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ARTICLE

Bats (Mammalia, Chiroptera) from Yuscarán in Eastern Honduras: Conservation and acoustic characterization for the insectivorous species

Wilson Noel Gómez-Corea[1,4,6]; **Farlem Gabriel España**[2,7]; **David Josué Mejía-Quintanilla**[3,4,8] & **Andrea Nicole Figueroa-Grande**[4,5,9]

[1] Universidade Estadual de Santa Cruz (UESC), Programa de Pós-Graduação em Zoologia. Ilhéus, BA, Brasil.
[2] Mesoamerican Development Institute (MDI). Lowell, Massachusetts, United States and Tegucigalpa, Honduras.
[3] Fundación en Ciencias para el Estudio y Conservación de la Biodiversidad (INCEBIO). Tegucigalpa, Francisco Morazán, Honduras.
[4] Programa de Conservación de los Murciélagos de Honduras (PCMH). Tegucigalpa, Francisco Morazán, Honduras.
[5] Instituto Nacional de Desarrollo y Conservación Forestal, Áreas Protegidas y Vida Silvestre (ICF), Programa Nacional de Reforestación. Tegucigalpa, Francisco Morazán, Honduras.
[6] ORCID: http://orcid.org/0000-0003-3145-258X. E-mail:wilsongomezcorea@gmail.com (corresponding author)
[7] ORCID: http://orcid.org/0000-0002-1395-8096. E-mail: efarlem@yahoo.com
[8] ORCID: http://orcid.org/0000-0002-2774-4849. E-mail: davidmejia93@hotmail.es
[9] ORCID: http://orcid.org/0000-0002-2729-5967. E-mail: anfigueroa16@gmail.com

Abstract. In Honduras, most bat inventories have been carried out with mist nets as the main sampling method, skewing knowledge towards the Phyllostomidae family, therefore the diversity and distribution of insectivorous bats is underrepresented. In order to have a more complete knowledge of the diversity of bats in the municipality of Yuscarán and mainly in the Yuscarán Biological Reserve, an inventory was carried out using the techniques of mist-netting and acoustic monitoring. The samplings were carried out between 910 and 1,827 m.a.s.l., covering agroecosystems, broadleaf forest, pine forest and urban environment. A total of 32 species of bats were registered, which represents 28% of the species diversity present in Honduras. Species belonging to five families were recorded: Emballonuridae (6.25%), Mormoopidae (15.22%), Phyllostomidae (56.25%), Molossidae (9.37%) and Vespertilionidae (12.5%). With the mist nets, a sampling effort of 7,128 m^2/h was reached, which allowed the capture of 20 species and 186 individuals. Through the acoustic method, with 84 h/r, 13 species of insectivorous bats were recorded. The values of the acoustic parameters analysed from the search phase of each insectivorous species are provided, which can serve as a reference for the identification of species from Hondurans. To advance our understanding of the distribution patterns, composition, and vocal signatures of insectivore bats, we suggest the complementary use of mist nets and acoustic recorders in the inventories.

Keywords. Biological Reserve; Central America; Insectivorous bats; Acoustic sampling; Echolocation.

INTRODUCTION

Bats (order Chiroptera), with more than 1,400 species are after of rodents, the second-largest group of mammals in the world (Simmons, 2005; Fenton & Simmons, 2014; Solari *et al.,* 2019). They represent approximately half of the diversity of mammalian species in tropical forests (Simmons & Voss, 1998; Estrada & Coates-Estrada, 2001; Aguirre, 2002). Bats carry out important and complex ecological processes in forests, since they act as important pest controllers, pollinators and seed dispersers, including several plant species used by humans (Medellín & Gaona, 1999; Aguirre, 2002; Kunz *et al.,* 2011). Furthermore, they are used as indicators of the quality, biodiversity and disturbance of ecosystems and due to their ecological role, they contribute to the restoration of disturbed areas, secondary succession and re-establishment of primary forest species (Stevens & Willig, 2000; Botto-Nuñez *et al.,* 2019; García-Luis *et al.,* 2019). Despite their importance, they face multiple threats: habitat loss and fragmentation, loss of roosting sites, disease, pesticide use, wind farms and rabies control (Mickleburgh *et al.,* 2002; RELCOM, 2010; Botto-Nuñez *et al.,* 2019).

In the Neotropical region, monitoring of bats depends to a great part on the use of mist nets, skewing knowledge towards the Phyllostomidae family (Kalko *et al.,* 1996). Therefore, many aspects

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of the non-Phyllostomid species (insectivores), that are difficult to capture with traditional methods, or that are found in inaccessible sites, are still unknown (Kalko *et al.,* 1996). These aerial insectivorous bat species are a diverse group and typically use specialized echolocation calls and are able to easily detect and avoid mist nets or fly too high above the tree canopy (MacSwiney *et al.,* 2008; Marques *et al.,* 2015). The echolocation calls are emitted and used to orient themselves in flight, detect and capture prey; in addition the vocalizations have a social character, being the product of adaptations to specific environments, which provides valuable information for the knowledge of biology and ecology of insectivorous species (Arita & Fenton, 1997; Neuweiler, 2000). At present, in the Neotropical region, the number of ecological studies and inventory of bat species that involve the use of echolocation detectors has increased due to the greater availability and a wide variety of acoustic detection, recording and analysis equipment (Jung *et al.,* 2007; Barataud *et al.,* 2013; Jung *et al.,* 2014).

In Honduras, the eastern region and specifically the municipality of Yuscarán, constitutes an area of great importance for the conservation of Honduran biodiversity due to this it was included within the Union Biological Corridor (JICA, 2018), and was designated as an "Área de Importancia para la Conservación de los Murciélagos" (AICOM), with 37 registered species (Mejía *et al.,* 2019). Although the municipality of Yuscarán is forested, currently the landscape has been modified due to the pressures exerted on the forested areas, with the loss of habitat being the main threat. Promoting the management and conservation of bats in Yuscarán requires knowledge of the species found there. For this reason, our objective was to record the taxonomic diversity of bats present in this region, using mist nets and acoustic recording, in different ecosystems. This allows us to know and qualitatively and quantitatively characterize the calls of insectivorous bats in the search phase, which can serve as a reference for future bioacoustics studies in Honduras.

MATERIAL AND METHODS

Study area

Honduras has a territorial extension of 112,492 km² divided into 18 departments. In the eastern zone of Honduras is located the department of El Paraíso, to which the Municipality of Yuscarán belongs with a territorial extension of 348.9 km² and has a protected area, the Yuscarán Biological Reserve (YBR), (Fig. 1). Originally the YBR had a total area of 41.87 km², however this was extended, since approximately 21 km² were added (JICA, 2018). The average annual temperature is ~ 24.5°C

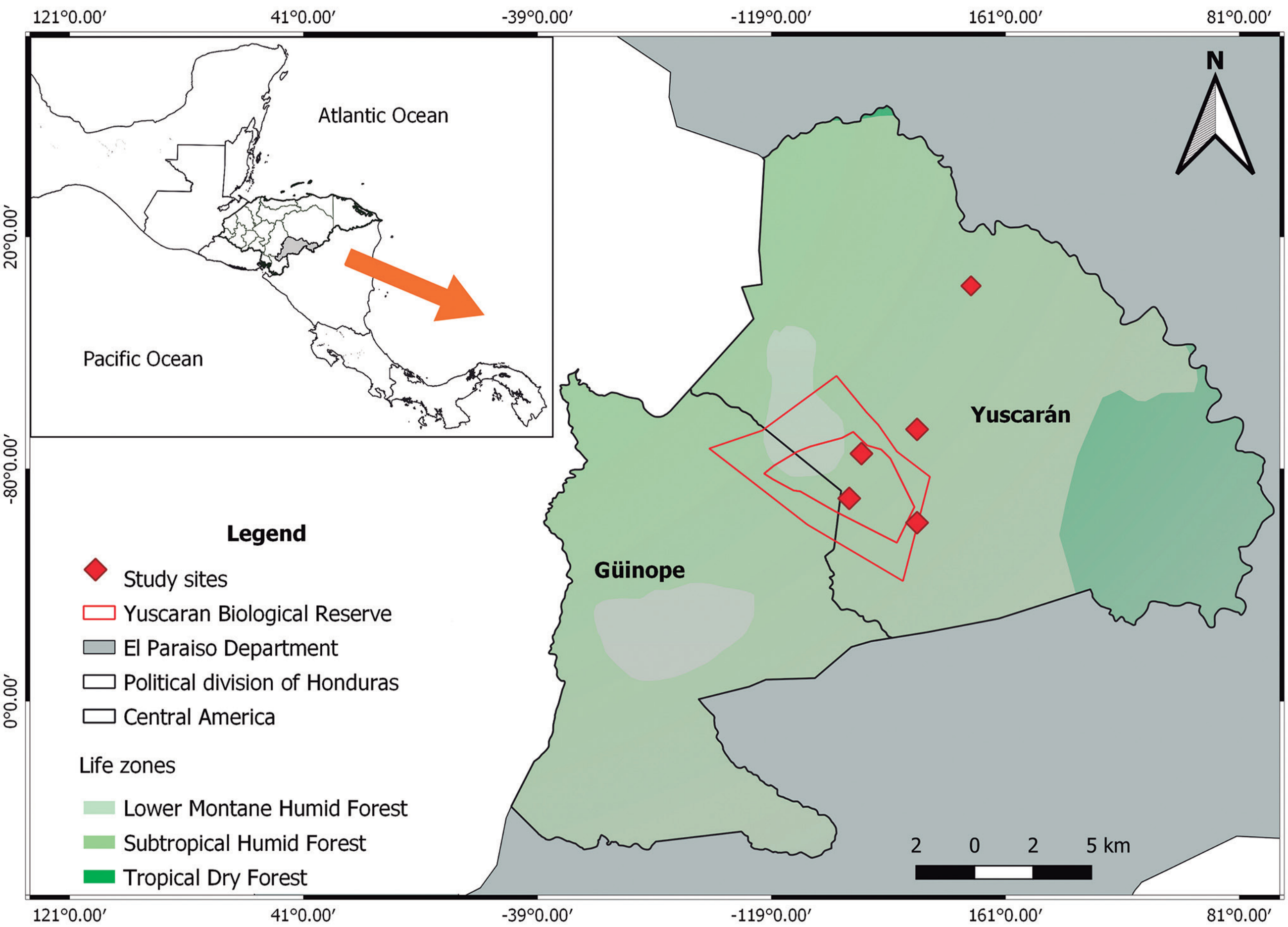

Figure 1. Geographic location of the study sites in the Yuscarán Biological Reserve and Municipality of Yuscarán, Department of El Paraíso, Honduras, Central America. Geographic coordinates and other details are in Table 1.

Table 1. Sampling locations with their respective geographic information, sampling efforts m^2/h (SE), number of individuals captured by each sampling site (IC), Richness observed (RO) and Average richness (AR).

Locality	Latitude N	Longitude W	m.a.s.l	SE	IC	RO	Chao 1	Jack 1	Bootstrap	AR
Laínez	14°00′8.28″	86°49′12″	929	972	14	4	8,06	7,78	7,78	7,87
Antennas	13°55′0.53″	86°52′28″	1827	2592	30	7	14,36	19,18	16,5	16,68
Cave	13°55′59.3″	86°52′06″	1716	2592	36	7	17,9	24,18	20,58	20,88
Agro-ecosystem	13°54′39.1″	86°50′56″	1151	972	106	16	20,6	28,25	23,75	24,2
Urban environment	13°56′42.0″	86°51′02″	910	84*		13				

* Acoustic sampling effort (hours recording).

and the average annual precipitation in the area is ~ 1,562.2 mm (elevations > 1,000 meters above sea level m.a.s.l., with ranges greater than 2,000 mm), (AFOCO, 2001; JICA, 2018). Less than 14% of the total area of the reserve is covered by dense broadleaf forest, 21% by intervened broadleaf forest, 10% by mixed forest and about 14% corresponds to pine forest; the remaining area destined for subsistence agriculture or coffee growing (AFOCO, 2001). In this area, five field samplings were carried out, in five previously defined study sites, in an altitudinal range of 910 to 1,827 m.a.s.l., covering different ecosystems: agroecosystems, broadleaf forest, pine forest and urban environment (Table 1).

Mist nets and species classifications

The field data were obtained between December 2017 to November 2019. We used the mist-netting technique, with a length of 12 m and 9 m long × 3 m high, (38 mm mesh), ground-level (~ 0.5-4 m height). We surveyed two nights at each site with an operating time of 17:00 to 24:00 hours, with nets cheked for captured bats at 15 minute intervals. The mist nets were located *ad libitum,* taking into account the characteristics of the sampled locations, trails, glades, near streams, caves, and shelters (Kunz *et al.,* 2009) and avoiding the days close to the full moon (Saldaña-Vázquez & Munguía-Rosas, 2013). Standard morphometric measurements (mm) and body mass (g) were taken from each captured bat. The measurements were made in millimetres, with a digital caliper to 0.01 mm accuracy, and body mass, using a Pesola spring scale (20, 50 and 100 g capacity). The bats captured in the mist nets were individually placed in cloth bags for later handling. For taxonomic identification, we used reference descriptions, comparing external measurements with the works of Timm *et al.* (1999); Medellín *et al.,* (2008); Reid (2009) and Medina-Fitoria (2014). Each individual captured was classified according to the sex class (males and females), age (adults and juveniles), determined by the degree of ossification of the pharyngeal epiphyses (Brunet-Rossini & Wilkinson, 2009) and reproductive status (female: non-reproductive, pregnant, lactating, post-lactating; male: enlarged and descended testicles: scrotal or non-scrotal) Racey (2009). After their identification and taking a photograph, the bats were released. Bats were captured and handled in the field following guidelines approved by the American Society of Mammalogists (Sikes & the Animal Care and Use Committee of the American Society of Mammalogists, 2016).

For bat species named in our study, we followed the currently valid taxonomic nomenclature as outlined in Wilson & Mittermeier (2019) and Simmons & Cirranello (2020). For the genus *Sturnira,* Velazco & Patterson (2013) was followed, *Dermanura* is considered as a separate genus from *Artibeus* (Hoofer *et al.,* 2008), and *Glossophaga soricina* represents a taxonomic complex and the valid species is adopted, Calahorra-Oliart *et al.* (2021). The arrangement of the family Mormoopidae follows Pavan & Marroig (2016) and the genus *Molossus* follows Loureiro *et al.* (2019, 2020). The species were classified according to the primary feeding guild based on the works of Kalko *et al.* (1996), Schnitzler & Kalko (2001), Schnitzler *et al.* (2003), and the guilds are: Insectivore (I); Animalivore (A); Frugivore (F); Nectarivore (N); Omnivore (O); Hematophagous (H). The conservation status was assigned according to the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN), considering the following categories: Critically Endangered (CR); Endangered (EN); Vulnerable (VU); Threatened (NT); Least Concern (LC); No data (NC) (IUCN, 2021).

Bioacoustic recording and analysis

A bat recorder (Song Meter SM3Bat +) was used with an omnidirectional ultrasound microphone SMX-US (Wildlife Acoustics, Maynard, Massachusetts, USA). The recorder was installed in open urban areas at a height of approximately one meter above ground level, with an inclination of 45° (Adams *et al.,* 2012) oriented towards the flight path of the bats. All vocalizations were recorded through the heterodyne system, with frequencies between 20 and 100 kHz and in a WAV (Waveform Audio Format) audio format. The recordings started at 18:00 hours and ended at 6:00 hours, on nights without strong winds or rain (Parsons & Szewczak, 2009). The Kaleidoscope software (version 5.1.9) (Wildlife Acoustics, Maynard, Massachusetts, USA) was used to visualize the spectrograms. Kaleidoscope settings were as follows: FFT size 256, window size 128 and cache size 256 MB. Recordings with very weak bat passes or indistinguishable callout passes were excluded. We define the sample unit as a 'bat pass', to a sequence with a minimum of two recognizable echolocation pulses per species (Azam *et al.,* 2015; Millon *et al.,* 2015).

Bat calls were manually identified through a series of acoustic characteristics and standard measurements: maximum frequency (Fmax), minimum frequency (Fmin), mean frequency (Fmean), characteristic frequency (Fc), characteristic slope (Sc), duration of each pulse (Dur). Finally, the structure (shape) and quantitative characteristics of the vocalizations were compared with reference works by Kalko (1995); O'Farrell & Miller (1997); Miller (2003); Barataud *et al.,* 2013; Jung *et al.* (2007, 2014), MacSwiney *et al.* (2008); Orozco-Lugo *et al.* (2013); Gómez-Corea *et al.* (2021) and the records of a reference acoustic library, obtained from previous monitoring in different regions of Honduras, using the hand release method in open areas; recordings at the exit of the shelters and in flight rooms. Functional traits of insectivorous bats that will be used to describe the structure of calls: frequency modulated (FM), quasi-constant frequency (QCF), constant frequency (FC) and frequency maximum energy (FME) (Schnitzler & Kalko, 2001; Schnitzler *et al.,* 2003).

Analysis of data

The species accumulation curve was calculated to measure the effectiveness of the inventory (Fig. 2). Previously, the samples were randomized 100 times with the software Estimates version 9.1.0 (Colwell, 2013) to avoid the order effect and smooth the curve (Moreno & Halffter, 2000). The percentage of representativeness was calculated using the Chao 1 (Chao & Lee, 1992), Jacknife 1 (Walther & Moore, 2005) and Bootstrap estimators these being regarded as particularly suitable for extrapolating richness estimates within biological communities (Colwell & Coddington, 1994; Walther & Moore, 2005; Hortal *et al.,* 2006). The completeness of the inventory was obtained by calculating the percentage of species observed with respect to those calculated by the estimators (Moreno & Halffter, 2000). Following Sodhi *et al.* (2005), we take the mean value of these three estimators (rounded to the nearest integer value) as an estimate of "true" species richness, since the effectiveness of the different estimators varies according to the composition of the data set. The sampling effort was calculated by multiplying the number of hours that the nets were kept open by the area of the nets (m^2), (Straube & Bianconi, 2002).

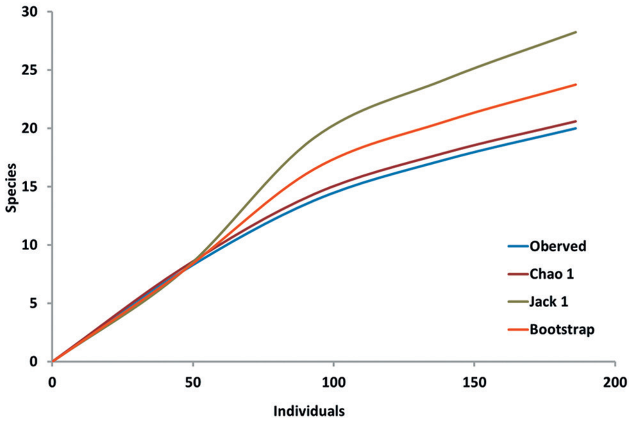

Figure 2. Species accumulation curve using mist nets, in the Yuscarán Biological Reserve and Municipality of Yuscarán, Department of El Paraíso, Honduras, Central America.

RESULTS

Registered species

Thirty-two species of bats belonging to 5 families and 19 genus were recorded (Table 2). The Phyllostomidae family was the most diverse (18 species; 56.25%), followed by the Mormoopidae Family (5 species; 15.22%). The least diverse families were Vespertilionidae (4 species; 12.5%), Molossidae (3 species; 9.37%) and finally the Emballonuridae family (2 species; 6.25%). Using mist nets with a sampling effort of 7,128 m^2/h, 20 species were captured, with a total of 186 individuals, and the proportion of the sex class favoured males with 103 males and 83 females (Fig. 3). Regarding the age class, 149 adults and 37 juveniles were captured. The species accumulation curve obtained with the mist nets did not show a stabilizing trend, indicating that the number of species would have increased if we had sampled more nights. The relationship between the observed wealth and the estimated wealth according to the estimators: Chao 1, 97.69%; Jacknife 1, 70.79% and Bootstrap 84.21%.

According to the qualitative and quantitative characteristics of the ultrasounds emitted by the bats, 13 species belonging to the families Emballonuridae, Mormoopidae, Molosidae and Vespertilionidae were identified. The Fig. 4 shows the pulses of the echolocation calls emitted by insectivorous species during the foraging phase and Table 3 the quantitative characteristics of the pulses. Species from 6 trophic guilds were recorded: insectivorous (I = 14), frugivorous (F = 10), nectarivorous (N = 4), animalivorous (A = 2), omnivorous (O = 1), and haematophagous (H = 1).

Acoustic characterization of aerial insectivorous species

Family Emballonuridae: based on their vocal signatures, we identified two species, which do not show overlap in the average frequency measurements and are characterized by emitting QCF pulses, with an initial or terminal FM component and with 3 harmonics, of which the second is the fundamental. *Balantiopteryx plicata,* emits pulses, with a final FM segment, the averages of the Fmax-Fim are 41.09-39.94 kHz and a Fc of 40.33 kHz, while *Peropteryx macrotis* for the same frequencies 39.18-37.82 kHz and 38.55 kHz in the dominant harmonic. Family Mormoopidae: we identify and describe four species, with a basic component arrangement, CF-FM-QCF. For the identification of *Pteronotus gymnonotus* and *P. fulvus,* the range of Fmax-Fmin was considered since the calls of the first species were located on average between 53.99-45.04 kHz and the second be-

Table 2. List of species captured using the mist-netting technique (individuals captured IC) and acoustic recordings (indicated with *), also shows the respective primary feeding guild (GT) and the state of conservation of the IUCN.

Family	Sub Family	Species	IC	♂	♀	FG	IUCN
Emballonuridae	Emballonurinae	*Balantiopteryx plicata* Peters, 1867*				I	LC
		Peropteryx macrotis (Wagner, 1843)*				I	LC
Mormoopidae		*Mormoops megalophylla* (Peters, 1864)	2	2	0	I	LC
		Pteronotus fulvus (Thomas, 1982)*				I	LC
		Pteronotus gymnonotus (J.A. Wagner, 1843)*				I	LC
		Pteronotus mesoamericanus Smith, 1972*				I	LC
		Pteronotus psilotis (Dobson, 1878)*				I	LC
Phyllostomidae	Micronycterinae	*Micronycteris hirsuta* (Peters, 1869).	2	1	1	A	LC
	Desmodontinae	*Desmodus rotundus* (É. Geoffroy, 1810)	10	5	5	H	LC
	Lonchorhininae	*Lonchorhina aurita* Tomes, 1863	1	1	0	A	LC
	Phyllostominae	*Phyllostomus discolor* Wagner, 1843	2	2	0	O	LC
	Glossophaginae	*Anoura geoffroyi* Gray, 1838	3	3	0	N	LC
		Choeroniscus godmani (Thomas, 1903)	1	1	0	N	LC
		Glossophaga mutica Merriam 1898	30	10	20	N	LC
		Glossophaga leachii Gray, 1844	10	5	5	N	LC
	Carolliinae	*Carollia perspicillata* (Linnaeus, 1758)	20	10	10	F	LC
		Carollia sowelli Baker, Solari & Hoffmann, 2002	10	5	5	F	LC
		Carollia subrufa (Hahn, 1905)	24	14	10	F	LC
	Stenodermatinae	*Artibeus jamaicensis* Leach, 1821	19	14	5	F	LC
		Artibeus lituratus (Olfers, 1818)	17	9	8	F	LC
		Chiroderma salvini Dobson, 1878.	1	1	0	F	LC
		Dermanura azteca K. Andersen, 1906.	2	2	0	F	LC
		Dermanura phaeotis (Miller, 1902)	9	5	4	F	LC
		Sturnira hondurensis Goodwin, 1940	12	6	6	F	LC
		Sturnira parvidens Goldman, 1917	8	4	4	F	LC
Molossidae	Molossinae	*Molossus alvarezi* Gonzalez-Ruiz, Ramirez-Pulido & Arroyo-Cabrales, 2011*				I	LC
		Molossus molossus (Pallas, 1766)*				I	LC
		Molossus nigricans Miller, 1902*				I	LC
Vespertilionidae	Myotinae	*Myotis nigricans* (Schinz, 1821)*				I	LC
	Vespertilioninae	*Eptesicus brasiliensis* (Desmarest, 1819)*				I	LC
		Eptesicus furinalis (d'Orbigny, 1847)*				I	LC
		Eptesicus fuscus (Beauvois, 1796)**	3	3	0	I	LC

** Acoustic recording and mist net capture.

Table 3. Acoustic parameters analysed from echolocation calls during the search phase of insectivore bats.

Species	Number of calls	Fmax (kHz)	Fmin (kHz)	Fmean (kHz)	Fc (kHz)	Sc	Dur (ms)
Emballonuridae							
B. plicata	12	41.09	39.94	40.49	40.33	7.47	4.04
P. macrotis	33	39.18	37.82	38.36	38.55	1.61	6.05
Mormoopidae							
P. fulvus	18	57.13	48.26	53.20	55.93	-5.87	4.78
P. gymnonotus	17	53.99	45.04	50.72	52.81	6.36	5.55
P. mesoamericanus	15	61.30	59.18	59.18	60.47	-3.03	6.94
P. psilotis	10	74.09	64.02	68.22	68.59	23.06	4.32
Molossidae							
M. alvarezi	12	34.79	32.83	33.69	33.51	5.17	6.51
M. molossus	18	32.50	30.31	31.51	31.87	1.81	5.52
M. nigricans	17	31.20	25.66	28.05	25.42	21.18	6.63
Vespertilionidae							
M. nigricans	10	63.28	49.04	51.96	49.26	12.38	3.60
E. brasiliensis	12	35.59	31.49	32.84	31.87	24.74	2.96
E. furinalis	7	59.53	46.45	49.75	46.79	43.78	2.39
E. fuscus	20	45.51	30.53	34.95	31.27	47.73	4.76

tween 57.13-48.26 kHz, with an Fc of 55.93 kHz and the pulse with a "Z" shape. *Pteronotus mesoamericanus* has a range of Fmax-Fim of 61.30-59.18 kHz and pulses lasting 6.94 ms. *Pteronotus psilotis,* presents averages of Fmax 74.09 and Fim 64.02 and the pulses have a "Z" shape.

Family Molossidae: we identify and characterize three species of the genus *Molossus,* which are characterized by emitting FM or CF pulses and by presenting an overlap of the calls in the search phase in the Fmax and Fmin, but with well-marked differences in the Sc. The averages of the parameters of Fmax-Fmin and Sc: 34.79-32.82, 5.17 for *M. alvarezi;* 32.50-30.31, 1.81 for *M. molossus* and 31.20-25.66, 21.18 kHz for *M. nigricans,* the pulses of the latter species are modulated in FM in the first part of the call, while the rest is CF. Family Vespertilionidae: based on their vocal signatures, we identify and characterize four species, one species from the genus *Myotis* and three from the genus *Eptesicus,* which are characterized by emitting pulses of FM structure, sometimes with an QCF ending. For the identification of the species of the genus *Eptesicus,* Fmin was considered and later the shape of the pulses. The Fmax-Fmin and Dur was: 63.28-49.04 kHz and 3.60 ms for *M. nigricans;* 35.59-31.49 kHz and 2.96 ms for *E. brasiliensis;* 59.53-46.45 kHz and 2.39 ms for *E. furinalis* and 45.51-30.53 kHz and 4.76 ms for *E. fuscus,* this last species the Fmin presents a partial overlap with *E. brasiliensis.*

DISCUSSION

Vocalizations of insectivorous bats

The species registered in the YBR and the municipality of Yuscarán, represent 28% of the 113 species of bats reported for Honduras (Turcios-Casco *et al.,* 2020; Mora *et al.,* 2021). It is evidenced once again that the mist net is the best method to capture bats of the Phyllostomidae family (nectarivorous, frugivorous and hematophagous), (Kalko *et al.,* 1996) and that acoustic detectors are devices very useful for registering insectivorous bats. The calls of aerial insectivorous bats are highly variable due to numerous factors, such as the type of activity, the surrounding environmental disorder, search behaviour, the acoustic complexity of the emitted sound, the number of sequences analysed and the availability of material for comparison (Faure & Barclay, 1994; Simmons & O'Farrell, 1977). According to Jiang *et al.* (2010), some geographic or intraspecific variations of the calls show allelic discontinuity between populations, which suggests a genet-

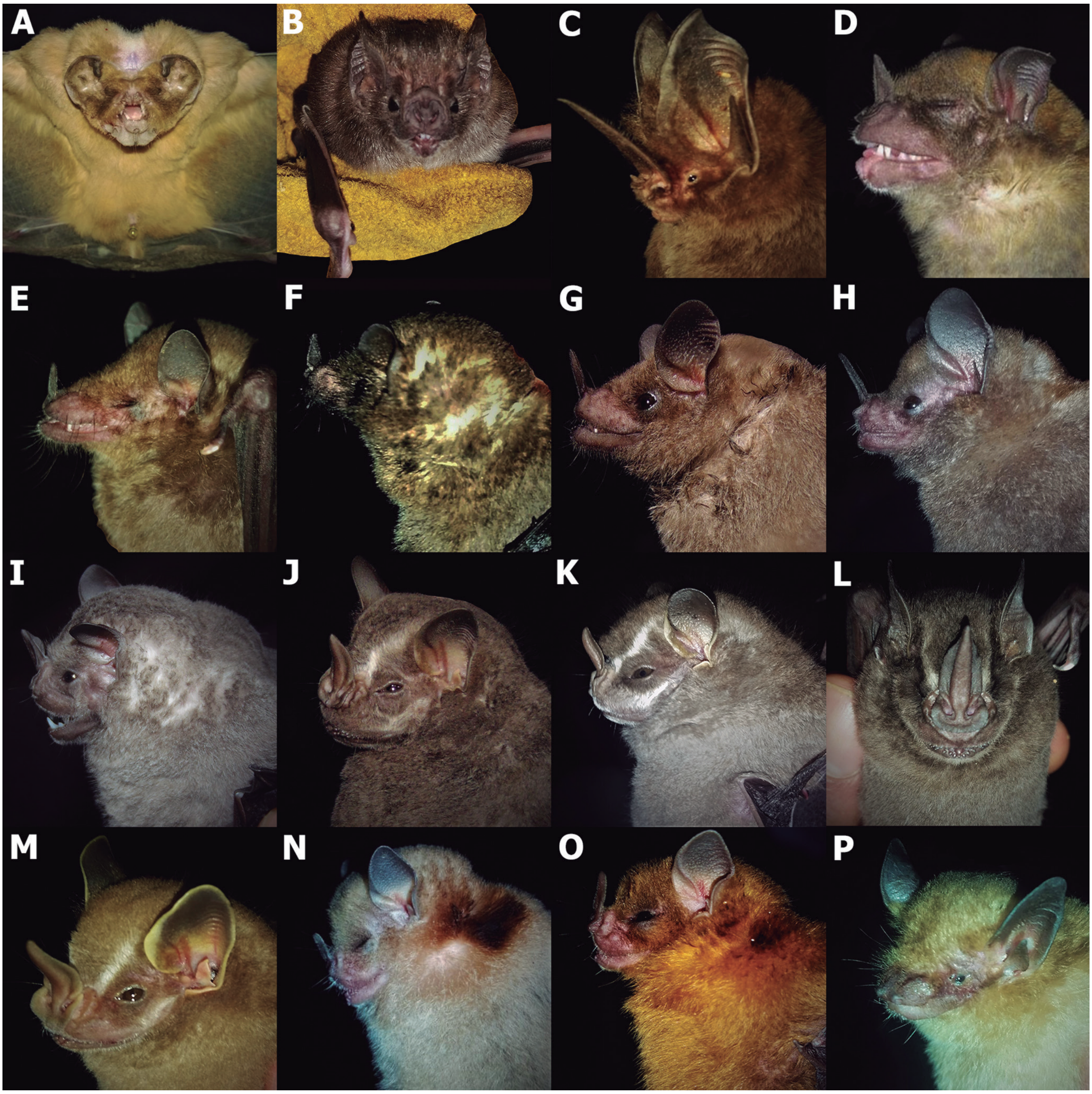

Figure 3. Part of the bat species captured with mist nets, in the Yuscarán Biological Reserve and Municipality of Yuscarán, Department of El Paraíso, Honduras, Central America. (A) *M. megalophylla;* (B) *D. rotundus;* (C) *L. aurita;* (D) *P. discolor;* (E) *A. geoffroyi;* (F) *G. leachii;* (G) *G. mutica;* (H) *C. perspicillata;* (I) *A. jamaicensis;* (J) *A. lituratus;* (K) *C. salvini;* (L) *D. azteca;* (M) *D. phaeotis;* (N) *S. hondurensis;* (O) *S. parvidens;* (P) *E. fuscus.* Photos: (C) D.J.M.Q.; (A-P) W.N.G.C.

ic basis for these differences. These variations can lead to the overlapping of the characteristics of the calls of certain species that can complicate their identification. The complexity tends to be even greater with forest species, which mostly emit low intensity broadband FM signals (Siemers & Schnitzler, 2004). For the families Emballonuridae and Mormoopidae, the acoustic identification of the species seems to be simple (O'Farrell & Miller, 1997, 1999), whereas it can be complicated for the family Vespertilionidae and Molossidae.

The echolocation calls in the family Emballonuridae are multi-harmonic; however, the energy distribution differs strongly since the FME of the search calls resides mainly in the second harmonic or in the higher harmonics (Jung *et al.,* 2007; Arias-Aguilar *et al.,* 2018). *B. plicata* and *P. macrotis* exhibit a different Fc that allows them to be discriminated from the rest of the emballonurid bats (Williams-Guillén & Perfecto, 2011). For *B. plicata,* the Fmax and Fim that we document is within that recorded by Briones-Salas *et al.* (2013) and partially coincides with that of García-Luis & Briones-Salas (2017), both are sequences of individuals in Mexico. While Jung *et al.* (2007), for this same species in Costa Rica and Panama register maximum frequencies around 42.60 ± 0.80 kHz, (Peak frequency). In *P. macrotis,* the averages of the parameters fit with the frequencies documented by Miller (2003), in Belize, the Fmax and Fmin fit the one documented by Barataud *et al.* (2013) in Guyana, partially with those registered by Briones-Salas *et al.* (2013) in Mexico and with the FME values around 38.9 ± 0.91 kHz in Panama and Costa Rica (Jung *et al.,* 2007). The bandwidth of search calls for emballonurid bats flying in rim space is still narrow compared to molossid and vespertilionid bats where it can extend 30-50 kHz or more (*e.g.,* Schnitzler & Kalko,

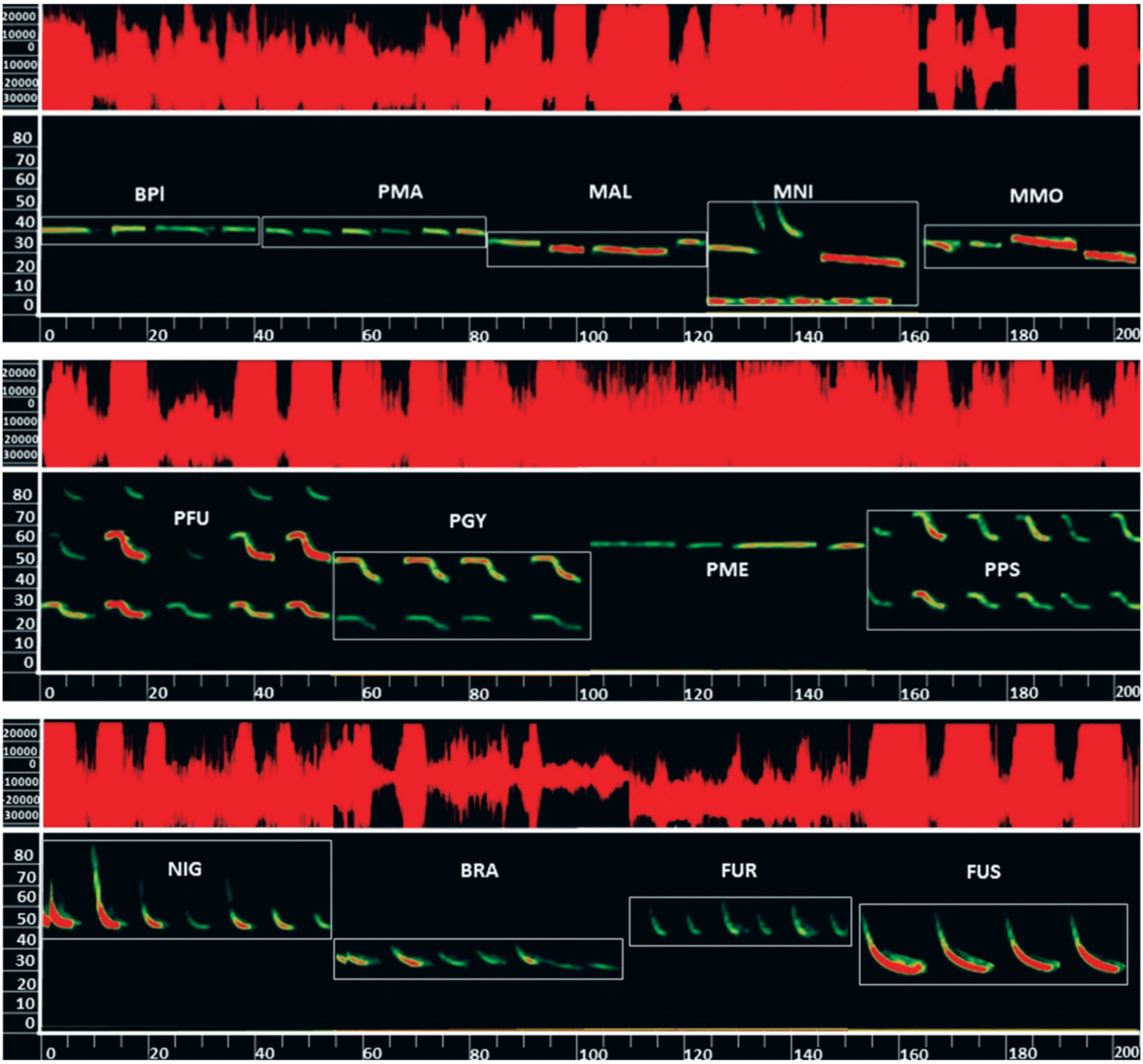

Figure 4. Echolocation pulses of aerial insectivorous bats. Spectrograms (bottom) and oscillograms (top) correspond to search calls. X axis milliseconds (ms) and Y axis Kilohertz (kHz). Emballonuridae: (BPI) *B. plicata,* (PMA) *P. macrotis;* Molossidae: (MAL) *M. alvarezi,* (MNI) *M. nigricans,* (MMO) *M. molossus.* Mormoopidae: (PFU) *P. fulvus,* (PGY) *P. gymnonotus,* (PME) *P. mesoamericanus,* (PPS) *P. psilotis.* Vespertilionidae: (NIG) *M. nigricans,* (BRA) *E. brasiliensis,* (FUR) *E. furinalis,* (FUS) *E. fuscus.*

2001; Siemers *et al.,* 2001). The frequency stratification between the species of this family constitutes a perfect example of acoustic niches within this group; the vocalizations of each species have undergone a displacement to avoid interspecific overlap (Barataud *et al.,* 2013).

The echolocation in the species of family Mormoopidae, can be easily recognized, since their vocalizations are considered as acoustic signatures. They can exhibit pulses made up of an easily recognizable CF or FM, with very characteristic shapes of each species, and there is practically no problem during the assignment of species (Miller, 2003; Williams-Guillén & Perfecto, 2011). In general, the calls we recorded were multi-harmonic, the FME is in the second harmonic and the pulses have at least one CF section. *Pteronotus fulvus* differs 7 kHz below the range documented in Belize and in Mexico by O'Farrell & Miller (1997), Miller (2003) and Ibáñez *et al.* (1999) (cited as, *P. davyi* Gray, 1838). *Pteronotus psilotis,* fits with respect to the frequencies previously described in the region, (Fmax between 72-87 kHz and Fmin between 60-69 kH), (O'Farrell & Miller, 1997; Miller, 2003) (cited as *P. personatus* (Wagner, 1843)).

In the family Molossidae, the species are characterized by emitting low FM pulses and have an FME in fundamental harmonics with long surface modulation signals emitted at quite low frequencies (Jung *et al.,* 2014). These species usually show irregular frequency alternation, narrowband, variable amplitude and great plasticity (Arias-Aguilar *et al.,* 2018). According to Jung *et al.* (2014), the wide frequency range emitted by some molossid bats is related to foraging strategies, allowing these species a greater feeding success. Regarding some species, identification at the species level is complicated due to the lack of reference calls in the literature and the overlap of frequency parameters (Williams-Guillén & Perfecto, 2011). Even so, the genus *Molossus* is easily identified by the paired pattern of the pulses (Miller, 2003). In this study, *M. molosus,* presented frequencies similar to those of low frequencies registered by Miller (2003), nevertheless the frequencies found here contrast with the descriptions of O'Farrell & Miller (1999), which provide a mean frequency of 34.1 kHz for the lowest pulses and 39.7 kHz for the highest frequency. *M. nigricans* emitted vocalizations that are within the range documented by Gómez-Corea *et al.* (2021) in different regions of Honduras, and conforms to the portion of high frequency recorded by Miller (2003), the Fmax and Fmin coincide with that reported by Kraker-Castañeda *et al.* (2013) in Mexico, (cited as *M. rufus*). It should be noted the presence of these three species in urban environments, it can be attributed to the fact that some species of this genus have a certain adaptive plasticity to disturbance conditions (Jung & Kalko, 2011).

The differentiating of the species in the Vespertilionidae family is particularly difficult, and some authors recommend using Fmin, FME and Dur as diagnostic characters (O'Farrell & Miller, 1999; Williams-Guillén & Perfecto 2011; Arias-Aguilar *et al.,* 2018). According to Williams-Guillén & Perfecto (2011), the genus *Myotis* emits Fmin between 48 kHz and 58 kHz and the identification is complicated due to the superposition of parameters, in this case the Fmin that we found for *M. nigricans* was 49.04 kHz. *M. nigricans* presents a call structure, with narrow bandwidth and quite long signals adapted to the search for food predominantly in open spaces, it is also capable of searching aerial food with short broadband signals in edge and space situations, emitting an average maximum energy peak frequency of 54.2 (Siemers *et al.,* 2001). In general, the calls of the genus *Eptesicus* are bilinear, with a clear break in the slope that precedes the flat part of the call, and they are also separated by the Fmin (Miller, 2003). The frequencies recorded here for *E. furinalis* fit with studies carried out by Miller (2003), and Kraker-Castañeda *et al.* (2013), who record a range of Fmax and Fmin between 64.02-32.78, in this regard Rydell *et al.,* (2002) mentions that most of the energy is between 36 ± 41 kHz. The average of Fmax and Fmin that we report for *E. fuscus* was 45.51-30.53 kHz, similar to that reported by León-Tapia & Hortelano-Moncada (2016) of 53.44-32.79 kHz in Mexico. The variations in the Fmax and Fmin can be attributed to the adaptation of the vocal repertoires, to the feeding sites in different habitats and prey, and the reproductive status of females (Gillam & McCracken, 2007).

Conservation efforts

In the study area, the main threat to biodiversity and in particular to bat species is the loss of habitat (JICA, 2018). The population increase in the municipality of Yuscarán from 2013 to 2018 was from 14,144 to 15,572 inhabitants (INE, 2013), and this causes enormous pressure on diversity, through the development of livestock activities and the advancement of traditional agriculture and technified (Mejía-Ordoñez, 2013; JICA, 2018). The YBR is part of the Union Biological Corridor (UBC), where the forest cover has decreased, and the agricultural area increased (JICA, 2018), further worsening the situation the high rates of erosion due to the absence of protection practices and soil improvement (AFOCO, 2001; Martinez-Garcia, 2002). The dense pine forest area decreased 7,850.6 ha (11.2% of the UBC area) and the scrub area 7,139.6 ha (10.2% of the UBC area) during the last 25 years, while the agricultural area increased 11,347.9 ha (16.2%) from 1987 to 2011 (JICA, 2018) and the pine forest was affected by the pine bark beetle (Mejía *et al.,* 2019).

In this fragmented landscape framework, the diversity of bats recorded here plays an important role, since they must be considered as part of the solution to connect patches of vegetation. Phyllostomid bats, especially the frugivorous and nectarivorous species, are fundamental in these ecosystems for the dispersal of seeds and pollination of plants both in disturbed and conserved areas (Stevens & Willig, 2000; García-Luis *et al.,* 2019). Insectivorous bats are using Yuscarán as a feeding site since we record hunting events determined from feeding trains (Griffin *et al.,* 1960) have been recorded, indicating the possible capture of prey (Bogdanowicz *et al.,* 1999). Especially the presence of the species of the genus *Myotis* and *Eptesicus* since their diet may include insects that are considered pests (Tuttle & Moreno, 2005). Therefore, the conservation of forest remnants is of utmost importance to sustain the pest control ecosystem service that bats are providing to crops in the area (Boyle *et al.,* 2011). For this region, some conservation efforts have already been established, as it is recognized as an AICOMS, however, to develop more effective conservation, the unified effort of local, regional and national organizations is necessary. Being necessary to strengthen the institutional framework (both public and private), which is explicitly associated with environmental issues, (universities, companies, NGOs, etc.) and develop an educational communication strategy linked to environmental education.

Undoubtedly, it is necessary to carry out more studies in the YBR and surroundings to know the possible fluctuations of the bat populations, implementing monitoring in the medium and long term, in addition in this sampling design did not include several months of the year and therefore, effort sampling may be insufficient to detect some species and in some calls analysed the samples are not representative to see the full range of vocalization of insectivorous bats. In Honduras, new studies should employ include methods for the upper stratum, using a combination of acoustic methods and mist nets (Gómez-Corea *et al.,* 2020), focusing on standardized acoustic monitoring that provides information to create an acoustic reference library. The range of variation inherent in the vocal repertoire of each species should be established, given the relevance of geographic variation and intraspecific and interspecific variations. This will allow the knowledge of the vocal signatures of the species which will be a useful tool to carry out fast and accurate inventories, establishing patterns of activity, habitat use and other aspects of the behavioural ecology of bat communities (Kalko, 1995).

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AUTHORS' CONTRIBUTIONS

W.N.G.C.; **F.G.E.** and **D.J.M.Q.**, design and implementation of the investigation. **W.N.G.C.**, formal analysis, writing – original draft. **W.N.G.C.**, **F.G.E.**, **D.J.M.Q.** and **A.N.F.G.**, carried out the field work, writing and revision. All authors contributed to the analysis of the results, reviewed and approved the final version of the paper.

CONFLICTS OF INTEREST

Authors declare there are no conflicts of interest.

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