the leaves were the substrate with the largest number of records with 83% and the branches with 17%. The species *T. spinosa*, *E. callistomma* and *H. colymbiophyllum* presented a greater number of individuals in the leaves showing a greater preference for this substrate, which could be due to the fact that the leaves are more preferred sites where the centrolénidos perform the amplexus, the so-called or singing of the males, parental care and the deposition or postures of their eggs so that they fall directly into the water and complete their development cycle (Rojas-Morales *et al.* 2011; Ortega-Andrade *et al.* 2013).

According to the IUCN (2018), the species *H. fleischmanni*, *H. colymbiophyllum*, *T. spinosa*, and *C. euknemos* are in a state of “Least Concern” (LC) however *E. callistomma* is in the category of “Deficient Data”(DD), and *H. aureoguttatum* is registered as an “Almost Threatened” species, which shows the importance of conserving the habitats of these species, since their low representativeness or abundance could indicate that the anthropic actions would be affecting populations of these species.

Acknowledge. We thank the research group in Herpetology of the Universidad Tecnológica del Chocó, for their collaboration in the field and laboratory in the identification of the specimens and information and tutoring provided, and we also thank the Ph.D. Wilmar Bolivar Garcia for the support, documentation, and identification of specimens in the laboratory. **Conflict of interests:** The article was prepared and reviewed with the participation of all authors, who declared that there is no conflict of interest that jeopardizes the validity of the results presented. **Founding:** The research was funded by the Herpetology Research Group, through the Universidad Tecnológica del Chocó “Diego Luis Córdoba”.

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