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ABSTRACT

The aim of this study in the Republic of Congo was to test a new method to conduct a quantitative study of cave-dwelling bat communities using acoustics. This area is characterised by limited knowledge of the acoustic repertoire of bats. Over a period of 19 months for a total of 398 nights, we carried out 11 individual capture sessions to build a species inventory, record reference sounds and set up a passive acoustic monitoring protocol (PAM) at the exit of two caves. We used the Tadarida automatic sonotype classifier to classify acoustic vocalisations of bats in caves. For this, we enhanced the Tadarida classifier library with reference recordings of bats captured in both caves. Due to the acoustic overlap of several species, we grouped them into five distinct acoustic units using *a posteriori* classification based on four distinct parameters: call shape, acoustic frequency, a harmonics index and the identification probability. A random manual control stratified by sonotype showed an accuracy ranging from 82% to 98% depending on the group. This study is the first local application of a bat sonotype classifier designed and developed to function globally. It confirms the possibility of undertaking quantitative assessments of bat communities with relatively minimal effort, even in areas with limited knowledge of their acoustic repertoires.

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Introduction

The current biodiversity crisis triggered by human encroachment on natural habitats is threatening the extinction of many wild species that require conservation attention. Bats

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are of particular concern as 15% of bat species worldwide are classified as threatened by the IUCN (Voigt and Kingston 2016; Ceballos et al. 2017; Frick et al. 2020; Tanalgo et al. 2023). In addition, 52.9% of bat species have unknown population trends and 17.7% of bat species are data deficient, which is much higher than other mammals and birds (Frick et al. 2020; IUCN 2023). The lack of knowledge on bats is partly due to their elusive and nocturnal behaviour, which makes them difficult to monitor.

More than 46% of all threatened bat species use caves as roosts (IUCN 2023) and this subterranean habitat supports some of the largest population of bats species in the world (Haest et al. 2021). Caves are a key habitat used by numerous bat species because they maintain a stable microclimate for roosting, offer protection against predators, weather and daylight during the day (Kunz et al. 2012; Furey and Racey 2016). Moreover, caves are also used by bats for mating, raising their offspring, performing social interactions and hibernating (McCracken and Wilkinson 2000; Kunz and Fenton 2005; Kunz et al. 2012; Tvrtkovic 2012). Yet these key habitats, vital for bats, are under anthropogenic pressure as they are located in progressively anthropised landscapes and are used for multiple purposes (e.g. hunting, cultural events) (Tanalgo et al. 2022).

The most common techniques used to study bat ecology include capture (i.e. harp traps and mist nets) with the possibility to mark them (i.e. capture marking recapture (CMR) with forearm ring or microchips) (Lobato-Bailón et al. 2023), direct visual counts at roosts (Krutzsch 1955; Gaisler 1979) and multimedia observations (i.e. photographic, video and thermal imaging) (Skalak et al. 2012; Koger et al. 2023; Krivek et al. 2023; Robinson et al. 2023). However, these methods require time and resources, are difficult to standardise and can cause stress, injury and mortality to bats. Over the last few decades, studies using passive acoustic monitoring (PAM) of ecosystems has become an increasingly popular method worldwide (Gibb et al. 2019; Sugai et al. 2020). Yet, in African countries, acoustic studies on bats are still scarce, despite their high diversity (Walters et al. 2013). Bat families primarily use echolocation for orientation and foraging, except for most of the *Pteropodidae* (large frugivorous bats) (Neuweiler 1989; Schnitzler et al. 2003). Bioacoustics therefore offers a different approach to answer various ecological questions, such as species or communities diversity and abundance of, spatial and/or temporal distributions and bat behaviours, including the impact of human activities on bat behaviour (O'Farrell and Gannon 1999; King et al. 2013; Kalan et al. 2015; Lucas et al. 2015; Merchant et al. 2015; Petrusková et al. 2015; Pirotta et al. 2015; Campos-Cerqueira et al. 2016; Astaras et al. 2017; Davis et al. 2017; Wrege et al. 2017; Darras et al. 2018; Lehnen et al. 2018; Mcloughlin et al. 2019; Stowell and Sueur 2020). PAM was also demonstrated to be very promising to monitor bat populations in caves (Kloepper et al. 2016; Revilla-Martín et al. 2020). PAM offers many insights, such as understanding the annual phenology of the roost occupancy, which cannot be monitored by direct human observers (for example, due to nocturnal behaviour or large number of individuals).

However, identifying acoustic calls to family and/or species level requires comparison with a reference database of bat calls. A number of techniques for creating reference libraries are currently being tested in the Northern Hemisphere, with the aim of minimising stress on individuals and acquiring quality sounds for referencing a species. For example, Zamora-Gutierrez et al. (2020) created a reference library of fifty percent of the bat species present in Mexico (e.g. 69 species) using five different methods to record them (hand release, zip lining, in bag, flight cage and flying from perch). To our knowledge, there is no consensus in the scientific community on the use of any of the methods as a reference protocol. Furthermore, these methods should be adapted to the environment in which the recordings take place (clutter vs. open field), as a given bat species can adapt its echolocation call characteristics depending on the clutter density (Jones and Holderied 2007; Schaub and Schnitzler 2007). In addition, echolocation calls are known to vary according to sex and age within species (Taylor et al. 2005; Barclay and Jacobs 2022).

The use of PAM limits disturbance to species while collecting a large amount of data over a long period, regardless of the environmental conditions or behavioural factors associated with the animals (Blumstein et al. 2011; Marques et al. 2013; Sugai et al. 2020).

Technological advances and their increasing use have also reduced the cost of acoustic devices, even if they remain expensive and require some expertise for data analysis (Hill et al. 2018; Gibb et al. 2019; Sugai et al. 2020).

However, until recently, the time required for the analysis of the usually large amounts of data collected by PAM (acoustic files), was considerable. In recent decades, the development of semi-automated and custom-built classifiers have facilitated the analysis of bat acoustic data through the development of machine learning techniques (Thessen 2016; Valletta et al. 2017; Kwok 2019; Tabak et al. 2019; Borowiec et al. 2022). The scientific community has developed several classifiers using different approaches or methodologies to automate the recognition of acoustic call features (López-Baucells et al. 2019; Chen et al. 2020; Yoh et al. 2022). Among the available software, Tadarida (Bas et al. 2017) is a versatile program that can detect sound events and classify them. For geographic areas such as Central Africa which are not covered by a bat classifier at the species level, two sonotype classifiers (i.e. shape associated to frequency) have been developed to analyse bat calls worldwide (Roemer et al. 2021; Yoh et al. 2022). In this study, we chose to use the sonotype classifier of Roemer et al. (2021), which has the advantage of having been already trained with a reference library of bat calls covering sonotypes worldwide, including Africa. To our knowledge, no method about the local application of such a classifier has yet been published. Indeed, questions remain such as how the results of a sonotype classifier must be handled in the case of acoustic overlaps between species.

The main objective of this study was to test whether it is possible to make a quantitative monitoring of bat acoustic activity using a universal sonotype classifier in an area where reference data are scarce and species classifiers are not available. To this end, we have developed an automatic classification method based on annual passive acoustic monitoring and applied our method to two caves in the Republic of Congo. We proceeded in three steps: (1) establish the list of species present in the two caves (2) describe the acoustic repertoire of these species and how they overlap, and (3) define how to apply the sonotype classifier, that is, which species can be identified as one mono-specific group using *a posteriori* classification based on several parameters.

Materials and methods

Ethics statements

A permit to capture and handle bats was validated by the Ministry of Forest Economy and the Ethics Committee of the Ministry of Scientific Research and Technological Innovation of the Republic of Congo (N°212/MRSIT/IRSSA/CERSSA and N°687/MEF/

CAB/DGEF-DFAP). All animals were handled with care and as quickly as possible during morphometric measurements and sample collection, following the recommendations of Kunz and Parsons (2009). Fragile individuals, pregnant or nursing females were handled as quickly as possible or released immediately, depending on their condition. In the event of incapacitating injury (less than 0.1% of individuals captured during eleven sessions), individuals were euthanised as quickly as possible to limit suffering, and their specimens (currently being analysed in the Institute of vertebrate biology – Czech Academy of Sciences) preserved for taxonomic analysis.

Study sites

Our study focussed on insectivorous bat populations in two caves located in the south of the Republic of Congo: Mont Belo Cave and Boundou Cave, in the Bouenza and Niari departments (administrative division of the country), about 50 km apart, near the town Dolisie town (Figure 1).

In this region, the year is divided in four seasons: the short dry season spans from January to February, the short-rainy season from March to May, the long-dry season from June to August, and the long-rainy season from September to December (Samba et al. 1999). The landscape is mountainous and contains limestone, favouring the presence of numerous caves and cavities. It is mainly composed of grassy savannah with patches of secondary forests and a patchwork of crops close to village.

The Mont Belo Cave (N site: GTCO 01) is surrounded by a small tertiary forest in a farming complex close to the small village of the same name. The cave has one main chamber and two secondary chambers. The Boundou Cave (N site: GTCO 03), dug into the cavity of a rocky outcrop, is smaller, with one main chamber and another secondary cavity (not easily accessible by humans) (Appendices A and B). It is also surrounded by a patch of tertiary forest in a predominantly grassy savannah habitat with forest patches. In the two caves studied, we observed the presence of between a hundred to a thousand individuals of insectivorous bats, depending on the season. Fruit-eating bats, on the other hand, had a limited presence, ranging from zero individuals per month to a maximum of around thirty.

Passive acoustic monitoring (PAM)

At each study site, an SM4BAT acoustic recorder with a U1 microphone (Wildlife Acoustics, Maynard, MA, USA) was installed 5 m outside the cave. It was oriented towards the cave exit, to capture bats calls while they exit or enter the cave, and to avoid recording calls while bats are inside (Figure 1). The microphone was placed on a tree or pole at a minimum height of one metre to avoid ground noise and reduce ‘echo’. The microphone was deployed at a minimum distance of 1.5 m from any obstacle that might obstruct the sound (vegetation, water) (Newson et al. 2015). The microphone cables were protected from insects and wildlife with PVC tubing, and small bags of desiccant were added to the battery compartment to minimise humidity in the case. Two SD memory cards with a minimum capacity of 128 GB were used to store the sound recordings. Over a period of 19 months (September 2021 to February 2023) at the two study sites, automatic recording was triggered 30 minutes before sunset and up to 30 minutes after sunrise. The SM4BATs were set up

Figure 1. Photograph of the two study caves and the microphones attached to an SM4 acoustic recorder: (a) Mont Belo Cave, (b) the microphone placed just in front of the main entrance to Mont Belo Cave, (c) boundou Cave and (d) the microphone placed high above at the main entrance to boundou Cave. The yellow star represents the approximate location of the microphone.

according to the French National Museum Natural History's fix points protocol for the Vigie-chiro project (Mariton et al. 2023). For sixteen of the nineteen months, we used batteries (four LR14 batteries) to operate the recorders, for periods varying from 5 to 28 nights, depending on the month and the cave, until the batteries were exhausted. An external battery (12 V) was used for a period of three months (November 2021, December 2021 and January 2022). However, the abrupt shutdown of the recorders on several occasions, probably due to the poor quality of the batteries, resulted in a loss of data, necessitating a return to the four-battery system for the remaining 16 months. Every month, the batteries and SD cards were changed by the field team when they were present for other research activities or specifically by a member of the field team.

Bat capture, morphometric and genetic data

Over the 19-month period, 11 capture sessions (spread over several seasons) were carried out using a Harp trap (Ecotone, Poland) placed at the entrances of the two study sites, in order to identify the most common cave-dwelling species. Depending on field constraints, we were able to collect several types of data, such as morphometric data, genetic samples (wing punches or faecal sample) and/or a reference acoustics sound.

Species identification was carried out using the morphological criteria referenced for species supposedly found in the Republic of Congo (Bates et al. 2013; Kingdon 2014; Monadjem et al. 2020). However, identification using morphological criteria was difficult due to the absence of an identification key for Congo species and the lack of information on many insectivorous species. Morphometric data for all individuals caught during our study are presented and available in Appendix C. In addition, 156 genetic samples were collected by taking skin samples (wing punch) or faeces.

Reference sound recordings

For 173 individuals, acoustic calls were recorded with a Pettersson M500–354 USB portable recorder (Pettersson Elektronik AB, Sweden), when bats were either in their pouch (Zamora-Gutierrez et al. 2020) before being handled, or on release after data collection as they flew away. However, 47 of these acoustic recording were not usable due to poor recording quality or technical problems during recording (including the only recording for *Coleura afra*).

To avoid recording calls of other bat species and to reproduce the conditions of a semi-enclosed environment (cave exit), we decided to change the methodology. We thus performed reference recordings for the next 30 individuals including a minimum of one male and one female for each genus except for *Macronycteris gigas* (only one female recorded) and for *Hipposideros sp*. (as the females captured were pregnant, we preferred not to keep them for this potentially stressful stage). Early in the morning, individuals were placed in a room in a building and allowed to fly freely, while acoustic calls were recorded using an SM4 recorder and its U1 microphone. We harmonised the recording parameters between the SM4 and the Pettersson to allow comparison and use of the acoustic recordings of all the individuals recorded.

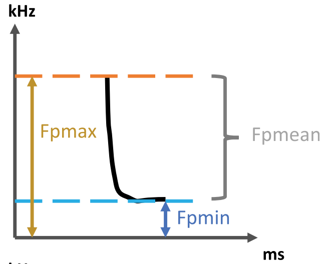
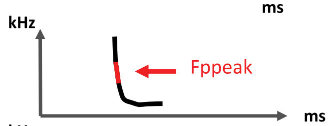
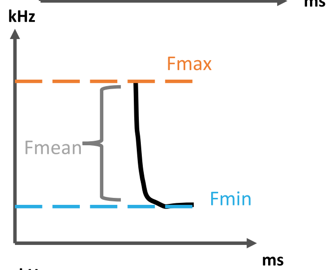
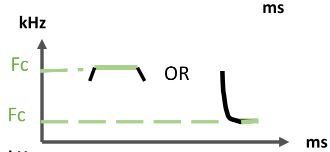
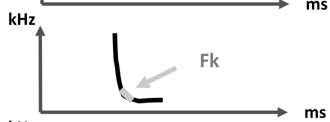
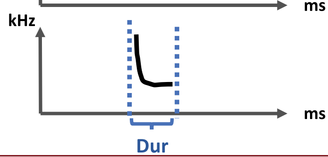
Using Kaleidoscope 5.6.2 software (Wildlife Acoustics, Maynard, MA, USA), we automatically measured manually selected acoustic calls on each reference acoustic file based on the acoustic parameters presented in the Table 1. We then calculated the mean and standard deviation of each of the acoustic parameters with the standard deviation, grouped by species and sex. By using this automatic method, we attempted to overcome the differences between operators that can be found in the acoustic data of bibliographic references for a given bat species.

Acoustic analyses

Automatic classifier and classifier training

The Tadarida classifier classifies bat calls into different sonotype categories (and 2) thanks to a random forest model trained on a reference library. For a given acoustic recording containing several calls, the classifier groups the calls displaying the same sonotype (i.e. shape) and the same frequency at the maximum energy with a tolerance of five kHz (Figure 3). The classifier then calculates several parameters for each group of calls such as the mean frequency at the maximum energy, the mean call duration and the presence of harmonics (Figure 3). It

Table 1. Summary of the different acoustic parameters calculated in each recorded bat reference file using Kaleidoscope (Wildlife Acoustics).

Acoustic parameter	Unit	Definition according to Kaleidoscope	Example
Fpmin	kiloHertz (kHz)	Estimate of minimum signal frequency. The estimate is determined by following signal amplitude starting from the peak frequency up until the noise floor.	
Fpmax	kiloHertz (kHz)	Estimate of the maximum signal frequency. The estimate is determined by following signal amplitude starting from the peak frequency down until the noise floor.	
Fpmean	kiloHertz (kHz)	Amplitude-weighted mean (average) frequency of the energy (amplitude) within the selection.	
Fppeak	kiloHertz (kHz)	Frequency which has the highest (peak) energy within the selection	
Fmin	kiloHertz (kHz)	Average minimum frequency of call pulses	
Fmax	kiloHertz (kHz)	Average maximum frequency of call pulses.	
Fmean	kiloHertz (kHz)	Time-weighted average frequency of call pulses.	
Fc	kiloHertz (kHz)	Average characteristic frequency of call pulses. This is the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call.	
Fk	kiloHertz (kHz)	Average knee frequency of calls. This is at the beginning of the call body.	
Duration (Dur)	Millisecond (ms)	Average duration of call pulses within the selection.	

also provides the probability of correct sonotype classification by the classifier (Ind), from zero for low probability to one for high probability (Roemer et al. 2021) (Figure 3). More details on the classifier can be found in Bas et al. (2017) and Roemer et al. (2021).

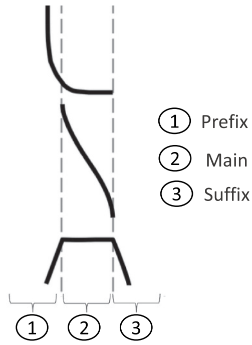
We only trained the classifier with the bat species from the two study caves. In an initial test of the classifier published in Roemer et al. (2021), we obtained a poor performance for the CF-FMd sonotype, which was displayed by several species in our study (*Hipposideros sp., Triaenops sp., Macronycteris sp.*) (Figure 3). The CF-FMd sonotype was poorly represented in the database used to train the classifier (i.e. 5,346 calls labelled for the CF-FMd sonotype while the mean number of calls labelled per sonotype was 39,067). Fortunately, the classifier offers a degree of flexibility, and reference sounds can be added. With 'Tadarida L' (Bas et al. 2017), we manually labelled

A)

The upper sonotype is a call divided into a frequency modulated (FM) prefix and a main quasi-constant frequency (QCF) element.

The sonotype in the middle is a call containing only a main FM element.

The lower sonotype is a call divided into an FM prefix, a main constant frequency (CF) element and an FM suffix.



B)

Sonotype	Prefix	Main element	Suffix	Sonogram	Exemple of species from the Republic of Congo
FMd- QCF	Downward FM or none	QCF	_		*Miniopterus sp. (minor,inflatus, ..); Neoromicia sp.*
Fmu-QCF	Upward FM	QCF	_		*Coleura afra*
QCF - FMd	_	QCF	Downward FM or none		Not detected to our knowledge in the Republic of Congo
CF-FMd	_	CF	Downward FM		*Hiposideros sp.* (*caffer/ruber*); *Triaenops sp.* ; *Macronycteris gigas*
FMu-QCF-FMd	Upward FM	QCF	Downward FM		Not detected to our knowledge in the Republic of Congo
Fmu-CF-FMd	Upward FM	CF	Downward FM		*Rhinolophus sp.* (*landeri,alcyone,denti*, ..)
FMd	_	Downward FM	_		*Nycteris sp.* ; *Myotis sp.* ; *Kerivoula sp.* ; ..
CF-FMd-CF	CF	Downward FM	CF		Not detected to our knowledge in the Republic of Congo

FM: Frequency modulated; CF: Constant frequency; QCF: Quasi-constant frequency; d: downward; u: upward.

c)

Other Sonotype	Prefix	Main element	Suffix	Sonogram	Taxon
Other mammals	These categories were not put in subcategories such as for bats			For example	Rodents, ..
Insects				For example	Crickets, …
Birds				For example	Crow, …
Noise				For example	Human voice, ..

Figure 2. (A) Illustration of the method for the definition of bat sonotypes with three examples on a sonogram (time as a function of frequency), (B) description of bat sonotypes from the Republic of Congo and (C) example of sonotype categories (other than bats) present in Tadarida. Inspired by Roemer et al. (2021).

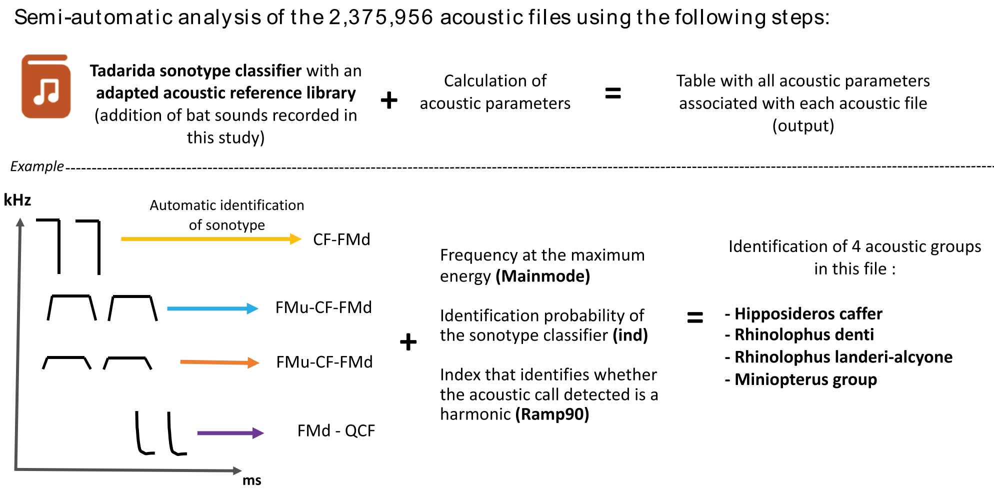


Figure 3. Diagram explaining how the Tadarida sonotype classifier works (logo from Flaticon.com).

a total of 145 acoustic files (reference files, see previous section, or cave recorder files) with 695 calls with different sonotype shapes, 50% of which belonged to the CF-FMd category. These files were then added to the classifier database and a new classifier was trained. During the training, two parameters, SubSamp and GradientSamp, define how the calls in the database will be sampled to train each tree of the random forest model. SubSamp defines the minimum number of calls subsampled, and it was set to 0.02, corresponding to a subsampling equal to 0.02 times the average number of calls available per species in the database. At each new tree, the call sampling gradient increases so that the final result is a large forest mixing a gradient of trees, from trees using a maximum number of sound events for high performance on common species to trees using more and more balanced sound events per species to decrease bias towards common species. GradientSamp defines the strength of this gradient and it was set to −0.1, which is the default. This classifier is available by following the link in the Appendix D.

Acoustic species identification and accuracy check

We fragmented the acoustic files collected into files of five second maximum using Kaleidoscope Lite software (Wildlife Acoustics, Maynard, MA, USA). We used the adapted Tadarida classifier to identify the sonotypes present in each acoustic file collected from the two caves in our study. The classifier returns a table in which each line corresponds to a group of calls with similar shape and frequency. Each group is associated with a sonotype, a summary of the acoustic parameters, and a probability of identification (Ind). In order to check the accuracy of the classifier and identify the probability of identification threshold under which the classifier had an unsatisfying performance (e.g. sonotype misclassification), we carried out a manual check of 5,461 files, spread over the two caves and two different months (September 2021 and February 2022).

Classification *a posteriori*

Due to the presence of several bat species with similar call characteristics, the results of the sonotype classifier needed to be refined with *a posteriori* classification. The goal was to try and separate species, or else place them in acoustic groups. We compared the collected recordings with our reference recordings (see dedicated section above) and the literature to identify the various key parameters needed to achieve greater accuracy in automatic identification. For this purpose, we selected the MainMode used by Tadarida to build groups of calls based on their frequency at the maximum energy (see Figure 3), the identification probability of the sonotype classifier (Ind) and Ramp90 (an index that identifies whether the acoustic call detected is a harmonic or a fundamental frequency). If the Ramp90 index is below zero, it is a fundamental frequency; an index close to zero is a fundamental frequency with a harmonic and a number greater than zero is a harmonic. Furthermore, in order to take into account the potential variations in acoustic frequency due to the environmental and methodological conditions of our study, we decided to define the acoustic frequency range of our acoustic species based on two different data sources. For each acoustic interval of the species group, we used: (i) the acoustic data from the literature (Table 2) and (ii) the minimum and maximum of the reference acoustic data we measured using Kaleidoscope (Fppeak, Fpmin, Fpmax for all the species groups with the addition of Fmax for the *Miniopterus* species) (Table 3).

Results

Species identification

A total of 680 individuals were captured with harp traps over 11 sessions. In Mont Belo and Boundou caves, we regularly captured three different bat genera: *Miniopterus*, *Hipposideros* and *Rhinolophus*. In addition to these, in Boundou Cave, we also regularly captured three other genera: *Triaenops*, *Macronycteris* and *Coleura*.

The species of each individual was first identified using morphometric data, followed by a second validation based on acoustic data. We identified the presence of five species in both caves: *Miniopterus cf. inflatus*, *Miniopterus cf. minor*, *Rhinolophus cf. landeri-alcyone*, *Rhinolophus cf. denti* and *Hipposideros cf. caffer*. In the Boundou Cave, we also identified *Triaenops cf. afer*, *Macronycteris gigas* and *Coleura afra*. At Boundou, *Coleura afra* was only caught once during the April 2022 session and *Macronycteris gigas* was caught three times out of the 11 sessions (December 2021, April 2022, November 2022). Details for each species and the distribution of sexes according to capture sessions are presented in Appendix E. Morphometric species identification is detailed in another paper (submitted).

Acoustics characteristics of the species communities

Of the 680 individuals captured, 159 (23.5%) were recorded for reference purposes, and their main acoustic characteristics are presented in Table 2. The acoustic parameters found in the literature are reviewed in Table 3. The acoustic data for the various species found in the literature are fairly consistent with the data collected in the field. The overview of both our reference recordings and the literature allowed us to establish the following characteristics for the eight species present in our study caves. Among *Rhinolophus* (FMu-CF-FMd,

Table 2. Mean (± standard deviation) echolocation parameters of 159 bats caught in two caves in the Republic of Congo (BD =boundou and MB = Mont Belo) depending on sex (F = female and *M* = male), using Kaleidoscope Lite software (Wildlife Acoustics). *N* = number of bats in sample; Fpmin : estimate of minimum signal frequency; Fpmax : estimate of maximum signal frequency; Fpmean : mean frequency of the spectrum within the selection; Fppeak : frequency which has the highest (peak) energy within the selection; Fmax : average maximum frequency of call pulses; fc : the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; fk : average knee frequency of calls; Fmin : average minimum frequency of call pulses, Fmean : average frequency of echolocation pulses and dur (ms) : average duration of call pulses within the selection. An underscore indicates that the software is unable to calculate due to harmonics or poor recording quality.

Species	Cave	Sex	N	Fpmin (kHz)	Fpmax (kHz)	Fpmean (kHz)	Fppeak (kHz)	Fmax (kHz)	Fc (kHz)	Fk (kHz)	Fmin (kHz)	Fmean (kHz)	Dur (ms)
Hipposideros cf. caffer	BD	F	7	141 ± 4.2	138.3 ± 13.2	133.2 ± 11.6	135.7 ± 15	_	73.3 ± 9.7	72.7 ± 9.4	_	73 ± 9.7	4.3 ± 1.1
	BD	M	8	136.1 ± 15.1	149 ± 7.8	139.6 ± 5.7	142.8 ± 9.1	_	79.7 ± 9.2	79.7 ± 9.4	_	79.7 ± 9.3	4.2 ± 0.7
	MB	F	3	133.5 ± 0	145.2 ± 8.8	134.9 ± 2.1	109.7 ± 47.2	_	55.3 ± 0.2	39.5 ± 27.8	_	56.6 ± 0.1	6.2 ± 2.5
	MB	M	5	126.3 ± 23.4	147.7 ± 3.6	132.3 ± 20.7	134.5 ± 21.7	_	52.8 ± 3.9	53 ± 3.4	_	52.5 ± 4.3	5.2 ± 3.6
Miniopterus cf. inflatus	BD	M	1	_	55.5 ± 0	54.2 ± 0	51.2 ± 0	105.3 ± 0	98.8 ± 0	104 ± 0	44 ± 0	69.2 ± 0	2.2 ± 0
	MB	F	7	39.6 ± 3.3	69.6 ± 11.1	54.6 ± 6.2	47.6 ± 2.7	82 ± 17.8	53.5 ± 16.6	56.2 ± 16.2	44.6 ± 2.9	58.7 ± 9	4.1 ± 0.2
	MB	M	24	37.3 ± 8.6	62.5 ± 9.9	52.5 ± 7.7	48.1 ± 7.5	77.1 ± 14.5	47.3 ± 8.4	51 ± 8.2	44.9 ± 5.2	55.4 ± 7.6	2.8 ± 0.5
Miniopterus cf. minor	BD	F	5	53.2 ± 1	76.2 ± 6.7	67.9 ± 5.8	64.8 ± 6	107 ± 12.9	60 ± 3.2	64.4 ± 5.7	58 ± 3.1	75 ± 6	2.4 ± 0.4
	BD	M	10	52.5 ± 2.1	78.1 ± 5	68.9 ± 4.9	68.7 ± 13.1	97.9 ± 21.9	60.3 ± 6.9	58.5 ± 21.5	57.2 ± 2.7	73 ± 7.3	2.9 ± 0.8
	MB	F	10	46 ± 3.8	71.1 ± 11.9	62.7 ± 8.8	56.7 ± 5	93 ± 15.1	55 ± 6.5	61.3 ± 8	51.7 ± 4.6	66 ± 9	2.8 ± 0.8
	MB	M	8	47.3 ± 3.2	72.9 ± 7.6	66.8 ± 7.3	61.1 ± 4	96.4 ± 20.3	59 ± 6.6	68.6 ± 19.3	55.3 ± 3.9	69.7 ± 8.7	2.6 ± 0.5
Rhinolophus cf. denti	BD	F	6	88.2 ± 8.6	110.5 ± 3.9	101.5 ± 1.2	101.7 ± 1.2	_	101.2 ± 1.3	100.6 ± 1.4	_	101 ± 1.4	32 ± 17.5
	BD	M	1	85.5 ± 0	103.5 ± 0	99.3 ± 0	99.3 ± 0	_	98.4 ± 0	99.2 ± 0	_	99.4 ± 0	18.5 ± 0
Rhinolophus cf. landeri-alcyone	BD	F	1	49.5 ± 0	58.5 ± 0	54.3 ± 0	54.3 ± 0	_	54.4 ± 0	54.2 ± 0	_	54.3 ± 0	9.2 ± 0
	MB	F	29	46.4 ± 4.6	60.6 ± 1.9	54.7 ± 0.7	55 ± 0.6	_	55 ± 0.5	54.7 ± 0.6	_	54.8 ± 0.6	23 ± 10.2
	MB	M	14	45 ± 4.8	61.9 ± 2.2	54.4 ± 2.7	55.4 ± 0.5	_	55.3 ± 0.6	55.3 ± 0.4	_	55.1 ± 0.5	19.5 ± 8.9
Triaenops afer	BD	F	9	79.12 ± 1.9	87.7 ± 5.3	80.4 ± 3.2	82.2 ± 1.6	_	81.7 ± 1.4	82.1 ± 1.9	_	81.1 ± 1.5	6.2 ± 2
	BD	M	10	69.3 ± 7.1	82 ± 2.9	75.1 ± 2.9	75.7 ± 2.6	_	75.6 ± 2.5	75.5 ± 2.9	_	75.2 ± 2.5	7.5 ± 1.7
Macronycteris gigas	BD	F	1	51 ± 0	_	54.2 ± 0	54.3 ± 0	_	54 ± 0	54.8 ± 0	_	54.3 ± 0	12.4 ± 0

Table 3. Summary of the acoustic frequency ranges of bat species identified in the two study caves using available reference works or studies. Fmin = average minimum frequency of call pulses; fmax = average maximum frequency of call pulses; fc = the point at the end of the body of the call pulse which is defined as the flattest part (lowest absolute slope) of the call; fk = average knee frequency of calls and dur (ms) = average duration of call pulses within the selection.

Species	Sex	Fmin (kHz)	Fmax (kHz)	Fc (kHz)	Fk (kHz)	Dur (ms)	Comments	References
Hipposideros caffer	Both	121.2–139	143.8–159	130–145.9	141.4–156	4.5–10.6	The fundamental harmonic may be present on the spectrogram at ~ 71 kHz	(Taylor et al. 2005; Wright 2009; Kingdon 2014; Monadjem et al. 2017, 2020; Webala et al. 2019; Moir et al. 2020; Brinkley et al. 2021)
Miniopterus inflatus	Both	47.2	58.1	47.4	50	3.2		(Kingdon 2014; Monadjem et al. 2020)
Miniopterus minor	–	–	–	–	–	–	Data deficient	
Rhinolophus landeri	Both	73–100	102–110	101–122	108–109	21–67		(Taylor et al. 2005; Tanshi et al. 2019; Monadjem et al. 2020)
Rhinolophus denti	Both	–	–	110–111	–	23.4		(Jacobs et al. 2008, 2016; Monadjem et al. 2020)
Rhinolophus alcyone	Both	53.6	54.4	50 (45*) – 59	53.7	29–67	*Perhaps misidentified by Pye and Roberts (1970) if we refer to the commentary by Monadjem et al. (2020)	(Pye and Roberts 1970; Adams and Kwiecinski 2018; Monadjem et al. 2020; Weier et al. 2020; Brinkley et al. 2021)
Triaenops afer	F	–	–	82–88	77	7.6–12.2	Sexual differences in echolocation call frequency	(Happold and Happold 2013; Kaipf et al. 2015; Webala et al. 2019; Monadjem et al. 2020)
	M	–	–	70–78	77	7.6–12.2		
Triaenops persicus or Triaenops p. majusculus	Unspecified	–	–	75–80.5	–	–	Perhaps sexual differences in echolocation call frequency, as in other *Triaenops sp.*	(Pye and Roberts 1970; Monadjem et al. 2020)
		–	–	85.5–89	–	–		
Coleura afra	Both	29.5–35.4	30.7–38.4	–	30–36.4	2.9–13		(Taylor et al. 2005; Kingdon 2014; Monadjem et al. 2020)
Macronycteris gigas	F	–	–	53	–	10.4–19.2	Sexual differences in echolocation call frequency	(Tanshi et al. 2019; Webala et al. 2019)
	M	–	–	53–54	–	10.4–19.2		

see Figure 4A,B), the two different species have distinct frequency ranges: *Rhinolophus cf. landeri-alcyone* with a frequency (FMu-CF-FMd or other mammals) ranging from 45 to 62 kHz and *Rhinolophus denti* with a frequency (FMu-CF-FMd or other mammals) between 95 and 110 kHz (Figure 4A,B). For *Miniopterus* (FMd – QCF, see Figure 4A,B), the two species have frequency ranges between 39 and 107 kHz (Tables 2 and 3, Figure 4A,B). At Mont Belo, *Hipposideros caffer* (CF-FMd, Figure 4B) has an acoustic frequency ranging from 120 to 155 kHz and no overlap with other species. But at the Boundou Cave, three FMu-CF-FMd species have overlapping frequencies: *Rhinolophus landeri-alcyone* (between 45–62 kHz), *Triaenops sp.* (between 69 and 87 kHz) and *Macronycteris gigas* (between 51 and 56 kHz) (Tables 2 and 3, Figure 4A).

Acoustic dataset obtained from the passive acoustic monitoring

We obtained a total of 2,375,956 wav files collected with an average of 60,927 files per month collected in Boundou Cave and 64,123 files per month in Mont Belo Cave. A total of 398 nights were recorded, with 74.6% (297 nights) collected simultaneously in both caves. Details for each month are shown in the Appendix F. Our acoustic analysis focused on the bat species that roost in the two caves and that we regularly observed.

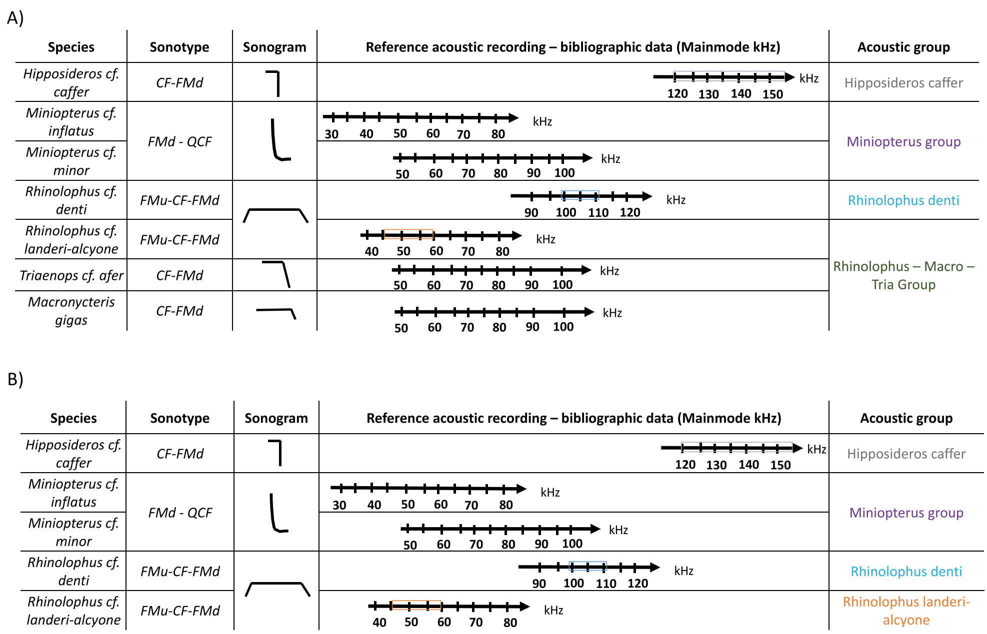


Figure 4. Acoustic characteristics of the different bat species captured at the two study sites and categorisation into different acoustic groups or species. Detail of sonotype, sonogram and acoustic frequency recorded or listed in reference works for each bat genus and species captured in the two caves. (A) In the Boundou Cave, there are four acoustic groups (*Hipposideros caffer, Miniopterus group, Rhinolophus denti* and Rhinolophus – macro – tria group) and in (B) The Mont Belo Cave there are four acoustic groups (*Hipposideros caffer, Miniopterus group, Rhinolophus denti and Rhinolophus landeri-alcyone).*

Automatic classification of acoustic recordings

Due to the overlap of certain calls for *Rhinolophus – Macronycteris – Triaenops* at Boundou or for the two *Miniopterus* species in both caves, we decided to group the seven species into five different groups: (1) *Rhinolophus landeri-alcyone*, (2) *Rhinolophus denti*, (3) Miniopterus group (*M. cf minor/M. cf. inflatus)*, (4) *Hipposideros caffer (H. cf. caffer)* and (5) Rhinolophus – Macro – Tria group (*R.cf. landeri-alcyone/M. gigas/ Triaenops sp.)* (only present in Boundou Cave). Figure 3 shows the sonotypes and frequency ranges of species from both caves. The acoustic characteristics of each individual are presented in Appendix G.

In Mont Belo Cave, we manually randomly checked 1,043 acoustic files from September 2021 and 1,051 files in February 2022. For Boundou Cave, we checked 2,133 acoustic files from September 2021 and 1,234 files in February 2022. In total, we verified the accuracy of Tadarida using *a posteriori* classification on 5,461 files. Tables 4 and 5 show the parameters chosen for each group at each study site, enabling a group to be identified with over 95% accuracy. For instance, in the two caves, for the *Miniopterus* group, we used a filter with the FMd-QCF sonotype, a Mainmode ranging from 39 to 107 kHz, a confidence probability index greater than 0.15 for Mont Belo (and 0.20 for Boundou) and a harmonic index (Ramp90) less than zero. For *Hipposideros caffer*, in both caves, we filtered using two different filters: CF-FMd and FMd, for a frequency of 120 to 155 kHz, a harmonic index less than or equal to 0.1 and a confidence probability index of 0.2. These *a posteriori* acoustic parameters allowed us to identify the *Hipposideros caffer* group with an accuracy of over 96%.

For *Rhinolophus denti*, we could not find parameters allowing an identification over 95% accuracy, and attained only 82% accuracy (see Tables 3 and 4). Some of the *Rhinolophus denti* sonotypes were confused by the classifier with ‘other mammals’ rather than ‘bat calls’.

During peak periods of simultaneous emergence of numerous individuals of several species, we observed a time and frequency overlap of acoustic calls. This type of scenario was quite frequent, especially during the peak emergence times for the various species (6.30–7.30pm) or during periods when there was a large abundance of individuals in the caves (the main rainy season: November, December or during mating periods, that is, in July for *Rhinolophus*). This overlap could lead to a misidentification of the sonotype by the classifier or to a mismeasurement of the frequencies. Concrete examples of these scenarios are shown in Table 6.

Identification of other species

Acoustic recordings also enabled us to detect the regular presence of at least one other species in the Boundou Cave: *Coleura afra*. The acoustics characteristics of this species detected by the Tadarida classifier are either i) a FMd-QCF sonotype with an acoustic frequency between 36–65 kHz, a confidence probability index greater than 0.4 and a harmonic index between one and eight or ii) a CF-FMd sonotype with a frequency of 49–75 kHz, a confidence probability index greater than 0.4 and a harmonic index greater than one. As mentioned above, this species was only captured once during the April 2022 capture. However, the acoustic characteristics of this species (see sonogram Figure 5) were identified by the classifier during the 19 months of acoustic monitoring (about 70% accuracy).

Table 4. *A posteriori* classification of the different species or groups of bats in the Mont Belo Cave using the different acoustic parameters and manual verification of the acoustic files.

Species or Group name	Sites and period	Acoustic parameters used for *a posteriori* classification	Number of files checked manually	Number of files that do not correspond to the corresponding bat guild
Miniopterus Group	Mont Belo Cave – September 2021 Mont Belo Cave - February 2022	Sonotype: FMd-QCF Frequency (Mainmode): 39–107 kHz Harmonic index (Ramp90) ≤ 1 ID Probability ≥ 0.15 Sonotype: QCF-FMd Frequency (Mainmode): 55–62 Harmonic index (Ramp90) ≤ 0 ID Probability ≥ 0.2	361 152	0 0
Rhinolophus landeri-alcyone	Mont Belo Cave – September 2021 Mont Belo Cave - February 2022	Sonotype: FMu-CF-FMd Frequency (Mainmode): 45–62 kHz Harmonic index (Ramp90) ≤ 0 Sonotype: Other mammals Frequency (Mainmode): 45–62 kHz Harmonic index (Ramp90) ≤ 0	428 224	1 0
Rhinolophus denti	Mont Belo Cave - September 2021 Mont Belo Cave - February 2022	Sonotype: FMu-CF-FMd Frequency (Mainmode): 95–110 Harmonic index (Ramp90) < 0 ID Probability ≥ 0.35 Sonotype: Other mammals Frequency (Mainmode): 95–110 Harmonic index (Ramp90) ≤ 0	28 3	5 0
Hipposideros caffer	Mont Belo Cave - September 2021 Mont Belo Cave - February 2022	Sonotype: CF-FMd Frequency (Mainmode): 120–155 Harmonic index (Ramp90) ≤ 0 Sonotype: FMd Frequency (Mainmode): 120–155 kHz Harmonic index (Ramp90) ≤ 0.1 ID Probability ≥ 0.2	167 138	0 12

Table 5. *A posteriori* classification of the different species or groups of bats in Boundou Cave using the different acoustic parameters and manual verification of the acoustic files.

Species or Group name	Sites and period	Acoustic parameters used for a posteriori classification	Number of files checked manually	Number of files that do not correspond to the corresponding bat guild
Miniopterus Group	Boundou Cave - September 2021 Boundou Cave - February 2022	Sonotype: FMd-QCF Frequency (Mainmode): 39–107 kHz Harmonic index (Ramp90) ≤ 1 ID Probability ≥ 0.2 Sonotype: QCF-FMd Frequency (Mainmode): 55–62 kHz Harmonic index (Ramp90) ≤ 0 ID Probability ≥ 0.2	183 197	2 6
Rhinolophus Macro-Tria Group	Boundou Cave - September 2021 Boundou Cave - February 2022	Sonotype: FMu-CF-FMd Frequency (Mainmode): 45–85 kHz Harmonic index (Ramp90) ≤ 0 Sonotype: CF-FMd Frequency (Mainmode): 45–89 kHz Harmonic index (Ramp90) ≤ 0 Sonotype: Other mammals Frequency (Mainmode): 45–85 kHz Harmonic index (Ramp90) ≤ 0	248 366	0 0
Rhinolophus denti	Boundou Cave - September 2021 Boundou Cave - February 2022	Sonotype: FMu-CF-FMd Frequency (Mainmode): 95–110 kHz Harmonic index (Ramp90) < 0 ID Probability ≥ 0.35 Sonotype: Other mammals Frequency (Mainmode): 95–110 kHz Harmonic index (Ramp90) ≤ 0	179 113	2 1
Hipposideros caffer	Boundou Cave - September 2021 Boundou Cave - February 2022	Sonotype: CF-FMd Frequency (Mainmode): 120–155 kHz Harmonic index (Ramp90) ≤ 0 Sonotype: FMd Frequency (Mainmode): 120–155 kHz Harmonic index (Ramp90) ≤ 0.1 ID Probability ≥ 0.2	291 127	11 0

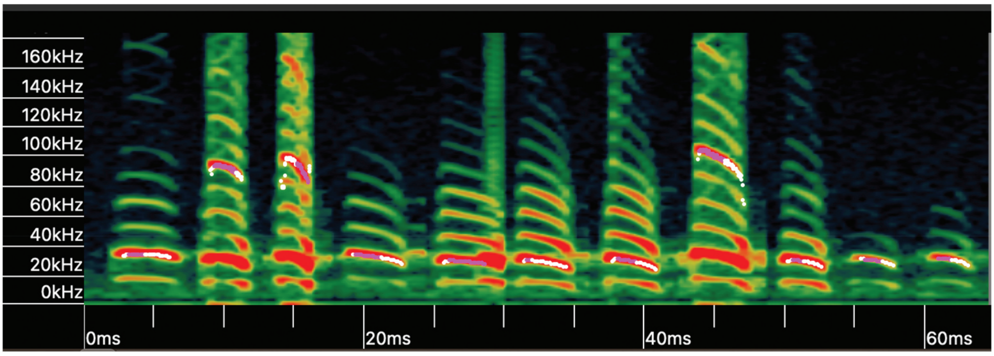


Figure 5. Acoustic call of a bat of the Emballonuridae family (probably *Coleura afra*) recorded by the Boundou acoustic recorder (20 September 2021 at 23:05). Visualised with Kaleidoscope (*Wildlife Acoustics*).

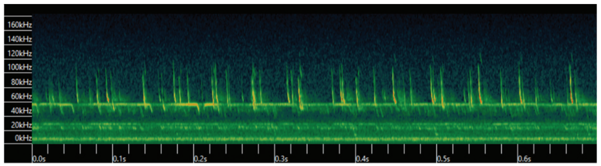
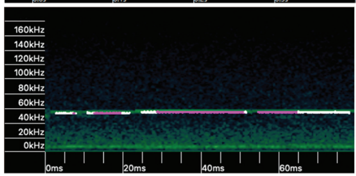
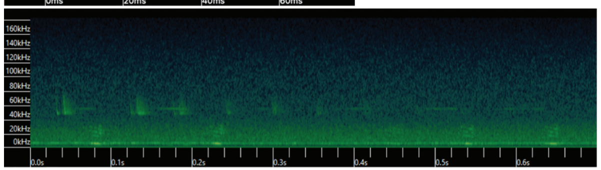
Discussion

This study is the first in Africa and one of the first worldwide to apply a sonotype classifier in a country without any species classifier. We were able to accurately identify the presence of cave-dwelling insectivores of interest (captured species) at our study sites using a universal sonotype classifier. The applied semi-automatic method with *a posteriori* classification allows to analyse a large amount of data while overcoming most of the problems of overlapping acoustic characteristics of different species.

With the exception of Europe and North America, acoustic studies on bats are still scarce, especially in tropical countries despite their high bat diversity (Walters et al. 2013). This study improves the acoustic knowledge of cave-dwelling bats in Central Africa thanks to the recording of 145 reference sequences. These data will greatly facilitate future acoustic studies in Central Africa and strengthen the capacity to identify bats in Africa.

The applied semi-automatic method can help researchers to quickly identify the bat species present in a given habitat while testing ecological hypotheses. Two challenges had to be overcome to implement this method. First, it was necessary to record acoustic reference calls. To tackle this first challenge, we had neither a morphological identification key for local bat species, nor acoustic reference data from a recent complete inventory of species present in the Republic of Congo. In addition, the scientific community has not yet reached a consensus on a reference acoustic recording method that can achieve interference-free sound quality while considering individual well-being. To overcome this challenge, we produced reference recordings using three different methods (free flight in a large room during the day, recording during the release of the individual and in the pouch) in order to maximise the quality of the acoustic recordings. In our study, the best reference acoustic recording quality (without the presence of other bats and the absence of ancillary noise) was achieved using the method of recording bats during flight in a large room. The advantage of this method was that it replicated the

Table 6. Example of errors detected in the Tadarida classifier.

Example of a Sonogram	Description of the problem identified	Example of consequences in the Tadarida classification
	Presence of several sonotypes at the same time, some of which intersect or overlap.	One out of the two sonotypes is identified and the main mode is the mean of both sonotypes
	Acoustic call of a *Rhinolophus* bat of poor quality because only the constant frequency (Fc) is present.	The Tadarida classifier did detect the calls, but they were categorised as other mammal instead of FMu – CF - FMd.
	Some recorded acoustic calls may be of poor quality (e.g. incomplete sonotype).	The Tadarida classifier is able to detect them, but their categorisation in the different sonotypes may be erroneous or complex due to the absence of part of the acoustic call.

conditions of acoustic calls in a semi-open environment that bats experience when emerging from caves, while limiting the presence of other acoustic calls.

The second challenge was to limit the number of errors in the automatic classifier for bat species. Even with manual identification, it was still difficult to classify certain species using acoustics only. For example, it was difficult to differentiate the two *Miniopterus* species using the automatic classifier because of overlaps in acoustic characteristics. This problem has also been highlighted in other studies involving the family Vespertilionidae (Russo and Voigt 2016; Zamora-Gutierrez et al. 2016). We decided to use two processes to limit identification errors. Firstly, the data was analysed according to acoustic species or groups when there was a doubt. This notion of acoustic group is closely related to the notion of acoustic guild that was defined by Denzinger and Schnitzler (2013) such as 'a group of bat species with similar acoustic characteristics and wing morphology adapted to a dominant foraging behavior'. In our case, the two *Miniopterus* species belong to the same guild. The second process used to limit identification errors was *a posteriori* classification to bring the acoustic groups together and thus limit classification errors. We then carried out a multi-criteria sorting for each of these species or groups, defining the *a priori* acoustic criteria and checking the effect of their introduction *a posteriori*. For example, we initially sorted for *Hipposideros cf. caffer* acoustics calls by sonotype type and acoustic frequency only. However, this did not prevent us from obtaining *Rhinolophus* acoustic calls in this selection. We therefore refined our selection by excluding harmonics to obtain a better result. We obtained accuracies similar to those found in other studies (>80%), although our results varied between acoustic groups (MacSwiney et al. 2008; Britzke et al. 2013; Zamora-Gutierrez et al. 2016; López-Baucells et al. 2019; Chen et al. 2020; Yoh et al. 2022). This back-and-forth between raw data, *a priori* and *a posteriori* classification enabled us to obtain an identification performance of over 98% for most species or acoustic groups. This back-and-forth process is similar to the last steps of developing a regional classifier from the start, with the difference being in the first steps, where there is no need to gather a complete collection of reference sounds. This back-and-forth process is time demanding, although it saves time once the *a posteriori* classification criteria are defined, and this should be considered when applied to other studies.

We were unable to identify all bat species down to species level. However, the use of sonotypes may be sufficient to answer ecological questions such as activity patterns or habitat use, especially if the group can be considered as a guild. In future studies, the Tadarida sonotype classifier could be improved (i.e. distinction between CF-FMd/FMu-CF-FMd, see) by increasing the species reference database, e.g. reference sounds with different noises and species or greater variation in reference sound quality. In this way, we could refine the accuracy of the classifier and use Tadarida outputs with additional acoustic criteria such as the duration (Dur) of each acoustic call, which can help to differentiate species (e.g. *Rhinolophus* or *Coleura afra).*

Tadarida is an open-source software package associated with a reference acoustic database of sonotypes of many bat families from around the world, which can be enhanced with additional data, as we did in this study, contributing to more open, accessible and reproducible research (Hampton et al. 2013). Even though the sonotypes classifiers are open, the reference database used to build the classifier is unfortunately not freely available to the research community. However, Tadarida's reference files are labelled using software that currently only runs on the Windows operating system. On the other hand, the classifier analyses are based on R scripts that can be used on any operating system. Yoh et al. (2022) published both their classifier and reference database, but so far these are limited to a restricted geographical area. Compared to the classifier of Yoh et al. (2022), Tadarida offers an additional functionality that was not exploited in this study, as it can also detect bat buzzes, identify other animal species (birds, insects) and human noises. Therefore, Tadarida software offers flexibility for a wide variety of studies.

Our study was focused on cave roosts. Acoustic recordings at populated bat roosts are a challenge because during peak bat emergence periods several hundreds of individuals may emerge from the cave at the same time. Overlapping acoustic calls from different species interfered with the automatic identification of sonotypes by Tadarida and distorted the measurement of frequencies, both used to separate species from one another. Nonetheless, this problem was limited to periods of high species abundance (e.g. breeding season or peak emergence periods common to all species). Despite this, the use of passive acoustic recording monitoring at roost exits allows valuable data to be collected to study activity patterns, the presence and absence of certain species, or the impact of human disturbance, while limiting disturbance to bat populations. These disturbances can have a major impact on bat colonies, particularly on the roosts where females give birth. In addition, the detection by the classifier of the regular presence of *Coleura afra* in the Boundou Cave underlines the importance of combining acoustics and capture (Appel et al. 2021). Acoustics is more sensitive and allows for detection of rare or elusive species (Silva and Bernard 2017; Appel et al. 2021; Carvalho et al. 2023).

Conclusion

We present a 'step by step process' to support researchers studying the acoustic communities of species in geographical areas where species classifiers do not exist. It can be applied and replicated worldwide by adapting the method to species complexes present in other study areas. The widespread use and automation of acoustic monitoring methods will help to improve ecological knowledge of chiropterans and contribute to their conservation.

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Disclosure statement

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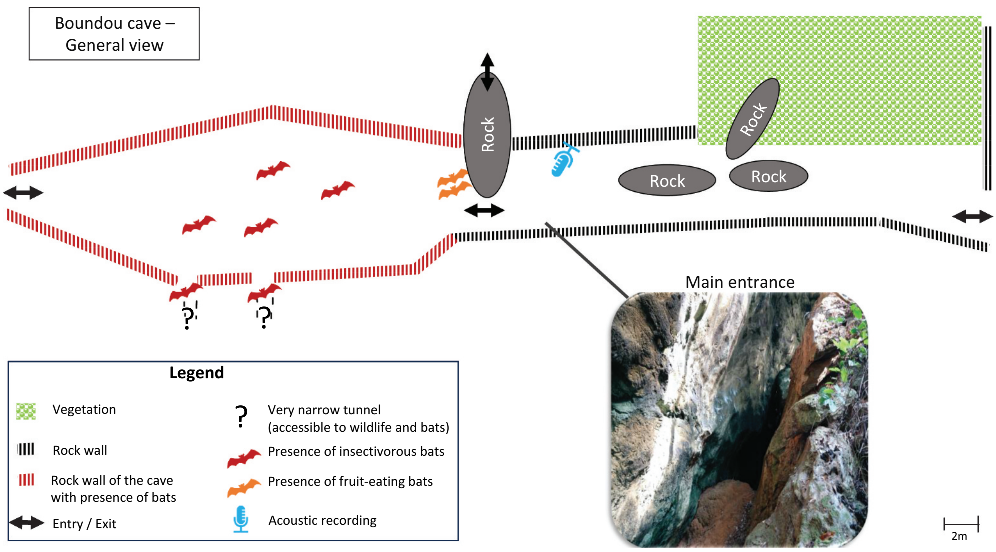
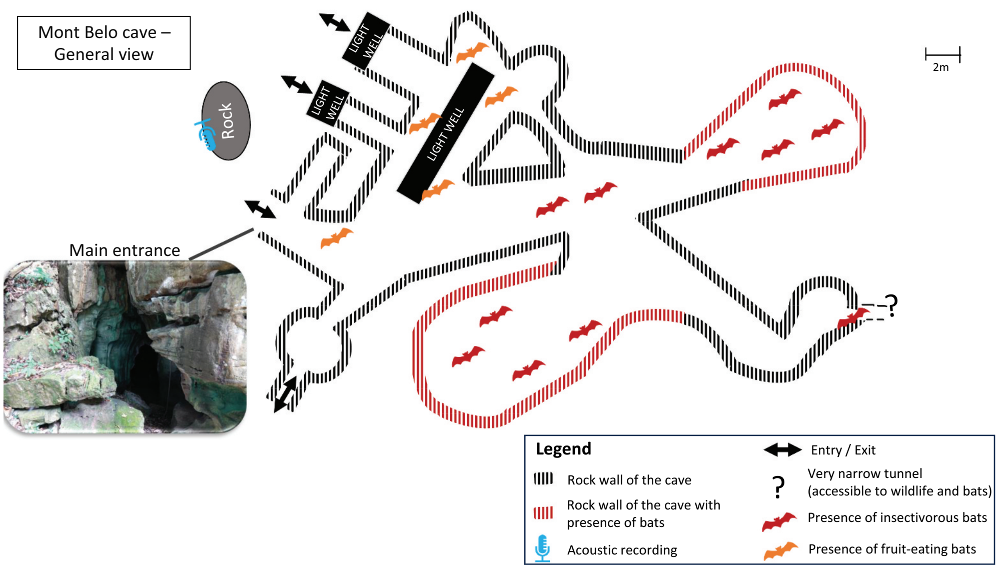
Appendices

Appendix A. Diagram of Boundou cave topography with location of acoustic recorder and bats



Appendix B. Diagram of Mont Belo Cave topography with location of acoustic recorder and bats



Appendix C

Morphometric data of individual captured at Mont Belo Cave and Boundou Cave in Republic of Congo https://doi.org/10.18167/DVN1/M2J0TY.

Appendix D

Download link for the classifier database used in this study https://doi.org/10.5281/zenodo.14678783.

Appendix E

Presence of various insectivorous species in the two study caves (Mont Belo and Boundou).

Capture sessions	Sites	*Triaenops sp*	*Hipposideros cf caffer*	*Rhinolophus cf denti*	*Rhinolophus cf landeri_alcyone*	*Miniopterus cf minor*	*Miniopterus cf inflatus*	*Macronycteris sp*	*Coleura afra*
18 September 2021	Boundou Cave	♀ ♂	♀ ♂			♀ ♂			
	Mont Belo Cave	-*	-*	-*	-*	-*	-*	-*	-*
18–19 October 2021	Boundou Cave	♀ ♂	♀ ♂	♀		♀ ♂			
	Mont Belo Cave		♂	♀ ♂	♀ ♂	♂	♀ ♂		
3–4 December 2021	Boundou Cave	♀ ♂	♀ ♂	♂	♂	♂		♂	
	Mont Belo Cave		♀ ♂		♀ ♂	♂	♂		
5–6 February 2022	Boundou Cave	♀ ♂	♀ ♂		♂	♀ ♂			
	Mont Belo Cave		♀ ♂	♂	♀ ♂	♀	♂		
3–4 April 2022	Boundou Cave	♀ ♂	♀ ♂	♀		♀ ♂	♂	♂	♂
	Mont Belo Cave		♀ ♂	♂	♀ ♂		♀ ♂		
8–9 June 2022	Boundou Cave	♀ ♂	♀ ♂			♂	♀ ♂		
	Mont Belo Cave		♀ ♂		♀ ♂	♀ ♂	♀ ♂		
12–13 September 2022	Boundou Cave	♀ ♂	♀ ♂			♀ ♂			
	Mont Belo Cave		♂	♀ ♂	♀ ♂	♀ ♂			
19–22 October 2022	Boundou Cave	♀	♀ ♂	♀	♀	♀ ♂			
	Mont Belo Cave		♀ ♂	♂	♀ ♂	♂	♀ ♂		
29–30 November 2022	Boundou Cave	♀ ♂	♀ ♂	♀ ♂	♀			♀	
	Mont Belo Cave		♂	♀	♀ ♂	♀	♂		
5–6 January 2023	Boundou Cave	♀ ♂	♀ ♂	♀		♀ ♂			
	Mont Belo Cave		♀ ♂	♀	♀ ♂	♀	♂		
7–8 March 2023	Boundou Cave	♀ ♂	♀ ♂	♀		♀ ♂			
	Mont Belo Cave		♀ ♂	♂	♀ ♂	♀ ♂	♂		

*No capture at Mont Belo Cave due to the presence of a slash-and-burn farming in the area around the cave ♀ ♂: capture of males and females; ♀: capture of females only, ♂: capture of males only, grey box: absence of this species in the captures.

Appendix F

Number of raw acoustic files collected over the 19-month period at the two study sites (Boundou Cave and Mont Belo Cave)

Month and year	Boundou Cave (number of files and period of collect)	Mont Belo Cave (number of files and period of collect)	Comments
September 2021	78,554 files (from 20/09 to 19/10)	68,962 files (from 19/09 to 6/10)	
October 2021	71,901 (From 19/10 to 10/11	66,420 files (From 20/10 to 06/11	
November 2021	12,222 (from 11/11 to 14/11)	26,007 files (from 11/11 to 19/11)	Battery problem in both recorders
December 2021	15,354 files (From 5/12 to 7/12)	58,381 files (From 5/12 to 19/12)	Battery problem in both recorders
January 2022	39,128 files (From 10/01 to 17/01)	389 files (From 5/01 to 5/01)	Battery problem in both recorders
February 2022	90,352 files (From 7/02 to 2/03)	80,044 files (From 7/02 to 2/03)	
March 2022	36,987 files (From 2/03 to 9/03)	30,954 files (From 2/03 to 10/03)	
April 2022	98,654 files (From 6/04 to 8/05)	55,315 files (From 6/04 to 8/05)	
May 2022	1,08,758 files (From 8/05 to 8/06)	1,05,300 files (From 8/05 to 8/06)	
June 2022	94,552 files (From 9/06 to 2/07)	89,080 files (From 10/06 to 3/07)	
July 2022	1,02,927 files (From 3/07 to 29/07)	72,890 files (From 3/07 to 13/08)	
August 2022	85,502 files (From 13/08 to 2/09)	75,754 files (From 13/08 to 3/09)	
September 2022	74,995 files (From 12/09 to 7/10)	1,28,880 files (From 14/09 to 13/10)	
October 2022	60,076 files (From 21/10 to 5/11)	85,781 files (From 22/10 to 15/11)	
November 2022	23,233 files (From 30/11 to 8/12)	59,527 files (From 29/11 to 18/12)	
December 2022	49,949 files (From 21/12 to 2/01)	61,355 files (From 18/12 to 5/01)	
January 2023	42,850 files (From 6/01 to 10/01)	39,357 files (From 5/01 to 15/01)	
February 2023	25,941 files (From 5/02 to 17/02)	75,895 files (From 5/02 to 26/02)	
March 2023	45,674 files (From 27/02 to 9/03)	38,056 files (From 26/02 to 9/03)	Only few days in March 2023
Total	1,157,609 files	1,218,347 files	2,375,956 files

Appendix G

Acoustic parameters calculated for bats captured in the Republic of Congo
https://doi.org/10.18167/DVN1/FC61JQ