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# Acoustic characterization of new species of bats for the State of Oaxaca, Mexico

Miguel Briones-Salas<sup>1\*</sup>, Mario Peralta-Pérez<sup>2</sup>  
and Margarita García-Luis<sup>1</sup>

## Resumen

Los inventarios de la biodiversidad en ocasiones se ven sesgados por los diversos métodos de colecta utilizados. Para el caso de los murciélagos, la técnica de monitoreo acústico ha demostrado ser complementaria a los métodos convencionales de muestreo. De julio de 2007 a junio de 2008, se estudió la composición de la comunidad de murciélagos en la región sur del Istmo de Tehuantepec, Oaxaca, México. Registramos 32 especies de murciélagos que pertenecen a seis familias. Trece especies de la Familia Phyllostomidae se capturaron en redes de niebla, mientras que tres especies de la Familias Emballonuridae, una especie de la Familia Noctilionidae y seis de la familia Molossidae se registraron a través del monitoreo acústico. Las cuatro especies de la Familia Mormoopidae y cinco de la Vespertilionidae se registraron con ambos métodos. A través del monitoreo acústico se suman dos nuevas especies a las 93 ya reconocidas para el estado de Oaxaca: *Molossus molossus* y *M. sinaloae*, se registraron en zonas con bosque tropical caducifolio y vegetación de galería dentro de dos áreas naturales protegidas por comunidades indígenas. Estos sitios protegidos por iniciativas locales, resulta ser una buena estrategia de conservación para los murciélagos y otros grupos taxonómicos.

**Palabras clave:** conservación, insectívoros, inventarios, Istmo de Tehuantepec, monitoreo acústico, murciélagos.

## Abstract

Biodiversity inventories are sometimes biased by the various collection methods applied. In the case of the bats, the acoustic monitoring technique has proven to be complementary to conventional sampling. From July 2007 to June 2008, we studied the composition of a bat community in the southern region of the Isthmus of Tehuantepec, Oaxaca, Mexico. We registered 32 species of bats belonging to six families. Thirteen species of the family Phyllostomidae were captured in mist nets, while three species of the family of Emballonuridae, one species of family Noctilionidae and six of the family Molossidae were recorded by acoustic monitoring. Four species of the family Mormoopidae and five

<sup>1</sup>Laboratorio de Vertebrados Terrestres (Mastozoología), Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Oaxaca (CIIDIR-OAX), Instituto Politécnico Nacional. Hornos 1003, Santa Cruz Xoxocotlán, Oaxaca, Oaxaca, 71230. E-mail mbriones@ipn.mx (MB-S) ma\_ga\_lu@hotmail.com (MG-L)

<sup>2</sup> Licenciatura en Biología, Escuela de Ciencias, Universidad Autónoma Benito Juárez de Oaxaca. Avenida Universidad s/n Ex -Hacienda Cinco Señores. Oaxaca, México, 68120. E-mail mariociidir@yahoo.com.mx (MP-P)

\*Corresponding autor

of the Vespertilionidae were registered with both methods. Through acoustic monitoring two new species are added to the 93 already known to the state of Oaxaca: *Molossus molossus* and *M. sinaloae* were registered in areas with tropical deciduous forest and gallery vegetation in two areas protected by native communities. These sites protected by local initiatives, turns out to be a good strategy of conservation for bats and other taxonomic groups.

**Key words:** acousting monitoring, conservation, insectivorous bat, inventory, Tehuantepec Isthmus.

## Introduction

Oaxaca is one of the states that protect a great amount of the biodiversity in Mexico. Many of its biological groups show a great species richness, among others butterflies, mammals and flowering plants (García-Mendoza 2004; González Pérez *et al.* 2004). In this biological richness, bats present a highly diverse group. Comprising a total of 200 species of mammals in the state (Briones-Salas and Sánchez-Cordero 2004; Alfaro *et al.* 2005; Lira-Torres and Sánchez-Cordero 2006; Botello *et al.* 2007; García-García *et al.* 2007), 93 species (46.50%) are flying mammals (García-Grajales and Buenrostro 2012). However, the knowledge about this is still incomplete because there are areas difficult to access due to complicated topography and geological origin (Briones-Salas and Sánchez-Cordero 2004; Centeno-García 2004; Ortiz-Perez *et al.* 2004). Also traditional methods, allow only to register certain groups leaving biological inventories incomplete.

Different studies of bats made in the Neotropic (Fleming *et al.* 1972; Findley 1993) and in Mexico, particularly (Íñiguez 1993; Medellín 1993; Sosa-Escalante 1997; Chávez and Ceballos 2001; Briones-Salas *et al.* 2005; Vargas-Contreras *et al.* 2008) have focused on accounting species richness, by collecting specimens mainly through mist nets, which effectively help only with those species that fly at relatively low altitudes (Tuttle 1976; Kunz and Kurta 1988; Jones *et al.* 1996; Kunz *et al.* 1996; Hodgkison *et al.* 2002). Those flying at high altitudes, however such as the families Emballonuridae and Molossidae, are poorly represented in scientific collections and inventories.

Ultrasonic detectors are equipped for the detection and recording of high frequency ultrasonic sounds emitted by bats (Kunz and Brock 1975; Kunz *et al.* 1996; O'Farrell and Gannon 1999), and turn out to be an effective tool for obtaining data about the presence, relative abundance and / or activity of insectivorous bats which emit high-intensity calls (Rydell *et al.* 1999; O'Farrell *et al.* 1999; Swystun *et al.* 2001).

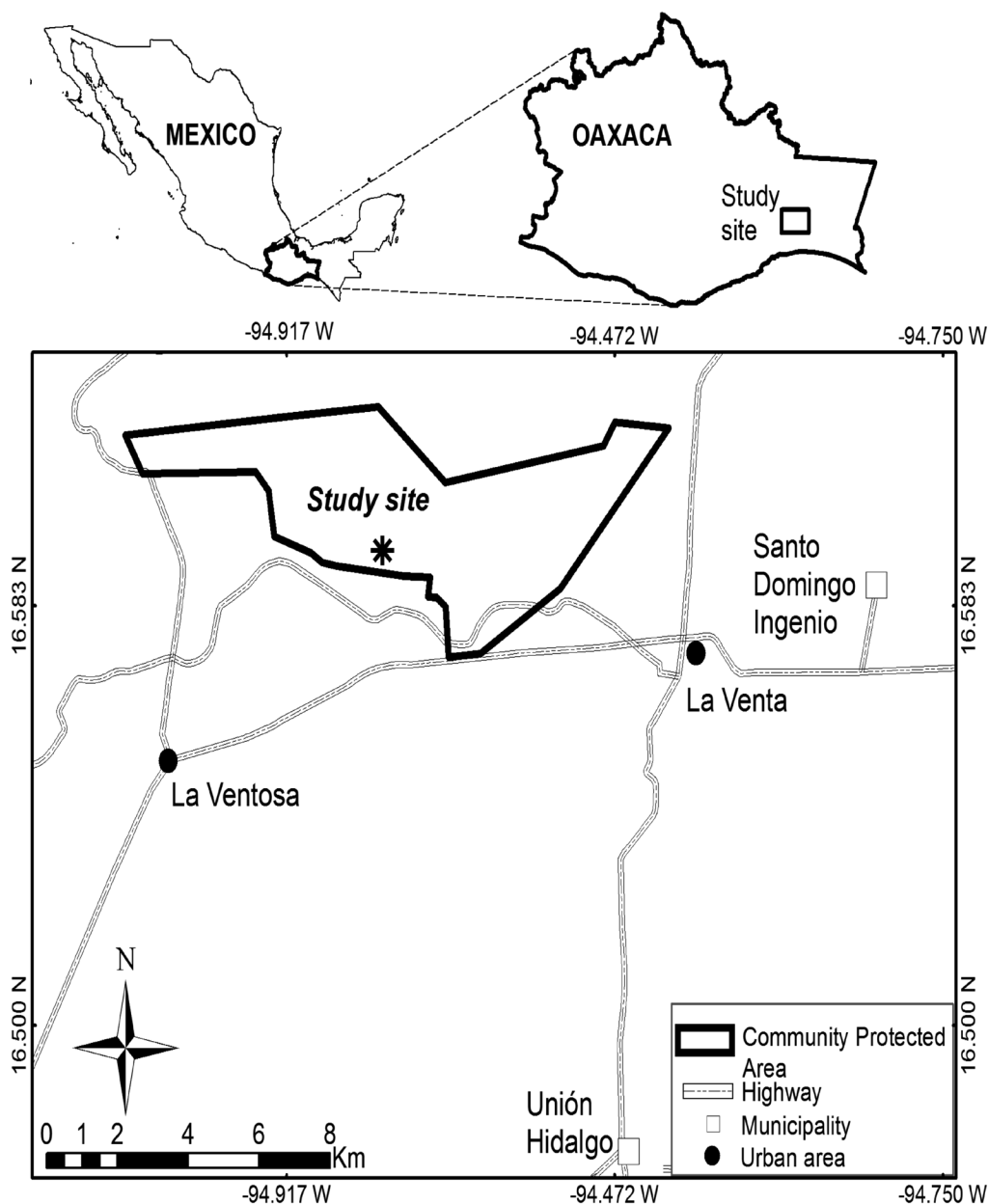
Results obtained to date using this method indicate that there are families and even species with vocal signature features that make them easily recognizable in the field. This also emphasizes the need for libraries of sounds that permit the implementation of comparisons with recordings obtained in the field (Fenton 1994; O'Farrell and Miller 1997; Ibáñez *et al.* 1999; O'Farrell and Miller 1999; Gannon and Sherwin 2002; Rydell *et al.* 2002; Siles *et al.* 2003; Waters y Gannon 2002).

The purpose of this paper is to present information on the vocal signatures of the echolocation within the community of insectivorous bats found in a communal area protected in the Tehuantepec Isthmus, Oaxaca, particularly of two new species registered for the state of Oaxaca, as well as corroborating that inventory taken with complementary

techniques (nets and acoustic) can provide accurate information to assist the knowledge and conservation of bats in tropical environments.

## Material and Methods

**Study Area.** The study took place in a protected communal area called “Zona de uso común Ojo de agua del Cerro Tolistoque”, 5 km NW of the Ejido La Venta in the municipality of Juchitán de Zaragoza, in the physiographic subprovince of Planicie Costera de Tehuantepec, in the state of Oaxaca, within the coordinates 16.584311° N and -94.873392° W (Fig. 1; Ortiz Pérez et al. 2004; Ortega et al. 2010).



**Figure 1.** Location of the study area in the northwest of La Venta, Juchitan, Oaxaca.

The terrain is flat, with altitudes averaging 200 m above sea level. The climate is markedly seasonal, with a long dry period. Average monthly temperatures range between 25° C and 29° C and rainfall occurs from June to October. The site has strong gusts of wind

from the air masses of the Pacific Ocean during the last and the first months of the year (García 1988; Trejo 2004).

The vegetation on the site is mainly dominated by tropical deciduous forest and thorn scrub, the latter prevails in the area and is characterized by being a habitat with dense thorny closed vegetation, where mainly herbaceous and shrub strata can be observed, some isolated trees are also distinguished in the area (Torres-Colin 2004). Some agricultural plots irrigated by a network of irrigation canals are located a few kilometers south of the study area.

The field work consisted of hanging mist nets from July 2007 to June 2008 as well as acoustic monitoring from January to June of 2008 with four nights visits per month to each site. In each period six mist nets were randomly arranged in different places (12 x 2.5 m) in the flight paths at the edge and within the fragments of vegetation, near streams and in general in places where flying bats were observed.

Bats caught in nets were identified using specialized keys (Álvarez *et al.* 1994; Medellín *et al.* 1997). We obtained data on age (young or adult), sex and reproductive status (active: for males when they had their testicles in their scrotum and females when they had large breast development or lactating, vagina open or pregnant; inactive: for males when they had their testicles in their abdomen and females when they had no breast development or closed vagina), later the same night they were released on the same site of capture.

The collection effort was obtained for each sampling period and for the entire year. It was calculated by summing the product of the total number net meters worked (adding those of every night) by the total number of recording hours worked. The sum of the values of each period showed total net meters per hour (mn/h). With this value, we estimate the relative abundance (number of individuals collected) of each species, by dividing the number of animals caught between mn/h. The data are expressed in number of bats per mn/h (Medellín 1993). Some specimens were collected and prepared as a museum specimen, and subsequently deposited in the Colección Mastozoológica (OAX. MA.026.0497) Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Oaxaca (OAXMA).

For acoustic monitoring the Anabat SD1 Bat Detector (Titley Electronics, Ballina, New South Wales, Australia) was used. This device is equipped with a wide range microphone and uses frequency division techniques and Zero-Crossing analysis to make the calls both audible and visible ([www.titley-scientific.com](http://www.titley-scientific.com); Parson *et al.* 2000). The Anabat recording equipment has been used extensively in monitoring bats (O'Farrell *et al.* 1999), in the description of echolocation calls (O'Farrell and Miller 1997) or temporal variation in the activity of bats (Hayes 1997; Gehrt and Chelvig 2003, 2004; Brooks and Mark 2005; Williams and Perfecto 2011).

The Anabat was placed vertically to a height of 30 cm above the ground in the same sites where mist nets were placed (O'Farrell and Gannon 1999). The height was chosen at low 30 cm to avoid the tripod that supported the detector from toppling in days with strong winds. For six months we used the method of passive monitoring, where we recorded the calls of bats during eight hours starting from sunset. The data were stored on a memory card and later transferred to a computer. All recordings were performed in the absence of rain and avoiding the full moon phase.

The spectrograms of the recordings were visualized with the software Analook © v4.9g as the primary representation of the relationship between the time, measured in milliseconds (ms) and frequency, expressed in kilohertz (kHz). The quantitative measures were obtained from the calls used to discriminate species (Simmons *et al.* 1979).

In the recordings reference is made to calls which are defined as individual and discrete pulses of which characteristic parameters can be quantified (maximum frequency, minimum frequency and duration) and to a sequence, which is the set of calls from the same species in a single file recorded (O'Farrell *et al.* 1999; Gannon *et al.* 2004).

The identification of the calls of the species was done by comparing sequences of previous recordings of individuals released in networks of virtual libraries (<http://www.msb.unm.edu/mammals/batcall/>) and by checking literature (Barclay 1983; Kalko 1995; O'Farrell 1997; O'Farrell and Miller 1997, 1999; O'Farrell *et al.* 1999; Fenton *et al.* 1998; Ibáñez *et al.* 1999; Ochoa *et al.* 2000; Granados 2001; Ibáñez *et al.* 2002).

The qualitative characteristic used in the identification of the species was the form of the signal in the graph and it can be of modulated frequency (FM, the signals of calls abruptly changes in the frequency and their duration is short in time), constant frequency (CF, the calls are located in a narrow bandwidth; Schnitzler and Kalko 2001; Limpens and McCracken 2002). While quantitative characteristics were the maximum frequency (Fmax), defined as the highest frequency of the call, the minimum frequency (Fmin), defined as the lowest frequency of the call, both expressed in kilohertz (kHz) and the duration is the interval of time covered by the call and is expressed in milliseconds (ms) (Gannon *et al.* 2004). In all cases the combination of qualitative and quantitative features enabled better identification of the species.

## Results

*Capture nets.* We worked 368 hours in 46 nights of sampling, with a total of 3,420 meters of net. The nets stayed open for eight hours every night and in average we used 74.35 meters of net per night. The total effort amounted to 27,360 net meter hours (Table 1).

We captured 425 individuals representing three families, 11 genera and 19 species. The best represented family was Phyllostomidae with 13 species and 343 individuals, representing 81% of the total sample. From the family Mormoopidae 76 individuals of three species were captured and lastly the family of Vespertilionidae was collected in lowest number with only five individuals of three species (Table 2).

The species with the highest relative abundance were *Artibeus jamaicensis* (0.0031) and *Pteronotus parnellii* (0.0024), three species were rarely captured. Only one specimen of *Dermanura phaeotis*, *Lasiurus blossevillii* and *Lasiurus intermedius* were captured resulting in a low relative abundance of 0.00003 each (Table 2).

*Acoustic monitoring.* In a total of 22 recording nights, a total of 1,791 recordings could be sampled for analysis. There were 13 species that were not captured in nets corresponding to three embalonurids, a noctilionid, a mormopid, six molossids and two vespertilionids. Five species were recorded by both methods. Of the species *M. megalophylla*, *P. davyi*, *P. parnellii* and *M. molossus* there were a higher number of sequences (73% of the total analyzed, Table 2).



**Table 1.** Collection effort total of bats with mist nets at La Venta, Juchitan, Oaxaca from July 2007 to June 2008.

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total	Average period	Average night
Nets (n)	9	6	6	6	6	6	6	6	6	6	6	6	75	6.25	1.63
Meters (m)	108	72	72	72	72	72	72	72	72	72	72	72	900	75.00	19.57
Nights	3	3	4	4	4	4	4	4	4	4	4	4	46	3.83	1.00
Hours	24	24	32	32	32	32	32	32	32	32	32	32	368	30.67	8.00
m net	324	216	288	288	288	288	288	288	288	288	288	288	3420	285.00	74.35
m net*h	2592	1728	2304	2304	2304	2304	2304	2304	2304	2304	2304	2304	27360		
Num. Individuals	11	34	37	41	51	4	31	4	41	44	66	61	425		
Num. species	8	9	9	11	10	4	9	2	8	9	12	12			

**Table 2.** Relative abundance of bat species collected in mist nets at La Venta, Oaxaca. We note the absolute number of individuals collected and the number of animals per net meters per hour (mm/h); and average characteristics (X + SD) search pulses recorded by acoustic monitoring (Anabat SD1 Bat Detector) in La Venta, Oaxaca (in parentheses indicates the coefficient of variation; CV). The taxonomic arrangement is based on Ramírez-Pulido et al. (2005).  $n^1$ = individuals caught in mistnet,  $n^2$  = number of calls, RA= Relative abundance (mistnet/h), Fmax = maximum frequency, Fmin = minimum frequency, D = Duration; OAX = New species for Oaxaca, Pr = Subject to special protection A = Threatened (NOM-059-SEMARNAT 2010).

Family/ species	Guild *	$n^1$	RA	$n^2$	Frequency (kHz)		D (ms)
					F max	Fmin	
Emballonuridae							
<i>Balantiopteryx plicata</i>	Insec			95	45.17 ± 0.83 (1.8375)	39.99 ± 2.48 (6.2016)	9.03 ± 2.38 (26.3566)
<i>Centronycteris centralis</i> , <b>OAX, Pr</b>	Insec			24	41.63 ± 0.37 (0.8888)	39.29 ± 0.61 (1.5526)	6.98 ± 0.73 (10.4585)
<i>Peropteryx macrotis</i>	Insec			23	38.81 ± 0.41 (1.0564)	36.56 ± 0.86 (2.3523)	6.14 ± 1.98 (32.2476)
Noctilionidae							
<i>Noctilio leporinus</i>	Pis			1	57.14	29.63	13.95
Familia Mormoopidae							
<i>Pteronotus davyi</i>	Insec	4	0.00014	585	72.96 ± 1.32 (1.8092)	59.64 ± 1.06 (1.7773)	5.92 ± 0.99 (16.7230)
<i>Pteronotus parnellii</i>	Insec	66	0.0024	388	64.73 ± 1.42 (2.1937)	54.93 ± 1.61 (2.9310)	24.42 ± 3.7 (15.1515)
<i>Pteronotus personatus</i>	Insec			12	83.72 ± 1.44 (1.7200)	66.75 ± 1.61 (2.4120)	4.40 ± 0.57 (12.9545)
<i>Mormoops megalophylla</i>	Insec	6	0.0002	329	54.05 ± 2.31 (4.2738)	47.87 ± 1.63 (3.4051)	5.55 ± 2.19 (39.4595)
Familia Phyllostomidae							

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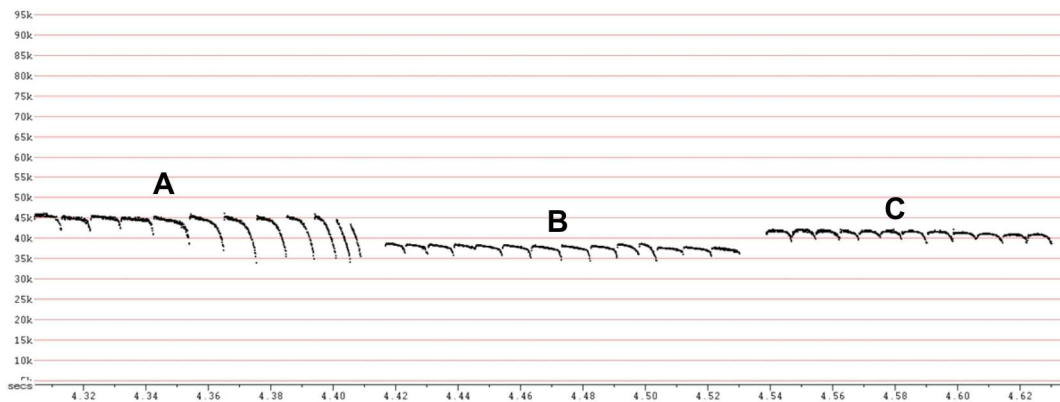
<i>Desmodus rotundus</i>	Hema	5	0.00018						
<i>Glossophaga commissarisi</i>	Nect	35	0.0012						
<i>Glossophaga leachii</i>	Nect	48	0.0017						
<i>Glossophaga morenoi</i>	Nect	29	0.0010						
<i>Glossophaga soricina</i>	Nect	37	0.0013						
<i>Leptonycteris yerbabuenae</i> , <b>A</b>	Nect	5	0.00018						
<i>Carollia subrufa</i>	Frug	2	0.00007						
<i>Sturnira lilium</i>	Frug	11	0.0004						
<i>Sturnira hondurensis</i>	Frug	43	0.0015						
<i>Uroderma bilobatum</i>	Frug	3	0.00010						
<i>Artibeus jamaicensis</i>	Frug	87	0.0031						
<i>Artibeus lituratus</i>	Frug	38	0.0013						
<i>Dermanura phaeotis</i>	Frug	1	0.00003						
Familia Molossidae									
<i>Cynomops mexicanus</i> , <b>Pr</b>	Insec	44		27.17 ± 0.55 (2.0243)	21.15 ± 0.77 (3.6407)	57.32 ± 16.05 (28.0007)			
<i>Tadarida brasiliensis</i>	Insect	3		34.04 ± 3.16 (9.2832)	27.32 ± 0.97 (3.5505)	8.69 ± 2.72 (31.3003)			
<i>Nyctinomops laticaudatus</i>	Insect	21		19.15 ± 0.85 (4.4386)	17.67 ± 0.67 (3.7917)	14.49 ± 1.50 (10.3520)			
<i>Molossus molossus</i> <b>OAX</b>	Insect	160		38.55 ± 3.17 (8.2231)	33.65 ± 2.82 (8.3804)	9.54 ± 2.06 (21.5933)			
<i>Molossus rufus</i>	Insect	59		29.70 ± 3.74 (12.5926)	25.16 ± 4.05 (16.0970)	11.08 ± 3.55 (32.0397)			
<i>Molossus sinaloae</i> <b>OAX</b>	Insect	18		40.78 ± 2.86 (7.0132)	37.09 ± 3.12 (8.4120)	6.66 ± 2.44 (36.6366)			
Familia Vespertilionidae									
<i>Rhogeessa parvula</i>	Insec	3	0.00010						
<i>Lasturus blossevillii</i>	Insec	1	0.00003	62.89 ± 8.14 (12.9432)	43.58 ± 1.36 (3.1207)	6.11 ± 1.67 (27.3322)			
<i>Lasturus intermedius</i>	Insec	1	0.00003	47.50 ± 8.12 (17.0947)	29.31 ± 1.32 (4.5036)	8.30 ± 1.91 (23.0120)			
<i>Lasturus xanthinus</i>	Insect	4		42.34 ± 3.33 (7.8649)	29.91 ± 1.07 (3.5774)	7.07 ± 2.33 (32.9562)			
<i>Eptesicus fuscus</i>	Insect	14		55.19 ± 9.19 (16.6516)	31.43 ± 1.61 (5.1225)	6.19 ± 1.83 (29.5638)			
TOTAL		425							

\* Frug: Frugivorous, Nect: Nectarivorous, Hema: Hematophagous, Insect: Insectivorous, Pis: Piscivorous.



From the family Emballonuridae 142 sequences were obtained. The form of the call in *B. plicata* and *P. macrotis* is similar although the first Fmax is the average of 45.17 kHz, while the second is 38.81 kHz (Fig. 2A, B; Table 2).

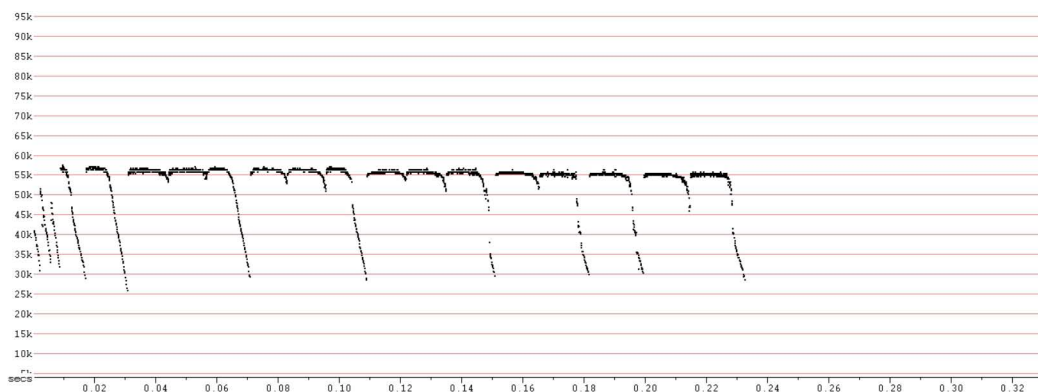
*Centronycteris centralis* is reported for the first time with this technique for the state of Oaxaca, and is the second record for the state, previously was collected in mist nets in the region of Chimalapas, Oaxaca, Mexico (Santos-Moreno *et al.* 2010). In the identification of this species the form of the call (Fig. 2C) and Fmax which on average is 41.63 kHz were considered. The recordings were made mainly in habitats dominated by grazing land and deciduous tropical forest edges in the communal protected area "Ojo de agua del Cerro Tolistoque".



**Figure 2.** Spectrogram with calls of the family Emballonuridae in La Venta, Oaxaca. **A)** *Balantiopteryx plicata*, **B)** *Peropteryx macrotis* and **C)** *Centronycteris centralis*. The time between calls is compressed to allow more calls per screen, ms= milliseconds, kHz = kiloHertz.

The closest registration of this species is located approximately at 102 km distance, 35 km NW of Jesus Carranza in Veracruz, Mexico (17.290839°N and -94.726610°W) (Hall, 1981), which corresponds to a humid tropical area, covered by semi-deciduous tropical forest and secondary vegetation. The second closest record is from Cachumbo, 9.7 km NW Santa Maria Chimalapa (16.859171° N and -94.748496° W) in tropical rain forest (Santos-Moreno *et al.* 2010). This town is located 79.5 Km NE of the recent registration site. The species is subject to special protection (PR) under Mexican law (NOM-059; SEMARNAT 2010).

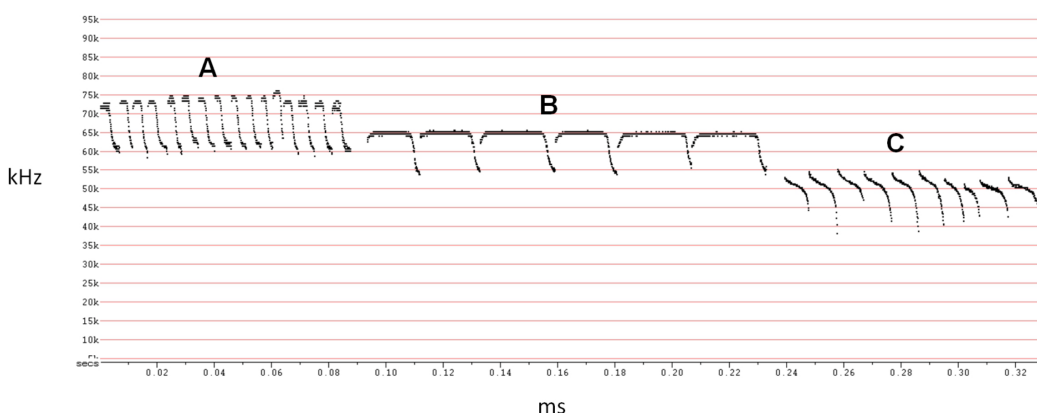
For the family Noctilionidae only one sequence was obtained. In the identification we considered the form (Fig. 3) and Fmax of 57.14 kHz was also comparable with recorded files from *N. leporinus* of Huatulco, Oaxaca (Trejo 2011). The recording took place in a gallery vegetation habitat.



**Figure 3.** Spectrogram with calls of *Noctilio leporinus* (Family Noctilionidae) in La Venta, Oaxaca. The time between calls is compressed to allow more calls per screen, ms= milliseconds, kHz = kiloHertz.

The Mormoopidae family provided the greatest number of sequences (73.37%; Table 2). For the identification of *P. davyi* and *P. personatus*, the range of Fmax-Fmin was considered as calls of the first species were located on average between 72.96 and 59.64 kHz and the second between 83.72 and 66.75 kHz, about 10 kHz apart (Table 2, Fig. 4A, B). *P. parnellii* vocalizations were very constant in form and frequency and the latter reaches its maximum value in the long flat part of its calls that was observed on average at 64.73 kHz (Fig. 4C).

**Figure 4.** Spectrogram with calls of the Family Mormoopidae in La Venta, Oaxaca. **A)** *Pteronotus davyi*, **B)** *P. parnellii* and **C)** *Mormoops megalophylla*. The time between calls is compressed to allow more calls per screen, ms= milliseconds, kHz = kilo-Hertz.

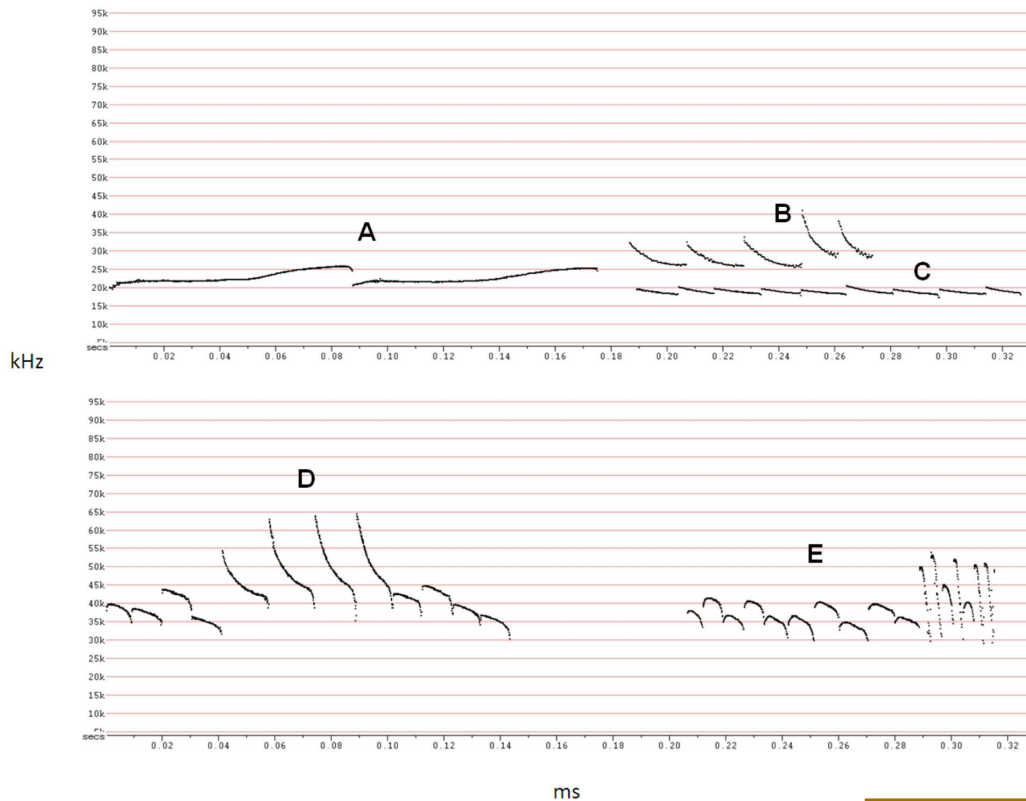


The family Molossidae presented 284 recorded sequences (Table 2). The identification of *C. mexicanus* was based primarily on the form of the spectrograms, where it was possible to identify three patterns, two differing little in its Fmax-Fmin, 27.17-21.15 kHz and 26.45-23.57 kHz respectively, both of search (Fig. 5A, B) and a short one in the approaching phase (called more steep FM, Fig. 5B). In the case of so-called search phase calls, the difference lies in duration, reaching in average 57.32 ms (the longest of all the species), while in approach phase calls are in average 17.41 ms long. Search phase calls were quantified in Table 2 because of their highest usefulness in identifying species.

For identification of *T. brasiliensis* and *N. laticaudatus* we considered the form and Fmin that averaged in the search phase to 27.32, 17.67, 12.32 and 15.58 kHz respectively (Table 2). Considering the form of the spectrogram patterns, we differentiated three species of the genus *Molossus*, which all alternated in the search phase calls with differences in range between Fmax-Fmin of 38.55 - 33.65 kHz in *M. molossus* of 29.70 - 25.16 kHz in *M. rufus* and 40.78 - 37.09 kHz in *M. sinaloae* (Table 2).

*Molossus molossus* and *M. sinaloae* represent two new bats species registered for Oaxaca (Fig. 5F, H). For *M. molossus* a large number of sequences were obtained (fourth in number of recorded sequences,  $n = 160$ ) throughout the sampling period and in different plant communities as tropical deciduous forest, thorn scrub, secondary vegetation and grazing. The nearest town to the registration site lays 15 mi SW Las Cruces, Chiapas (16.311723° N; -93.845507° W), located at 102 km SE of Ojo de agua del Cerro Tolistoque, Oaxaca (Hall 1981). Although the species is distributed from northern Mexico in Coahuila, Durango and Nuevo Leon to Chiapas in southeastern Mexico (Sánchez-Hernández et al. 1999; Muñiz-Martínez et al. 2003), it had not been registered by any method in the state. Also, García-Grajales and Buenrostro (2012) cited the species; however, when to reviewing the original quotes, the species has not recorded previously in Oaxaca.

For *M. sinaloae* few sequences were recorded, these were during the months of January, April and May, in habitats corresponding to grazing land and tropical deciduous forest edges near the protected communal area Ojo de agua del Cerro Tolistoque. The closest town to this record corresponds to: Puerto Arista Chiapas, approximately 35 km southeast of Arriaga, Chiapas (15.933889° N and -93.807500° W), with vegetation of tropical dry forests, tropical deciduous forests and agricultural areas, 130 km SE of study site (La Venta). Four individuals of this locality are deposited in the collection of the American Museum of Natural History (AMNH: M249129, M249130, M249131 and M249132).

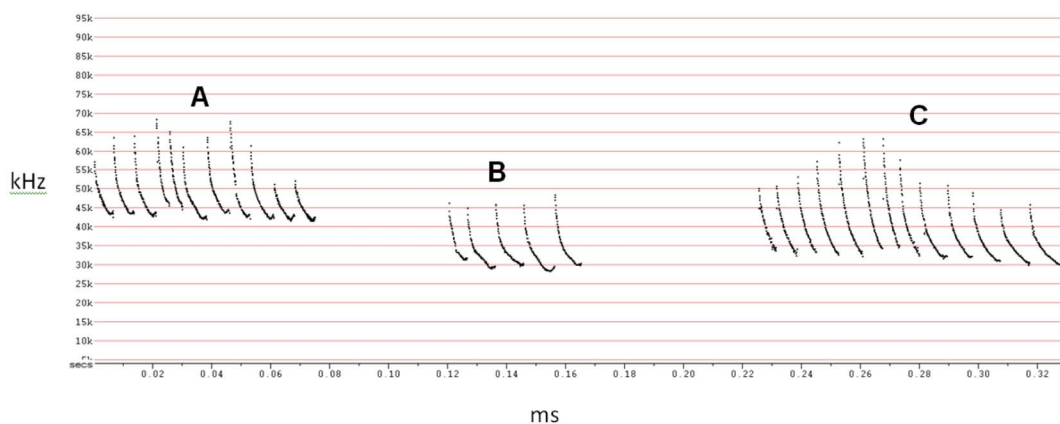


**Figure 5.** Spectrogram with calls of the Family Molossidae in La Venta, Oaxaca. **A)** *Cynomops mexicanus*, **B)** *Tadarida brasiliensis*, **C)** *Nyctinomops laticaudatus*. **D)** *Molossus molossus* and **E)** *M. sinaloae*. The time between calls is compressed to allow more calls per screen, ms= milliseconds, kHz = kilo-Hertz.

For the family Vespertilionidae, we obtained a total of 50 sequences (Table 2). For identification of vespertilionids Fmin was considered and subsequently the form. Species of the genus *Lasiurus* were separated considering their average Fmin. For *L. intermedius* its Fmin was 29.31 kHz, *L. xanthinus* 29.91 kHz, finally *L. blossevillii* was 43.58 kHz. For the first two species, their average Fmin is very similar; the individual values of each of their calls tend, in the case of *L. intermedius*, to go below 28 kHz, while *L. xanthinus* are closer to 30 kHz. Additionally, the Fmax of these species show greater differences in average frequency (47.50 kHz vs. 42.34 kHz; Table 2; Fig. 5).

The Fmin and form of the spectrograms were the characteristics considered in the identification of *E. fuscus*. Their calls were an average of 31.43 kHz Fmin was less than *Lasiurus* (Fig. 6), this due in part to the bandwidth exhibited in call of *E. fuscus*: between Fmin and Fmax is a width of 23.76 kHz, while none of the species *Lasiurus* reaches 20 Khz.

**Figure 6.** Spectrogram with calls of the Family Vespertilionidae in La Venta, Oaxaca. **A)** *Lasiurus blossevillii*, **B)** *L. xanthinus* and **C)** *Eptesicus fuscus*. The time between calls is compressed to allow more calls per screen, ms= miliseconds, kHz = kiloHertz.



## Discussion

The total species richness in a particular location may vary and it is usually due to the method or methods used for registration, the time spent for sampling, the extent of the sampled area, and the amount of prior information that the site has as well as the knowledge of the surveying person (Voss and Emmons 1996; Moreno and Halffter 2000). The use of acoustic monitoring allowed detection of 59% of the species known for the region. Previous similar studies have reported percentages ranging from 51% (MacSwiney *et al.* 2008) to 100% (O'Farrell and Gannon 1999; Siles *et al.* 2005). We must emphasize the importance of complementary methods in order to have better represented the bat fauna of a particular site.

For the humid tropics this bias has been studied and it is known that using a single method may underestimate the number of species present in a site (Kunz and Kurta 1988; Voss and Emmons 1996; Kalko and Handley 2001; Bernard and Fenton 2002; Hodgkison *et al.* 2002). This same statement can be applied to dry tropics where the number of species and vespertilionids and molossids flying beyond the height of mist nets is high.

There were species with few registrations from nets such as *L. borealis* and *L. intermedius*. A possible explanation for the paucity of registrations of these species of vespertilionids is their flight at high altitude, above the canopy (Kalko *et al.* 1996), but the acoustic recordings showed the presence of these species in the study area with intermediate values for the number registered calls ( $n = 20$  and  $43$ , respectively). It is also important to mention that there were slight differences in the calls of *L. blossevillii* to previously register in Belize that showed higher characteristic frequency (Miller 2003).

Thanks to acoustic monitoring two new species were recorded in the state of Oaxaca (*M. molossus* and *M. sinaloae*) and one more for the second time (*C. centralis*), in addition to the 93 previously reported (Briones-Salas and Sánchez-Cordero 2004, Alfaro *et al.* 2005; García-García *et al.* 2007), with these new records, the list of bats for Oaxaca increased to 95 species.

Of the species registered in this study we would like to highlight *L. yerbabuenae* a species considered threatened by the Mexican law (NOM-059; SEMARNAT 2010) and considered as vulnerable by the IUCN. Although their relative abundance was low ( $0.00018$ ,  $n = 5$ , Table 2) there are several registrations of the species in large parts of Oaxaca (Briones-Salas and Sánchez-Cordero 2004). *C. centralis* is another species that

is cataloged under the category of special protection (PR) under Mexican law (NOM 059; SEMARNAT 2010). Of the 18 species recorded with acoustic monitoring, *C. centralis* was eighth in descending order for the number of known sequences ( $n = 24$ , Table 2).

It is worth mentioning that all species in the assemblage of bats were registered within a communal protected area, covered mainly by tropical deciduous forest vegetation with a body of water that gives the name to the area: Ojo de agua del Cerro Tolistoque.

This small lake has permanent running water from the top of Cerro Tolistoque. Possibly the great majority of insectivores species registered during this study prefer this place because of the constant presence of insects, some studies have reported several species of insectivores habits in abundance near rivers and streams (O'Farrell and Miller 1997; Rydell *et al.* 2002; García 2003; MacSwiney *et al.* 2006). It is important to mention that community conservation strategies have shown effectiveness in protecting many species of wild mammals in the state of Oaxaca (Duran *et al.* 2012).

It is important to develop acoustic libraries of the Neotropical region, as suggested by Ochoa *et al.* (2000), Siles *et al.* (2003), MacSwiney *et al.* (2008) that help in the trusted identification of species of bats.

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