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Amphibians from the cloud forest of El Silencio de Los Ángeles, San Ramón de Alajuela, Costa Rica

Anfibios del bosque nuboso de El Silencio de Los Ángeles, San Ramón de Alajuela, Costa Rica

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Abstract

We present the list of amphibians for the El Silencio de Los Ángeles Cloud Forest (Villa Blanca Hotel and Reserve) in San Ramón de Alajuela, Costa Rica, Central America. We performed nocturnal visual and acoustic surveys in the trail system, mainly from 2013 to 2014. We also received data from the local guides up to 2019. We compared the similarity of amphibian richness of our site against other premontane reserves in Costa Rica with a cluster analysis (Jaccard index, single linkage). We recorded 27 species distributed in 17 genera, nine families and three orders. *Notrotiton gamezi* was the only threatened species detected. The El Silencio de los Ángeles Cloud Forest site is around 80 % similar to the San Lorencito River Station and 55% to Nectandra Reserve. Containing 12% of the Costa Rica's amphibian richness, this premontane woodland should be considered among the most important clouded forest in Central Costa Rica for amphibian conservation.

Key Words: Biological inventory, Central American Herpetofauna, citizen science, herping, Neotropical region, *Nototriton gamezi*.

Resumen

Presentamos la lista de anfibios para el Bosque Nuboso de El Silencio de Los Ángeles (Hotel y Reserva Villa Blanca) en San Ramón de Alajuela, Costa Rica, Centro América. Realizamos estudios nocturnos visuales y acústicos en el sistema de senderos, principalmente de 2013 a 2014. También recibimos datos de los guías locales hasta 2019. Comparamos la similitud de la riqueza de anfibios de nuestro sitio contra otros sitios premontanos en Costa Rica con un Análisis Clúster (índice de Jaccard, enlace único). Registramos 27 especies distribuidas en 17 géneros, nueve familias y tres órdenes. *Notrotiton gamezi* fue la única especie amenazada detectada. El sitio del Bosque Nuboso de El Silencio de los Ángeles presenta una similitud de alrededor del 80% a la Estación del Río San Lorencito y 55% a la Reserva Nectandra. Con un 12% de la riqueza de anfibios de Costa Rica, este bosque premontano debe considerarse entre los bosques nublados más importantes del centro de Costa Rica para la conservación de los anfibios.

Palabras Clave: ciencia ciudadana, inventario biológico, herpetofauna centroamericana, *Nototriton gamezi*, observación de anfibios, región Neotropical.

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I. Introduction

Costa Rica contains nearly 215 species of amphibians in a relative small area (51100 km2) (Zumbado-Ulate et al., 2019). The diversity and distribution of amphibians in Costa Rica is considered among the best known in comparison with other Neotropical countries (Savage, 2002), but despite this, there is an absence of amphibian and reptile checklists from the Western region of Central Valley in comparison with areas such as Sarapiquí, Monteverde, San Vito or Fila Costeña (Rovito *et al.*, 2015). Bolaños and Ehmcke (1996) inventoried the herpetofauna of "Alberto Manuel Brenes" Biological Reserve (hereafter AMBBR) in Northwest San Ramón from 1986 to 1996, and later that effort was updated by Morera-Chacón and Sánchez-Porras (2015), Morera-Chacón and Jiménez (2017), and Acosta-Chaves *et al.*, (2019) to obtain 41 species of amphibians; unfortunately, some were enigmatically extirpated from the reserve. More recently the Nectandra Reserve was inventoried with 17 species of amphibians recorded (Rovito *et al.*, 2015). Other recent central Costa Rica checklists between 500 to 1800 meters above sea level (m asl) by Arias and Bolaños (2014) in San Isidro de Dota from the Pacific slope of San José where 30 species were documented, and later by Acosta-Chaves *et al.*, (2015) and Acosta-Chaves *et al.*, (2016) in Río Macho Biological Station of the Orosi Valley with 12 amphibians species reported. Recently, with data obtained by citizen science Gómez-Hoyos *et al.*, (2018) generated a checklist of 14 amphibian species for the surrounded premontane areas of La Amistad International Park in Costa Rica. Currently, other survey efforts to understand the diversity of herpetofauna from middle elevation forests in Costa Rica are limited to museum specimens collected (Acosta-Chaves, obs.pers.).

By means of multiple surveys and citizen science submissions a checklist of amphibians observed in the premontane woodland in the El Silencio de Los Angeles Cloud Forest (hereafter ESLA Cloud Forest) of San Ramón, Costa Rica has been produced and is presented here. The purpose of this checklist is to draw attention to the ESLA Cloud Forest amphibian diversity and to enhance conservation efforts through further research.

Additionally, we compared the richness for ESLA Cloud Forest against other reserves located within the foothills of central Costa Rica in order to understand the similarities and differences between compositions. Based on similar elevation and life zones, we predicted that ESLA Cloud Forest amphibian diversity will be comparable to the richness of the Nectandra Reserve and San Lorencito River Station of AMBBR. In the future this research will be useful to compare with other checklists to determine which species are currently colonizing and/or disappearing from the middle elevations in Costa Rica (Acosta-Chaves and Cossel, 2016; Acosta-Chaves *et al.*, 2019).

II. Material and methods

II.1. Study area

The ESLA Cloud Forest is within the Caribbean basin, close to the continental divide, and located approximately 16 km north the town of San Ramón, San Ramón canton, province of Alajuela. Our study site is about nine kilometers east from the highway to La Fortuna (Route 702), that also dissects the Nectandra Reserve and later travels adjacent to the entrance of the AMBBR (Rovito *et al.*, 2015). This type of forest is a wet premontane belt according to the Holdridge life zones classification (Holdridge, 1987). Our work was carried out in the private reserve of the Villa Blanca Hotel and Cloud Forest Reserve (1127803,742m N; 446907,055m W, datum WGS84; 1100 m. ASL Figure 1). Annual precipitation varies between 2000 to 4000 mm (Morera and Granados, 2013), and the average temperture is of approximately 19° C. The reserve encompasses 70 hectares, it is mostly vegetated with secondary forest with at least 20 years of regeneration that are surrounded by fragments of primary forest, and it has several bodies of standing water and streams (Appendix 1).

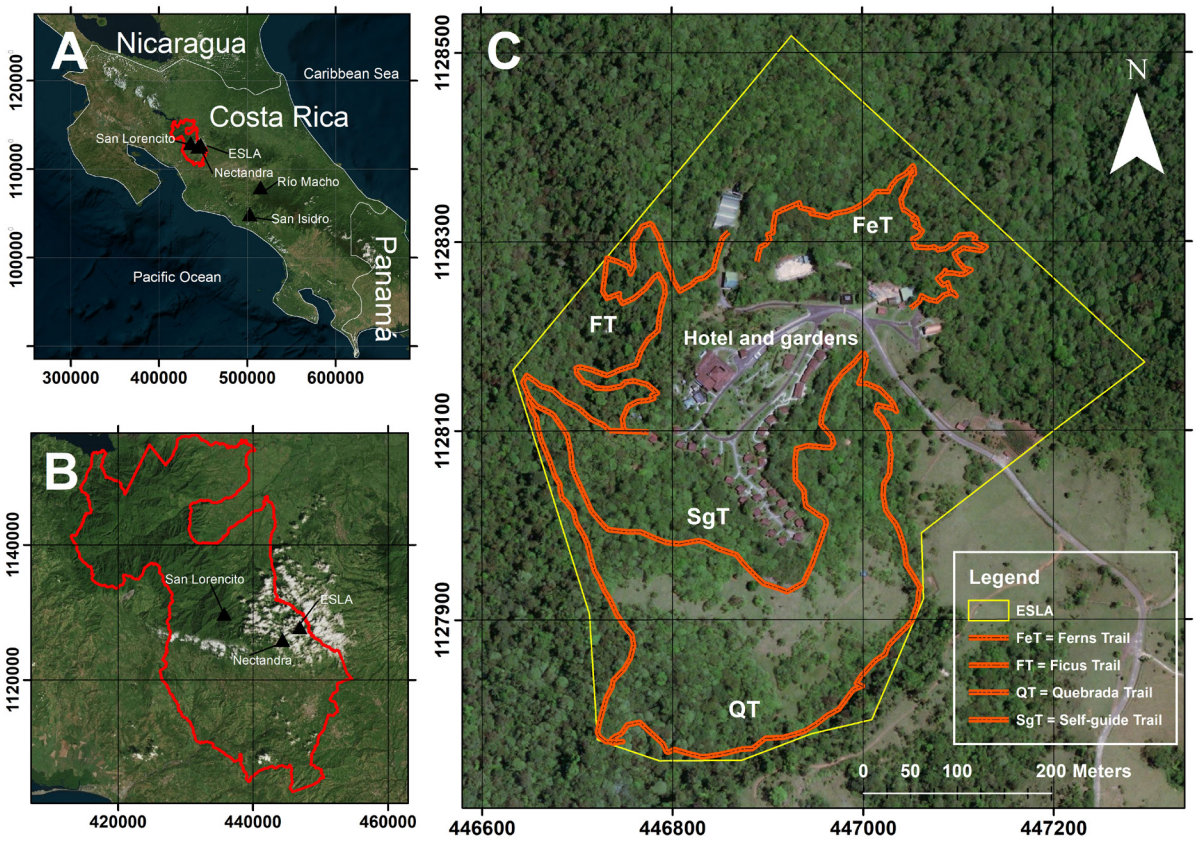

Figure 1. Map of Costa Rica showing the location of El Silencio de Los Ángeles Cloud Forest (Villa Blanca Hotel), Nectandra Reserve, San Lorencito River Station of Alberto Manuel Brenes Biological Reserve, San Isidro de Dota and Río Macho Biological Station. Additionally, there is a close up of the trail system in Villa Blanca Hotel and Cloud Forest Reserve.
Source: Own elaboration.

II.2. Data collection and analysis

From August 2013 to March 2014, July 2018 and September-november 2019, we conducted visual and acoustic encounter surveys (Heyer et al., 1994). We sampled the trails (Quebrada Trail, Self-guided Trail, Ficus Trail and Ferns Trail) and the gardens in Villa Blanca Hotel and Cloud Forest Reserve (Figure 1, Appendix 1), searching for amphibians in areas with primary and secondary forest, streams, and ponds. The sampling effort consisted of two-hour survey once a month. We additionally utilized the reports of species with photographic records made by the local tour guides and random observations. No individuals were collected during surveys. All animals were identified based on preview experience (e.g. Acosta-Chaves et al. 2015, 2016, 2019), and field guides such as Savage (2002) and Leenders (2016). Our checklist was produced following Amphibian Species of the World (Frost, 2018). We identified endangered species following the IUCN Red List of Threatened species (IUCN 2018) and the Conservation Priority levels of endemic amphibian and reptiles of Central America (Mata-Silva et al. 2019).

To understand what the level of similarity in amphibian richness between, the ESLA Cloud Forest and other recent checklists from premontane localities is, we compared our results against those reported for the Nectandra Reserve (1000-1200 m ASL), the San Lorencito River Station of AMBBR (500-1000 m ASL), the San Isidro de Dota (680-800 m ASL) and the Río Macho Station (1500-1800 m ASL). A cluster analysis (single linkage, Jaccard index) using the software PAST 3.2 (Hammer *et al.*, 2001) was calculated to obtain the level of similarity between sites.

III. Results

The total species richness for the ESLA Cloud Forest consisted of 27 species distributed in three orders, nine families, and 17 genera (Table 1, Appendix 2). Currently, the ESLA Cloud Forest contains about 12% of the amphibians registered for Costa Rica. Following the classification of Frost (2018), both Hylidae and Craugastoridae families had the highest number of species in the ESLA Cloud Forest, each with six species (Table 1). However, if Strabomantidae is a valid family (Heinicke et al., 2018) then Craugastoridae, Strabomantidae, Bufonidae, Ranidae, and Centrolenidae contained a similar number of species (Table 1).

Table 1. List of amphibians found in the El Silencio de Los Ángeles cloud forest, Costa Rica, Central America. Sampled areas and trails: QT = Quebrada Trail, SgT = Self-guided Trail, FT = Ficus Trail, FeT = Ferns Trail, G = Gardens.

Taxon	QT	SgT	FT	FeT	G
Order Gymnophiona					
Family Dermophiidae					
Gymnopis multiplicata Peters, 1874					**x**
Order Caudata					
Family Plethodontidae Gray, 1850					
Oedipina uniformis (Keferstein, 1868)					x
Nototriton gamezi (García-París & Wake, 2000)				x	
Order Anura					
Family Bufonidae J. E Gray 1825					
Incilius coniferus (Cope, 1862)	x			x	x
Incilius melanochlorus (Cope, 1877)	x	x	x	x	
Rhinella horribilis (Wiegmann, 1833)					x
Family Centrolenidae Taylor, 1951					
Cochranella granulosa (Taylor, 1949)					x
Espadarana prosoblepon (Boettger, 1892)	x	x		x	x
Teratohyla pulverata (Peters, 1873)					x
Family Craugastoridae Hedges, Duellman & Heinicke, 2008					
Craugastor crassidigitus (Taylor, 1952)	x	x	x	x	
Craugastor fitzingeri (Schmidt, 1857)	x	x	x	x	
Craugastor underwoodi (Boulenger, 1896)		x		x	x
Pristimantis cerasinus (Cope, 1875)	x	x		x	
Pristimantis cruentus (Peters, 1873)	x	x	x	x	
Pristimantis ridens (Cope, 1866)	x	x	x	x	
Family Eleutherodactylidae Lutz, 1954					
Diasporus diastema (Cope, 1875)	x	x	x	x	x
Family Hylidae Rafinesque, 1815					
Dendropsophus ebraccatus (Cope, 1874)	x			x	x
Duellmanohyla rufioculis (Taylor, 1952)	x			x	
Scinax elaeochrous (Cope, 1875)					x
Smilisca manirosum (Taylor, 1954)				x	
Smilisca phaeota (Cope, 1862)	x			x	x
Smilisca sordida (Peters, 1863)				x	

Familia Leptodactylidae Werner, 1896 (1838)					
Leptodactylus savagei Heyer, 2005				x	
Family Phyllomedusidae Gunther, 1858					
Agalychnis callidryas (Cope, 1862)	x			x	
Family Ranidae Rafinisque, 1814					
Lithobates taylori (Smith, 1959)			x		
Lithobates vaillanti (Brocchi, 1877)				x	
Lithobates warszewitschii (Schmidt, 1857)	x	x	x	x	x
Total number of species	14	10	7	20	11

Source: Own elaboration.

The species richness was different between trails: the Ferns Trail contained the highest number of species, while the Ficus Trail contained the lowest (Table 1). Some species such as *Craugastor underwoodi, Diasporus diastema,* and *Lithobates warszewitschii* were found everywhere, while others including *Gymnopis multiplicata Oedipina uniformis, Nototriton gamezi, Rhinella horribilis, Cochranella granulosa, Teratohyla pulverata, Scinax elaeochrous* and *Smilisca sordida, Leptodactylus savagei,* and *Lithobates vaillanti* were captured only at one location (Table 1).

Most of the species detected in the ESLA Cloud Forest are considered as Least Concerned according to the IUCN Red List of Threatened Species (UICN 2018); only the salamander *N. gamezi* is classified as Vulnerable. However, the 42% of the amphibians of ESLA Cloud Forest are endemic to Central America, especially from the Isthmian Central American highlands and Caribbean lowlands from Nicaragua to Panama (Mata-Silva et al., 2019). According with the Conservation Priority levels established recently by Mata-Silva et al. (2019) the species *N. gamezi, O. uniformis, C. underwoodi, D. rufioculis, S. manisorum* are in priority one, *P. cerasinus* is in priority three, *D. diastema* is in priority four, G. multiplicata *C. granulosa* is in priority five, *I. melanochlorus* and *L. taylori* are in priority seven, and *L. warszewitschii* is in priority ten.

After comparing the amphibian richness of the ESLA Cloud Forest against other localities from San Ramón, we found that it was roughly 80% similar with the San Lorencito River Station and less than 55% similar with the Nectandra Reserve. When the ESLA Cloud Forest was compared with localities outside San Ramón the contrast was higher; for the San Isidro de Dota and the Río Macho Station, the relationship was lower than 35% and 30 % respectively (Figure 2).

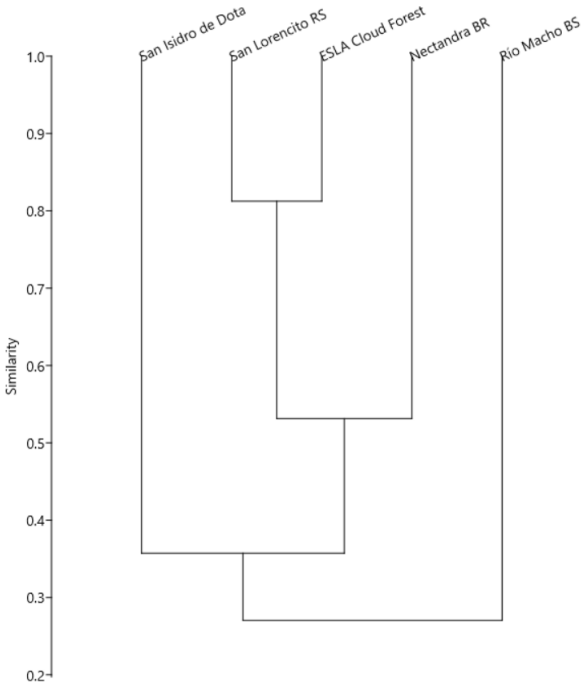

Figure 2. Cluster analysis (Jaccard index, single linkage) showing the percent of similarity between amphibian richness of recent checklists from different premontane and lower montane locations in central Costa Rica (San Isidro de Dota, Nectandra Biological Reserve, San Lorencito River Station of Alberto Manuel Brenes Biological Reserve, El Silencio de Los Ángeles Cloud Forest and Río Macho Biological Station).

Source: Own elaboration.

IV. Discussion

There is a strong association between environment variation and amphibian diversity at the ESLA Cloud Forest. Microhabitats (primary and secondary forest, streams and ponds) along the Fern Trail correlated to greater amphibian richness. In contrast, fewer species were observed in the primary forest of the Ficus Trail. Common species such as *C. underwoodi, D. diastema* and *L. warszewitschii* were detected in all the sites due to their wide habitat parameters: *C. underwoodi* utilizes suitable secondary and primary forest (Acosta-Chaves *et al.*, 2015; 2016), *D. diastema* inhabits the understory and canopy layers of forest and borders, using bromeliads as breeding sites (Savage, 2002; Acosta-Chaves *et al.*, 2015); while *L. warszewitschii* breeds in streams and ponds, and can forage on the interior forest floor (Savage, 2002). The least common species were microhabitat specialist occupying: salamanders (*N. gamezi* and *O. uniformis*) were observed on streambanks with accompanying mosses *R. horribilis* inhabits open and disturbed sites and *D. rufiocollis*, *C. granulosa, T. pulverata* and *S. sordida* were restricted to streams and rivers (Savage, 2002; Leenders, 2016). In general, the trail system in the ESLA Cloud Forest provides abundant natural and artificial ponds that are used by frogs and toads from the families Hylidae, Phyllomedusidae, Bufonidae and Ranidae for reproductive activities.

Additionally, several species that are common at lower elevations have been gradually appearing in the ESLA Cloud Forest. An example of potential successful colonization involves *Agalychnis callidryas,* because it was recorded until 2014 in the ponds next to the "José Miguel Alfaro" Biological Station; since then a well-established population has been observed in succeeding years. Two other species, *L.savagei* and *L. vaillanti*, were also recorded until 2019, then we cannot discard a current colonization process occurring in the ESLA Cloud Forest, as it have been documented for other middle elevations (Acosta-Chaves and Cossel, 2016; Acosta-Chaves et al. 2019).

The Costa Rican endemic and endangered salamander *N. gamezi* observed along the Ferns Trail was previously only registered for the Monteverde region (Pounds *et al.,* 2008) and San Ramón (previously named *N. abscodens* by Bolaños and Ehmcke (1996), Acosta-Chaves et al. 2019). According to the literature (Bolaños and Ehmcke, 1996; Savage, 2002; Leenders, 2016, Acosta-Chaves et al. 2019), we expect that other rare amphibians with secretive arboreal and/or fossorial habits could occur in the ESLA Cloud Forest. Some examples of expected species are *Bolitoglossa alvaradoi, Craugastor andi, Craugastor fleischmanni, Pristimantis caryophyllaceus, Anotheca spinosa* and *Ecnomiohyla* spp. Other species such as *Craugastor ranoides, Atelopus varius* and *Agalychnis lemur* should have occurred in the past within the ESLA Cloud Forest, but to date those species have been extirpated from that region (Morera-Chacón and Sánchez-Porras, 2015, Acosta-Chaves *et al.,*2019.).

Although the similarity with the San Lorencito River Station was predicted after the conclusions of Rovito *et al.* (2015) for the Nectandra Reserve and our own surveys in AMBBR (Acosta-Chaves *et al.,* 2019.), originally we expected a higher similarity between the ESLA Cloud Forest and the Nectandra Reserve. Nectandra and ESLA are similar in elevation and proximity between reserves than with the San Lorencito River Station. Other similarities include the resemblance in the form of the forest patch, size of the patch and matrix structure can determine the structure and composition of species in a site (Herrera, 2011). Additional surveys are needed for detection of species absent from their list; we believe that the amphibian richness of the Nectandra Reserve should be greater than the reported by Rovito *et al.* (2015). Due to the confluence of the widespread species of the premontane elevation and the herpetofauna from the Pacific slope, the premontane forest of San Isidro de Dota (Arias and Bolaños, 2014) has richer species diversity than the ESLA Cloud Forest. Finally, the lower montane forest of Río Macho is in a different and higher life zone that naturally limits the distribution of some species common in the other analyzed localities, but additionally Río Macho also lost a greater part of its amphibian species richness because the enigmatic decline of populations (Acosta-Chaves *et al.*, 2015).

Our surveys of the ESLA Cloud Forest complements the inventories of the Nectandra Reserve and the San Lorencito River Station, helping to provide a better

understanding of the amphibian richness in the western Central Valley. The ESLA Cloud Forest can be considered the second richest cloud forest in the San Ramón area for conservation and amphibian watching, and among the better clouded forests in central Costa Rica. However, the significance of the ESLA Cloud Forest as reservoir of herpetofauna is currently underestimated and the likelihood for exploitation is high. Species such as *A. callidryas, D. ruficollis, L. warszewitschii* and the three glass frogs (Centrolenidae) are attractive animals, an eye-catching commodity for photographers. The other species, which is restricted to premontane and montane belts, may also be focal point for specialized herpetological tours. In order to reduce perturbation and disturbance of all species and ecosystems in the ESLA Cloud Forest, we recommend that ethical field caution should occur during human interaction with the herpetofauna.

Finally, we strongly suggest continuing the amphibian surveys in unexplored areas of the ESLA Cloud Forest, for example the recently open sector "Pittier Trail" that encompass pristine forest in a better conservation status that the others we surveyed for this manuscript. Continued surveys and research is still needed at premontane and middle elevations throughout Costa Rica in order to monitor amphibian population dynamics and community structure in relationship to habitat fragmentation, climate change, intra- and interspecific competition, colonization and/or extinction of Costa Rican clouded forest species.

VI. Acknowledgements

The Villa Blanca Hotel and Cloud Forest provided us the facilities. We are especially grateful to Roy Valverde and Esteban Hernández from the "José Miguel Alfaro" Biological Station, and other staff members, for sharing their observations and resources. Finally, we thank Robert Goodman, and two anonymous reviewers, for their valuable comments improving the manuscript.

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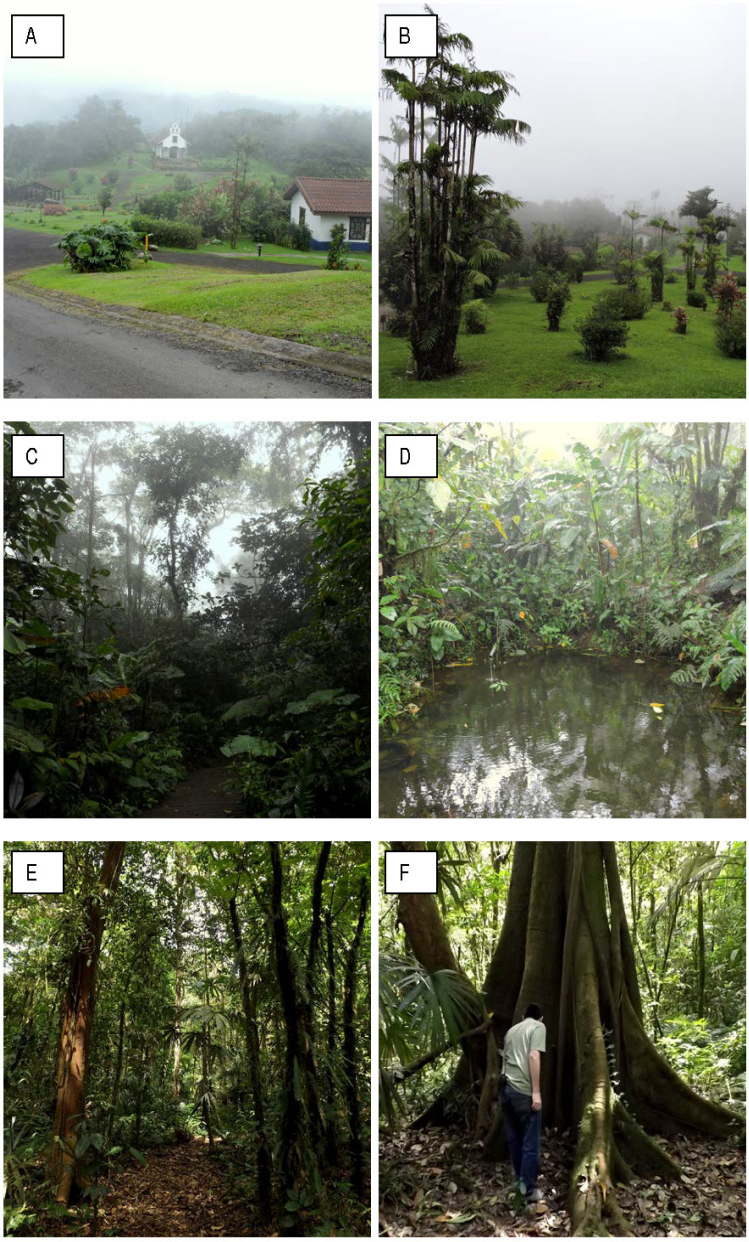
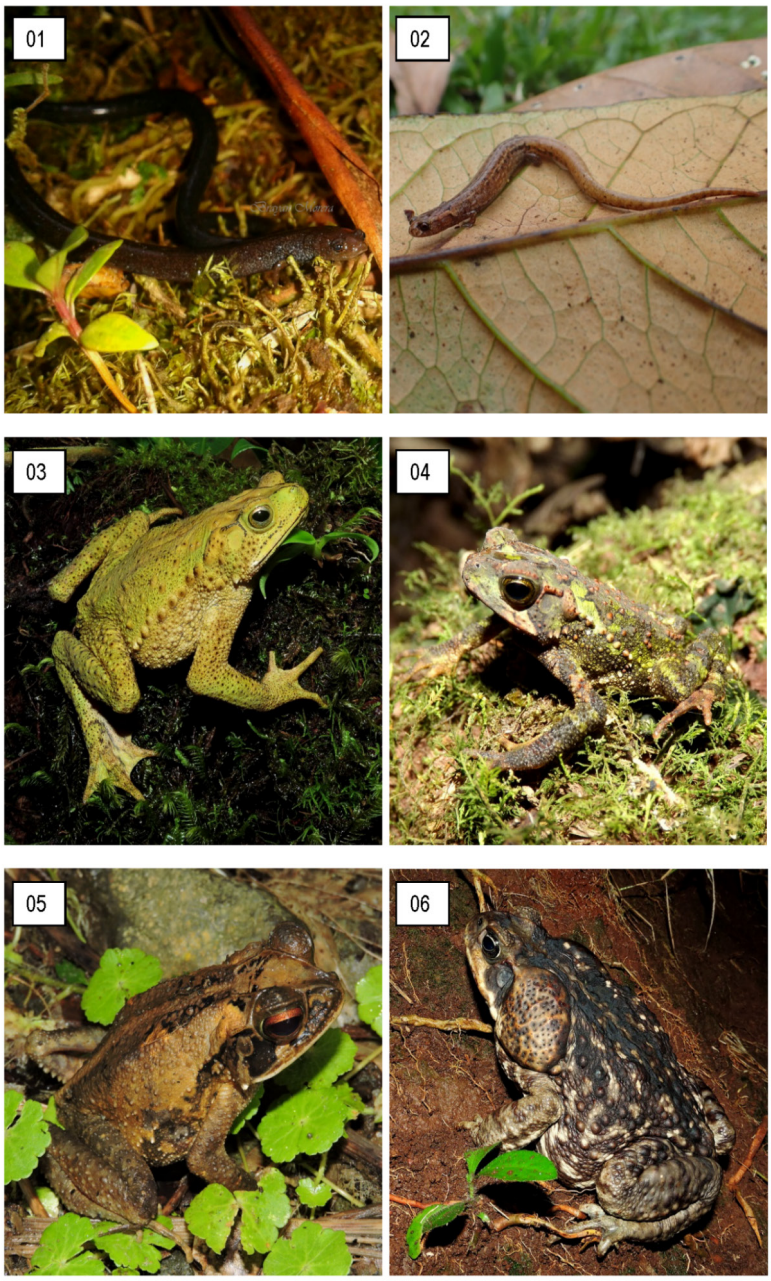
Annexes

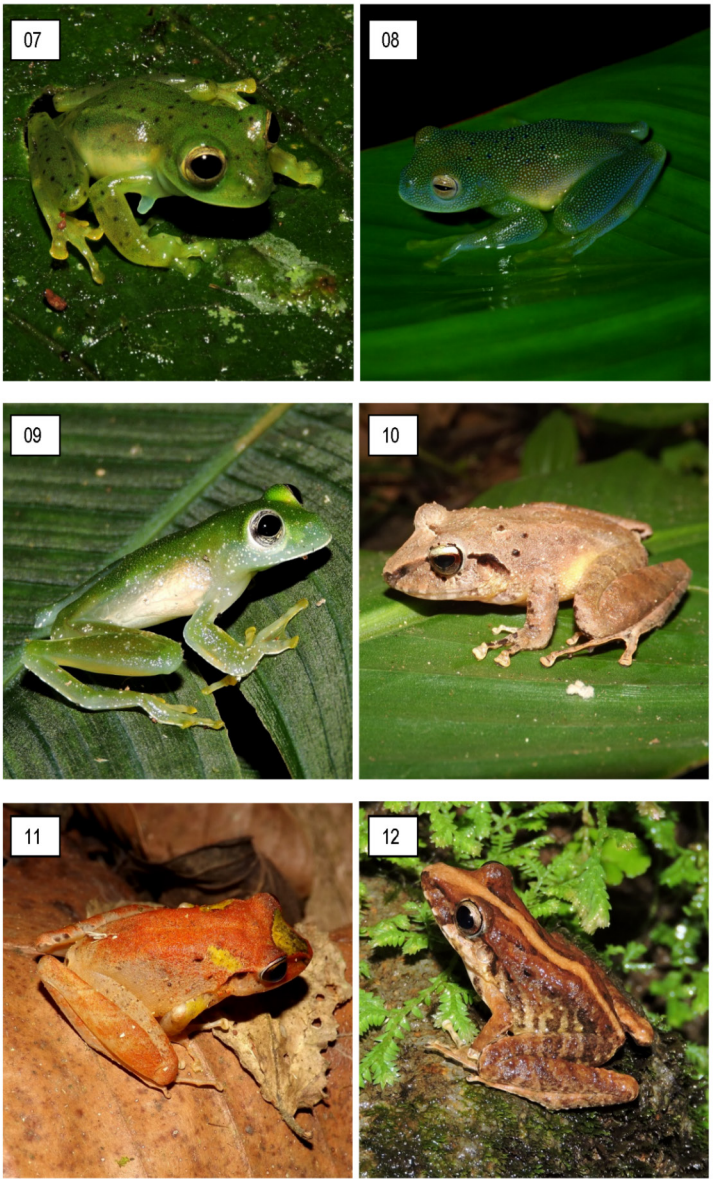
Appendix 1. Types of microhabitats in Villa Blanca Hotel. Gardens and open areas (A) & (B). Secondary forest (Ferns trail) (C). Artificial pond (Ferns trail) (D). Primary forest (Ficus trail) (E). Brayan Morera sampling in primary forest (Ficus trail) (F). Photos by Víctor Acosta.



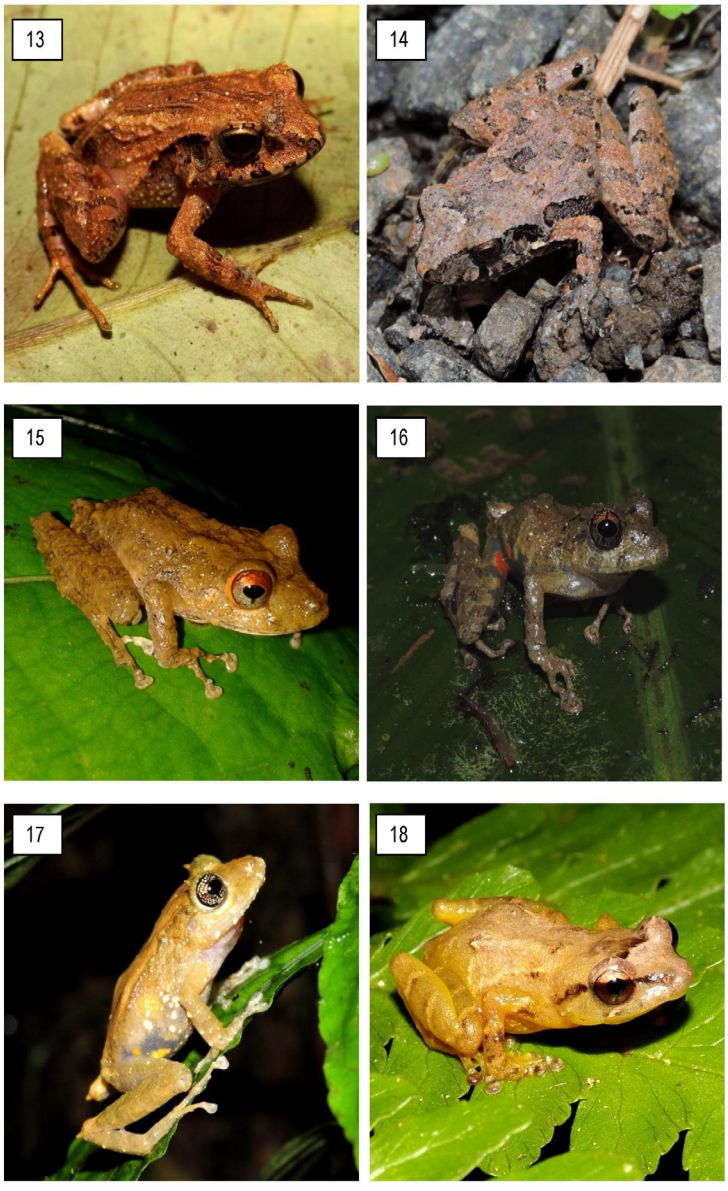
Appendix 2. Amphibian species found in the ESLA cloud forest. *Oedipina uniformis* (01). *Nototriton gamezi* (02). *Incilius coniferus* (adult) (03). *Incilius coniferus* (juvenile) (04). *Incilius melanochlorus* (05). *Rhinella horribilis* (06). Photos 01, 03, 05 and 06 by Brayan Morera, Villa Blanca; 02 by Esteban Hernández, Villa Blanca; 04 by Víctor Acosta, Villa Blanca (Figure continued in following page).



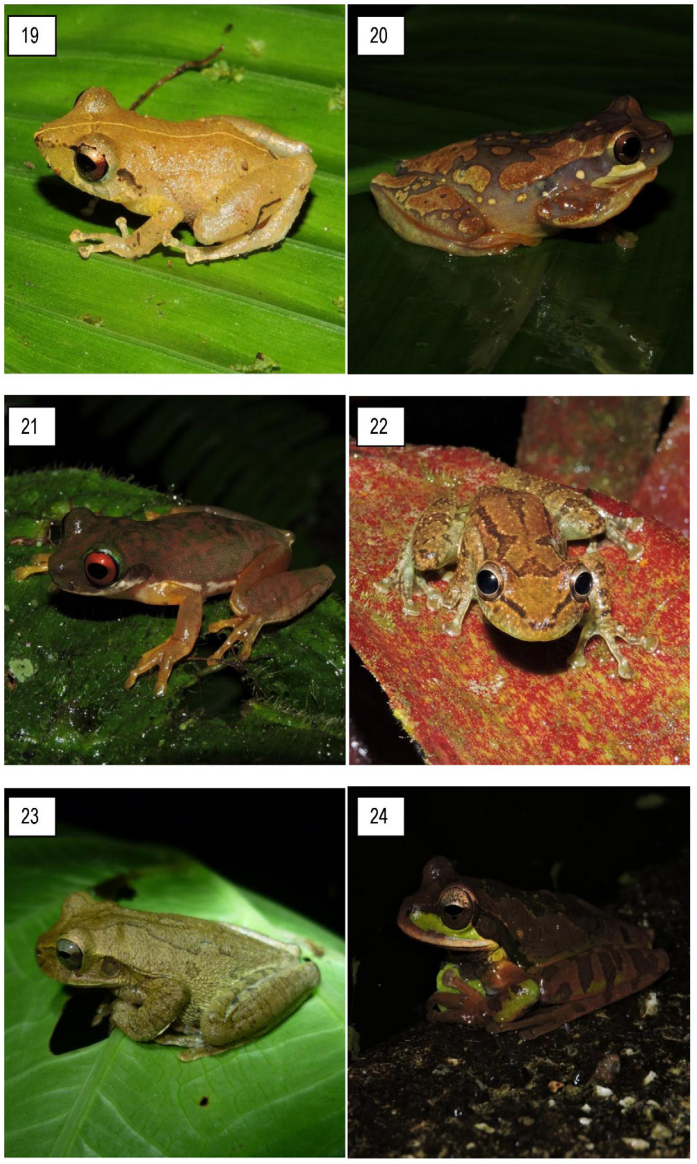
Appendix 2, continued. Amphibian species found in the ESLA cloud forest. (7) *Espadarana prosoblepon*. (8) *Cochranella granulosa*. (9) *Teratohyla pulvarata*. (10) *Craugastor crassidigitus* (morph1) (11) *Craugastor crassidigitus* (morph2). (12) *Craugastor fitzingeri*. Photos 7,10 and 11 by Víctor Acosta, Villa Blanca; 9 by Víctor Acosta, Peñas Blancas de San Ramón; 8 and 12 by Brayan Morera, Villa Blanca. (Figure continued in following page).



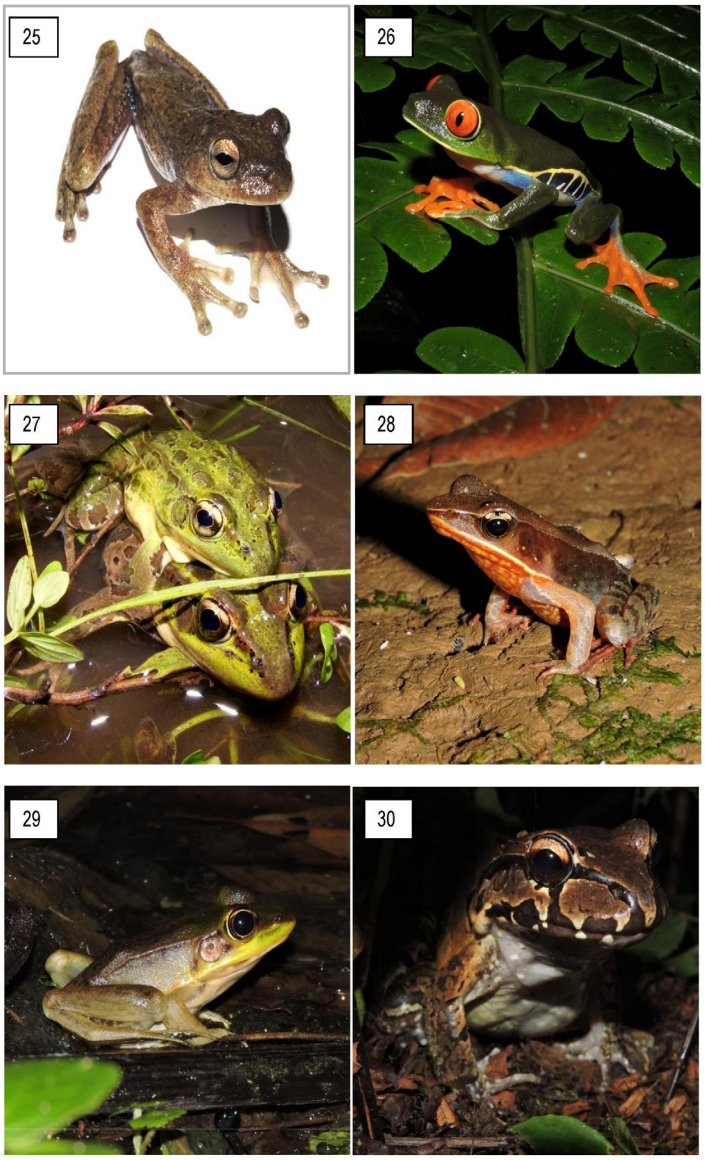
Appendix 2, continued. Amphibian species found in the ESLA cloud forest. *Craugastor underwoodi* (morph1) (13). *Craugastor underwoodi* (morph2) (14). *Pristimantis cerasinus* (morph1) (15). *Pristimantis cruentus* (morph1) (16). *Pristimantis cruentus* (morph1) (17). *Diasporus diastema* (18). Photos 13, 16 and 18 by Víctor Acosta, Villa Blanca; 14, 15 and17 by Brayan Morera, Villa Blanca *(Figure continued in following page).*



Appendix 2, continued. Amphibian species found in the ESLA cloud forest. *Pristimantis ridens* (19). *Dendropsophus ebraccatus* (20). *Duellmanohyla rufiocollis* (21). *Scinax elaeochrous* (22). *Smilisca manisorum* (23). *Smilisca phaeota* (24). Photos 19, 22 and 23 by Brayan Morera, Villa Blanca; 20, 21 and 24 by Víctor Acosta, Villa Blanca (Figure continued in following page).



Appendix 2, continued. Amphibian species found in the ESLA cloud forest. *Smilisca sordida* (25). *Agalychnis callidryas* (26). *Lithobates taylori* (27). *Lithobates warszewitschii* (28). *Lithobates vaillanti* (29). *Leptodactylus savagei* (30). Photos 25, 26, 28 and 30 (Villa Blanca), 27 (Río Macho) and 29 (Soltis Center) by Víctor Acosta.



Appendix 2, continued. Amphibian species found in the ESLA cloud forest. *Gymnopis multiplicata* from Villa Blanca, from Víctor Acosta.