

Bioacoustical characterization of Phyllostomidae bats in Colombian low montane rain forest

Caracterización bioacústica de murciélagos Filostómidos en un bosque húmedo montano bajo colombiano

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Abstract

The emission of ultrasound allows bats make decisive tasks associated with the interpretation of their surroundings, displacement, orientation and obtaining food. In the case of Phyllostomidae bats, the emissions show high frequencies and low intensities, similarly they complement largely by the sense of smell and in a less proportion, by sight. **Objective:** This study presents the characterization of bioacoustics emissions for seven species: *Artibeus jamaicensis* (Leach, 1821), *Carollia brevicauda* (Schinz, 1821), *Carollia perspicillata* (Linnaeus, 1758), *Phyllostomus hastatus* (Pallas, 1767), *Phyllostomus discolor* (Wagner, 1843), *Sturnira luisi* (Davis, 1980), *Sturnira lillium* (E. Geoffroy, 1810). **Methodology:** The acoustic records have been reported in a humid forest in San Francisco, Cundinamarca, Colombia, using the autonomous recorder Song Meter SM2 Bat (Wild Life Acoustics). The emissions were obtained applying closed recording booth space flight method and after, they were visualized using Sonobat 2.9.4 Software. **Results:** Spectrograms were obtained and a consensus among the spectral values recorded by each specie, with the mean and the standard deviation, then some values are contrasted. **Conclusion:** Recognition of modulated structures, numerous harmonics and high frequencies. Likewise, after making contrast of spectral values, which are mainly referred to the high or initial frequency (Hif), low or initial frequency (Lof) and duration (ms), with different authors, it study recognizes the need to expand studies that take into account characteristics of Phyllostomidae individuals in field or under controlled conditions.

Keywords: Acoustic record, Flying mammals, Spectral parameters, Spectrogram, Ultrasounds.

Resumen

La emisión de ultrasonidos permite a los murciélagos efectuar labores determinantes, asociadas con la interpretación de su entorno, desplazamiento, orientación y obtención de alimento; para el caso de murciélagos Filostómidos las emisiones presentan altas frecuencias y bajas intensidades, de igual manera se complementan en gran parte por el sentido del olfato y en menor proporción por la vista. **Objetivo:** En este estudio se presenta la caracterización de emisiones acústicas para siete especies: *Artibeus jamaicensis* (Leach, 1821), *Carollia brevicauda* (Schinz, 1821), *Carollia perspicillata* (Linnaeus, 1758), *Phyllostomus hastatus* (Pallas, 1767), *Phyllostomus discolor* (Wagner, 1843), *Sturnira luisi* (Davis, 1980), *Sturnira lillium* (E. Geoffroy, 1810). **Metodología:** Los registros acústicos han sido reportados en un bosque húmedo en San Francisco, Cundinamarca, Colombia, empleando la grabadora autónoma Song Meter SM2 Bat (Wild Life Acoustics). Las emisiones fueron obtenidas aplicando el método de grabación en espacio cerrado en caseta de vuelo y luego visualizados a partir del paquete de software Sonobat 2.9.4. **Resultados:** Se obtienen espectrogramas y un consenso entre los valores espectrales registrados por especie, con la media y la desviación estándar, luego algunos valores son contrastados. **Conclusión:** Se han reconocido estructuras moduladas con múltiples armónicos y elevadas frecuencias; de igual manera, luego de efectuar el contraste de valores espectrales referidos principalmente a la frecuencia inicial o mayor (Hif), frecuencia menor o inicial (Lof) y duración (ms) con distintos autores, se reconoce que es necesario ampliar estudios que tengan en cuenta características de las emisiones de individuos Filostómidos en campo o bajo condiciones controladas.

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Introduction

After of research developed in 1973 by Lazzaro Spallanzani, the contribution of Louis Jurine associated with creation of a detector capable of detecting vibrations ultrasound in 1938 and other researchers, interested in the displacement of bats in the dark, it is Donald Redfield Griffin who in 1944 designated the term ecolocalization (Hill and Smith 1985), to describe the mechanism for guiding and hunting bats from the emission of high sounds frequency (Hill and Smith 1985 Neuweiler 1980).

Bats have successfully used their Larynx and ears for sound production and the subsequent detection and discriminalization of objects in darkness. Other bats emit ultrasound through open mouth or through the nostrils, which allows them to interpret their surroundings (Neuweiler 1980). From this, bat can determine distance, direction, size, shape and texture of objects, which are part of their habitat; similarly, they can maneuver and avoid obstacles that have been identified by them, from the surroundings in their nocturnal or crepuscular activities (Hill and Smith 1985).

Chiropteran night-flying mammals are the only ones who explore their environment through ultrasonic signals (Hill and Smith 1985). Ultrasonic emissions show particular events individuals in a given time and differ according to bats activity in search phase, calling orientation or feed, also known as approach phase (Hill and Smith 1985). Another well-differentiated phase in insectivorous bats, is known as feeding buzz or terminal phase ensues when the bat is fairly close to the insect and emits a burst of pulses at a very high rate. The buzz of an insect catch seems typically to last longer and to include more closely spaced pulses (Griffin *et al.* 1960).

The emissions in search phase are linked to environment in which the individual forages (Rivera and Burneo 2013). This kind of callings are relatively uniform throughout transmission and they are the most common used in the behavior of individuals, and from them are obtained the parameters describing these emissions, providing the enough information to carry out various studies; therefore, they are taken

into account when making descriptions are associated with the acoustic identity of many species (Rydell *et al.* 2002, Murray *et al.* 2001).

In the case of Phyllostomidae individuals, their distribution is widely known in tropics, particularly in forest habitats that are home to the biggest number of bats in this family (Miller 2002), where individuals perform functional roles associated to seed dispersal, pollination, insects control, among others (Kalko 2004); the impact of these roles in the ecosystems generates priorities for conservation and knowledge of these bats, therefore, the acoustic monitoring and methods propose significant contributions to knowledge, distribution and other aspects related to the natural history of these species. Therefore, it is necessary to make progress in acoustic sampling, since traditionally; sampling of these individuals has been done mainly through mist nets (Kalko 2004).

Similarly, as it stated by Ochoa *et al.* (2000), the researchers that do not apply recordings to track bats in field may provide incomplete results, because many species are poorly studied or viewed, likewise, the researchers that only take into account acoustic monitoring may be fragmented and be inappropriate. This study presents the characterization of bioacoustics emissions for seven species considered common in Neotropical forests.

Echolocation in Phyllostomidae. The echolocation system of these individuals reports low intensities and high frequencies that are often difficult to record in field. These ultrasonic emissions of this family reflect an adaptation to structurally complex spaces such as forests, where foraging activities have been reduced to canopy level and individuals can find many obstacles (Thies *et al.* 1998).

Many Phyllostomidae individuals as *Carollia perspicillata* or *Philostomus hastatus* have structures modulated type (FM) or broadband emissions search, which are known in this way for contain a wide range of frequencies in a short time (Thies *et al.* 1998); they often report components known as harmonics, which are repetitions or dominant or characteristic frequency, in which the frequency value associated with higher emission energy is concentrated (Hill and Smith 1985). They serve to individuals to get better representations of a particular habitat place, in which they develop foraging activities and they also are part of the acoustic identity to them and are

involved in their taxonomic determination (Rivera and Burneo 2013).

The design of echolocation signal shown at the species level, is related to or depends on key variables according to Kalko and Schinitzler (1993), Fullard and Dawson (1997), Jones and Waters (2000).

Habitat type used by individuals, for example: open lands, border areas, dense and with obstacles vegetation areas.

Diet habits (arthropods, small vertebrates, blood, fruit or nectar).

Hunting or getting food habits, if they flight feeding, looking for objects on fixed surfaces.

After the term of echolocation has been coined, many applications have been made in complex tasks performed by man according to the principles associated with that term, an example of this was navigation and robotic topics (Seco and Jiménez 2006). In the present case, these principles have made significant progress in the bioacoustic technique. This technique focuses on the study of sounds emitted by different animals and on the resolution of different concerns based on this information. In Colombia, advances have been made on the use of this technique in different taxonomic groups, mainly in birds, but there are few antecedents that reflect the study of ultrasonic emissions of flying mammals as a biological model in an ecosystem such as the humid forest; In addition, when we refer to Phyllostomid bats, the background is also scarce given that these bats, known acoustically as whispering bats (Miller 2002), have high frequency and low intensity ultrasonic emissions, which makes it difficult to report their emissions in the field.

The contribution of this research consists in establishing a contrast between the existing antecedents on the phyllostomid species presented with respect to other investigations in other Neotropical countries, taking into account that in Colombia there is no acoustic report of these species in an ecosystem like the humid forest.

Methodology

The study area is a low humid forest located in the municipal rural settlement San Miguel, located in the northern part of the municipality of San Francisco, Cundinamarca $4^{\circ} 59' 14.193''$, $- 74^{\circ} 17' 10.532''$ (Figure 1). This municipality borders at the north with La

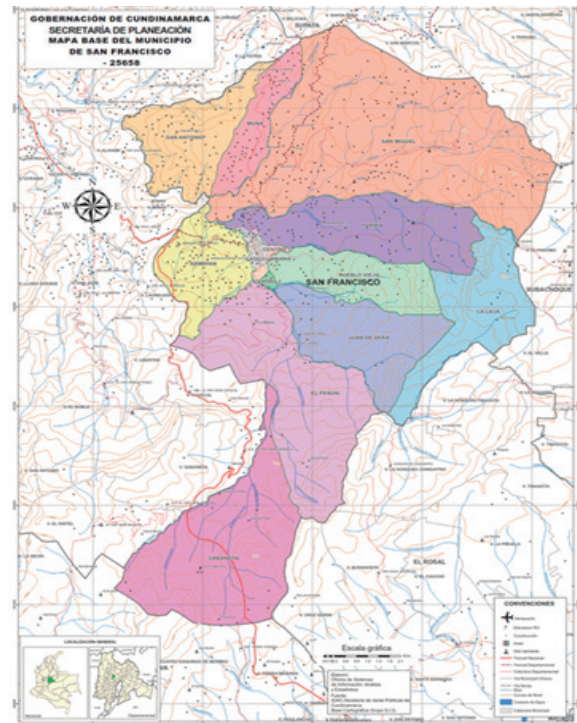


Figure 1. Location of San Francisco in Cundinamarca and in Colombia (On line) 2017.

Retrieved on november24th, 2017 (http://sanfrancisco-cundinamarca.gov.co/mapas_municipio.shtml).

Vega and Supatá towns, in the east with Subachoque town and South with Facatativá town. San Francisco has an elevation range between 2300 and 2600 meters above sea level, with huge lands of montane rain forest, which show fertile and rocky mountain areas, broken relief where the permanent vegetation and representative aquifers are predominant. The annual rainfall varies between 900 and 1800 mm, rains are often irregular, but they achieve to keep moisture in the land. The temperature is moderate and typical of temperate climate, which varies between 12 and 20°C.

Samplings. The sampling was carried out in the of November and December months at 2013. Mist nets from 6 m to 12 m were used, between 18:00 to 00:00 hours, with 20 minutes frequency monitoring. These mist nets were located in areas partially open to the interior of the forest (Frick 2013, Ossa 2010).

Recording flight cage. According to Figure 2, for this study was used, a recording booth space o flight cage made with shading polysombra with dimensions of 4 m x 4 m. To make easier controlled flight on the booth roof, it has a wooden stick, from which is tied one end of an elastic strand semi-coarse of an

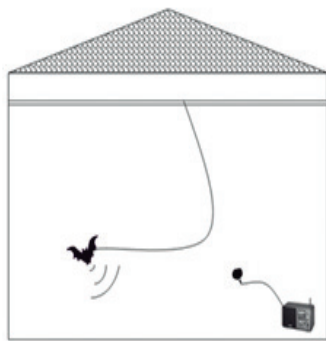


Figure 2. Recorded procedure from the recording booth (flight cage).

Adapted by authors

approximate length of 2.5 m, on the other strand end, was carefully adjusted the neck of each individual reported, the enough tight so that bat do not escape and likewise, it could not affect it.

In separated experiments, each individual was released into the flight cage where the recordings were made, while they were raising flight and sending out their callings in search phase, for one or two minutes, while the autonomous recorder stored their emissions. After acoustic record, individuals were collected and were put in the Natural History Museum of University Distrital Francisco José de Caldas (MUDFJD), Bogotá, where it was verified the taxonomic identity of individuals.

Acoustic reported. In this study, an individual of each species was recorded in the room space. The ultrasound report was made by the autonomous recorder Song Meter SM2 Bat (Wild Life Acoustics) (Frick 2013, Ossa 2010) in a sampling rate of 192 kHz. The recorder was manually adjusted and to optimize the records, it was connected to the omnidirectional microphone; in each experiment, the microphone was manually inclined to 45° in order to optimize the emission range in each record, the files were stored on SD memory by the time expansion system in WAV format and after, they were changed into expanded time (TE) (Ossa 2010). The registration time for each species has been two minutes.

Visualization and selection of ultrasound. We have selected emissions in the search phase, the selection criteria of these emissions through the spectrograms recorded for each species, consists of the presence of consecutive pulses modulated showing a homogeneous pattern, with a duration less than 0.2 ms according to principles proposed by Schnitzler and

Kalko (2001), Thies *et al.* (1998). In general, search calls are the most frequent of bats and these should be used as a starting point for different studies and taking into account that calls can be variable even between species (Kalko and Condon 1998), it is feasible use frequencies with search emissions belonging to an individual, which must have a good signal-to-noise ratio (Aguirre 2007).

Initially we have employed software package AviSoft SAS Lablite, it made a preliminary display of the reported files (Frick 2013), in the generation of these spectrograms, have been used as a display parameter a Hamming window with a 256 FFT a 100% frame size and 50% overlap according to Thies *et al.* (1998), Kalko and Condon (1998), Kalko (2004). After that, it was used the software Sonobat 2.9.4, a program designed to process spectrograms and ultrasonic records which are particularly associated with bats. This process was done to evaluate properties of each ultrasound in search phase and to make an adequate selection of pulses given quality rules stated by Barboza-Marquez (2009); these criteria, allow to classify an acoustic report in excellent, good, regular or bad depending on the level of noise and interference reported in the field. Then to get quantitative and qualitative values that represent in the best way, the emissions of each individual, according to Waters and Gannon (2004) we choose a pulse that would present the appropriate characteristics in terms of resolution and spectral values.

The sequences are part of selections made and for this study; those sequences showed a minimum of three and a maximum of six consecutive pulses based on image quality they showed, however, it is emphasized that a total of 10 consecutive pulses have been selected from each species to perform descriptive statistics. In this study, we present the acoustic characterization of seven individuals, each individual represents a different species, in other words, for each species the acoustic characterization has been made taking as sample an individual as indicated by Table 1.

Qualitative and quantitative parameters. The qualitative description refers to the structure or shape of the pulse that sequences show. The structure is classified according to the nature of emission of a particular individual. In the case of phyllostomid bats, they show frequencies modulated (FM), these signals are usually short (<2 ms) and cover a large number of

Table 1. Reported species information given sex and number of pulses analyzed per species

Specie	Individual sex	Number of	
		individuals analyzed	pulses analyzed
<i>Artibeus jamaicensis</i> (Leach, 1821)	Male	1	10
<i>Carollia brevicauda</i> (Schinz, 1821)	Male	1	10
<i>Carollia perspicillata</i> (Linnaeus, 1758)	Male	1	10
<i>Phyllostomus hastatus</i> (Pallas, 1767)	Male	1	10
<i>Phyllostomus discolor</i> (E. Geoffroy, 1810)	Female	1	10
<i>Sturnira liliium</i> (E. Geoffroy, 1810)	Male	1	10
<i>Sturnira luisi</i> (Davis, 1980)	Male	1	10

frequencies and are precise to measure short distances and to obtain accuracy on the position of fixed objects within the forest (Schnitzler and Kalko 2001).

Quantitative parameters or spectral parameters are measurable attributes of the emissions in the search phase that have been obtained in each individual; these parameters are usually expressed in terms of frequency, intensity and duration in milliseconds. A pulse selection in search phase was done, showing a high quality per each reported specie. This allowed establishing a qualitative characterization refers to frequencies modulation and a quantitative characterization getting spectral parameters. It is necessary to clarify that the parameters have been described from ten pulses selected per each specie based on the mean and the standard deviation standard ($X \pm SD$) as statistical descriptors for each spectral parameter analyzed and its description for further studies.

The spectral parameters that were taken into account to this study and were obtained from Sonobat 2.9.4 Software were the following Rivera y Burneo (2013).

Hif. It represents the frequency value at the widest point emission and it is known as initial frequency.

Lof. It is the lowest frequency at the lowest point of the call, it is known as final frequency.

Fc. Characteristic frequency, it is the common frequency at which the animal emits (this parameter is set by the default software, according to each spectrogram or individual reported).

Knee. It is the inflection point frequency that represents the transition point frequency. given in a change of angle or curvature during a call. It is also known as gradient frequency modulation.

Fmax. Highest energy frequency; it is the frequency which covers the greatest emission intensity.

Freq (Ldg). Frequency in which falls to the

greatest intensity pulse.

Bandwidth (kHz). Difference between initial frequency and final frequency is typical for type-modulated emissions (Hif-Lof)

Duration (ms). It is the time between the start and the end of the pulse is measured in milliseconds (ms).

Finally, other studies concerning the acoustic characterization of the phyllostomid species have been taken into account, in order to establish a contrast between qualitative and quantitative variables previously proposed and those obtained for the humid forest from this research.

Results

Sampling time was from October to December 2013, avoiding rainy or full moon nights, seven individuals who belong to Phyllostomidae family were collected. They are represented in seven species (Table 1). Visualization of ultrasonic records was made to give an answer to the objective, which was suggested previously, therefore the description and analysis is proposed from a sample of 10 pulses in search phase by each specie. Taking into account that the recording time for each species by applying the report method in the flight cage, corresponds to two minutes, at this time, two to three sequences are obtained per individual, these sequences are characterized by presenting from 3 to 4 consecutive pulses in short periods of time, according to these sequences, the ten pulses have been selected for each individual that is part of the analysis of this study.

The spectrograms recorded, there are not ultrasonic records to nose leaf individuals related to kind of forest in the study area in the country. According to Barboza-Marquez (2009), the emissions of reported individuals were good and excellent quality. They

were excellent because in the moment to do visualization using software, there is a mild background noise and it is easy to distinguish components that are part of transmission; there is not any overload and some are good because although there is a background noise, it is still easy to distinguish and to measure components that are part of the emission.

The calls echolocation for bats species of this study are:

Artibeus jamaicensis (Leach, 1821). The spectrogram shown in Figure 3 shows a good quality of three pulses sequence; each pulse has two harmonics, being the second of them, which reflects the higher intensity of the emission, and spectral parameters that are part of the acoustic identity for each specie.

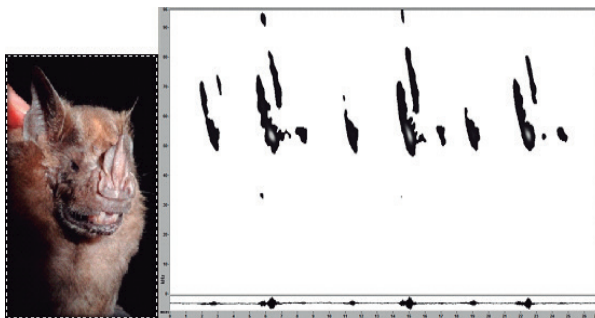


Figure 3. Pulse sequences in search phase of *A. jamaicensis*.

The structure of the pulses is fully modulated, covering frequencies from 50 to 70 kHz and a bandwidth near to 27 kHz. The frequency scale produced by spectrogram shows some variations which fall on the initial frequency of pulses, while the less frequency appears to keep similar values along the issuance. A summary of the qualitative characteristics associated to the pulse structure and the consensus of the spectral values obtained ($X \pm SD$) of *Artibeus jamaicensis* is shown in Tables 2 and 3.

Carollia brevicauda (Schinz, 1821). The Figure 4 shows a spectrogram with five consecutive pulses of good quality and low gradient of modulation, because

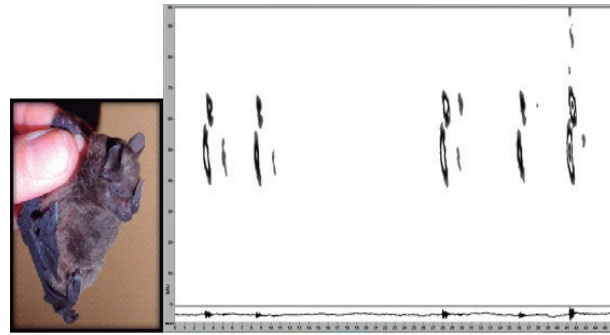


Figure 4. Pulse sequences in search phase of *C. brevicauda*.

the structure of pulses is seen markedly horizontal, covering frequencies from 38 kHz to 60 kHz with fully modulated structures (FM), a bandwidth near to 20 kHz. Each pulse is made up of two harmonics, being the first one, which to see spectral parameters that are part of the acoustic identity of individual and the highest emission intensity. A summary of the qualitative characteristics associated to the pulse structure and the consensus of the spectral values obtained ($X \pm SD$) of *Carollia brevicauda* is shown in Tables 2 and 3.

Carollia perspicillata (Linnaeus, 1758). In the Figure 5 sequences are seen five excellent quality pulses. In this spectrogram, each pulse in search phase, reported to *C. perspicillata* has two characteristic harmonics, which, the second reflects the highest emission intensity of the species. The calls show a frequency range between 45 and 68 kHz in a relatively short time (1.86 ms) and therefore the structure of the pulses is fully modulated. A summary of the qualitative characteristics associated to the pulse structure and the consensus of the spectral values obtained ($X \pm SD$) of *Carollia perspicillata* is shown in Tables 2 and 3.

Phyllostomus discolor (E. Geoffroy 1810). Emissions in search phase reported for *P. discolor* have two characteristic harmonics, which the second

Table 2. Summary of qualitative aspects of species

	<i>Artibeus jamaicensis</i>	<i>Carollia brevicauda</i>	<i>Carollia perspicillata</i>	<i>Phyllostomus hastatus</i>	<i>Phyllostomus discolor</i>	<i>Sturniralilium</i>	<i>Sturnira luisi</i>
Structure	FM	FM	FM	FM	FM	FM	FM
Number of harmonics	2	2	2	4	2	2	2
Higher intensity harmonic (h)	2	1	2	2-3	2	1	1

Table 3. Summary consensus of spectral parameters obtained by species (X±SD). A total of 10 pulses per specie was taken into account to obtain temporal and spectral variables

	<i>Artibeus jamaicensis</i>	<i>Carollia brevicauda</i>	<i>Carollia perspicillata</i>	<i>Phyllostomus hastatus</i>	<i>Phyllostomus discolor</i>	<i>Sturniralilium</i>	<i>Sturnira luisi</i>
HiF (kHz)	68,48±2,13	60,2±2,8	70±2,56	49,78±4,95	67,41±2,84	65,89±2,28	66,8±2,0
LoF (kHz)	47,47±3,32	43,23±1,69	47,20±3,67	39,48±0,31	47,31±3,14	41,54± 1,67	42,31±2,73
Fmax	55,83±3,83	49,84±3,03	56,6±3,67	40,13±2,49	52,19±3,46	55,51±5,63	52,67±3,4
Fc (kHz)	52,7±2,04	46,5±3,21	47,97±3,53	36,81±0,47	51,7±3,20	48,38±2,71	45,9±3,0
Knee (Khz)	57,8±2,84	51,5±3,25	57,88±5,52	40,37±2,97	58,8±4,54	61,30±3,13	57,7±2,36
FreqLdg (kHz)	56,3±2,9	49,2±2,8	50,29±4,25	38,166±0,89	60,17±5,17	53,85±7,54	57,5±3,62
Duration (ms)	1,02±0,36	0,77±0,52	1,86±0,52	1,16±0,36	0,81±0,42	0,90±0,36	1,31±0,27
Bandwidth (kHz)	27,3±4,71	21,0±1,55	26,1±3,64	15,93±1,58	22,23±1,99	27,08±2,44	28,3±2,08

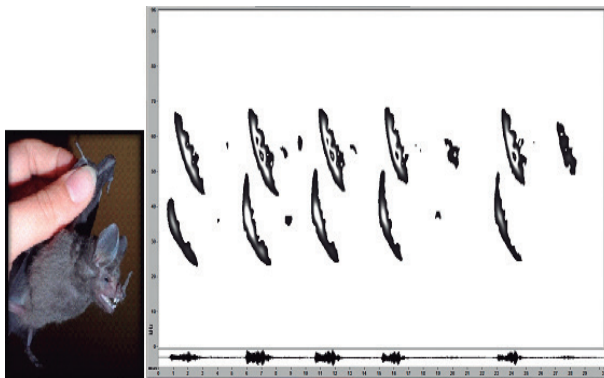


Figure 5. Pulse sequences in search phase of *C. perspicillata*.



Figure 6. Pulse sequences in search phase of *P. discolor*.

one has the highest emission intensity according to the Figure 6. The pulses show FM with a bandwidth close to 24 kHz. It shows a sequence of four pulses in search phase. Depending on the scale, in terms of provided frequency by spectrogram, a variation in the values given by initial frequency (Hif) can be seen, while values related to the lowest or final frequency (Lof) appear to show few variations. A summary of the qualitative characteristics associated to the pulse structure and the consensus of the spectral values

obtained (X±SD) of *Phyllostomus discolor* is shown in Tables 2 and 3.

***Phyllostomus hastatus* (Payas 1767).** Emissions of *P. hastatus* in search phase which are shown in Figure 7 have multiharmonic pulses and scanning modulated (FM). The spectrogram shows a sequence of six quality pulses in search phase; the pulses that have been displayed by the software show from three to four harmonics and the highest intensity emission is concentrated in the second of them.

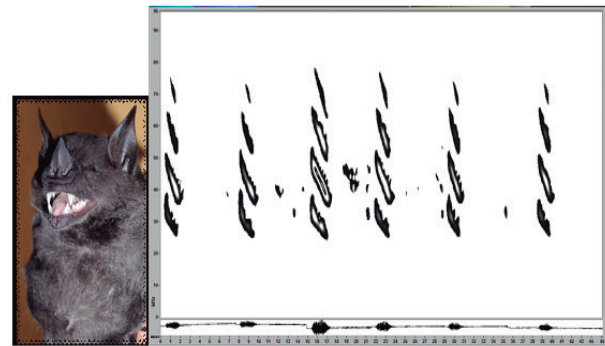


Figure 7. Pulse sequences in search phase of *P. hastatus*.

According to scale provided by spectrogram, frequency values are in a range between 38 and 50 kHz. The comparison between pulses obtained in the sequence shows that there are not changes or significant variations between values based on the value related to the highest frequency (Hif) and the lowest frequency (Lof). A summary of the qualitative characteristics associated to the pulse structure and the consensus of the spectral values obtained (X±SD) of *Phyllostomus hastatus* is shown in Tables 2 and 3.

***Sturnira lilium* (E. Geoffroy, 1810).** According to the Figure 8, the spectrogram demonstrates a three consecutive pulses sequence with excellent quality covering a frequency range between 45 and 68 kHz.

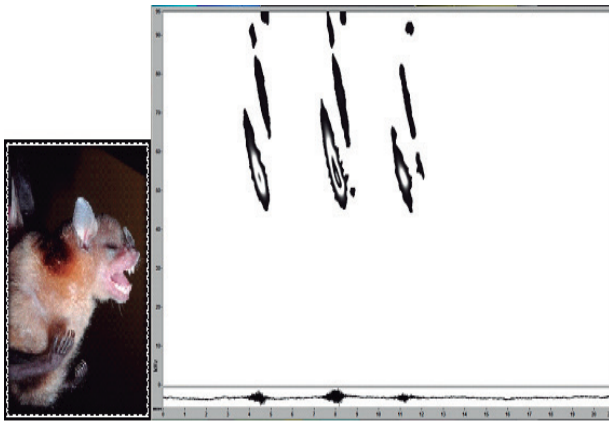


Figure 8. Pulse sequences in search phase of *S. liliium*.

It is seen that the structure of the pulses is fully modulated (FM), each pulse has two characteristic harmonics which the first one falls on the greatest emission energy. From the provided scale by the spectrogram in terms of frequency can be visually seen a variation related to values showed by the higher or initial frequency (Hif), while values related to the lowest or final frequency (Lof) appear to show few variations up to the 45 kHz. A summary of the qualitative characteristics associated to the pulse structure and the consensus of the spectral values obtained ($X \pm SD$) of *Sturnira liliium* is shown in Tables 2 and 3.

***Sturnira luisi* (Davis, 1980).** In the case of *S. luisi*, the sequence of spectrogram in the Figure 9 demonstrates four consecutive pulses with good quality. Each pulse has a modulated structure that covers a frequency range between 40 and 70 kHz and a bandwidth around 30 kHz, likewise the acoustic identity that takes this specie indicates that each pulse is accompanied by two harmonics, which the first one falls on the largest energy emission. A summary of the qualitative characteristics associated to the pulse structure and the consensus of the spectral values obtained ($X \pm SD$) of *Sturnira luisi* is shown in Tables 2 and 3.

Discussion

This research has been dedicated to recognition and allocation of ultrasonic reports associated to seven different species, which were reported in the study area, however the main purpose is to show the description of the aspects and characteristics that are part of the acoustic identity of them, particularly

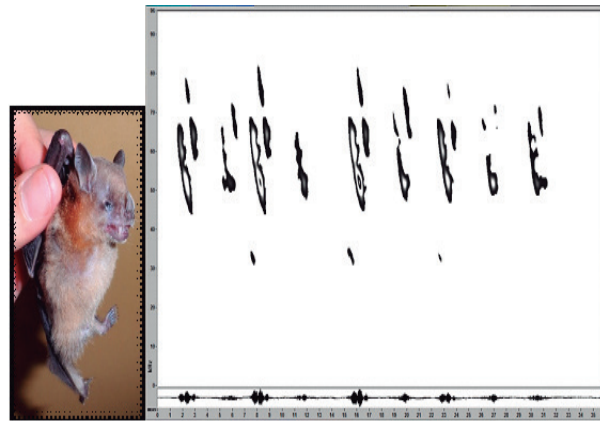


Figure 9. Pulse sequences in search phase of *S. luisi*.

when their emissions are in search phase. As has been already mentioned, ultrasonic signals provide special information related to natural history, acoustic identity of each individual and assessment of differences among species, and others. In this study, a sample of these qualities is presented taking as reference the individuals reported in the study area.

In this research the discussion is generated from the data generated by the seven individuals reported in the field, other investigations such as that of Rizo (2008), who presents the characterization of the echolocation pulses taking as sample 14 individuals of different families of insectivorous air bats in Mexico, after fulfilling aims associated with the production of a catalogue of recordings, to propose a description of the ultrasounds by species, the author suggests for future studies, to increase the number of samples and species, as well as to analyze pulses of echolocation from individuals recorded in different moments, which will allow to generate a major number of references, for the correct identification of species in the acoustic area. Orozco-Lugo *et al.* (2013), present the description of the pulses of echolocation, from eleven species of insectivorous air bats in Morelos, México. These researchers also emphasize the importance of increasing the number of acoustic records in both number of species and number of individuals, including recording in different flight situations to have a greater acoustic representation.

We agree with the authors, when recognizing the importance of having representative data when the acoustic characterization of an individual is being carried out, since this allows corroborating proposed inferences.

In this investigation, was observed that the calls of echolocation associated with species of the family Phyllostomidae that have been reported in this study, include pulses with FM, are emissions that begin with values of high frequencies and that in a relatively short time, the value of these frequencies decrease. The emissions have a bandwidth between 20-30 kHz, the duration less than 2 ms and are also accompanied by multiharmonics, as have been reported by Hill and Smith (1985), Kalko and Condon (1998), Aguirre (2007), Kalko (2004) and Estrada *et al.* (2014). In studies carried out in countries such as Bolivia, Panama and Venezuela; the patterns reported by the authors, are consistent with the acoustic sample by individual presenting this study.

According to Kalko (2004) the modulated emissions are specially adapted for foraging individuals in enclosed and complex spaces; there is too much variation related to structure and spectral values at species level. This principle is adequate to describe the type of phyllostomid bat emissions that have been obtained in the Colombian rain forest ecosystem in which this research was carried out and although the reports were obtained in a flight room, these reports generate antecedent that can be taken into account in other investigations that also apply this methodology in forest habitats, given the difficulty of recording emissions in open spaces due to the reduced reach of its high frequencies and the high degree of attenuation it imposes the air.

The modulated emissions (FM) are reported only within the forest and taking into account the role that the modulated frequencies play in the finding of fixed objects by the individuals, this can be compared with the dietary habits of the species reported in the studied forest that they have dietary habits associated with fruits that are fixed or nested between the branches of plants (Kalko 2004, Thies *et al.* 1998).

The frequency modulated emissions that characterize Phyllostomids, are usually accompanied by harmonics, these are part of the nature of these emissions, however many of these harmonics are filtered by the vocal tract of individuals or there is also the risk that may be attenuated by the air which prevents an adequate recording of them in the field. (Kalko 2004). Modulated frequency signals to Phyllostomidae individuals show from two to four harmonics (Hill and Smith 1985), however due to factors that have been

already mentioned, in many cases it is only possible to record the first and the second one (Lisón 2011). The seven individuals that this study has taken into account as a sample to perform an acoustic characterization, are of different species, but belong to the same family and in general, when comparing the harmonics of these individuals and according to the mentioned authors, we have reported two Harmonics by species, taking into account that the report has been made in a flight cage except for *Phyllostomus hastatus* whose ultrasonic records showed four harmonics.

In the following lines, the discussion focuses on establishing a contrast between both qualitative and quantitative parameters associated with the Phyllostomid species found in this research and those that have been reported by other authors taking into account acoustic monitoring of these species and according to the methodology proposed. It is clarified that the contrast was made taking into account only the spectral parameters that the other authors presented.

With the intention to start the contrast of qualitative aspects refers to the structure or shape of the pulse that sequences show and spectral parameters (Brinklov *et al.* 2009) describe some parameters to *Artibeus jamaicensis* after doing records in a box flight, getting bandwidth position pulses and multiharmonic with signal duration of 1.0-3.9 ms, which are emitted out in a continuous way in foraging activities, these data are consistent with the species reported in this study because the average time of emission of the species is 1.02 ms. These authors report the greatest frequency (Hif) values corresponding to 90.4 ± 0.7 , the smallest frequency (Lof) of $66 \text{kHz} \pm 0.7$ and a bandwidth near to 24.4 ± 1.0 kHz; in contrast to study that brought values of $47.4 \text{kHz} \pm 3.32$, 68.48 ± 2.13 kHz y 27.3 ± 4.71 respectively.

By noting a pulse which belongs to this specie in a proposed study by Carter (2014) the authors recognize that emissions in search phase show a greater intensity over the third harmonic, the first and second being less intense energy. In contrast observing the spectrogram that this research revealed, *A. jamaicensis* presents two harmonics, being the second of them which reflects the higher intensity of the emission. The Table 4 shows a contrast of values, taking into account mean values obtained from each spectral parameter and according to the authors mentioned.

In general, discriminated the number of individu-

Table 4. Comparison between spectral values reported for *A. jamaicensis*

Parameter/Author(s)	Brinklov <i>et al.</i> (2009)	Carter <i>et al.</i> (2014)	This research
Duration (ms)	1,0-3,9	---	1,02
Bandwidth (kHz)	24,4	25,0	27,3
Initial Frequency – (HiF) (kHz)	90,4	75,0	68,48
Final frequency – (LoF) (kHz)	66,0	50,0	47,4
Highest intensity harmonic (h)	---	3	2

als that can report one studio to the other we consider that the differences in the spectral values of the reported species are mainly due to the methodological design carried out, for example acoustic detector, software used, inclination or distance of the microphone to the individual, if the individual was reported or not in a room of flight; In general, it must be recognized that there are variables that are very particular depending on the development of each investigation. In the same way and according to Waters and Gannon (2004), Heffner *et al.* (2003), it is necessary to take into account that many species of bats have a flexibility when producing different calling structures which are coupled to different situations of foraging.

To continue with the description, Koay *et al.* (2002) recognize that is essential to study frugivorous species as *Carollia perspicillata* because their acoustic characteristics have been poorly described despite their lifestyle and abundance in Neotropical forest. Emission of this specie consist of short modulated frequency calls which has a duration less 1 ms and with presence of harmonics, of which the second is usually who has the highest emission intensity (Cloutier and Thomas 1992). In contrast to the author and for this study, *C. perspicillata* presented an average duration per pulse emitted corresponding to 1.86 ms and in fact, the second harmonic presented the highest intensity of emission.

Thies *et al.* (1998), explores roles of echolocation in two frugivorous species (*C. perspicillata* y *C. brevicauda*), feeding of *Piper sp.* (Pipeareceae), they are given to know some spectral parameters were brought to comparison with this study for *C. perspicillata*, which are related to pulse duration and a bandwidth. Later in 2004, it has added the description of values over initial frequency (the highest) and final frequency (the lowest) because of a spectrogram provided

Table 5. Comparison between spectral values reported for *C. perspicillata*

Parameter/Author (s)	Thies <i>et al.</i> (2008)	This research
Duration (ms)	1,5 ±0,4	1,86±0,52
Bandwidth (kHz)	43,9 ±7,8	26,0 ± 3,64
	Kalko (2004)	This research
Initial frequency- (HiF) (kHz)	70	70
Final frequency- (LoF) (kHz)	50	47
Structure of pulse	FM	FM
Highest intensity harmonic (h)	2	2

in a research proposed by Kalko in 2004 (Table 5).

Although it does not evidence a concrete acoustic comparison in front of *Carollia brevicauda* given previous precedents about emissions of frugivorous bats of tropical forests with which it can be proposed a comparison. It is necessary to highlight the report of emission and descriptions were presented for the species in this study. High similarity was expected in front of pulse structure between *C. brevicauda* and *C. perspicillata* given the way in which they use signals to fly in forest and get food, however between both species has shown a high difference which is also evident over spectral values. As a result of the descriptive statistics applied on spectral parameters it is found that the most dissimilar parameters among species respond to: initial frequency (Hif): 60.2±2.8 for *C. brevicauda* and 70±2.56 for *C. perspicillata*, Duration (ms) 0.77±0.52 for *C. brevicauda*, and 1.86±0.52 for *C. perspicillata*, and in bandwidth: 21.0±1.22 for *C. brevicauda* and 26±3.64 for *C. perspicillata*. These values can be contrasted in Table 3.

For example, species belonging to genus *Sturnira*

which are founded in tropical, sub-tropical and temperate forests often prefer tropical rain forests and they are rare in dry forests (Tirira 2008). Likewise, with a comparison related to geographical distribution at Latin American level over species of this genus and which are part of this study. It was founded that *Sturnira lilium* covers since Lesser Antilles, North of Argentina, Uruguay and Eastern Brazil, Trinidad and Tobago (Tirira 2008), distribution pattern confirmed by Albuja (1999), while *Sturnira luisi* is a specie that can be founded from Panama, Costa Rica, Colombia, Ecuador and Northwestern Peru (Albuja 1999) and it was confirmed by Tirira (2008). Therefore, we consider that the distribution spectrum of the species to be restricted to areas of the tropics also in some way restricts or influences the development of research associated with acoustic monitoring of these species, also taking into account the few antecedents associated to the Acoustic characterization of Phyllostomid bats for the Neotropic.

Given the previous information, a more restricted distribution pattern for *Sturnira luisi* was identified. For this specie there is not particular description at least reported for an individual reported at Colombian Andean forests, however for this study, it has achieved to verify in both pulse shape and spectral values in relation to *Sturnira lilium*, which can be associated to foraging style, diet and functional significance of echolocation calls which share these two species.

With regard to acoustic precedents for *Sturnira lilium* it has recognized little specialization in the structure of its ultrasonic emissions. It uses low intensity frequencies and its emission is accompanied by four harmonics (Anderson 2009, Jennings *et al.* 2004). These authors make a characterization of two species: *Sturnira lilium angeli* and *Sturnira lilium paulsoni* in a forest habitat of Puerto Rico; in front of these precedents, the closest values in comparison with the report obtained to this study, values corresponding to *S. lilium angeli*.

According to Jennings *et al.* (2004), a spectrogram is reported with a pulse composed of two harmonics; these authors points out the highest intensity emission is concentrated over the second harmonic, while in this study the highest intensity falls over the first one. Authors report characteristic which are contrasted with the results of this research in the Table 6. However, according to Jennings *et al.* (2004), it is

Table 6. Comparison between spectral values reported to *S. lilium*

Parameter/Author (s)	Jennings <i>et al.</i> (2004)	This research
Initial frequency (HiF) (kHz)	92±8,8	65,8±2,28
Final frequency (LoF) (kHz)	46±10,3	41,5±1,67
Characteristic frequency Fc (kHz)	70±11,5	48,3±2,71
Duration (ms)	2,7±0,8	0,90±0,36
Structure of pulse	FM	FM
Highest intensity harmonic (h)	2	1

necessary to take into account these values not have been previously reported.

The pulses were reported to *Sturnira lilium* have proved to be of better quality than others which were reported to *Sturnira luisi*, by compare the spectral values of those species as a result of the descriptive statistics applied on spectral parameters it is found that these values are very close to each other; three spectral parameters are relevant: initial frequency (HiF): 65.89±2.28 for *S. lilium* and 66.8±2.01 for *S. luisi*, the final frequency (LoF): 41.54±1.67 for *S. lilium* and 47.20±3.64 For *S. luisi* and in Bandwidth: 27.08±2.44 for *S. lilium* and 28.30±2.08 for *S. luisi*. These values can be contrasted in Table 3.

In fact the values are close to each other. Both species show search pulses, which are composed by two clearly distinct harmonics, being the first one in which falls on the highest emission energy.

In the case of geographic distribution among reported species of Phyllostomidae to this study, *Phyllostomus hastatus* can be found from Honduras, eastern Brazil, northern Argentina and Peru, also island of Trinidad and Tobago (Tirira 2008, Albuja 1999), similarly *Phyllostomus discolor* can be found in Oaxaca and Veracruz, until Guianas, Southeastern Brazil, Northern Argentina and Peru, Trinidad and Margarita Island (Tirira 2008, Albuja 1999).

Unlike previous studies to those species, the acoustic tracking of *P. hastatus* has been wider. Regarding to ultrasonic emissions, Kalko (2004) recognizes that they have an appreciable intensity which can be registered using ultrasonic equipments at a distance of 5-10 m approximately. This causes that characteristics of emission can be reported easier than other species. In fact, in her researches, she points out

Table 7. Comparison between spectral values reported for *P. hastatus*

Parameter/Author(s)	Baker <i>et al.</i> (1977)	Santos <i>et al.</i> (2003)	This research
Maximum frequency (HiF) (kHz)	42-50	30-35	42-45
Minimum frequency LoF (kHz)	25-30	30-35	35-36
Duration(ms)	0,5-4,0		
Characteristic frequency Fc (kHz)	---		

that peculiar emission for this specie makes good for monitoring and acoustic assessments.

Table 7 shows values of spectral parameters were reported out by species in contrast to other studies ranges proposed. This research can complement and assess similarities between some acoustic presented in search phase by pulses belong to *P. hastatus*.

Pulses of *P. hastatus* are described as short duration signals and modulated frequency. For the specie reported in Cundinamarca, information was provided about five variables which were found enough data and they can be reached (Pinilla *et al.* 2013). This study shows coherence with reported data from Baker *et al.* (1977), related to maximum frequency value and duration of the pulse, while in comparison with reported data from Santos *et al.* (2003) it shows a range of similar values associated to maximum frequency, the characteristic frequency which falls on the highest emission intensity is concentrated.

Finally, in the case of *P. discolor*, Fenzl and Schuller (2002) point out emissions in search phase can reflect from 3 to 5 harmonics, covering a frequency range since 45 kHz until 100 kHz with a duration of 0.76 ms. For this specie, Kwiecinski (2006) described modulated frequency pulses with many harmonics, which the third over falls on the higher intensity. The reports show duration of approximately 0.3-2-5 ms and a range of emission that covers frequencies between 45 and 70 kHz. On the other hand, Genzel and Wiegrebe (2013) mention range of emission for this specie is between 90 and 40 kHz and duration of 3 ms. According to Firzlaff and Schuller (2003), the social calls sent out by *P. discolor* to communication among species, covers a range of frequency between 11 and 54 kHz.

Besides number of harmonics reported to this study, the values related to spectral parameters are according to those reported by Kwiecinski (2006), because the range of given frequencies reported to spectral parameters also covers a range between 45 and 70 kHz and a emission time of 0.81 ms in relation to pulses structure and precedents researched, they recognize frequencies accompanied of multi-harmonics.

Conclusions

In this research, we report the description of the emissions in search phase of the species found in the study area, this description includes qualitative characteristics referring to the structure of the pulses, temporals and spectral values after performing the methodological design. From the qualitative aspect, similar and sometimes stereotyped characteristics are observed, but the main variation is reported at a quantitative level, since in comparisons with other studies and in particular on the spectral values there are high differences, which can be explained by from the recording techniques used, the nature of the emission of the species, the atmospheric attenuation and the situation to which the field report was made.

There are many questions or concerns associated with the natural history of the individuals that can be approached using the bioacoustic technique, some of these concerns are associated to the monitoring of species, animal activity and / or behavior, study of acoustic variations between species, questions related to the use of habitat, contributions to the diagnosis or taxonomic determination at the species level, establishment of contrasts between emissions reported to different geographic ranges, among others that can be raised according to the curiosity of the researcher and as long as the technique allows.

The contributions generated from the spectrograms and digital files represent a given moment in the natural history of the individual and are part of the repertoire of emissions of a particular species, they are valuable for the dissemination and knowledge of the species in a specific geographical area. Some risk is assumed when proposing acoustic inferences from a single individual, because when it comes to biological research, the ideal is to have data that allow a better contrast as this enriches the characterizations

and contributes in a more complete knowledge of the natural history of animals. For this reason, it is highly recommended to work in each investigation to have a good representation of the data.

Each question implies an investigative approach according to a scope and degree of complexity, in the same way the development of these questions may require antecedents in acoustic terms, these antecedents allow advancing in the knowledge and application of the technique in general, and according to the objective that has made this study, it is considered that an adequate starting point in studies with flying mammals implies a diagnosis and recognition of acoustic identities, taking into account that, in the case of Colombian forests, there is a very small number of acoustic investigations, particularly when dealing with Phyllostomid bats.

The importance of the findings of this research is to make visible some attributes that are part of the acoustic identity of the nasal leaf species taken as a sample in the wet forest taken as study area and although it is necessary to present samples that show a higher degree in terms of acoustic representativeness in other ecosystems, in contrast with other studies that also refer to this type of characterization at the level of the Phyllostomidae family, it is found that the temporal and spectral parameters are not reported by the authors in a standardized or complete way, neither there is a consensus on the number of pulses that speak of the acoustic representativeness of the species, before which this study makes an effort to present both the same number of spectral parameters as the number of pulses for each individual reported and also has increased the number of parameters if contrasted with others and studies in order to be taken into account in other investigations.

Some challenges associated with the acoustic monitoring of Phyllostomid individuals involve applying techniques and instruments that can combat the attenuation of emissions in the air, which is why it is recommended to use an omnidirectional microphone which is communicated to the ultrasonic detector by means of a cable of departure. The use of microphones increases the possibility of retaining the attributes of the recorded emissions. When manual recording is performed, it is suggested that the microphone be directed to 45 degrees in order to increase the range of the report.

As regards the storage of acoustic records, it is recommended to do so in full spectrum - Expanded Time and in wav format because this format is compatible with many software packages and allows to make adequate visualizations of the reports taken in the field (Ossa 2010). The use of recording booths as a strategy to record Phyllostomids is considered adequate because it facilitates the researcher to maintain control conditions while performing the acoustic report and this guarantees that there will be no acoustic interference with other species that are not part of the study (Rivera and Burneo 2013).

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