

<https://doi.org/10.15517/jm7kr371>

Acoustic characterization of the threatened bat *Neoptesicus innoxius* (Chiroptera: Vespertilionidae) and two sympatric species in Western Ecuador

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Received 04-II-2025. Corrected 31-III-2025. Accepted 02-IX-2025.

ABSTRACT

Introduction: Bioacoustics allows the study of the ecology and behavior of bats through the analysis of echolocation signals. In insectivorous bats, foraging strategies, prey preference, and habitat use are closely related to the emission patterns of echolocation signals. Identifying significant bat habitats and improving conservation efforts can benefit from understanding these relationships.

Objectives: To describe the echolocation signals of an endangered species *Neoptesicus innoxius*, and other commonly detected species in the study area, *Myotis nigricans* and *Molossus molossus*, to contribute to the construction of a bat echolocation call reference library in Western Ecuador.

Methods: Mist nets were used to capture bats, and reference recordings were subsequently obtained using the Anabat Swift ultrasonic detector. Echolocation pulse selection for each species was carried out using Kaleidoscope Pro 5.6.8 and BatSound 4.2.1, measuring the following spectral and temporal parameters of the echolocation signals in the search phase: initial, final, and maximum energy frequency, pulse duration, and interpulse interval.

Results: *N. innoxius* presented the echolocation signals with the greatest variability of the spectral and temporal parameters, emitting pulses with high modulation speed (with one prominent FM component), and of low modulation speed (with one prominent QCF component); *M. nigricans* emissions were characterized by broadband signals of very short duration; and *M. molossus* presented alternation in its echolocation signals, where both high and low pulses had very little variability.

Conclusions: The acoustic description of an endangered species like *N. innoxius* alongside the descriptions of other species frequently detected at the same study site, contributes to the construction of a bat echolocation call reference library. This input is the basis of future research of the ecology and behavior of the insectivorous bats that inhabit the Western Ecuador, which in turn is very valuable for designing tools and strategies for bat conservation.

Keywords: insectivorous bats; echolocation; Isla Santay; Ecuador; Molossidae; Vespertilionidae.



RESUMEN

Caracterización acústica del murciélago amenazado *Neoptesicus innoxius* (Chiroptera: Vespertilionidae) y dos especies simpátricas en el occidente de Ecuador

Introducción: La bioacústica permite estudiar la ecología y el comportamiento de los murciélagos mediante el análisis de las señales de ecolocalización. En los murciélagos insectívoros, las estrategias de alimentación, la preferencia de presas y el uso del hábitat están estrechamente relacionados con los patrones de emisión de las señales de ecolocalización. La comprensión de estas relaciones puede ayudar a identificar los hábitats importantes de los murciélagos y mejorar los esfuerzos de conservación.

Objetivos: Describir las señales de ecolocalización de la especie en categoría Vulnerable *Neoptesicus innoxius*, en conjunto con las de otras dos especies detectadas frecuentemente en el mismo sitio de estudio, *Myotis nigriscans* y *Molossus molossus*, para contribuir a la construcción de una biblioteca de referencias ultrasónicas de murciélagos insectívoros en el Occidente del Ecuador.

Métodos: Para la captura de murciélagos se utilizaron redes de niebla y posteriormente se obtuvieron grabaciones de referencia con el detector ultrasónico Anabat Swift. Mediante los programas Kaleidoscope Pro 5.6.8 y BatSound 4.2.1 se seleccionaron los mejores pulsos grabados de cada especie y se midieron los siguientes parámetros espectrales y temporales de las señales de ecolocalización en fase de búsqueda: frecuencia inicial, frecuencia final, frecuencia de máxima energía, duración del pulso e intervalo entre pulsos.

Resultados: *N. innoxius* presentó señales con mayor variabilidad en sus parámetros espectrales y temporales, emitiendo señales de alta velocidad de modulación con un componente FM predominante, y de baja velocidad de modulación con un componente QCF predominante; las emisiones de *M. nigriscans* se caracterizan por ser de banda ancha y de muy corta duración; y *M. molossus* presentó alternancia en sus señales de ecolocalización, donde tanto las señales altas como las bajas presentan muy poca variabilidad.

Conclusiones: La descripción acústica de una especie en categoría Vulnerable como *N. innoxius* al igual que las de otras especies que se detectan y capturan frecuentemente en el mismo sitio de estudio, contribuyen a la construcción de una biblioteca de referencia de los sonidos que emiten estos animales para hacer uso del hábitat. Este aporte es la base de futuras investigaciones para entender mejor la ecología y el comportamiento de los murciélagos insectívoros que habitan en el Occidente Ecuatoriano, lo que a su vez es crucial para diseñar instrumentos de conservación adecuados.

Palabras clave: murciélagos insectívoros; ecolocalización; Isla Santay; Ecuador; Molossidae; Vespertilionidae.

INTRODUCTION

Traditionally, the study of the ecology of bats (order Chiroptera) has been based on their capture with mist nets or direct roost observation. However, not all species are susceptible to being captured or can be easily observed, such as the case of insectivorous bats, since they are extremely agile and small, and their high-frequency echolocation emissions make it difficult to observe their behavior and monitor their movements (Jones & Rayner, 1989). Insectivorous bats use echolocation as the primary sensory modality, which aids in orientation, object classification, and search for potential resources (Schnitzler & Kalko, 2001). This trait enables their study with bioacoustics using ultrasonic detectors, which usually record species of bats that fly close to the ground and at

high altitudes, therefore the complementary use of mist nets and ultrasonic detectors increases the probability of species detection, helping to better estimate bat species richness in each area (Orozco-Lugo et al., 2013).

In Ecuador, bat bioacoustics studies have been around for more than a decade, and surprisingly the number of publications remain scarce. Nonetheless, one of the most relevant efforts for building Ecuador's first reference library of bat echolocation calls took place in the Amazon, at the Yasuní National Park, grouping the acoustic description of 28 species of bats recorded by active (Pettersson 240 x ultrasound detector) and passive detection (BioAcoustic Technology AR125 ultrasound detector) (Rivera-Parra & Burneo, 2013). The first technique is performed on a mobile transect, better suited for detecting bats using understory flight

paths or forest edges; and the latter is based on the deployment of detectors for extended periods over fixed points at variable heights, better suited for detecting bats flying over the canopy. This highlights the importance of combining different methodological approaches for working in the Amazon rainforest with its high diversity of habitats and species.

Less than a decade ago a second effort for describing bat echolocation signals was carried out in Western Ecuador, specifically in Cerro Blanco Protected Forest, a remnant of dry forest close to the city of Guayaquil, reporting a total of 17 phenotypes belonging to several families: Emballonuridae, Molossidae, Mormoopidae, Noctilionidae, Phyllostomidae and Vespertilionidae, with the latter being the most abundant and diverse family (Tinajero, 2017). This proves bioacoustics methods valuable for enhancing our understanding on bat ecology and behavior over a wide range of habitats, ecosystems and perturbation gradients (Stahlschmidt & Brühl, 2012).

Insectivorous bats occupy different habitats such as dry forests and mangroves, along the coastal profile and in marine-coastal areas in Ecuador (Paz-Ramírez et al., 2018; Paz-Ramírez & Salas, 2019; Salas, 2019). Both ecosystems are known for their species diversity, regional endemism, and presence of threatened species (Burneo et al., 2015), making it necessary to implement study techniques and tools that would allow identifying suitable habitats to improve conservation efforts based on a more comprehensive understanding of diversity, ecology, and behavior of species (Torres-Domínguez et al., 2022). The use of bioacoustics would help to partially solve this problem, especially in ecosystems such as mangroves, where the height of the canopy and the difficulty of access to firm ground do not allow the use of classic techniques such as mist netting (Estrada-Villegas et al., 2018).

The use of complementary methods to strengthen the ability to study and understand bats is currently recognized (Mancini et al., 2024). A holistic view of the bat community can be achieved by combining different

recording and capture techniques, especially in Neotropics where the insectivorous species that are very difficult to capture with mist nets converge with other species that are better represented with this capture method, such as the frugivorous and nectivorous bats of the Phyllostomidae family (Pech-Canche et al., 2011). This, in turn, provides less biased information on their ecology, life history, and other aspects relevant to conservation (Carvalho et al., 2023; Fenton et al., 1992). Additionally, the advantage of using acoustic methods lies in the fact that they are non-invasive and allow obtaining more precise information on the relative activity and richness of species (Barlow, 1999).

Nevertheless, the use of acoustic methods applied to bat ecology and conservation poses significant challenges for researchers given the need for standardization of different protocols and procedures for obtaining valuable information, from capturing and handling the specimens, to managing, systematizing and analyzing the data on acoustic parameters from the echolocation signal recordings. Nonetheless, the advancement of technologies for the processing and analysis of acoustic data and the automated identification of species (Cao et al., 2023; Mora et al., 2002; Yoh et al., 2022), together with the apparition of low-cost ultrasonic detection devices, have allowed more affordable and better quality recordings of bat echolocation signals, which in turn led to an increase in the number of reference libraries and descriptions of emission patterns of different bat species in America, Europe, and Asia (Arévalo-Cortés et al., 2024; Görföl et al., 2022; Rydell et al., 2017; Zamora-Gutierrez et al., 2020).

Changes in echolocation signal emission patterns and habitat use estimated from ultrasonic detection can be used to design and implement conservation actions, since changes in persistence, activity patterns, distribution, or habitat use, and selection of insectivorous bats can be estimated (López-Bosch et al., 2021). However, to achieve it, it is essential to generate sound reference libraries for each species, since there is an intrinsic variation in acoustic parameters due to sexual, developmental,



geographical or individual differences, among others (Barclay & Brighman, 2004; Frick, 2013; Jacobs et al., 2007; Jones, 1997; Jones & Hold-eried, 2007).

In this work, the echolocation signals of three species of insectivorous bats in Western Ecuador are described: *Neoptesicus innoxius*, an endemic species shared between Western Ecuador and Northwestern Peru (Cláudio et al., 2023; Salas et al., 2023), and two species with a wide geographical distribution, and with whom *N. innoxius* frequently occurs on Santay Island: *Myotis nigricans* and *Molossus molossus*. With these descriptions, we seek to contribute to a reference library of bat echolocation calls on the Ecuadorian coast, that serves as a baseline for future work that could prescind of direct bat capture, while enabling researchers to identify threatened and sensitive species in free flight.

MATERIALS AND METHODS

Study area: The field work was carried out under the research permit MAAE-DBI-CM-2022-0234 from the Ministry of the Environment of Ecuador, in the Isla Santay and Isla Gallo National Recreation Area, commonly called “Isla Santay”, created in 2010 and located between two highly populated urban centers: Guayaquil and Durán, province of Guayas, Western Ecuador. This protected area has an area of approximately 2 215 hectares, with an elevation that ranges from zero to ten m.a.s.l., presenting a variety of habitats: mangroves, dry forest, and grasslands. The mangrove habitat is composed primarily by species like red mangrove (*Rhizophora mangle*); red crawling mangrove (*Rhizophora racemosa*); red knight mangrove (*Rhizophora harrisonii*); jelí mangrove (*Conocarpus erectus*); black mangrove (*Avicennia germinans*) and white mangrove (*Laguncularia racemosa*) (Cruz-Cordovez, 2019).

Bat Capture: The capture of bats was carried out during 30 nights between September 2019 and March 2022 (14 nights for September-December 2019; five nights for

January-February 2020; four nights for October-December 2020; four nights for January-February 2021; three nights for January-March 2022), sampling simultaneously each night at four sites (Guayaquil-Durán route, Huaquillas Trail, Guayaquil-Comuna Santay route, and Ecoaldea) located along the mangrove edges adjacent to open areas mostly comprised by grasslands and wetlands, considering the presence of known hollowed trees that serve as roosts. These sites were selected based on previous capture success at the general study area, as they are almost entirely edge habitats laying between highly cluttered spaces and open spaces, hence allowing for a better chance to capture species that fly fast in open spaces, and species with more maneuverable flights exploiting the mangrove interior. At each sampling site, four nylon mist nets (six m long x 2.6 m wide and with a 38 mm mesh opening) were deployed per night, remaining active from approximately 18:00 to 00:00, located at ground and sub-canopy levels and separated from each other at least by 200 m, in places near water sources over the trails with open spaces along the mangrove edges. Environmental variables such as air temperature, wind speed, moon phase and relative humidity were collected once per night (data not provided). Once the individuals were captured, the basic morphometric measurements of each specimen were obtained for *in situ* identification (Tirira, 2017), along with sex and reproductive status.

Recording echolocation signals: An Anabat Swift ultrasonic detector (Titley Scientific, E.U.) was used to record the echolocation signals from bats on hand release and free flight, which were then analyzed with the software BatSound 4.2.1 (Pettersson Elektronik AB, 2013). The detector was configured to record in continuous mode and Full Spectrum, saving the files in WAV format, at a sampling rate of 250 kHz, with the omnidirectional ultrasonic microphone oriented at a position of approximately > 90° with respect to the ground and aiming towards the bats flight path, with 5 ms minimum trigger time, 16 dB

sensitivity level, and with a recording trigger configured at 10 kHz minimum frequency. Once the recordings were obtained, individuals were released close to their capture site (Martínez-Medina et al., 2021).

Data analysis; description of echolocation signals: Recordings were filtered with SeaWave 2.0 (Centro Interdisciplinare di Bioacustica e Ricerche Ambientali, 2013) to separate recorded files containing noise from files containing pulses or echolocation signals. The files containing the bat echolocation signals to be described were then selected based on its signal-to-noise ratio (S/N), only keeping files whose signals had an overall amplitude difference of at least 25 dB regarding the background noise of each file. BatSound 4.2.1 was used to reconstruct the spectrograms based on a Fast Fourier Transformation (FFT) of 1 024 samples, allowing a better resolution in the frequency domain, given the specific need of separating two species with overlapping bandwidths, reconstructing also the power spectra which corresponds to the

energy profile of the component frequencies of a given sound, yielding information on what frequencies are concentrating the most energy (Martínez-Medina et al., 2021).

Some search phase signals were selected from the echolocation sequences obtained for the three species, to measure the following parameters: Initial Frequency (FINI), Final Frequency (FFIN) and Maximum Energy Frequency or Peak Frequency (FME), and Bandwidth (AB, subtracting the final frequency from the initial) measured in kilohertz (kHz) and Pulse Duration (DUR) and Interpulse Interval (IPI), measured in milliseconds (ms) (Martínez-Medina et al., 2021). The average value and its standard deviation were calculated for all these parameters, except for the IPI, for which the median was calculated. The calculation of the median in this case responds to the need to avoid biases in the central distribution of the duration of the IPI, since this can be overestimated when ignoring the fact that sometimes aerial insectivorous bats can omit the emission of a pulse while traveling between sites, making the intervals between emissions twice as long in such cases (Adams, 2017; Stidsholt et al., 2021) (Fig. 1).

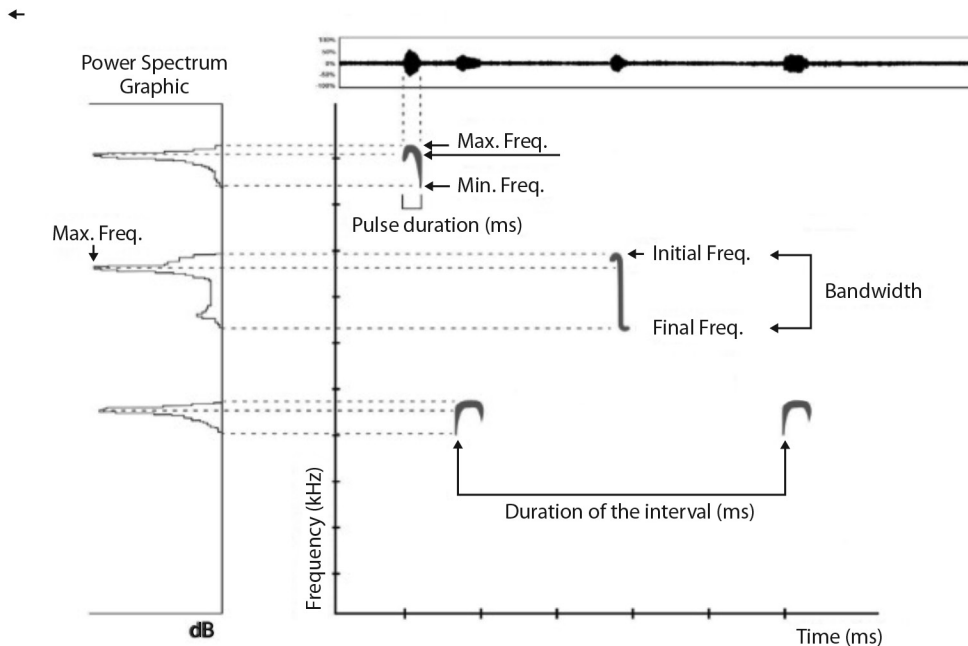


Fig. 1. Acoustic parameters for the description of echolocation signals, adapted from “Standards for recording echolocation signals and building bat reference libraries in Colombia” (Martínez-Medina et al., 2021).



Additionally, *N. innoxius* and *M. nigricans* modulation rates were calculated for their two main components: Modulated Frequency (FM) and Quasi-Constant Frequency (QCF) by dividing the bandwidth of each component by its duration; where each component was separated at the inflection point of the signal so that the measurement of the FM structure was obtained from the beginning of the signal to its inflection point and the measurement of the QCF component was taken from the inflection point to the end of the signal. The inflection point frequency is also compared for both species. The modulation rate refers to the speed at which the component frequencies of an echolocation signal are changing, with high change speeds associated with the emission of FM pulses, and low change speeds associated with the emission of QCF pulses. Furthermore, when bats combine both types of emissions (FM-QCF) there is a point of transition between both component structures, called the inflection point, where the modulation rate changes abruptly (Martínez-Medina et al., 2021).

Subsequently, a modulation speed proportion was estimated between both components to support qualitative descriptions of the pulse shapes, where high modulation speed pulses will have a preponderance of deep and very fast modulation FM structures, being the most prominent components of the signal. While pulses with relatively lower modulation rates will tend towards curvilinear shapes, and the QCF components will be predominant within each signal. Although the use of frequency rate change measurements in echolocation signals is rare, understanding how the modulation of these and their components change is especially useful to classify their structure (Redgwell et al., 2009), discriminate species in different flight contexts (Limpens, 2004) and understand the sensory capabilities and limitations of the two species that, in this case, have been referenced under the same conditions, that is, subjected to the same structural complexity of the flight space (edge of mangrove) (Jones & Teeling, 2006).

Only echolocation signals with a high signal-to-noise ratio that allowed the signals of interest to be separated from background noise were included in the analyses; to do this, the power spectrum of each recording fragment with echolocation signals was examined, confirming that there was a difference of at least 25 dB between the power level of the background noise and the power level of the signals to be measured. As an exclusion criterion, recording segments that did not exceed 25 dB difference between power profiles of the noise and the signal of interest were omitted since the measurement of characteristics such as intensity or maximum frequencies may be affected (Martínez-Medina et al., 2021).

Statistical analysis: In this study, the presence of *N. innoxius* and *M. nigricans* is reported in the same acoustic monitoring and mist net capture sites, so we consider it convenient to make a comparison between the signals of these species to set the precedent on the spectral and temporal variables that characterize and differentiate them, given that in some cases variables such as the final frequency, the inter-pulse interval and the frequency of maximum energy of *N. innoxius* usually have values that are distributed in the same range as those of the same variables in *M. nigricans*. The statistical computing language R (R Core Team, 2024) was used to construct two-sample *t*-tests and compare the different spectral and temporal variables measured for *N. innoxius* and *M. nigricans*, testing the null hypothesis that there was no true difference between the means of IPI, DUR, FINI, FFIN, AB, FME, frequency at the inflection point of the signal, and modulation rates of the FM and QCF components of both species with a 95 % confidence interval. In the same way, the hypothesis that there was no true difference between the means of DUR and the IPI of the high and low pulses of *M. molossus*, as well as with the means of the AB of both types of pulses, was tested through a test Wilcoxon rank sum given the non-normal distribution of the data.

Additionally, given that *M. molossus* can emit alternatively between low or high pulses, we explored more about the variability that each of these scenarios of alternation in the emission of pulses can imprint on some variables such as the duration of these and their intervals, testing whether the distribution of the variances of the duration of the high and low pulses and their interpulse intervals come from the same distribution with an alpha of 0.05. Finally, a *post hoc* Tukey HSD multiple means comparison test was run at a 95 % confidence level to determine which data groups differed from each other.

RESULTS

Description of *Neoptesicus innoxius* echolocation signals: For this species, 163 signals from 11 search phase echolocation sequences were measured. This species emitted signals with prominent FM components

accompanied by a small QCF component at the end of each pulse with nearly linear modulation rates between 0.8-2.5 kHz/ms, and relatively short durations varying from 5.9-7.7 ms. Its FINI ranged between 77-61.6 kHz, and its FFIN ranged between 51.8-46.5 kHz, resulting in signals with bandwidths from 11.79-28.47 kHz.

The average MEF of *N. innoxius* echolocation signals was between 53.3-50.6 kHz, which corresponds to the frequency range between 10.1-3.6 kHz above the inflection point frequency, when the change in signal emission from a FM structure to a QCF structure occurs; although it also accumulated energy around the inflection point in signals with slower modulation. The IPI of the echolocation signals of *N. innoxius* had a median duration of 93.5 ms, being the longest of the two species of the Vespertilionidae family described in the present work (Table 1).

The average modulation speed of the FM structure of *N. innoxius* echolocation signals

Table 1

Spectral and temporal parameters (mean $\pm$ standard deviation) extracted from the search phase echolocation sequences of the three species of insectivorous bats studied on Isla Santay, Ecuador.

Family	Species	FINI (kHz)	FFIN (kHz)	FME (kHz)	ABAND (kHz)	DUR (ms)	IPI (ms)	SPEED (kHz/ms)
Vespertilionidae	<i>M. nigricans</i> N = 6; n = 109	96.10 $\pm$ 8.68	48.31 $\pm$ 2.14	a) 53.55 $\pm$ 2.67 b) 67.63 $\pm$ 5.95	47.78 $\pm$ 9.24	4.89 $\pm$ 0.70	84.3 $\pm$ 11.79	FM 14.08 $\pm$ 2.95 QCF 6.25 $\pm$ 2.37
	<i>N. innoxius</i> N = 11; n = 163	69.33 $\pm$ 7.73	49.20 $\pm$ 2.64	51.99 $\pm$ 1.30	20.13 $\pm$ 8.34	6.82 $\pm$ 0.88	93.5 $\pm$ 12.78	FM 6.02 $\pm$ 2.97 QCF 1.71 $\pm$ 0.84
Molossidae	<i>M. molossus</i> N = 7; n = 76	H: 43.70 $\pm$ 3.24	H: 36.81 $\pm$ 3.10	H: 40.69 $\pm$ 3.38	H: 6.89 $\pm$ 1.90	H: 9.82 $\pm$ 2.32	H-H: 112 $\pm$ 30.98	
		L: 39.02 $\pm$ 3.00	L: 35.56 $\pm$ 2.20	L: 37.38 $\pm$ 2.87	L: 6.46 $\pm$ 1.90	L: 0.38 $\pm$ 1.97	H-L: 136.6 $\pm$ 34.38	
							L-H: 84.65 $\pm$ 31.46	
							L-L: 81.6 $\pm$ 36.52	

FINI: initial frequency; FFIN: final frequency; FME: maximum energy frequency; DUR: pulse duration; ABAND: bandwidth; IPI: interpulse interval; N: number of recordings/sequences analyzed; n: number of signals analyzed (hand released and free flight). For *M. nigricans*, two maximum amplitude frequency values are reported, where a) corresponds to the maximum amplitude frequency of the QCF component, and b) corresponds to the maximum amplitude frequency of the FM component. The values of the alternating high (H) and low (L) signals of *M. molossus* are reported, as well as the specific interpulse intervals for when the interval is produced between a pair of high pulses (H-H), a high pulse and a low pulse (H-L), a low pulse and a high pulse (L-H), and two low pulses (L-L). Only the IPI values correspond to the median and standard deviation. SPEED: modulation speed of an FM or QCF element within the same signal.

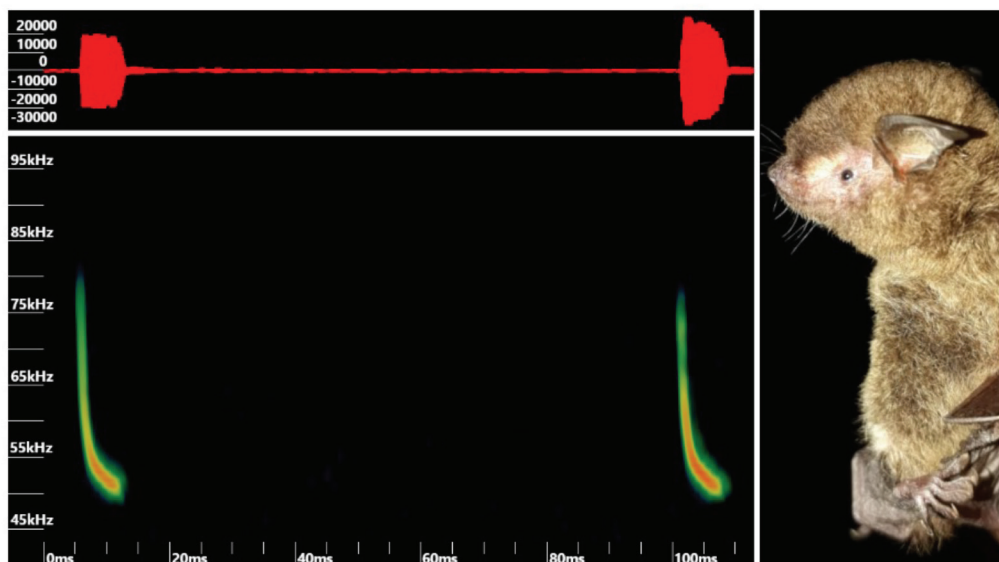


Fig. 2. Echolocation signals of *N. innoxius* from Isla Santay, Ecuador, which were reconstructed using Kaleidoscope Pro 5.6.8 (Wildlife Acoustics Inc., 2024) in a spectrogram of 1 024 points of FFT window size, with a Hanning window type and 85 % sample overlap. X-axis in milliseconds (ms); Y-axis in kilohertz (kHz). The oscillogram is shown in red in the upper box and the spectrogram in the lower box, with intensity (dB) on a color scale from green to orange (less intense to more intense respectively). Values of intervals between pulses, durations and frequencies are shown as recorded in nature.

ranged between 9-3 kHz/ms, while the average modulation speed of its QCF component had a linear trend, giving a more curvilinear and concave appearance to their echolocation signals compared to those of *M. nigricans*, which turned out to be more vertical than curvilinear (Fig. 2).

Description of *Myotis nigricans* echolocation signals: From this species, 109 search phase echolocation signals were measured from 6 echolocation sequences that may or may not contain approach and terminal phases. The *M. nigricans* signals were formed by a nearly vertical FM component that becomes curvilinear after the second third of the signal, or the final part of the signal that follows the inflection point where the modulation speed is slowed. Its FINI ranged between 87-104 kHz, and the FFIN ranged between 46-50 kHz, resulting in a usually broadband signal with a bandwidth of 38.5-57 kHz, and shorter durations (4.2-5.5 ms) compared to *N. innoxius* (Fig. 3).

The average MEF of the slowest modulated terminal part of the FM structure was between 50.8-56.2 kHz, while the average MEF of the FM component ranged between 61.6-73.5 kHz. In this way, *M. nigricans* accumulates the greatest amount of its energy in the last three kHz of the lower limit of the FM structure and towards the inflection point between the two types of structure. Furthermore, the greatest amount of energy accumulated in the final third of the *M. nigricans* signals was located between four-16 kHz after the signal inflection point. The IPI of *M. nigricans* have a median duration of 84.3 ms, which turns out to be the shortest of the two species of the Vespertilionidae family described here (Table 1).

The average modulation speed (kHz change per unit time) of the FM component of *M. nigricans* signals ranged between 17-11.1 kHz/ms, while the average modulation rate of its QCF component varied between 8.6-3.8 kHz/ms, indicating that although there is a two to three times reduction in the modulation speed compared to the FM component, the

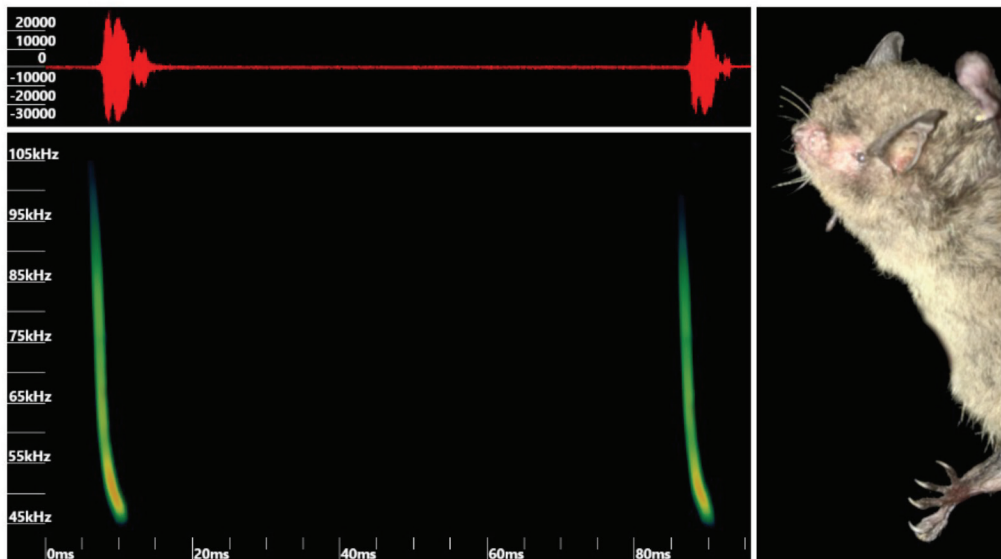


Fig. 3. Echolocation signals of *M. nigricans* from Isla Santay, Ecuador, which were reconstructed using Kaleidoscope Pro 5.6.8 in a spectrogram of 1 024 points of FFT window size, with a Hanning window type and 85 % sample overlap. X-axis in milliseconds (ms) and Y-axis in kilohertz (kHz). The oscillogram is shown in red in the upper box and the spectrogram in the lower box, with intensity (dB) on a color scale from green to orange (less intense to more intense respectively). Values of intervals between pulses, durations and frequencies are shown as recorded in nature.

modulation of the QCF component is still fast enough with modulation speeds above linear modulation (one kHz/ms), making its appearance more vertical than diagonal.

Description of *Molossus molossus* echolocation signals: For this species, 76 echolocation signals were measured from seven search phase sequences, where approach and terminal sequences were also present. This species presents two types of signals: high and low, both consisting of a prominent QCF element that was sometimes complemented by a very short FM element at the beginning or end of the signals; The FINI of the high signal varied between 46.9-40.4 kHz, and its FFIN ranged between 39.9-33.7 kHz. The FINI and FFIN of low signals were in a range between 42-36 kHz, and 34.7-30.3 kHz, respectively. Most of the energy of *M. molossus* echolocation signals is held between 34-44 kHz, depending on whether it is a high pulse or a low pulse. Both types of signals also have a similar bandwidth, ranging between four-eight kHz, with high

signals normally having relatively wider bands. It should be noted that high and low signals can overlap by two or three kHz in their bandwidth, although their bandwidths do not differ from each other (Wilcoxon rank sum test with continuity correction $W = 789$, $p\text{-value} = 0.4593$) (Fig. 4).

No significant differences were found (Welch Two Sample t-test $t = -1.1153$, $df = 74$, $p\text{-value} = 0.2683$) between the mean durations of high and low echolocation signals of *M. molossus*, showing similar ranges. (7.5 ms to 12.1 ms, or 8.4 ms to 12.3 ms respectively), although the duration of low signals tends to extend almost 1 ms longer compared to the duration of high signals. The IPI of the *M. molossus* signals differ (Wilcoxon rank sum test with continuity correction $W = 1\,028.5$, $p\text{-value} < 0.01$), and can be shorter or longer depending on the measured pair of pulses: a high and a low pulse, vice versa, two high pulses or two low pulses. The longest intervals have been observed from high to low signals (maximum 170.9 ms), while the shortest intervals

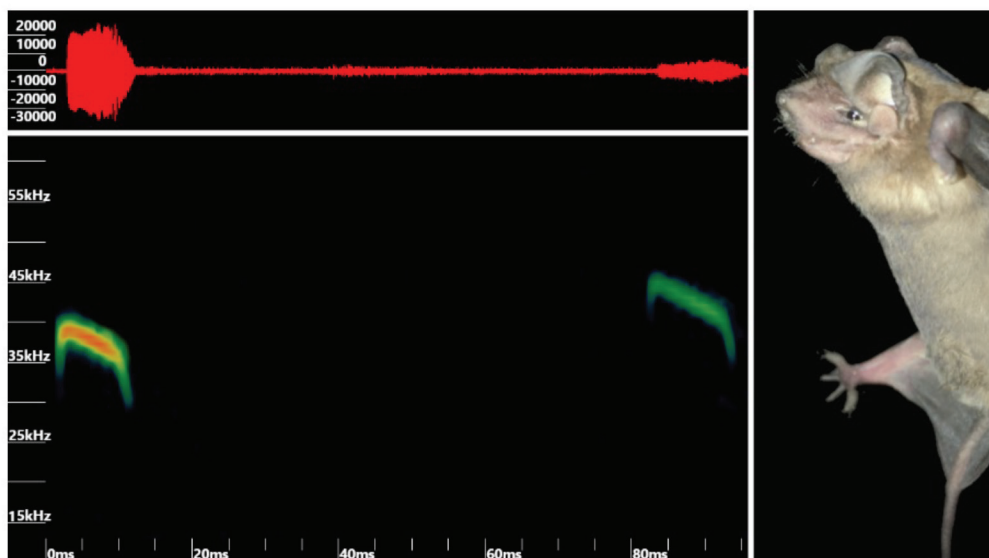


Fig. 4. Echolocation signals of *M. molossus* from Isla Santay, Ecuador, which were reconstructed using Kaleidoscope Pro 5.6.8 in a spectrogram of 1 024 points of FFT window size, with a Hanning window type and 85 % sample overlap. X-axis in milliseconds (ms) and Y-axis in kilohertz (kHz). The oscillogram is shown in red in the upper box and the spectrogram in the lower box, with intensity (dB) on a color scale from green to orange (less intense to more intense respectively). Values of intervals between pulses, durations and frequencies are shown as recorded in nature.

are observed between two low pulses (minimum 45 ms), or from a low pulse to a high pulse ($p < 0.01$).

Although the paired test based on the type of pulse (high and low) did not show significant differences in the average duration of the pulses, the *post hoc* test showed that the duration of a high pulse when followed by another high pulse is significantly shorter than the duration of the same high pulse when it is followed by a low pulse ($p < 0.005$). Similarly, the bandwidth of the low pulses when followed by other low pulses was significantly narrower than the bandwidth of the high pulses that are followed by other high pulses ($p < 0.05$).

Differences between *N. innoxius* and *M. nigricans* echolocation signals: After comparing the set of values of each variable describing the echolocation behavior of *N. innoxius* and *M. nigricans*, we found that IPI ($t = 5.6833$, $df = 243.74$, $p\text{-value} = < 0.001$), duration ($t = 19.873$, $df = 261.21$, $p\text{-value} < 0.001$) and FFIN ($t = 4.7223$, $df = 155.29$, $p\text{-value} < 0.001$) of *N.*

innoxius echolocation signals were significantly higher in comparison to those of *M. nigricans* signals. In contrast, the bandwidth ($t = -25.026$, $df = 215.01$, $p\text{-value} < 0.001$), FINI ($t = -25.905$, $df = 212.63$, $p\text{-value} < 0.001$), MEF ($t = -5.6356$, $df = 142.82$, $p\text{-value} = < 0.001$), and the modulation rates of the FM ($t = -21.882$, $df = 232.68$, $p\text{-value} < 0.001$) and the QCF ($t = -19.043$, $df = 126.42$, $p\text{-value} < 0.001$) components of *M. nigricans* were significantly higher compared to those of *N. innoxius* (Fig. 5).

DISCUSSION

The present study constitutes the most recent contribution towards the construction of a regional and national reference library of echolocation signals, adding three more species to the list of insectivorous bats inhabiting the lowlands of Western Ecuador. But more importantly, it provides the first acoustic description of *N. innoxius*, an endemic species distributed in the region comprised between Southwest Ecuador and Northwest Peru, which is listed as

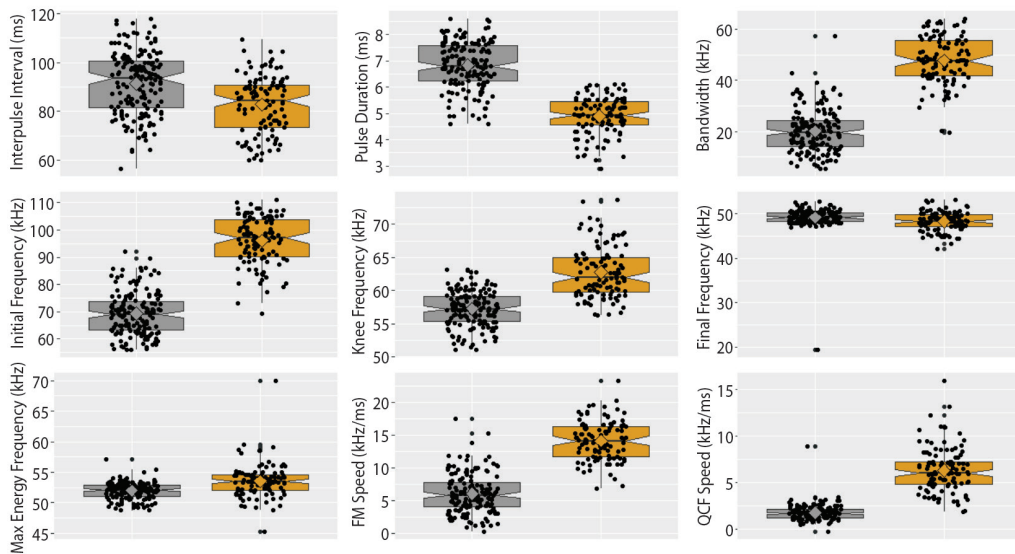


Fig. 5. Comparison of spectral and temporal parameters between *N. innoxius* (grey) and *M. nigricans* (orange). Boxes represent Q1 and Q3, the thick line represents median, whiskers represent maximum and minimum values, diamonds inside the boxes represent the mean, and black dots represent randomly jittered data points, allowing a better view of overlapping points distribution.

Vulnerable (VU) in the Red List of Mammals of Ecuador (Tirira, 2021), and as Near Threatened (NT) in the IUCN red list of species (Velazco & Aguirre, 2020), being rarely captured in the study area where this research is carried out, the coastal lowlands.

The species studied here have been reported sharing roosts or have been captured simultaneously in the same mist nets in the Guayas region, Western Ecuador (Linares & Zabala, 2018; Salas et al., 2014; Salas et al., 2023) and are part of the same soundscape of Isla Santay. Hence, we consider it relevant to include a detailed description of their emission patterns, even though *M. nigricans* and *M. molossus* are commonly recorded in Neotropical bioacoustics studies (Arévalo-Cortés et al., 2024; Barbosa et al., 2022). This is in response to the need of having enough diverse references for estimating how different sources of variation shape the echolocation signal designs that each insectivorous bats species employs, with the geographical variations and local spatial context being two of the most relevant factors altering their emission patterns (Barclay & Brighman, 2004; Schnitzler & Kalko, 2001).

As observed here, some spectral and temporal features in the signals of *N. innoxius* and *M. nigricans* tend to overlap, hence it would be useful to understand the differences that separate the signal designs of the two species to avoid potential identification errors and the subsequent generation of unreliable data, which can have serious consequences for species conservation (Russo & Voigt, 2016). Both *N. innoxius* and *M. nigricans* exploit the same habitat, specializing in forest edges, and exhibiting a convergent echolocation signal design, based mostly on FM signals, suited for rendering high spatial resolution at short distances (Jones & Holderied, 2007; Siemers et al., 2001; Schnitzler & Kalko, 2001), therefore the differences on the modulation rates of the FM and QCF components of these signals could be useful for separating both species, also having the potential to shed light on the resource partitioning experienced by these species along the mangrove edge at Isla Santay.

Even when they can overlap in the edge area for which both species emit signals of relatively broad band, the faster modulation of *M. nigricans* signals enables it to hunt for insects



closer to the clutter of the mangrove (~ 70 cm for a signal with a minimum duration of 4.19 ms, Table 1) compared to what *N. innoxius* can achieve with an echolocation signal of slower modulation and longer duration (~1.02 m for a signal with a minimum duration of 5.94 ms, Table 1) without experiencing backward masking, which happens when the echoes from the background clutter engulf those of the prey that sits on or fly near it, rendering it impossible for the bat to separate the relevant information from the background noise (Schnitzler & Kalko, 2001).

On the other hand, the signals of *N. innoxius* present a QCF structure which modulation speed ranges from 0.8 to 2.5 kHz/ms resulting in an elongated component that concentrates the vast majority of signal's energy in a narrow band of frequencies, thus having the potential to travel further in open spaces adjacent to border habitats where the faint signal of *M. nigricans* would decay in intensity rendering poorer information with greater distances, enabling *N. innoxius* to exploit open grasslands and wetlands more efficiently (Stilz & Schnitzler, 2012).

The emission pattern described here for *M. nigricans* mostly agrees with those reported in previous studies, although with variations in relevant features such as signal shape and final frequency. These differences could result from geographical, individual or flight context variations, since *M. Nigricans* is an edge specialist that can move between open areas and cluttered sites, changing the modulation rate of the FM and QCF components of its echolocation signals as seen in this study, being able to modulate the QCF component speed between 3-8 kHz/ms (Arias-Aguilar et al., 2018; Kraker-Castañeda et al., 2018; Surlykke & Kalko, 2008).

Although the spectral and temporal values described here for *M. molossus* agree with others reported for the same species, the comparison between its alternating pulses has not been approached until this study. Even though, this is a widely referenced species, and the alternation pattern of its echolocation signals is already well known (Arias-Aguilar et al., 2018; Jung et al., 2014; Kössl et al., 1999; Mora et al., 2004),

there is still a lack of clarity about the variation of temporal features between high and low pulses. Current literature does not address different signal emission patterns between different types of pulse pairs (high-low, low-high, high-high, low-low) and only reports two interval values, one for low pulses and one for high pulses (Arévalo-Cortés et al., 2024). As illustrated here, some traits vary between the different combinations of high and low pulses that could help better describe and identify this species.

Finally, Isla Santay is recognized as an Important Area for Bat Conservation or AICOM (Salas, 2022), so the records collected will be used as a reference library, which will be a key tool for the conservation of this group by facilitating the operation of monitoring programs. For instance, we now acknowledge that *N. innoxius* can exploit open areas besides the mangrove interior and edges, hence a monitoring program dedicated to this species should include all the potential habitats for foraging and commuting like grasslands and wetlands, in addition to only focusing monitoring efforts over mangroves (Zamora-Gutierrez et al., 2016). Nonetheless, while the references for *N. innoxius* presented in this work were acquired over a spatial context dominated by mangrove edges, we urge that more references in other spatial contexts need to be added to the repertoire of this species, since the combination of the FM and QCF components of its signals reflect a higher vocal complexity that will not be enough well represented by references from edge habitats only (Martínez-Medina et al., 2021).

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

ACKNOWLEDGMENTS

The authors would like to thank the Santay Island National Recreation Area, especially the administrator, park rangers and community for their support and accompaniment during the field phase. They would also like to thank Gabriela Gonzalez Olimón for her collaboration in the translation and Luciana Carrera for her collaboration. Funding: This research is part of the “Programa Biodiversidad Sostenible del Manglar al Coral 2021-2050”, funded by the Universidad de Especialidades Espíritu Santo, through its Research Center. Under the contract code: MAAE-DBI-CM-2022-0234.

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