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METHODOLOGICAL INSIGHTS

What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages

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Summary

1. Microchiropteran bats have the potential to be important biodiversity indicator species as they are distributed globally and are important in ecosystem functioning. Survey and monitoring protocols for bats are often ineffective as sampling techniques vary in their efficacy depending on the species involved and habitats surveyed. Acoustic sampling using bat detectors may prove an alternative or complementary technique to capture methods but is largely untested in the tropics.
2. To compare the efficacy of bat detectors and capture methods in surveys, we used ground mist nets, sub-canopy mist nets and harp traps to sample bats while simultaneously recording the echolocation calls of insectivorous bats in a diversity of habitats in the Yucatan, Mexico. We described echolocation calls, analysed call characteristics to identify species, and compared species inventories derived from traditional capture methods with those derived from acoustic sampling.
3. A total of 2819 bats representing 26 species and six families were captured; 83% were captured in ground nets, 13% in sub-canopy nets and 4% in harp traps. Fourteen species and five phonic types were identified based on five echolocation call characteristics. Discriminant function analysis showed a high level of correct classification of the calls (84·1%), indicating that identification of species by their echolocation calls is feasible.
4. In all habitats, acoustic sampling and capture methods sampled significantly more species each night than capture methods alone. Capture methods failed to sample 30% of the bat fauna, and aerial insectivores were sampled only by bat detectors.
5. *Synthesis and applications*. Given the importance of bats in ecosystem functioning, and their potential as indicator species, developing effective methodologies to survey and monitor bats is important for sustainable forest management and biodiversity conservation. Acoustic sampling should be used with capture methods to increase inventory completeness in bat assemblage studies, and could form part of a single standardized monitoring protocol that can be used globally in tropical forests, as this method detects aerial insectivores not sampled by capture methods.

Key-words: biodiversity, cenotes, echolocation, forest, insectivorous bats, Mexico, pastureland, time-expansion

Introduction

Bats are the second largest order of mammals with 1116 recorded species (Simmons 2005). Neotropical bats exhibit the greatest diversity of feeding habits and their key ecological roles as seed dispersers, pollinators and predators of insects and small vertebrates is well established (e.g. Heithaus, Fleming & Opler 1975; Lang *et al.* 2006). The different ecological requirements that bats exhibit, the relatively ease of identification of most species, their abundance in most habitats, and the ability of biologists to apply standard methods to sample them without jeopardizing their conservation, favour the use of bats as an indicator group (Favila & Halffter 1997). The species richness and abundance of gleaning animalivores, for example, are negatively affected in deforested, fragmented or selectively logged landscapes (e.g. Fenton *et al.* 1992; Gorresen & Willig 2004), whereas some frugivores such as *Sturnira* and *Carollia* species which feed largely on the fruits of pioneer plants, increase their populations

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in habitats dominated by such species (e.g. Estrada, Coates-Estrada & Meritt 1993; Clarke, Pio, & Racey 2005). Despite their value as bioindicators, at present there is a lack of long-term bat monitoring programmes globally and no common set of protocols developed to monitor bats. Determining effective and comprehensive methods for surveying and monitoring bats is an essential first step in developing monitoring protocols and conducting complete species inventories of habitats.

For more than 100 years, attempts have been made to inventory Neotropical bat faunas mainly by setting mist nets. However, mist nets set at ground level and in the forest canopy are heavily biased towards the capture of phyllostomid bats which are more easily captured than species in other families (Barnett *et al*. 2006). Thus, traditional sampling methods do not give a full representation of bat assemblages. Although studies of Neotropical bats assemblages using multiple capture techniques such as ground mist nets, canopy nets and harp traps (an array of stretched nylon lines that run vertically along the length of four metal frames, above an escape-proof collecting bag for bats; Francis 1989) have documented high species richness in some habitats, aerial insectivores are still under-represented among captures (Simmons & Voss 1998; Clarke, Rostant & Racey 2005; MacSwiney G. *et al*. 2007). Aerial insectivores often commute and forage above the forest canopy, and even when they are within the sampling range of nets and harp traps, they are able to easily detect and avoid such devices (Berry *et al*. 2004).

Bat detectors allow researchers to hear and subsequently visualize the ultrasonic echolocation calls of most species and, in Europe and North America, are regularly used to identify some species (e.g. Fenton & Bell 1979; Russ 1999). Acoustic sampling has rarely been conducted in the Neotropics (e.g. Rydell *et al*. 2002; Jung, Kalko & von Helversen 2007), and detailed descriptions of the echolocation calls of most of the Neotropical bat species are lacking. Bats are abundant and important components of vertebrate communities, and in particular, frugivores and insectivores play key roles in forest dynamics and regeneration. Their inclusion in management plans to conserve biodiversity and manage forests is hampered by incomplete species lists. The development of new sampling methods such as bat detectors, and the testing of their efficacy, is therefore essential and urgently required. Studies in a range of habitat types and environmental conditions are needed to test and compare the efficacy of acoustical sampling against traditional capture methods, in order to devise sampling methodologies and protocols which will increase inventory completeness (Moreno & Halffter 2000).

Ours is the first study to simultaneously compare acoustical sampling with the most commonly employed capture methods in a variety of natural and anthropogenic-modified habitats. We thus aimed to (i) describe the echolocation calls of free-flying insectivorous bats in the Yucatan Peninsula, (ii) test the efficacy of bat detectors at identifying species, and (iii) compare the effectiveness of the most commonly used sampling methods simultaneously with acoustical sampling in constructing species inventories in a range of habitat types.

Materials and methods

CAPTURE METHODS

A total of 96 nights of fieldwork were conducted in 2003, 2004 and 2005 at eight localities in the north of the Yucatan Peninsula: two cenotes (water-filled sink-holes) in pastureland, two cenotes in rainforest and two sites without cenotes in each of these two vegetation types (for description of sites, see Supplementary Material Appendix S1). Each locality was sampled for six nights during both the dry and wet season. Sampling on consecutive nights at the same location was avoided. Each night, five 12·6 m mist nets of 36 mm mesh size, two-ply, and with four shelves (AFO Mist Nets, Manomet, Inc., Manomet, MA, USA.) were erected at ground level (0–3 m). A 12·3-m mist net modified to become an 8·3-m sub-canopy mist net (Avinet Inc., Dryden, NY, USA) was set following the method described by Clarke *et al*. (2005). A four-frame harp trap (Francis 1989) with an area of 1·42 m^2 was set in trails. Nets and the harp trap were set at sunset, left open for an average of 7 h and monitored every 30 min. We estimated capture rate for each catching method by dividing the number of bats captured by the number of mist net hours (bats/mnh) or harp-trap hours (bats/hth). Mist net hours were calculated as the length of all nets set multiplied by the number of hours they were left open (Medellín 1993). We standardized catching methods to achieve an equal sampling effort among sites.

ACOUSTIC SAMPLING

Each night, echolocation calls were recorded with a Pettersson D980 bat detector (Pettersson Elektronik AB, Uppsala, Sweden) and stored using a Sony Walkman Professional WM-D6C recorder with TDK Type IV metal tapes. For a continuous period of 10 min, we set the bat detector to repeatedly record calls for 3 s of real time and time-expand these calls (10×). These time-expanded recordings were stored on Channel 1 of the Walkman. At the same time, the bat detector was also set to record in frequency division, and the output recorded on Channel 2 of the Walkman. Call characteristics were measured only from the time expansion recordings. This method of recording allowed only 9% of the real time to be recorded in each 10-min period (Jones, Vaughan & Parsons 2000). However, this protocol was standardized in all habitats which permitted comparison. The first recording period was carried out 30 min after sunset followed by the two subsequent sampling periods at hourly intervals. To minimize call variability and changes in detection due to habitat structure, we recorded the calls in open spaces. We are aware that recording the calls in open spaces reduces the detection of bats such as phyllostomids, which forage in cluttered habitats (Schnitzler & Kalko 1998). However, it is difficult to use bat detectors to sample phyllostomid bats because they produce low-intensity calls. In addition, their calls show low inter-specific variation (Vaughan *et al*. 2004) resulting in low classification rates in discriminant function analyses (D.V. Pio, F.M. Clarke and P.A. Racey, unpublished data). Consequently, we only used capture methods and not acoustic sampling to sample phyllostomid bats. In habitats with cenotes, the microphone was orientated towards the water and at the forested sites towards natural vegetation gaps. A tripod was used to maintain the bat detector at an angle of 45° from the horizontal at 1·3 m above-ground. To aid species identification, calls were analyzed with BatSound Pro 3·10 (Pettersson Elektronik AB, Uppsala, Sweden). Recordings from free-flying bats were identified by comparison with our echolocation call library constructed with captured individuals

(identified to species). Detailed description of the methods applied and species identification are summarized in Supplementary Material Appendix S1.

STATISTICAL ANALYSES

To assign confidence to identification of species, a discriminant function analysis (DFA) was performed. To construct the model, all call variables employed to visually separate and identify the species were used: frequency of maximum energy (FME), low frequency (LF), high frequency (HF), duration (DUR) and inter-pulse interval (IPI). We did not use data obtained from calls recorded from hand-released bats in the statistical analyses. Examination of covariance matrices with a Box's M test demonstrated that these were heterogeneous ($F = 10{\cdot}4$, $P < 0{\cdot}001$), therefore a quadratic discriminant analysis was performed. Cross-validation was used in the DFA. A multivariate analysis of variance (MANOVA) was conducted to detect statistical significance of DFA analysis. Wilk's λ statistic and naive ranking were used to explain the importance of each variable in classification. Species accumulation curves were constructed to assess completeness of sampling effort with capture methods only and with capture plus acoustic methods at each habitat and at the landscape scale. To eliminate the influence of the order in which nights were added to the total, the sample was randomized 100 times using Species Richness and Diversity version 2·6 (Pisces Conservation Ltd., UK). We used a non-parametric Wilcoxon test to compare species richness per night determined by capture methods only and with these methods plus echolocation call analysis. Statistical analyses were performed with Minitab 14 (Minitab Inc., State College, PA, USA) and Brodgar version 2·5·1 (Highland Statistics Ltd, Newburgh, UK).

Results

CAPTURE METHODS

A total of 2819 bats of 26 species and six families was captured (Table 1). The Phyllostomidae was the best represented family with 17 species and members of this family accounting for 95% of the captures, whereas the Emballonuridae, Natalidae and Molossidae were each represented by only one species and relatively few captures. *Artibeus jamaicensis* Leach, 1821 was the most abundant species in all sites with 61–65% of captures. Eighty-three per cent of the bats were captured in ground mist nets, 13% in sub-canopy nets and 4% in harp traps (Table 2). We captured 16 species with more than one method, but five species were exclusively recorded in the ground mist nets, three species in the sub-canopy nets and two species in the harp traps (Table 1). Fifteen species of our captured species (57% of the assemblage) can be considered as rare (where rare species are those contributing < 0·5% of the total captures), with four species being represented by a single individual (Table 1).

GENERAL DESCRIPTION OF CALLS

A total of 2280 recorded minutes of calls from free-flying bats was analyzed. Fourteen species belonging to the families Emballonuridae, Mormoopidae, Molossidae and Vespertilionidae, and five phonic types belonging to the family Molossidae were identified (Supplementary Material Table S1; Fig. 1). Most species consistently produced one type of call but some, such as *Saccopteryx bilineata* Temminck, 1838, *Molossus rufus* E. Geoffroy, 1805 and *Molossus sinaloae* J. A. Allen, 1906, displayed alternate high and low-frequency search phase calls. Molossid 5 produced two types of low frequency calls: one of short duration (Fig. 1c, M5c) and one of long duration (Fig. 1c, M5a), and a less common higher frequency call was also evident (Fig. 1c, M5b). These different types of calls are described but were not treated as different prior to the discriminant function analysis (see Supplementary Material Table S1).

Considerable variation in time and spectral characteristics was evident among species. The calls of *Pteronotus personatus* Wagner, 1843 had the highest FME with a mean of 80·1 kHz, whereas the call of Molossid 1 had the lowest FME with 15·7 kHz. Mean call duration of the species varied from 3·6 milliseconds (ms) for *Myotis keaysi* J. A. Allen, 1914 up to 65·6 ms for Molossid 5. Inter-pulse interval showed the greatest variation of all call characteristics measured, with coefficients of variation of up to 70%. *Nyctinomops laticaudatus* E. Geoffroy, 1805 showed the highest intraspecific variation in FME with a minimum of 23·3 kHz and a maximum of 31·1 kHz (Supplementary Material Table S1). For detailed description of species' calls, see Supplementary Material Appendix S2.

DISCRIMINANT FUNCTION ANALYSIS

Quadratic discriminant function analysis resulted in a 92·1% correct classification of the original data and 84·1% when cross-validated (Supplementary Material Table S2). MANOVA showed that the model was significantly different from random (Wilk's $\lambda = 0{\cdot}001$, $F = 93{\cdot}7$, $P < 0{\cdot}001$). The calls of *Pteronotus parnellii* Gray, 1843, *Pteronotus davyi* Gray, 1838 and *Myotis keaysi* were 100% correctly classified. In contrast, for *Lasiurus intermedius* H. Allen, 1862, only 35% of the calls were correctly classified. The first three discriminant functions explain 98·6% of the total variance. To determine the importance of variables for discriminating between groups, a backward selection was performed. Wilk's λ statistic and naive ranking showed that the duration of the call and frequency of most energy were the most important variables discriminating between groups, whereas inter-pulse interval was the least important. In a further DFA analysis, removal of variables with lower discrimination power reduced the classification to 81·3% for the original data and 78·8% for the cross-validated data; thus, the best model for classification of all species relied on all five variables.

INVENTORY COMPLETENESS

The analysis of echolocation calls of free-flying bats revealed the presence of 11 insectivorous species not sampled by any of the capture techniques used to sample habitats (Table 1). Thus, harp traps and mist nets at ground level and in the canopy failed to sample 30% of the bat fauna, and this component was only sampled by bat detectors. The average number of

Table 1. Checklist of species recorded in the north of the Yucatan Peninsula, Mexico, by capture methods (C) and acoustic methods (A). Species in boldface were recorded exclusively by acoustic methods. CENPAST, cenotes in pastureland; PAST, pastureland; CENFOR, cenotes in forest; FOR, forest

	Pastureland		Forest	
Taxon	Cenotes	No cenotes	Cenotes	No cenotes
Emballonuridae				
Peropteryx macrotis	A	A	A	A
*Saccopteryx bilineata**†		A	A	C, A
PHYLLOSTOMIDAE				
Desmodontinae				
Desmodus rotundus	C	C		C
*Diphylla ecaudata**‡				C
Glossophaginae				
Glossophaga soricina	C	C	C	C
Phyllostominae				
*Chrotopterus auritus**†	C			
*Lampronycteris brachyotis**†			C	C
Lonchorhina aurita			C	C
Micronycteris microtis	C	C	C	C
*Micronycteris schmidtorum**§	C			
*Mimon cozumelae**	C		C	C
Carolliinae				
Carollia sowelli			C	C
*Carollia perspicillata**		C	C	C
Stenodermatinae				
Sturnira lilium	C	C		C
Artibeus jamaicensis	C	C	C	C
Artibeus lituratus	C	C	C	C
Artibeus phaeotis	C	C	C	C
*Centurio senex**†	C		C	C
*Chiroderma villosum**	C	C	C	C
Mormoopidae				
Mormoops megalophylla	A	A	A	A
Pteronotus parnellii	C, A	C, A	C, A	C, A
Pteronotus personatus	A		A	
*Pteronotus davyi**	C, A	A	A	C, A
Natalidae				
*Natalus stramineus**§	C	C	C	C
Molossidae				
*Molossus rufus**‡	A	C, A		A
Molossus sinaloae	A		A	A
Nyctinomops laticaudatus	A	A	A	A
Molossid 1	A		A	
Molossid 2	A	A	A	A
Molossid 3	A	A	A	
Molossid 4	A	A	A	
Molossid 5	A	A		
Vespertilionidae				
*Eptesicus furinalis**†	A	A	C, A	
*Lasiurus ega**‡	A	C, A	A	
Lasiurus intermedius	A	A	A	
*Rhogeessa aeneus**†	C, A	C, A	C, A	
Myotis keaysi	C, A	A	C, A	C, A
	CENPAST	PAST	CENFOR	FOR
No. of species captured	17	14	17	20
Species recorded acoustically	18	16	17	10
Total no. of species	31	26	30	26

*rare species (contributing < 0·5% of the total captures). Exclusively captured in: †ground level mist nets, ‡sub-canopy mist net, and §harp trap.

species sampled per night with capture methods and capture methods plus acoustic sampling was 4·5 and 10·3, 3·0 and 5·6, 5·5 and 9·7 and 5·3 and 6·12 for pastureland with cenotes, pastureland without cenotes, forest with cenotes and forest without cenotes, respectively. The number of species recorded per night with acoustic sampling in combination with capture methods was significantly higher than that recorded with capture methods alone. This was true for all habitats sampled

Table 2. Sampling effort of the catching methods per habitat. *n*, number of bats captured; S, number of species; mnh, mist net hour; Bats/mnh, capture rate at number of bats captured divided by mist net hour; Bats/hth, capture rate at number of bats captured divided by harp-trap hour. CENPAST, cenotes in pastureland; PAST, pastureland; CENFOR, cenotes in forest; FOR, forest

	Ground mist net				Sub-canopy mist net				Harp trap			
Habitat	*n*	S	mnh*	Bats/mnh	*n*	S	mnh	Bats/mnh	*n*	S	hth	Bats/hth
CENPAST	437	13	239 760	0·0018	56	5	32 640	0·0017	23	8	159	0·14
PAST	158	9	241 920	0·0006	120	9	31 680	0·0036	8	3	164	0·04
CENFOR	873	16	240 048	0·0036	113	9	31 776	0·0035	45	9	161	0·27
FOR	859	15	246 240	0·0034	88	10	32 448	0·0027	39	9	163	0·23
Total	2327	21			377	16			115	13		

*mnh is the product of length of all nets in metres multiplied by the number of hours nets were set.

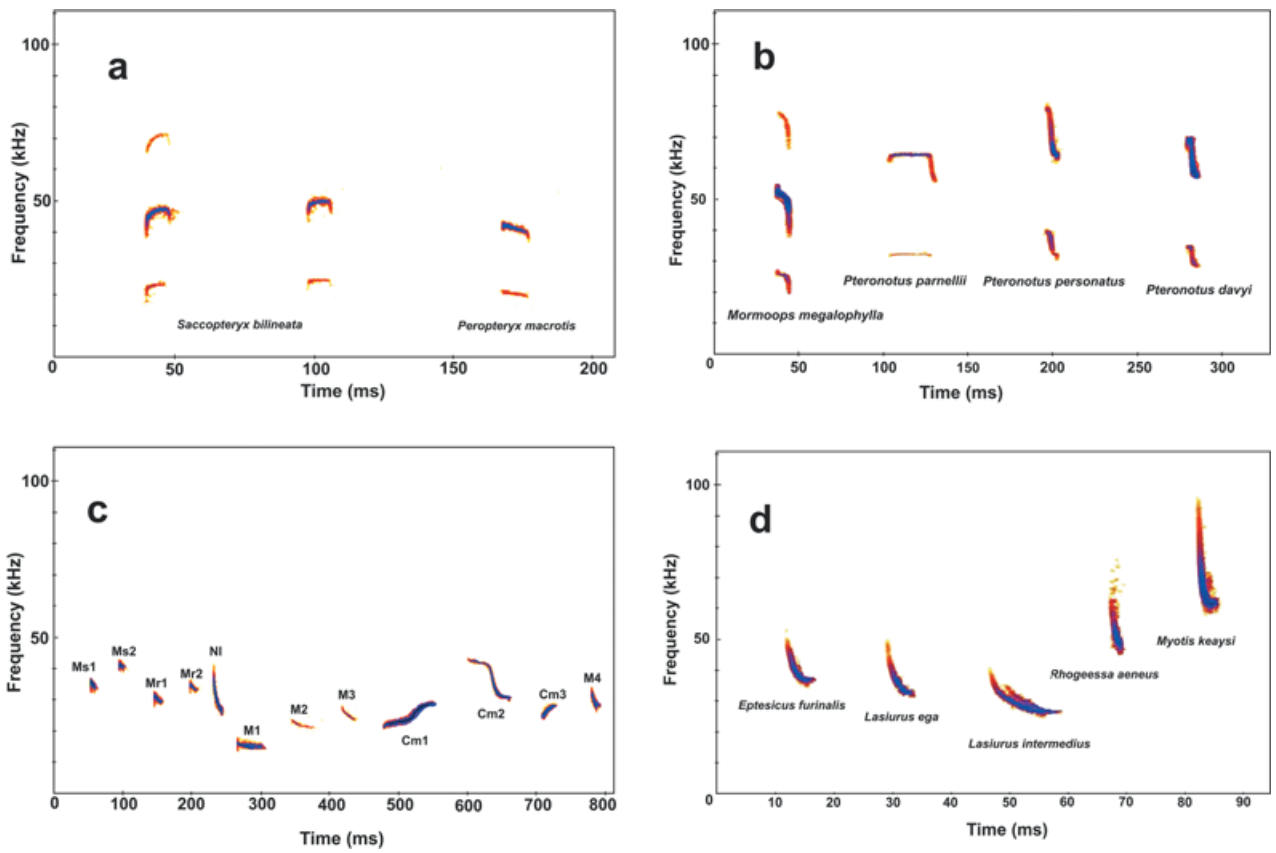

Fig. 1. Spectrogram with typical search calls of the four families identified in the north of the Yucatan Peninsula. (a) Emballonuridae, (b) Mormoopidae, (c) Molossidae: Ms, *Molossus sinaloae*; Mr, *Molossus rufus*; Nl, *Nyctinomops laticaudatus*; M1, phonic type molossid 1; M2, phonic type molossid 2; M3, phonic type molossid 3; M4, phonic type molossid 4; M5, phonic type molossid 5; and (d) Vespertilionidae.

(all comparisons $P < 0{\cdot}01$) (Fig. 2). Acoustic sampling demonstrated that locally rare insectivorous species (< 0·5% of total captures) captured in only one habitat were present in most habitats and much more common than suggested by analysis of capture methods. A combination of acoustic sampling and capture methods recorded 37 species for the north of the Yucatan Peninsula (Table 1; Fig. 2).

Discussion

We described and analysed the echolocation calls of bats and compared species inventories derived from traditional capture methods with those derived from acoustic sampling. Fourteen species and five phonic types belonging to the families Emballonuridae, Mormoopidae, Molossidae and Vespertilionidae were identified based on five echolocation call characteristics. Discriminant function analysis showed a high level of correct classification of the calls, indicating that identification of species by their echolocation calls is feasible. At all habitats, acoustic sampling and capture methods sampled significantly more species each night than capture methods alone. Capture methods failed to sample 30% of the bat fauna. This component, which consisted entirely of aerial insectivores, was sampled only by bat detectors.

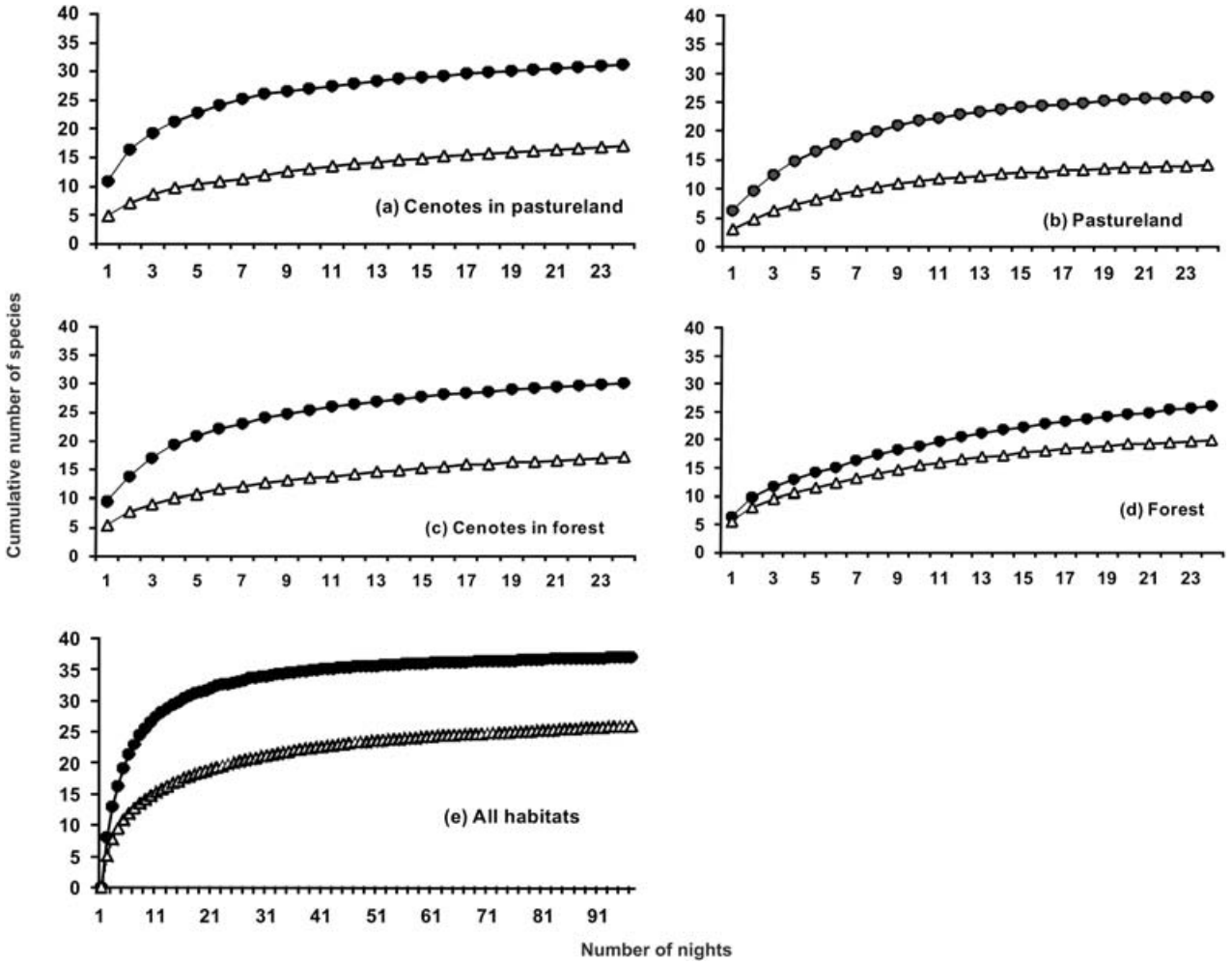

Fig. 2. Bat species accumulation curves for the four habitats sampled and landscape. Δ, species recorded with capture methods only; and •, species recorded with capture methods and acoustical sampling.

SPECIES IDENTIFICATION

The correct identification to bat species was possible from the echolocation call characteristics of the bats we recorded. Most call parameters were consistent with those reported in the literature for the few species whose calls have been analysed (O'Farrell & Miller 1997, 1999; Rydell *et al*. 2002). Some differences were detected between our recorded calls and those published, particularly in call duration, for example, *Mormoops megalophylla* Peters, 1864 and *P. personatus* (O'Farrell & Miller 1997). Such differences are probably due to calls being recorded in habitats that differ in levels of background clutter (forest vs. open-space habitats) since clutter affects intra-specific call variability. Alternatively, reported differences in calls may be attributed to researchers using different recording systems (frequency division vs. time-expansion).

In recent years, there has been a lively debate about the comparative efficiency of the frequency division (i.e. the Anabat system) and time expansion systems in detecting echolocation calls (O'Farrell, Gannon & Miller 1999; Fenton 2000). The bat detector's time expansion facility retains most information of the call in contrast to frequency division systems which often fail to detect all harmonics (Fenton 2000). This was particularly important in successfully identifying species in the present study, because observation of flight behaviour, an important factor discriminating species in the field (Kalko 1995), was not possible for many species. The strongest harmonics are, however, efficiently detected with frequency division recordings (O'Farrell & Miller 1999), and we were able to compare them with our time-expanded recordings.

ECHOLOCATION CALL DESIGN

Differences in call design among species can be explained by their different foraging habits. Within the Emballonuridae, *Peropteryx macrotis* Wagner, 1843, which forages mainly in open spaces, emits narrowband calls, whereas *S. bilineata*, which typically forages close to vegetation, also emits a narrowband call but with broadband components that helps to determine the position of prey relative to background clutter (Jung *et al*. 2007). Within the Mormoopidae, species displayed

multiharmonic calls of short duration, with the exception of *P. parnellii*. The calls of *P. parnellii* consist of a long narrowband component terminating with a broadband sweep, a signal design that gives this species not only high performance in target detection but also enables classification of targets in cluttered habitats (Jones & Teeling 2006). Species within the Molossidae displayed long narrowband or broadband–narrowband calls which are typically used for hunting in uncluttered or open spaces (Schnitzler & Kalko 1998). Finally, vespertilionids emitted broadband calls with a distinct shallow component (e.g. *Eptesicus furinalis* d'Orbigny 1847, *M. keaysi*) used for foraging when the background is cluttered (Schnitzler & Kalko 1998).

SAMPLING METHODS AND INVENTORY COMPLETENESS OF BAT ASSEMBLAGES

Exhaustive inventories of Neotropical bat faunas are not feasible objectives for short-term fieldwork: too many species are rare or hard to capture (Simmons & Voss 1998). Our results demonstrate that, with the addition of acoustic methods, new species are sampled at a faster rate than with the use of capture methods alone, and inventory completeness increased. This has important implications for sampling design as the use of bat detectors might reduce the time, effort and money required for surveys. Comparison between acoustic sampling and traditional sampling methods (mist netting and harp traps) conducted simultaneously every sampling night in the variety of habitats shows that insectivorous species, particularly within the family Molossidae, are under-sampled by netting methods. Mist nets were highly effective at capturing phyllostomids, whereas harp traps were effective at sampling mormoopids that forage close to the ground such as *P. parnellii* and *P. davyi*. However, species that fly and forage at higher altitudes, above the height of nets and harp traps (e.g. *M. sinaloae*, *N. laticaudatus*, molossid phonic types) were not caught. Other species such as *S. bilineata* and *P. macrotis* visually and/or acoustically detected the mist nets and harp traps, as we acoustically detected these bats flying close to but avoiding nets and harp traps.

It has been suggested that in the Neotropics where phyllostomids predominate, mist netting is the most appropriate sampling method (e.g. Fenton *et al*. 1992; Simmons & Voss 1998). Following the present analysis of the acoustic recordings of free-flying bats, the checklist of bats in northern Yucatan increased from 26 species, based on captures only, to 37 species. Twenty of these species are non-phyllostomids and were more easily recorded with acoustic sampling. These findings demonstrate that the use of traditional capture methods such as mist nets alone, would give a misleading picture of the bat assemblage and that only studies employing both multiple capture methods and acoustical sampling increase inventory completeness and produce representative species assemblages in habitats and landscapes. Accurate species lists and abundance estimates are essential to carry out effective forest management and conservation of bat diversity. In the Neotropics, this only can be achieved with the combination of both capture and acoustic methods. We strongly recommend the adoption of acoustic sampling in all future studies of bat assemblages in the Neotropics.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1. Detailed methods

Appendix S2. Description of calls by family

Table S1. Call characteristics of insectivorous bat species

Table S2. Discriminant function analysis results

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2664.2008.01531.x
(This link will take you to the article abstract).

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Appendix S1
Study area and sampling sites

Sampling was carried out at eight locations in the north of the Yucatan Peninsula: two cenotes in pastureland, two cenotes in rainforest and two sites without cenotes in each of these two vegetation types. Cenotes in pastureland were Cenote Azul (N21º13', W88º40') and Cenote Tortugas (N20º54', W88º51') which were approximately 50 to 65 m in diameter with a surrounding vegetation belt of 30 to 50 m width. Pastureland sites were Rancho Santa Teresa (N21º10', W88º53') and Rancho Noyaxché (N21º17', W88º36'). Cenotes in forest were Cenote Benita (N21º13', W87º12') and Cenote Km 4.5 (N21º04', W87º12') approximately 30 to 70 m in diameter. Forest locations were Selva Rancho Santa María (N21º06', W87º10') and Selva El Eden (N21º12', W87º12').

Echolocation call library

We constructed an echolocation call library with captured individuals (identified to species) hand-released approximately 5 m from the bat detector in the same open areas where we acoustically sampled free flying bats, and their calls recorded to form a reference library. Species caught and their calls recorded were: *Saccopteryx bilineata, Pteronotus parnellii, Pteronotus davyi, Molossus rufus, Lasiurus ega, Myotis keaysi, Eptesicus furinalis* and *Rhogeessa aeneus*. *Nyctinomops laticaudatus* was recorded near a known roosting site. Identification of the remaining species was based on comparison of previously published descriptions of calls from echolocation studies. Identification of the calls of *Peropteryx macrotis, Mormoops megalophylla, Molossus sinaloae* and *Lasiurus intermedius* was based on previously published calls of individuals captured in the Yucatan (Granados, 2001; Rydell *et al*., 2002), Belize (O'Farrell & Miller, 1997, 1999; Miller, 2003) and other Central American localities (Jung, Kalko & von Helversen, 2007). *Pteronotus personatus* was identified based on published data about

its echolocation (O'Farrell & Miller, 1997, 1999). We obtained forearm (fa) and body mass (bm) measurements from captured bats; whereas the morphometrics of species not captured were obtained from published sources: *P. macrotis, M. megalophylla and L. intermedius* (Rydell *et al*., 2002), and *P. personatus, M. sinaloae, N. laticaudatus and E. underwoodi* (Reid, 1997). Taxonomy of bat species follows Simmons (2005).

Species identification

To aid species identification, calls were analyzed with BatSound Pro 3.10 (Pettersson Elektronik AB, Uppsala, Sweden). Spectrograms were visually examined using a Hanning window at a sampling rate of 44100 Hz, Fast Fourier Transformation (FFT) size of 512. Because BatSound display settings affect the visual perception of the sonograms produced by the software, for standardization we used the same parameter levels for the spectrogram window (2,000-ms per plot; threshold = 10; amplitude contrast = 3) (Avila-Flores & Fenton, 2005). Only search-phase calls with enough intensity to be visually identifiable were considered for analysis. Within each 10-min file, a sequence of five calls from each species identified was selected for measurement and analysis. In each call or pulse, five characteristics were measured: call duration (DUR) and inter-pulse intervals (IPI) taken from the end of one call to the beginning of the next, from the oscillogram. Frequency with the most energy (FME) was measured from the peak of power spectrum (FFT 4096). Lowest frequency (LF) and highest frequency (HF) were calculated subtracting 10dB from FME (Fenton, 2002). Descriptive statistics of the call characteristics were calculated using the statistical software Minitab 14 (Minitab Inc.).

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Appendix S3.

Description of calls by family

Emballonuridae

Calls of *Saccopteryx bilineata* were characterized by search phase calls emitted in pairs that alternate in frequency with the main energy concentrated in the second harmonic. The first call of each pair was at 44.5 kHz and the second at 46.8 kHz (Table S1; Fig. 1a). Calls started with a steep, upward broadband component, followed by an upward narrowband portion and ending with a steep broadband component. *Peropteryx macrotis* echolocation calls were of short duration with intense narrowband pulses ending with a downward- sweep broadband (Fig. 1a). The FME was in the second harmonic at 39.6 kHz (Table S1).

Mormoopidae

Four species of mormoopids were identified. All displayed the maximum energy in the second harmonic in most of the sequences, but *Pteronotus davyi* and *P. personatus* emphasised the first harmonic in others. *Mormoops megalophylla* echolocation calls showed FME at 51.6 kHz (Fig. 1b; Table S1). This species' calls are very distinctive with curved downward sweeps, shallow at first and then steep towards the end. *P. parnellii* calls are unmistakeable as this is the only neotropical species that emits a short broadband upward sweep, then a long narrowband component followed by a downward broadband sweep (Fig. 1b). Maximum energy of the second harmonic was at 64.5 kHz, and call duration is the longest within the family, at 25.8 ms (Table S1). The calls of *P. personatus* consisted of two narrowband components at 80.1 and 65.9 kHz, respectively, joined by a steep downward-sweep with a mean duration of 7.1 ms (Table S1). The calls of *P. davyi* were very similar in

shape to *P. personatus,* but the CF components were around 10 kHz lower in frequency at 69.1 and 58.7 kHz, respectively (Fig. 1b). Calls were slightly shorter in duration than *P. personatus* with a mean of 6.8 ms. (Table S1). Inter-pulse interval in *P. davyi* (mean=66.8 ms) was longer than that observed in *P. personatus* at mean of 53.9 ms (Table S1).

Molossidae

Calls of *Molossus sinaloae* and *M. rufus* consisted in pairs of pulses that alternate in frequency. Calls in both species included a single harmonic with a steep upward broadband component, followed by a shallow broadband portion. Calls of *M. sinaloae* were higher in frequency and shorter in duration than the calls of *M. rufus* (Fig. 1c; Table S1). The calls of *Nyctinomops laticaudatus* showed the greatest variation in FME of all species recorded, ranging from 23.3 to 31.1 kHz. This species' calls showed the steepest broadband call among the molossids (Fig. 1c). Some calls of *N. laticaudatus* showed a steep FM portion followed by a shallow portion, while others show a narrowband-broadband shape, which became steeper as the bat approached the prey. Most calls consisted in a single harmonic, but occasionally a second was observed (Table S1).

In addition to the three species described above, five molossid phonic types were detected. Phonic types were assigned to the Molossidae as they displayed the typical characteristics of this family: long duration, low frequency and narrowbandw calls with the most energy is contained in the fundamental. Molossid 1 displayed long duration (19.5 ms) shallow broadband calls with the lowest frequency of all species in the present study (FME = 15.7 kHz). The phonic type of molossid 2, a narrowband

call had a call duration of 16.1 ms and the second frequency lowest call of all bats analyzed (FME = 21.6 kHz)(Fig. 1c, Table S1). Calls of molossid 3 consisted of a single harmonic with a FME of 25-27 kHz and a call duration of 13.9 ms (Fig.1c; Table S1). Molossid 4 had the steepest broadband shape and the highest frequency of all phonic types (mean FME = 29.3 kHz) but the shortest call duration of all phonic types at 11.9 ms (Fig. 1c; Table S1). Molossid 5 has one of the most distinctive calls found in the present study. The species displayed three types of calls. The first was a short-duration call with a mean FME of 26.5 kHz (Fig. 1c-M5c). The first pulse call in some sequences was elongated (mean DUR=65.6 ms) and emphasized energy in a lower frequency with a mean FME of 23.8 kHz (Fig. 1c-M5a). This long pulse was followed by the third type of call: a broadband downward sweeping pulse of mean FME 30.3 kHz (Fig. 1c-M5b; Table S1). Our observations, together with those of Granados (2001), suggest that the long duration calls emitted by this molossid might be involved in the detection of or approach to prey as they preceded "feeding buzzes".

Examination and comparison the calls of Molossid 5 with the unpublished calls recorded by A. Guillén and L. Aguirre (pers. comm.) suggest that this species may be *Promops centralis*. Because of uncertainty about the identity of Molossid 5, we will, for now, continue to classify it as a phonic type. Other phonic types could not be assigned to species. They had narrowband signals of long duration, typical of species which forage in open spaces such as molossids (Schnitzler & Kalko, 1998). Molossid call frequency scales negatively with body mass (Bogdanowicz, Fenton & Daleszczyk, 1999; Jones, 1999). As a result, we expect that the phonic type we recorded with the lowest FME (15.7 kHz) belongs to a large molossid species. *Eumops underwoodi* is the largest molossid (FA=74.7mm, body mass=65.5 g)

occurring in the Yucatan Peninsula (MacSwiney, Sosa-Escalante & Sélem-Salas, 2003). Echolocation calls of *E. underwoodi* recorded in Belize by Miller (2003) displayed a similar call frequency (16.5 kHz) to our phonic type but had shorter call duration. Field observations suggest that Phonic type 1 might correspond to *E. underwoodi,* as large bats were seen flying while recording these calls and recent comparison of these calls with recordings of *E. underwoodi* hand released and free-flying have similar characteristics (A. Guillén, pers. comm.). Phonic type 2 and phonic type 3 belong to medium-size molossid species, possibly *Eumops glaucinus* and *Eumops auripendulus,* both previously captured in the Yucatan Peninsula (Jones, Smith & Genoways, 1973; Bowles, Heideman & Erickson, 1990). Phonic type 4, which had the highest frequency, is probably the dwarf bonneted bat, *Eumops bonariensis*. This species occurs in the Yucatan (Bowles, Heideman & Erickson, 1990) and has been recorded with a FME at 28.6 kHz (M. Santos, pers. comm.), similar to that frequency observed for phonic type 4 (29.3 kHz).

Vespertilionidae

Species within the Vespertilionidae all displayed single harmonic calls of short duration and characterized by downward sweeping broadband pulses followed by a flat or narrow band portion of short duration. *Eptesicus furinalis* calls had a mean duration of 7.1 ms with FME at 37.6 kHz (Table S1). *Lasiurus ega* calls consisted of downward-sweeps with a narrow band portion and a FME of 32.2 kHz. The call duration showed the greatest variation within the family and ranged from 5 to 14 ms. *Lasiurus intermedius* calls had a FME of 28.3 kHz and the duration averaged 9.8 ms. In some sequences, a few calls of *L. intermedius* showed a FME which overlapped those calls of *L. ega*, but in general pulses averaged lower frequency and were more

curvilinear (Fig. 1d). The calls of *Rhogeessa aeneus* had a mean FME at 48.3 kHz and the call duration was 4.2 ms (Table S1; Fig. 1d). The calls of *Myotis keaysi* had the highest FME of all vespertilionids at 60.1 kHz and displayed the shortest duration calls (3.6 ms) of all species recorded in the present study (Table S1).

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Appendix S2. Descriptive statistics of the call characteristics of the insectivorous species in the north of Yucatan Peninsula. Means± standard-deviation, coefficient of variation (in parenthesis), and ranges of call parameters. fa= forearm (mm), bm=body mass (g) FME=Frequency of maximum energy; LF=Low frequency; HF=High frequency; DUR=duration; IPI=Inter-pulse interval.

Taxo		FME (kHz)	LF(kHz)	HF(kHz)	DUR(ms)	IPI (ms)	*n*	Other harmonics (kHz)
EMBALLONURIDAE								
Saccopteryx bilineata high	fa: 44.2	46.8 ± 0.8 (1.7)	46.0 ± 0.8 (1.8)	47.2 ± 0.8 (1.7)	8.8 ± 1.0 (12.1)	180.3 ± 39.07 (21.6)	25	1st: 23.4; 3rd: 69.7;
	bm: 4.1	45.0-48.7	44.3-47.4	45.4-49.1	5.8-10.9	121.4-276.1		4th: 91.6
Saccopteryx bilineata low		44.5 ± 0.7 (1.7)	43.7 ± 0.7 (1.7)	45.0 ± 0.8 (1.7)	9.2 ± 1.1 (12.0)	181.1 ± 33.56 (18.5)	25	1st: 22.3; 3rd: 66.3;
		42.7-45.9	42.1-44.9	43.3-47.2	5.8-11.4	126.0-272.6		4th: 87.7
Peropteryx macrotis	fa: 44.2	39.6 ± 1.8 (4.6)	39.1 ± 1.8 (4.7)	40.0 ± 1.8 (4.5)	8.8 ± 1.2 (14.0)	152.0 ± 59.5 (39.1)	79	1st: 19.7; 3rd: 60.7
	bm: 6.1	35.9-43.6	35.3-43.5	36.2-44.0	8.1-13.6	126.1-5-457.0		
MORMOOPIDAE								
Mormoops megalophylla	fa: 54.7	51.6 ± 1.1 (2.2)	48.9 ± 1.9 (4.0)	52.7 ± 1.4 (2.7)	6.8 ± 1.8 (26.4)	113.7 ± 71.9 (63.2)	16	1st: 25.8; 3rd: 76.2
	bm: 15.0	49.8-53.5	44.4-51.6	50.4-55.4	4.8-11.6	58.2-319.0		
Pteronotus personatus	fa: 40.6	80.1 ± 1.5 (1.9)	74.1 ± 4.2 (5.7)	80.9 ± 1.5 (1.9)	7.1 ± 0.5 (7.2)	53.9 ± 10.0 (18.5)	8	
	bm: 5.6	78.9-83.5	68.4-78.5	79.1-84.2	6.7-8.3	30.4-64.0		
Pteronotus parnellii	fa: 55.9	64.5 ± 1.0 (1.6)	64.2 ± 1.1 (1.7)	64.6 ± 1.0 (1.6)	25.8 ± 3.1 (12.0)	48.0 ± 21.1 (44.0)	25	1st:32.3; 3rd: 97.9
	bm: 16.9	62.3-66.4	62.1-66.3	62.5-66.7	17.1-30.1	23.1-109.7		
Pteronotus davyi	fa: 43.2	69.1 ± 1.2 (1.8)	68.1 ± 1.8 (2.7)	69.6 ± 1.2 (1.7)	6.8 ± 0.9 (13.8)	66.8 ± 20.1 (30.0)	41	
	bm: 7.2	67.2-73.4	61.6-72.7	68.0-73.9	5.4-9.3	36.3-124.5		
MOLOSSIDAE								
Molossus rufus high	fa: 51.4	33.0 ± 1.6 (4.6)	32.0 ± 1.7 (5.4)	33.6 ± 1.6 (4.9)	13.4 ± 3.6 (26.8)	344.6 ± 131.9 (38.2)	8	
	bm: 34.2	29.9-35.0	28.7-34.3	30.7-35.5	8.22-20.2	127.6-494.2		
Molossus rufus low		29.4 ± 1.9 (6.6)	28.1 ± 2.5 (9.1)	29.9 ± 1.8 (6.2)	13.2 ± 3.3 (24.9)	263.4 ± 82.5 (31.3)	8	
		26.0-32.1	23.8-31.4	27.1-32.6	9.5-19.4	99.9-363.9		
Molossus sinaloae high	fa: 51.5	39.0 ± 1.3 (3.3)	38.0 ± 1.2 (3.1)	39.5 ± 1.1 (2.8)	10.6 ± 2.2 (20.9)	250.1 ± 31.1 (12.4)	6	
	bm: 29.3	36.8-40.7	35.7-39.5	37.8-41.0	6.8-12.8	208.6-287.3		
Molossus sinaloae low		34.8 ± 1.8 (5.3)	33.6 ± 1.7 (5.1)	35.2 ± 1.6 (4.7)	10.6 ± 2.4 (22.7)	230.7 ± 16.8 (7.3)	6	
		31.9-36.7	30.6-35.2	32.7-37.1	6.9-12.6	209.6-245.3		
Nyctinomops laticaudatus	fa: 41-45	26.4 ± 2.5 (9.5)	25.1 ± 2.0 (8.2)	29.7 ± 3.1 (10.4)	12.5 ± 2.1 (17.3)	189.5 ± 19.5 (50.3)	24	2nd: 51.3
	bm: 9-16	23.3-31.1	22.1-28.6	26.5-35.3	10.6-17.4	42.5-390.5		
Molossid sp. 1		15.7 ± 0.8 (5.0)	15.1 ± 0.7 (4.8)	17.0 ± 1.1 (6.8)	19.5 ± 3.1 (16.0)	609.5 ± 131.7 (21.6)	11	2nd: 32.1
		14.8-17.1	14.1-16.6	15.7-19.1	13.9-24.4	395.9-823.5		
Molossid sp. 2		21.6 ± 1.1 (5.4)	20.8 ± 1.1 (5.4)	23.0 ± 1.5 (6.6)	16.1 ± 3.2 (20.1)	479.8 ± 3.2 (52.4)	15	
		18.7-22.9	18.1-22.2	20.0-25.6	10.3-21.7	250.3-1277.9		
Molossid sp. 3		25.3± 0.9 (3.8)	24.3 ± 1.0 (4.2)	27.4 ± 1.3 (4.8)	13.9 ± 2.4 (17.5)	361.0 ± 162.4 (44.9)	34	
		23.4-27.1	22.2-26.1	24.4-30.1	9.58-19.5	198.1-920.5		

Molossid sp. 4		29.3 ± 1.1 (3.8) 27.8-31.6	28.4 ± 1.1 (4.2) 26.4-30.3	31.3 ± 1.1 (3.5) 29.6-33.1	11.9 ± 1.7 (14.1) 9.2-14.7	310.7 ± 110.8 (35.6) 198.9-521.6	15	
Molossid sp. 5 high		30.0 ± 0.8 (2.8) 28.8-30.5	29.8 ± 0.8 (2.7) 28.6-30.3	30.0 ± 1.9 (6.3) 27.3-31.4	38.3 ± 18.4 (48.0) 25.4-65.2	235.0 ± 70.1 (29.7) 183.5-338.5	4	2^{nd}: 56.8
Molossid sp. 5 low long		23.8 ± 1.6 (6.9) 22.2-26.0	23.1 ± 1.4 (6.3) 21.5-24.8	24.7 ± 1.5 (6.4) 23.1-26.9	65.6 ± 21(31.9) 49.1-99.2	182.7 ± 39.6 (21.6) 131.5-238.9	5	2^{nd}: 47.4
Molossid sp. 5 low short		26.5 ± 1.0 (4.0) 25.0-27.4	25.1 ± 1.1 (4.6) 23.4-25.9	27.2 ± 0.8 (3.3) 26.0-28.1	17.7 ± 6.2 (35.4) 11.0-25.6	227.8 ± 160.8 (70.5) 135.7-468.5	4	
VESPERTILIONIDAE								
Eptesicus furinalis	fa: 40.4 bm: 7.0	37.6 ± 1.3 (3.4) 35.4-40.5	36.4 ± 1.4 (4.0) 33.0-39.5	40.4 ± 2.1 (5.2) 37.0-46.9	7.1 ± 1.9 (26.8) 3.8-11.1	175.8 ± 78.3 (44.5) 75.1-351.8	22	
Lasiurus ega	fa: 46.6 bm: 14	32.2 ± 1.2 (3.8) 29.7-34.6	31.1 ± 1.4 (4.7) 27.8-33.4	34.9 ± 1.6 (4.6) 32.5-39.5	8.7 ± 2.5 (28.9) 4.7-14.3	149.4 ± 64.7 (43.3) 55.0-312.7	34	
Lasiurus intermedius	fa: 54.4 bm: 20.2	28.3 ± 1.3 (4.8) 25.7-30.5	27.3 ± 1.2 (4.6) 25.0-29.7	30.7 ± 1.6 (5.5) 27.0-33.2	9.8 ± 2.5 (25.8) 6.5-16.5	231.6 ± 112.1 (48.3) 77.5-564.6	20	
Rhogeessa aeneus	fa: 26.8 bm: 3.6	48.3 ± 2.3 (4.7) 44.3-53.1	46.7 ± 2.4 (5.2) 42.7-51.9	52.3 ± 4.3 (8.2) 45.6-60.7	4.2 ± 1.0 (24.6) 2.7-6.5	80.9 ± 26.7 (33.0) 39.3-137.0	13	
Myotis keaysi	fa: 33.8	60.1 ± 1.3 (2.1)	59.2± 1.3 (2.2)	62.3 ± 2.1 (3.4)	3.6 ± 0.7 (21.2)	63.6 ± 17.5 (27.5)	48	

Appendix S4. Discriminant function analysis classification data for the 14 species and five phonic types recorded in the north of Yucatan Peninsula (92.1% of the original grouped cases and 84.1% of cross-validated cases were corrected classified). The model relied on five parameters: frequency of maximum energy, low frequency, high frequency, duration and inter-pulse interval.

	True group																		
Classified as	*S. bilineata*	*P. macrotis*	*M. megalophylla*	*P. personatus*	*P. parnellii*	*P. davyi*	*M. rufus*	*M. sinaloae*	*N. laticaudatus*	Molossid 1	Molossid 2	Molossid 3	Molossid 4	Molossid 5	*E. furinalis*	*L. ega*	*L. intermedius*	*R. aeneus*	*M. keaysi*
S. bilineata	47	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. macrotis	0	75	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
M. megalophylla	1	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
P. personatus	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. parnellii	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. davyi	0	0	0	1	0	41	0	0	0	0	0	0	0	0	0	0	0	0	0
M. rufus	0	1	0	0	0	0	11	2	0	0	0	0	0	4	1	0	0	0	0
M. sinaloae	0	1	0	0	0	0	1	8	0	0	0	0	0	0	0	0	0	0	0
N. laticaudatus	0	0	0	0	0	0	1	0	13	0	1	2	1	0	0	2	4	0	0
Molossid 1	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
Molossid 2	0	0	0	0	0	0	0	0	3	1	12	3	0	1	0	0	0	0	0
Molossid 3	0	0	0	0	0	0	0	0	3	0	1	25	0	0	0	0	2	0	0
Molossid 4	0	0	0	0	0	0	0	0	3	0	0	1	8	0	0	3	5	0	0
Molossid 5	1	0	0	0	0	0	3	0	0	0	1	2	0	8	0	0	0	0	0
E. furinalis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	19	0	0	0	0
L. ega	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	28	2	0	0
L. intermedius	0	0	0	0	0	0	0	0	2	0	0	1	4	0	0	1	7	0	0
R. aeneus	1	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	11	0
M. keaysi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	48
% correct	**94.0**	**94.9**	**87.5**	**87.5**	**100**	**100**	**68.8**	**66.7**	**54.2**	**90.9**	**80.0**	**73.5**	**53.3**	**61.5**	**86.4**	**82.4**	**35.0**	**84.6**	**100**