UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
Instituto de Biociências
Programa de Pós-Graduação emEcologia

Dissertação de Mestrado

The use of automated acoustic identification software for bat surveys in the Neotropics: Gaps and Opportunities

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“To Mali, Amma and Acha”

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Resumo

As populações de morcegos são conhecidas por serem afetadas por atividades antropogênicas,njá que os Chiroptera é um grupo extremamente diverso que ocupa quase todos os nichos disponíveis no meio terrestre. Assim, os morcegos são considerados bons bioindicadores para monitorar mudanças no meio ambiente, mas seu valor como tal também depende da facilidade de monitorar e detectar tendências demográficas em suas populações. O interesse a longo prazo dos pesquisadores na acústica dos morcegos resulta do fato de que é um método não-invasivo e eficiente em termos de tempo para monitorar os padrões espaço-temporais da diversidade e atividade de morcegos. A análise dos sons emitidos pelos organismos tem sido útil para a aquisição de conhecimento sobre as interações bióticas e abióticas específicas de cada espécie, e sua aplicação na conservação. Além das identificações manuais de chamados de morcegos, existe atualmente no mercado um conjunto de programas automatizados de identificação que utilizam bibliotecas regionais e se apresentam como uma ferramenta eficiente no monitoramento de populações de morcegos. A maioria desses programas não foi validada usando dados de campo. Este estudo avalia a confiabilidade de dois softwares automatizados, SonoChiro e Kaleidoscope Pro, em comparação com identificações manuais de dados de campo coletados da região Neotropical. Houve um baixo nível de concordância entre os dois métodos automatizados ao nível das identificações específicas, razoável ao nível do gênero e satisfatório ao nível a família. Houve também uma diferença significativa entre a proporção de chamados corretamente identificados entre os dois programas ao nível específico. Os principais desafios para o uso de software de identificação automatizada incluem a necessidade de bibliotecas de chamados abrangentes da diversidade existente nas regiões em foco dos estudos; as principais oportunidades, por outro lado, incluem a ampla possibilidade de monitorar os padrões espaço-temporais da atividade de morcegos. Existem ainda fortes lacunas que impedem uma aplicação generalizada de programas automatizados em estudos ecológicos e de conservação de morcegos, mas há potencial de melhoria. Considerando as limitações dos programas automatizados, é discutida uma estrutura para aplicação em estudos ecológicos e de conservação.

Palavras-chave: Bioacústica; Chiroptera; Kaleidoscope; SonoChiro.

Abstract

Bat populations are known to be affected by anthropogenic activities because bats are an extremely diverse group occupying almost all available niches in terrestrial environment. Hence, bats are considered bioindicators to monitor changes in the environment, but their value as such also depends on the ease to monitor and detect demographic trends in their populations. The long term interest of researchers in the acoustic of bats results from the fact that it is a non-invasive, time-efficient methods to monitor spatiotemporal patterns of bat diversity and activity.The analysis of sounds emitted by organisms has been considered useful to gain insight into species-specific biotic and abiotic interactions, which can further be applied to conservation. Besides manual identifications of bat calls, a number of automated species identification programs using regional call classfiers have been introduced into the market as an efficient tool in monitoring of bat populations. Most of these programs have not been validated using field data. This study evaluates the reliability of two automated softwares, SonoChiro and Kaleidoscope Pro, in comparison to manual identifications of field data collected from the Neotropical region. There was low agreement between the two automated methods at the species level, fair agreement at the genus level and moderate agreement at the family level. There was also a significant difference between the proportion of correctly identified calls of the two-automated software at the species level identifications. Major challenges for using automated identification software include the need for comprehensive call libraries of the regions under scope; major opportunities, on the other hand, include the widespread possibility to monitor spatiotemporal patterns of bat activity. Overall, there are serious gaps that preclude a widespread application of automated programs in ecological and conservation studies of bats, but there is a potential for improvement. Considering the limitations of the automated programs, a framework for application in ecological and conservation studies is discussed.

Keywords: Bioacoustics; Chiroptera; Kaleidoscope; SonoChiro.

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Figure 2. Stacked bar chart showing the level of agreement for species (κ=0.145, 23 agree, 579 disagree), genus (κ=0.326, 89 agree, 513 disagree) and family level (κ=0.456, 285 agree, 317 disagree). The y-axis represents the number of files analyzed.

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General Introduction

Conservation in the Neotropical region

The Neotropical region, which includes the tropical terrestrial ecoregions of America and the entire South American temperate zone, harbors a very high diversity of plants, mammals, birds, reptiles, amphibians and insects (Ceballos and Garcia 1995). The ecosystems of the Neotropics are responsible for essential goods and services (Calvo-Rodriguez et al. 2016). Conservation of the neotropical biodiversity is prudent because it has been and continues to be subjected to anthropogenic activities leading to deforestation, agricultural intensification, habitat fragmentation, introduction of invasive species, pollution and climate change (Ratter et al. 1997; Klink and Machado 2005; Nogueira et al. 2011; Escribano-Avila et al. 2017). Conservation measures and policies have traditionally been implemented only once information about different groups of species and specific threats are gathered (Cuarón 2000). Land management decisions should only be taken with reliable information about population trends and habitat use. However, there is an evident lack of data about species of the Neotropics. Anthropogenic activities are not going to be adjourned, nor are their impacts going to be effectively minimized, until researchers can compile sufficient information on those aspects of biological diversity. Therefore, efficient ways to monitor and predict changes in ecosystem and species populations must be undertaken immediately by using bioindicator species (Jones et al. 2009).

Bats as bioindicators of the qualitative status of an ecosystem

Bioindicators are usually defined as a species or group of species whose behavioral and population changes can provide information about the qualitative status of an ecosystem (Fränzle 2006), due to their moderate tolerance to environmental variability. Rare and sensitive or extremely ubiquitous and tolerant species or assemblages have less value as bioindicators. This is because rare species (or assemblages) with narrow tolerances are often too sensitive to environmental changes and are difficult to detect, while ubiquitous species (or assemblages) with broad tolerances are slightly insensitive to these changes that may disrupt the functioning of the rest of the community.

In the Neotropical region, bats are a highly diverse group of species occupying a wide range of ecological niches and accounting for over 40% of the mammal species present (Medellín et al. 2000). They have been considered good bioindicators (Jones et al. 2009; Stahlschmidt and Brühl 2012). Firstly, they provide certain ecosystem services such as pest control, plant pollination and seed dispersal, which benefits the agricultural industry directly and is essential to maintain functioning forests (Jones et al. 2009; Heer et al. 2015; Stathopoulos et al. 2017). Bats occupy higher trophic levels which makes them prone to bioaccumulation of toxic substances and further decline in abundance (Jones et al. 2009). Some bat populations decline rapidly in the presence of a wide range of stressors related to climate change, water pollution, agricultural intensification, habitat fragmentation, diseases, pesticide and wind energy farms (Jones et al. 2009; Adams and Pedersen 2013; Wordley et al. 2017; Bernard and Mccracken 2017; Frick et al. 2017); others are more tolerant (Law et al. 1999). Extensive research has been done to understand the behavior (Fenton 1986; Ulanovsky et al. 2004; Ahlén et al. 2009; Furmankiewicz and Kucharska 2009; Marques et al. 2016), distribution (Fenton et al. 1987; Moratelli and Wilson 2013; Michaelsen 2016), abundance (Fenton et al. 1987; Heer et al. 2015) and community assemblages of bats (Kalko and Handley 2001; Ramos Pereira et al. 2009; Mendes et al. 2014). Even though there is plenty of research on bats in the Neotropics, there is insufficient data available and the conservation status for many species is still unknown (Paglia et al. 2012). Besides a few studies, there is no evidence of long term monitoring of Neotropical bats (Faria et al. 2006; Bernard et al. 2011). On the other hand, Europe has one of the best monitoring programs for bats but accounts for only 40 species (Adams and Pedersen 2013; Barova and Streit 2014). More research efforts have to be applied to build databases for the Neotropical region, where there is 4 times the number of species in Europe (Medellín et al. 2000).

Monitoring bat diversity

A wide range of approaches have been used to measure biological diversity which extends from counts of species richness to functional diversity of species (Mendes et al. 2014; González-Maya et al. 2017). Bats have been monitored in the past using capture methods such as mist nests and harp traps, surveying of roost sites, radio telemetry and visual observations (Kalko and Handley 2001; Bernard and Fenton 2003; Zortéa and Alho 2008; Furmankiewicz and Kucharska 2009). These methods have been criticized, on their own, as they are unable to account for all species present in the area (O’Farrell and Gannon 1999). Hence, acoustic surveys have gained a lot of interest in the recent years and are being used currently to monitor bats globally. Acoustic methods can record high flying bats species which are not caught in nets and species with cryptic roosts that are not accounted for in roost site surveys (Miller 2001). Acoustic methods are particularly complete in regions where bat diversity is mostly comprised of aerial insectivore bats, easily detected by ultrasound detector machines (O’Farrell and Gannon 1999). In addition, acoustic methods are non-invasive, cheaper and time efficient method, may be used to survey areas where the continuous access is difficult or limited, and are not necessarily constrained by bad weather conditions (Skalak et al. 2012; Peri 2017) .

Bioacoustics

Acoustics forms the backbone of many animal societies as this is the way they communicate and exchange information amongst themselves (Laiolo 2010; Towsey et al. 2014a). Many years of research in animal sounds has led to the compilation of extensive and detailed information in the field of bioacoustics. Bioacoustics is defined as the study of emission, propagation and reception of sounds by animals (Sueur et al. 2014; Towsey et al. 2014a). Acoustics of birds, bats, marine mammals, anurans, reptiles, insects and even plants have been extensively explored in the last decades (Zimmerman 1983; Kunz et al. 1996; Au and Nachtigall 1997; Towsey et al. 2014a; Mishra et al. 2016). Acoustic studies have mostly been species-centered, exploring the acoustic interactions of an individual at the group or population levels (Sueur et al. 2014; Towsey et al. 2014a). Species that use acoustics for communication as a part of a larger and more complex structures like assemblages, communities, landscapes or ecosystems are constantly interacting vocally with their surroundings, their conspecifics and other species. These interactions have been incorporated in the field of soundscape ecology, where all biologically produced sounds are called biophony along with geophony (sounds produced geophysically) and anthrophony (sounds produced by humans) (Pijanowski et al. 2011). As such, bioacoustics is an interdisciplinary field of study with links to ethology, physiology, evolution and many other fields of biology (Ahlén et al. 2009; Fenton et al. 2012; Adams and Pedersen 2013; Jung et al. 2014).

The use of acoustics by animals to interact with their conspecifics and their environment can be a great insight into their behavioral ecology. The constant changes and disturbances caused by anthropogenic or natural events have led to the evolution of species vocalization in order to adapt (Endler 1993). Acoustic information has been able to provide warning indicators of anthropogenic perturbation effecting the individual fitness and population persistence of a species (Laiolo 2010). Furthermore, diversity indices derived from acoustics have been used in biodiversity assessment and monitoring (Sueur et al. 2014).

Recording and analysis of bat sounds

Almost 80% of total bat species are known to emit ultrasonic pulses, which can be recorded using bat detectors. Bat detectors are able to convert the ultrasonic pulses to audible recordings (Adams and Pedersen 2013) and were initially used by researchers to carry out transect surveys (Sattler et al. 2007; Furmankiewicz and Kucharska 2009). Currently, these bat detectors can save thousands of recorded sound files to be analyzed later. Sound files are then converted into spectrograms using acoustic software, making it possible to visualize the pulses of the calls and measure some characteristic parameters. These parameters are then used to either identify the species manually or automatically. Technological advances have led to the introduction of various such acoustic software that can classify/identify bat calls automatically.

Objectives

The general objective of this dissertation is to evaluate the use of automated acoustic software to identify bat species of the Neotropical region and to discuss any gaps. Ultimately, we will also explore some of the opportunities of these programs to be used in ecological and conservation studies.

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Are automated acoustic identification software reliable for bat surveys in the Neotropical region?

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To be submitted to *Bioacoustics* (http://www.bioacoustics.info)

Abstract

Bat populations are known to be affected by anthropogenic activities because bats are an extremely diverse group occupying almost all available niches in terrestrial environment. Hence, bats are considered bioindicators to monitor changes in the environment, but their value as such also depends on the ease to monitor and detect demographic trends in their populations. The long term interest of researchers in the acoustic of bats results from the fact that it is a non-invasive, time-efficient methods to monitor spatiotemporal patterns of bat diversity and activity.The analysis of sounds emitted by organisms has been considered useful to gain insight into species-specific biotic and abiotic interactions, which can further be applied to conservation. Besides manual identifications of bat calls, a number of automated species identification programs using regional call classfiers have been introduced into the market as an efficient tool in monitoring of bat populations. Most of these programs have not been validated using field data. This study evaluates the reliability of two automated softwares, SonoChiro and Kaleidoscope Pro, in comparison to manual identifications of field data collected from the Neotropical region. There was low agreement between the two automated methods at the species level, fair agreement at the genus level and moderate agreement at the family level. There was also a significant difference between the proportions of correctly identified calls of the two-automated software at the species level identifications. Major challenges for using automated identification software include the need for comprehensive call libraries of the regions under scope; major opportunities, on the other hand, include the widespread possibility to monitor spatiotemporal patterns of bat activity. Overall, there are serious gaps that preclude a widespread application of automated programs ecological and conservation studies of bats but it has the potential to serve as an effective tool.

Keywords: Bioacoustics; Chiroptera; Kaleidoscope; SonoChiro.

Introduction

Most bat species produce ultrasound for orientation, navigation and hunting prey (Adams and Pedersen 2013). Bats emit a signal (pulse) of a certain frequency and then perceive the reflected signal (echo) which returns after hitting a target or surrounding objects in the environment (Schnitzler and Kalko 2001; Fenton 2003; Adams and Pedersen 2013). These ultrasounds produced by bats are known as echolocation calls and have co-evolved over time depending on various ecological and physical factors (Murray et al. 2001; Obrist et al. 2007). When hunting for prey, bat echolocation calls are characterized by three phases: search phase, approximation phase and terminal buzz phase (Murray et al. 2001). Echolocating bats use tonal signals with structured changes in frequency over time ranging between 8 and 200kHz (Fenton 2003; Adams and Pedersen 2013). Bats also produce social calls when mating, foraging, and during distress, aggression and mother-offspring interactions (Wilkinson and Boughman 1998; Fenton 2003; Budenz et al. 2009; Furmankiewicz et al. 2011). Echolocation and social calls are species- specific and, in some cases, even colony-specific (Fenton 2003).

It is possible to visualize the calls with time-expanded recordings on a spectrogram created using acoustic software (Towsey et al. 2014b).The search phase calls, compared to feeding buzzes, are better to describe because they have longer intervals between pulses and longer duration (Murray et al. 2001; Fenton 2003). Feeding buzzes are formed by high pulse repetition when a target is perceived.

Biologists characterize bat calls using parameters of the pulse and further use it to identify the calls to species level. A bat call is composed of a sequence of pulses and the structure of each pulse is determined by frequency modulation (FM) or increase/decrease of frequency over time. Pulses could be composed of either upward frequency modulation (FM_u), downward frequency modulation (FM_d), constant frequency (CF) and quasi-constant frequency (qCF) or a combination of these (Suga 1990). For example, species of the Phyllostomidae have calls with steep frequency modulation (stFM) because they call at high frequencies between 100 and 180kHz with short pulse duration (Arias-Aguilar 2017). The Molossidae show high call plasticity, with some species showing two or three different types of calls with similar or differing structures (Jung et al. 2014). In general, call structure (FM, CF, qCF) gives family level information but more parameters are usually needed for further taxonomic identification.

Call harmonics refers to the different frequency levels of a call and multi-harmonic calls are characteristic of some families, genera or species (Hackett et al. 2016). The first harmonic is usually called the fundamental harmonic (HF). After that, there is second harmonic (H2), third harmonic (H3) and fourth harmonic (H4), which occur in the double frequency as the previous harmonic.

Other important call parameters in bat acoustic identification include: duration of the pulse (t), inter-pulse interval (IPI), frequency of maximum energy (FME), maximum frequency (F_{max}), minimum frequency (F_{min}) and bandwidth ($BW= F_{max}-F_{min}$) (**Figure 1**). The duty cycle of a call is defined as the ratio of inter-pulse interval to duration of pulse (IPI/t) (Fenton et al. 2012). In order to optimize sound transmission and exchange of information, some species are able to adopt strategies to avoid self-deafening (forward masking) by separating pulse and echo in time using low duty cycle (LDC) echolocation, or in frequency using high duty cycle (HDC) echolocation (Fenton et al. 2012; Adams and Pedersen 2013). HDC bats are specialized in detecting fluttering targets in cluttered environments and, to the present, was only found in the Old World bats of the Rhinolophidae and Hipposideridae and in the New World mormoopid, *Pteronotus parnellii* (**Figure 1**) (Jones and Teeling 2006; Schnitzler and Denzinger 2011; Fenton et al. 2012).

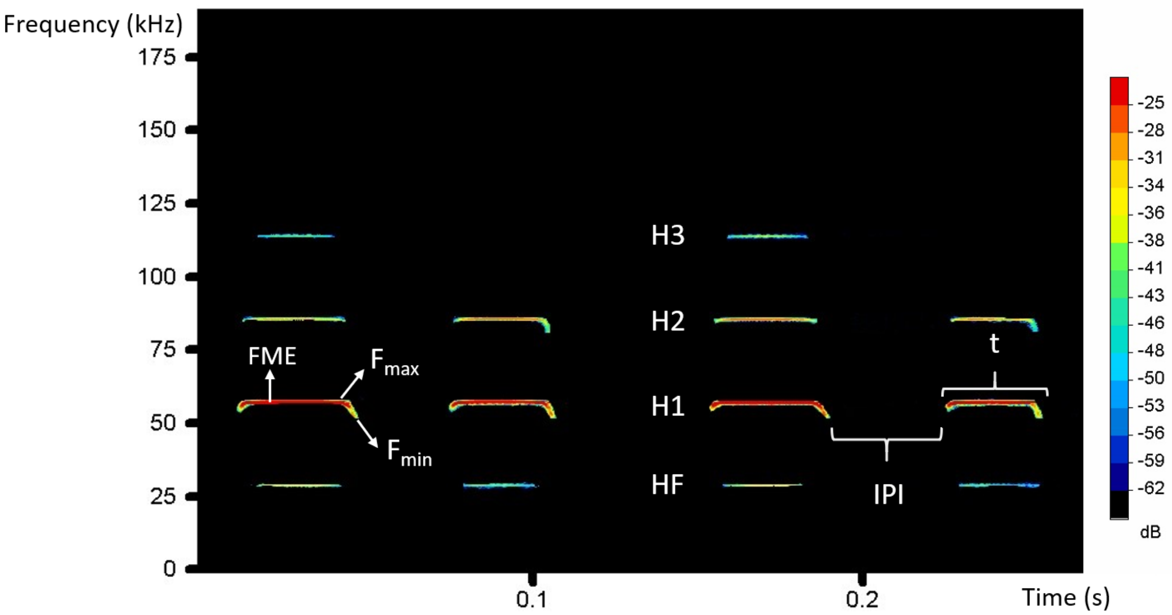

Figure 1. Typical spectrogram view of the echolocation call of *Pteronotus parnelli* where the y-axis is frequency in kilohertz and x-axis is time in seconds. The color scale represents the amplitude of sound in decibels (dB). The call parameters indicated are: maximum frequency (Fmax), minimum frequency (Fmin), frequency of maximum energy (FME), time duration (t), inter-pulse interval (IPI) and harmonics (HF, H2, H3, H4).

Bat acoustic monitoring

Bats are nocturnal mammals, difficult to catch and sensitive to anthropogenic intrusion which make them difficult to account for only using traditional capturing methods with mist nets or harp traps (MacSwiney et al. 2009; Russo and Voigt 2016). Acoustic monitoring has emerged as a non-invasive, time-efficient method which can be used to study spatiotemporal patterns of bat diversity and activity (Russo and Voigt 2016; Silva et al. 2017; Stathopoulos et al. 2017) and is not limited by inaccessible environments or bad weather conditions (Skalak et al. 2012; Marques et al. 2016). Acoustic monitoring have helped researchers gain knowledge about bat behavior, habitat preferences, foraging strategies, distribution, abundance, population trends and also about species that are difficult to capture (Miller and Degn 1981; Fenton et al. 1987; Vaughan et al. 1997; Verboom et al. 1999; Marques et al. 2016; Stathopoulos et al. 2017). Briones-Salas et al. (2013) found two new species of the Molossidae using acoustic methods to study the community composition of bats in Oaxaca, Mexico. Similarly, rare and common species never been captured by the traditional methods have been easily detected with acoustic monitoring (Fenton et al. 1987; Skalak et al. 2012), often leading to spectacular increases in the known distribution range of those species (e.g. *Promops centralis)* (Hintze et al. *in press*). Also using acoustic data records to build habitat suitability models for two cryptic species of the genus *Pipistrellus* in Switzerland, their conservation status was altered accordingly (Sattler et al. 2007). If earlier studies have focused mostly on extracting information about individual species (Miller and Degn 1981; Fenton at al. 1987; Barclay et al. 1999; Broders et al. 2004), the development of acoustic diversity indices allowed accounting for biodiversity at community, landscape and ecosystem levels (Vaughan et al. 1997; Parsons and Jones 2000; Adams et al. 2010; Briones-Salas et al. 2013; Mendes et al. 2014). These acoustic indices have been used to calculate evenness, richness, heterogeneity, abundance and activity of communities (Fenton at al. 1987; Miller 2001; Mendes et al. 2014; Sueur et al. 2014; Towsey et al. 2014a; Heer et al. 2015; Wimmer et al. 2010). Other studies have used acoustics data to record migratory behaviour of certain bat species in conjuncture with direct visual observations (Ahlén et al. 2009; Furmankiewicz and Kucharska 2009; Bernard and McCracken 2017). The use of acoustic surveys along with traditional capture methods and visual observations is encouraged to account for all bat species present in a region (O'Farrell and Gannon 1999; Wimmer et al. 2010; Skalak et al. 2012).

Identification methods of bat calls

Acoustic species identification done manually by experts using identification keys specific to an area is considered a reliable method but the problem arises with large data sets where identification becomes time consuming. The concept of automated species identification has been argued to have consistency, predictability, high levels of accuracy and measures of uncertainty (Jennings et al. 2008) which can be standardized over studies. The automated methods used in the past to quantify call parameters to classify animal calls include discriminant function analysis (Parsons and Jones 2000; Pfalzer and Kusch 2003; Broders et al. 2004; Preatoni et al. 2005; MacSwiney et al. 2009; Adams et al. 2010; Clement et al. 2014), cluster analysis (Preatoni et al. 2005), classification trees (Sattler et al. 2007), artificial neural networks (Preatoni et al. 2005; Jennings et al. 2008; Adams et al. 2010; Parsons and Jones 2000) and deep machine learning tools (Walters et al. 2012; Hackett et al. 2016). Jennings et al. (2008) compared identifications done manually with those of artificial neural networks (ANNs) and found that ANNs performed better than 75% of humans in the study. Walters et al. (2012) developed a continental-scale acoustic identification tool for European bats, which was confirmed to provide robust classification.

Using automated identification techniques on Neotropical bat species

The Neotropics show a very high diversity of bats with numerous gaps in knowledge about their ecology, behavior, acoustic classification and conservation status (Zortéa and Alho 2008; Adams and Pedersen 2013). Bats of this region, as well as other regions, are under threat due to changes caused by anthropogenic activities such as alteration of land-use, invasive species, air, water and noise pollution (Mendes and De Marco 2017). Therefore, the need for efficient and accurate species identification methods for larger areas has rapidly escalated and resulted in the availability of many automated software in the market. SonoChiro and Kaleidoscope are two such programs that have been used in previous studies for automated species identification with region specific call classifiers and careful speculation (Slough et al. 2014; Michaelsen 2016; Toffoli 2016). Even though, the producers of the software insist that the accuracy rates are high, researchers are aware of the inaccuracies and use manual identifications for certain species most of them have never actually been tested on field data (Russo and Voigt 2016). Lemen et al. (2015) used unidentified field data to compare the performance of 4 automated programs and found an average pair-wised agreement of 40%. More recently a study in Sweden showed poor performance of classifiers used by Kaleidoscope Pro and SonoChiro because the identifications were not reliable (Rydell et al. 2017).

The performance of such software has already been evaluated for temperate species, but the performance of the available Neotropical software and their respective classifiers has not been validated previously. The challenge of using automated identification for Neotropical species is that there is a lot of evidence showing inter and intraspecific variability of bat calls due to high species richness (Jones et al. 1992; Jones 1997; Barclay et al. 1999; Murray et al. 2001; Pfalzer and Kusch 2003; Broders et al. 2004; Russ et al. 2004; Jung et al. 2007; López-Baucells et al. 2017).

The aim of this study is to evaluate the reliability of two automated programs (SonoChiro and Kaleidoscope Pro) that are widely used for automated identifications, for Neotropical bat species. The agreement between the two automated and manual identifications for the same dataset was predicted to be low at species and genus level identification but not at the family level. Using the manual identifications as absolute true species, the second hypothesis was that there would be a difference in the proportion of correctly identified between the two-automated software. SonoChiro was predicted to perform better than Kaleidoscope because SonoChiro is able to give group (family and genera) and species level identifications separately while Kaleidoscope uses only species classifiers (Rydell et al. 2017).

Materials and methods

Field Collection

Our study species included eight out of nine families of Chiroptera found in Brazil, namely Emballonuridae, Furipteridae, Molossidae, Mormoopidae, Natalidae, Noctilionidae, Thyropteridae and Vespertilionidae. In Brazil, these families cover a total of 93 species (Arias-Aguilar et al. *submitted*), of at least 178 occurring in Brazil (Nogueira et al. 2014). The recordings were collected at two sites at 10 different sampling points at the National Park of Brasília in Federal district of Brasília, which is situated in the center of the Brazilian Cerrado.

The Cerrado is composed of woodlands, savannas, grasslands and dry forests and forms the second largest biome of Brazil (Klink & Machado 2005). The recording was made over two periods, August and September 2016, which correspond to the middle and the end of the dry season respectively. The SM2 Bat detector (Wildlife Acoustics, U.S.A; www.wildlifeacoustics.com) was used to record bat calls at the sites, without using any filter for the ambient noise.

Each recording had lasted four minutes. To carry out call analyses, the recordings had to be cut into 15-second intervals using Kaleidoscope, as the automatic identification software can only process files with a maximum duration of 15-seconds. A total of 49,783 WAVE files were extracted and again processed using the same software to filter out empty files. Finally, the remaining number of recordings added up to 3,465 15-second duration files.

Automated identification of recordings

For the automated identification, the 3,465 15-second duration files were analyzed using SonoChiro v.3.0 (Biotope, France www.biotope.fr) and Kaleidoscope Pro 3.14B (Wildlife Acoustics, U.S.A; www.wildlifeacoustics.com). The settings used were: for SonoChiro - type of recorder (SM2 Bat), region (Amazonian basin), time expansion (x1), maximum call duration (0.5), sensitivity (7), for Kaleidoscope Pro – filter noise files (keep noise files), signal of interest (8-120kHz, 2-500ms, minimum two calls), classifiers (Neotropical bats), (0 Neutral sensitivity). The sensitivity scale of SonoChiro ranges from 10 to 0 and that of Kaleidoscope is +1 to -1. They are calculated differently but essentially range between giving results for low quality pulses (more sensitive) and only high-quality pulses (more accurate). The output generated by the two automated programs is expected to show group and species level identifications. The identifications that may not be attempted result in “parasi” (SonoChiro), “no ID” or “Noise” (Kaleidoscope Pro).

Manual identification of recordings

The identifications were made manually on 44% of the recordings used for automated identifications (1506 WAVE files) using Avisoft SASLab Pro (Specht 2004). The spectrogram for each recording was created using the following parameters: FFT length (1024), frame size (100%), Overlap (87.5%) and Hamming window. The aforementioned parameters determine the frequency and time resolution of the pulse or sequence in the spectrogram. Frequencies below 10kHz were filtered out using noise filter for better identification. The recordings attempted to be manually identified required at least three clear pulses and any overlapping pulses were discarded to avoid any bias. The parameters that were observed and tabulated to identify the calls up to species level were: i) average call duration of at least three pulses; ii) number of harmonics and maximum energy harmonic; iii) number of call types; iv) pulse structure (FM, CF or qCF); v) frequency of maximum intensity (FME); vi) maximum frequency (Fmax); vii) minimum frequency (Fmin); viii) bandwidth (BW); and ix) inter-pulse interval (IPI) (**Figure 1**). Some additional parameters were measured when required, such as initial frequency (F_{intial}), end frequency (F_{end}) and individual parameters of different call types. The identification was done using an Illustrated identification key to the calls of Brazilian bats (Arias-Aguilar et al. *submitted*).

Statistical analysis

The data compiled for statistical analysis included family, genus and species level identifications for the automated programs (SonoChiro and Kaleidoscope Pro) and manual identifications. The agreement between the three sets of identifications for each of the levels (family, genus and species) was tested with Fleiss's kappa statistic (Dunn 1992) which is used to evaluate agreement between more than two methods. Further, the manual identifications were assumed as true identifications and the number of correctly identified recordings were recorded for each of the automated software. Overall difference in proportion of correctly identified files at each level (species, genus and family) between the two automated programs was computed using Chi-squared tests. True positives, false positives, true negatives and false negatives for each species were calculated for SonoChiro and Kaleidoscope Pro. True positives of each software were all the identifications of a species matched with manual identifications. False positives were those where the presence of species was identified incorrectly by the software while false negatives were those where the species was present but not perceived by the software. True negatives were calculated by accounting for all the recordings where other species were identified.

Results

A total of 643 and 274 WAVE files were not identified or did not have clear calls to identify by the automated programs and manually by an expert, respectively. Therefore, these were removed and the remaining 602 WAVE files were used for the further analyses.

Agreement between two automated and manual identifications

Following Dunn (1992) agreement level described as Poor if κ<0.00, Slight if 0.00 ≤ κ ≤ 0.20, Fair if 0.21 ≤ κ ≤ 0.40, Moderate if 0.41 ≤ κ ≤ 0.60, Substantial if 0.61 ≤ κ ≤ 0.80 and Almost perfect κ> 0.80, the Fleiss's kappa statistic value showed that there was low agreement between the three sets of identifications at the species level (κ=0.145), fair agreement at the genus level (κ=0.326) and moderate agreement at the family level (κ=0.456). The total number of recordings that were agreed on at the species, genus and family level was 23, 89 and 285 WAVE files respectively (**Figure 2**).

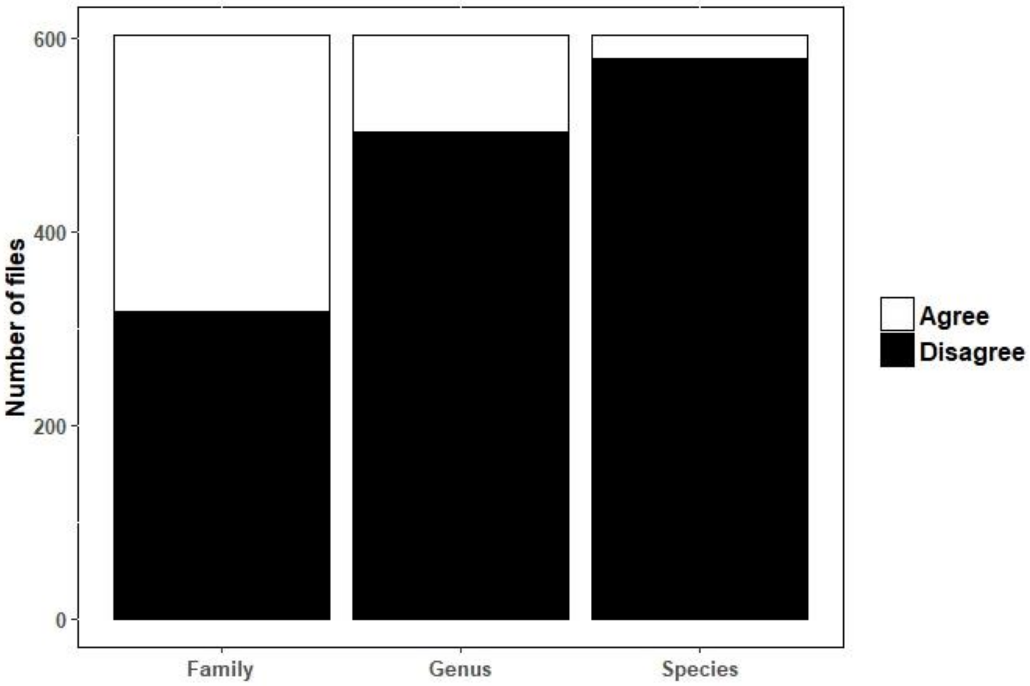

Figure 2. Stacked bar chart showing the level of agreement for species (κ=0.145, 23 agree, 579 disagree), genus (κ=0.326, 89 agree, 513 disagree) and family level (κ=0.456, 285 agree, 317 disagree). The y-axis represents the number of files analyzed.

Comparison of the proportion of correctly identified files

There was a significant difference between the proportion of correctly identified recordings by two automated programs at the species level (X^2 = 280.54, df =1, p = <2.2e-06) and family level (X^2 = 20.917, df =1, p = 4.796e-06) (**Figure 3**). The percentage of correctly identified species by SonoChiro and Kaleidoscope Pro was 5%. At the family level, 77% of the recordings were correctly identified by SonoChiro and 65% was correctly identified by Kaleidoscope Pro. There was no significant difference between the proportions of correctly identified files by the two automated programs at the genus level (X2 = 1.608, df =1, p >0.05). The percentage of correctly identified genera was 48% for SonoChiro and 52% for Kaleidoscope Pro.

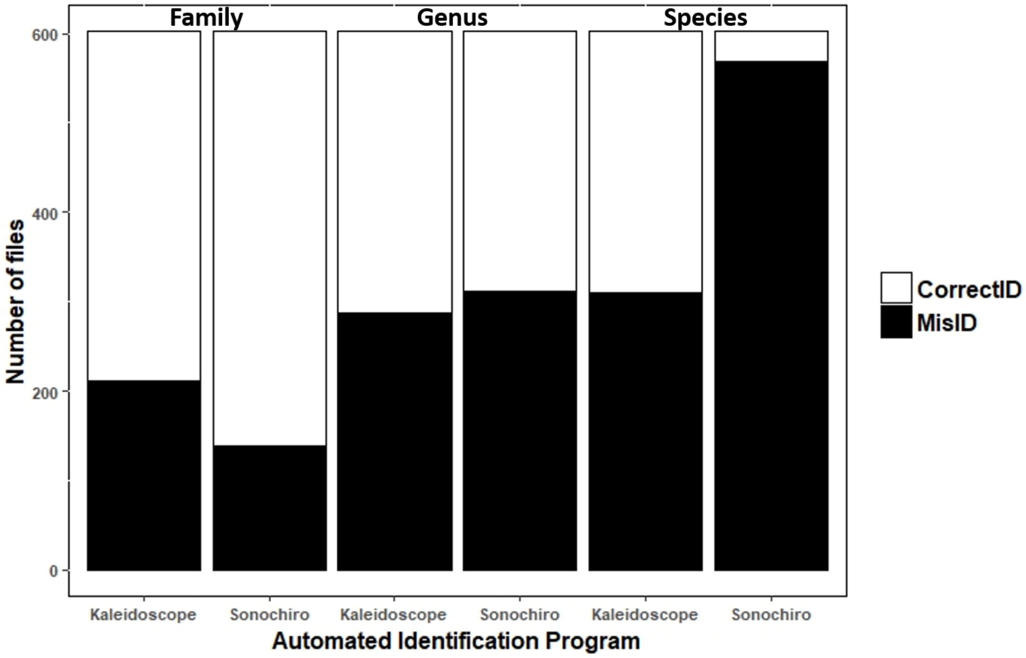

Figure 3. Stacked bar chart indicating the proportion of correctly identified files for Kaleidoscope (species= 48%, genus = 52%, family = 65%) and SonoChiro (species= 5%, genus=48%, family=77%).

They-axis shows the number of files and the x-axis is the two-automated software.and 48% respectively.

Correctly and misidentified species by automated software

In Table 1 is shown the number of true positives, false positives, true negative and false negatives calculated for each species manually identified from the 602 WAVE files: *Eptesicus brasiliensis*, *Eptesicus furnalis, Lasiurus blossevilli, Lasiurus ega*, *Molossos currentium, Molossus molossus, Molossops temminckii, Myotis lavali*, *Myotis nigricans, Myotis riparius, Peropteryx leucoptera/paldioptera,* *Peropteryx macrotis*, *Promops nasutus and Pteronotus parnellii*. The genera *Cynomops*, *Eumops*, *Nyctinomops* and *Tadarida* could not be manually identified to the species level because it has not been defined properly for this region. The species of genera *Myotis* and *Peropteryx* had no true positives for Kaleidoscope Pro but SonoChiro identified two out of five *Myotis riparius* and the only *Peropteryx macrotis* call correctly. *Eptesicus brasiliensis, Molossus currentium, Promops nasutus* and *Pteronotus parnellii* were misidentified by both programs. *Lasiurus ega* calls were identified correctly by Kaleidoscope Pro but not by SonoChiro in the two instances it was present. Most *Eptesicus furnalis calls* were identified correctly by SonoChiro (9 out of 10) and Kaleidoscope (7 out of 10) but they had 148 and 18 false positives respectively. Almost 88% of *Lasiurus blossevillii* calls were identified correctly by Kaleidoscope but none by SonoChiro. Species of Molossidae, *Molossus molossus* and *Molossops temminckii*, were identified correctly 80.5% and 84% of the time respectively. On the other hand, SonoChiro misidentified 80% *Molossus molossus* and all *Molossops temminckii calls.*

Table 1. True positives, false positives, true negatives and false negatives compared to the total number of manual identifications for each of the species.

	Total	**True positives**		**False positives**		**True negatives**		**False negatives**	
Species	**Manual**	**Kaleidoscope**	**SonoChiro**	**Kaleidoscope**	**SonoChiro**	**Kaleidoscope**	**SonoChiro**	**Kaleidoscope**	**SonoChiro**
Cynomops sp.	31	0	0	0	0	571	571	31	31
Eptesicus brasiliensis	4	0	0	0	0	598	598	4	4
Eptesicus furnalis	10	7	9	18	148	574	444	3	1
Lasiurus blossevillii	136	119	0	13	0	453	466	17	136
Lasiurus ega	2	2	0	0	0	600	600	0	2
Molossus currentium	4	0	0	0	0	598	598	4	4
Molossus molossus	103	83	21	8	3	491	496	20	82
Molossops temminckii	96	81	0	3	0	503	506	15	96
Myotis lavali	54	0	0	0	0	548	548	54	54
Myotis nigricans	11	0	0	0	28	591	563	11	11
Myotis riparius	5	0	2	0	32	597	565	5	3
Myotis sp.	1	0	0	0	0	601	601	1	1
Peropteryx leucoptera/paldioptera	15	0	0	0	0	587	587	15	15
Peropteryx macrotis	1	0	1	5	36	596	565	1	0
Promops nasutus	7	0	0	0	0	595	595	7	7
Pteronotus parenelli	25	0	0	0	0	577	577	25	25
Eumops/ Nyctinomops/ Tadarida sp.	97	0	0	0	0	505	505	97	97

Discussion

The low agreement between the three different methods, two automated and one manual, for species identification raises a concern about the reliability of automated species identification for bat monitoring and studies in the neotropics. Bats, unlike birds and other echolocating animals, alter certain parameters of their calls depending on their interaction with the environment or other species (Jones 1997; Kalko and Handley 2001; Chaverri et al. 2010). This would make it difficult to distinguish between individuals in species rich areas, such as the Neotropical region, where certain bats species might occupy similar niches and hence would have overlaps in call structures.

Classification methods

Lemen et al. (2015) suggested that the low levels of agreement between software could be because of recordings collected with different recording devices but in our study the call database was the same, and recorded using the same bat detector. This discrepancy could be attributed to the difference in sensitivity scale, classification method and the classifiers used by each of the methods. The sensitivity setting in the software allows researchers to manipulate the detectability of a call in the recording i.e. high sensitivity setting would detect even low quality pulses and low sensitivity setting would detect only high quality, clear pulses. Even though both the software were set at similar sensitivity, SonoChiro is able to detect more calls compared to Kaleidoscope Pro. In the presence of more than one species in one recording, SonoChiro has the ability to identify up to three species while Kaleidoscope identifies only what it perceives as the dominant call in the recording. Also, considering classification methods, SonoChiro detects any calls present on the recording and then classifies them using Random Forest classification method, which in this case uses active learning/ negative labelling (Bas et al. 2013). This method is supposed to have a powerful confidence index and can spot obvious errors in calls from diverse sources (Beard 2007; Cutler et al. 2007). On the other hand, the classification method of Kaleidoscope Pro uses error rates calculated from the confusion matrices of specific regional classifiers to determine the most likely distribution of the different species. The error rates for confusion matrices from different geographic regions and habitat types might be different leading to misidentifications (Agranat 2012). To reduce the misidentification rates, SonoChiro computes confidence levels for group and species level identification while Kaleidoscope is able to give possible alternative identifications for the data; both retrieve unknown classifications. Previously used automated identification methods were not able to provide confidence levels, alternative and unknown classifications; the lack of these variables might result in higher levels of misidentifications and has been criticized (Adams et al. 2010).

Reliable manual identifications are dependent on the level of expertise of the observer and the identification key used for species identification. There is a level of aptitude that can be acquired and applied, which allows the detection of certain patterns or variations when recordings are manually identified but this also adds an unquantifiable uncertainty in the identifications (Jennings et al. 2008; Rydell et al. 2017). An advantage of using automated identifications is that the results can be combined and a quantifiable uncertainty can be accounted for by using statistical methods (Russo and Voigt 2016).

Intraspecific variation and interspecific overlap

Although, SonoChiro showed discrepancies when compared to manual identification, there was a gradual improvement from species to genus to family level identifications. Kaleidoscope could correctly identify more species than SonoChiro but it only gives species level identification with no confidence indices. Therefore, SonoChiro might be at a better advantage as it is able to identify certain individuals at least up to the genus level. This information can be useful to survey and monitor specific focal genera (Rydell et al. 2017). At the species level, there were some species correctly identified by one or the other software but only *Eptesicus furnalis* and some *Molossus molossus* calls were correctly identified by both. *Eptesicus furnalis* was often misidentified as *Lasiurus blossevilli* probably because the two species have similar call structures and frequency ranges. The main difference noted while manually identifying these species is the transition of the downward frequency modulation (FM_d) to quasi constant frequency (qCF),that is highly marked by a sharp edge in *E. furnalis* as compared to a curved one for *L. blossevillii* (Arias-Aguilar et al. *submitted*). The species of the genus *Myotis* were mostly misidentified by both software programs. Previous studies using automated identifications also refer problems when distinguishing *Myotis* species; in fact this genus, while highly specious and widespread worldwide, tends to show very similar call designs level and suggest that *Myotis* species tend to have very similar frequency ranges, probably due to phylogenetic constraints (Parsons and Jones 2000; Rydell et al. 2017) and, eventually due to ecological convergence. *Myotis lavali* was only recently described as a separate species from *Myotis nigricans* complex and a possible sympatry of these species has been suggested (Moratelli and Wilson 2013). SonoChiro was able to identify the genera *Peropteryx* and *Pteronotus* correctly almost 100% of the time but at species level it failed to do so. Species of these genera as well share call design and frequency ranges; therefore we suggest that the call parameters considered for species level identification might be too similar for the software to classify. On the contrary, Kaleidoscope misidentified all the calls of the genera *Peropteryx* as *Centronycteris* and *Pteronotus* as *Noctilio*, possibly because of interspecific overlaps amongst these species. The genera *Peropteryx* and *Centronycteris* are from the family Emballonuridae and have similar call structure with qCF component (Jung et al. 2007). Similarly, genera *Pteronotus* and *Noctilio* have similar call structure with CF -FM component but are from different families (Suga 1990).

Misidentifications can be explained by the intraspecific variation in bat calls. Indeed, species show acoustic geographic variation (Barclay 1999; Murray et al. 2001; López-Baucells et al. 2017). Arias-Aguilar et al. (*submitted*) presents a revision of geographical call variation in Brazilian bats; according to these authors at least ten species of bats present regional variation above 10kHz difference in the FME parameter. At the intraspecific level, bats may also show variation according to habitat type (Surlykke and Moss 2000; Schnitzler and Kalko 2001; Broders et al. 2004; Guillén-Servent and Ibáñez 2007; Jung et al. 2007), foraging mode and diet (Fenton 1986; Jones 1997; Kalko and Handley 2001; Chaverri et al. 2017). All measurements for cryptic species *Pteronotus cf. rubiginosus* varied between individuals recorded in Central Amazon and French Guiana (López-Baucells et al. 2017). It has been shown that bats emit higher frequency, short duration calls when they are in areas of higher clutter or foraging at habitat edges as compared to their conspecific foraging in open spaces (Barclay et al. 1999; Surlykke and Moss 2000; Schnitzler and Kalko 2001; Broders et al. 2004; Jung et al. 2007; López-Baucells et al. 2017). Sex and age also have been shown to cause variation among individuals (Jones et al. 1992; Murray et al. 2001). Peak frequency of bat calls of species from the Vespertilionidae and Emballonuridae have shown to decrease with increase in body size (Barclay et al. 1999; Jung et al. 2007). Individuals also tend to alter their calls to differentiate their reflecting calls from their conspecifics (Obrist 1995; Ulanovsky et al. 2004; Adams and Pedersen 2013). Chaverri et al. (2017) showed also that certain species of the Molossidae modify their calls by decreasing frequency and increasing call duration in order to cancel out atmospheric attenuation, which is caused due to complex interaction between temperature and humidity.

Misidentifications may also be explained by interspecific overlap in call parameters. Interspecific overlap tends to occur amongst species that occupy similar ecological niches (Schnitzler and Kalko 2001) because they adopt similar call designs in order to navigate and forage in similar environments.

Classifiers used by automated software

Considering the intra and interspecific variation as one of the major source of misidentification, it would be appropriate to suggest that the classifiers used by the automated programs might not be reliable. They might not include calls from different region or habitat types which account for the variability discussed above. Also, they could be missing certain species that are not found in the region from where the reference calls were collected. For example, *Molossops temminckii*, *Pteronotus parnellii*, *Eptesicus brasiliensis* and *Molossus currentium*, which were largely misclassified by SonoChiro, are not included in the Neptropical classifier used by the software. Therefore, we argue that the classifiers used for automated identification should be specific to a region. Another factor which could jeopardise the accuracy of a classifier, i.e. the probability of correctly classifying a randomly selected recording (Fielding and Bell 1997), are the calls used as reference. Reference calls used for classifiers are of extremely good quality and should be that way, i.e. calls recorded from captured individuals and close to important roost sites (Lemen et al. 2015). However, field recordings often are of much lower quality. Classifiers should thus include calls recorded in a myriad of situations as to include the maximum variability acoustically expressed by a species. Currently, it is clear that the SonoChiro and Kaleidoscope Pro classifiers still do not account for the intraspecific variation required to make accurate species level identifications. The classification methods also need to include additional parameters for distinguishing acoustically similar species. Because classifiers are regionally or quantitatively limited (Adams et al. 2010), they should not be used as the only source of identification in monitoring and surveying of bats until this barrier is overcome.

The choice of relevant call parameters for species identification

Call structure and harmonics are usually enough for information about the family and often also genus. However, species identification implies measurements of additional parameters, ideally measured in several calls or pulses (Adams et al. 2010; Adams and Pedersen 2013). For example, the differentiation between *Peropteryx* species is based on FME. However, because FME intervals slightly overlap between species, FME measurements may often not be enough for species discrimination. Walters et al. (2012) established a continental scale tool for acoustic identification of European bats using 12 different parameters to characterize frequency and time course of the call and this tool was tested to give robust classifications. Still, it was unable to give reliable identifications in several occasions. This means that more parameters may be necessary for discriminating species with very similar calls.

Conclusion

The automated software programs has the potential to be used in ecological and conservation if the variability of bat calls and more parameters are included in the classifiers (Russo and Voigt 2016). The erroneous classification of species can result in inaccurate distribution mapping of species or selection of incorrect areas to protect. The current programs available in the market have not been tested on field data; relying on species identifications made by these programs for management decision-making may thus have negative conservation consequences. As of now, automated programs can and should be used to make a preliminary round of identification, while files with low confidence values should undergo manual confirmation, in what is called supervised automated identification. A combination of different automated programs used with caution might be able to give a reasonable level of accuracy but does not solve the need for efficient automated software to sample large data sets quickly.

The moderate performance of the two automated programs, namely SonoChiro and Kaleidoscope Pro, in identifying bats from the Brasília National Park should not disregard the ability of these programs to be used as essential tool in field of acoustics, ecology and conservation. Currently, Kaleidoscope Pro can be used to filter sound files containing bat calls and SonoChiro can be used to make identifications for most families and several genera. Incorporation of classifiers containing highly variable bat calls from species of different regions and better filters for extracting more specific call parameters can result in a powerful automated tool to make rapid species identifications.

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General Discussion

Application Framework

Considering the limitations of automated acoustic software, we provide an application framework, which can potentially be used to gain more information about species of bats in ecology and conservation field. **Figure 4** represents a schematic diagram of a possible application framework for automated bioacoustics software. The challenges that exist in applying acoustics to monitor biodiversity are the need for robust identifications to species level and the ability of acoustic surveys to provide reliable information about population trends (Walters et al. 2012; Adams and Pedersen 2013; Frick 2013). Ecological and conservational studies are complementary to an extent because information produced by the first would benefit the latter field and vice-versa.

Currently, automated identification programs are capable of providing preliminary information to focus research efforts in a certain area. Further improvements can be achieved by accounting for the intraspecific variability and interspecific overlap of bat calls (Russo and Voigt 2016). Using acoustic filters to extract more specific call parameters could also prove beneficial to differentiate at the species level (Clement et al. 2014). Other important aspects to consider before automated species identification is applied to the data collected, in particular the standardization of sampling methods, the implementation of statistically powerful sampling designs, and systematic and long-term sampling (Sampaio et al. 2003; Skalak et al. 2012; Adams and Pedersen 2013).

Bat detectors can be distributed over large areas over several days and can record several hours of data from different areas simultaneously. Automated species identification can be optimized and used as a very powerful tool to efficiently study and monitor spatiotemporal patterns of bats globally if all the above conditions are met. Good quality ultrasound recordings can be uploaded into these programs and some useful information can be extracted. While both software retrieves species identification, SonoChiro includes confidence indices with group and species identification, number of bat passes, records of feeding buzzes and the presence of social calls. An important aspect to consider is that the identification software should either be tested for the region or confirmed manually before being applied to the objectives described in the subsequent sections.

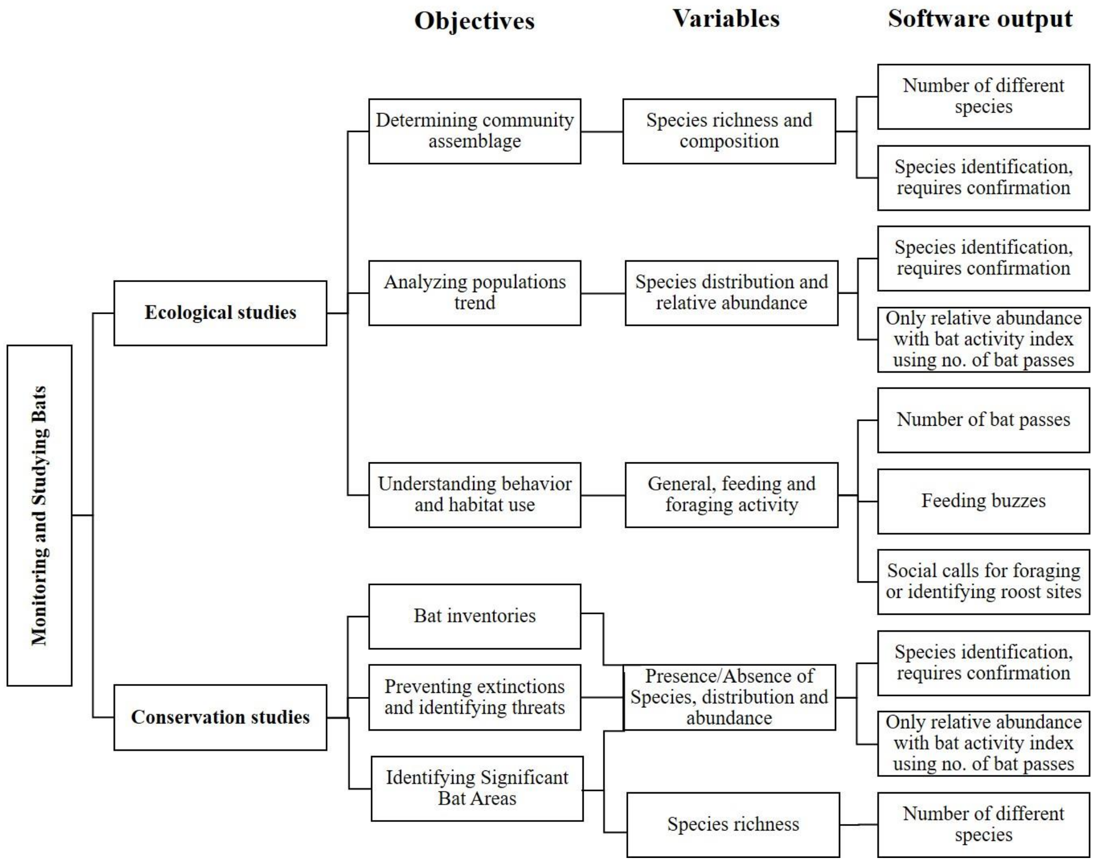

Figure 4. An application framework to use automated acoustic identification software in ecological and conservation studies of bats.

Species richness and composition

Studying the assemblage of bats in an area requires information about individual species to calculate species richness and to determine species composition (Briones-Salas et al. 2013; Mendes et al. 2014). Both the automated programs give species level identification. To calculate species richness, the number of species identified by the software might be sufficient; even if some species are misidentified, if there is a certain level of certainty that what is interpreted as two different species are indeed so, richness estimates may be reasonably accurate. For species composition, on the other hand, the identifications have to be accurate. In this case, it would be better to use the highest level of sensitivity in the program which will retrieve results only for only high-quality pulses. Further confirmation, in particular using supervised identifications of a certain percentage of randomly chosen calls, might be required before using this information.

Density, abundance and activity

One of the main challenges to overcome is monitoring bat populations with acoustics is gathering information on densities or abundances, as two bat-passes from the same species may result from two recorded individuals or from one individual flying twice over the bat detector. Until we develop means to individually identify each bat, only occurrence models and activity indexes may be attained.

Bat activity recorded from large number of sites may be used for determining habitat preferences by bats; similarly, bat activity recorded through time at the same site may reveal if there is a decrease or increase in the use of that site by bats, and indicate, a decrease or increase in the quality of the environment.

The number of feeding buzzes has been used as a proxy of foraging activity (Miller 2001; MacSwiney et al. 2009), may be especially relevant for determining foraging habitats and thus help in spatially prioritization for bat conservation. The presence of social calls has been considered an indication of a nearby roost (Chaverri et al. 2010; Furmankiewicz et al. 2011) or swarming sites (Furmankiewicz et al. 2013). Data retrieved from the automated software may provide information on specific behavioural patterns like mating, mother-infant interactions and territoriality.

Conservations implications

According to Bat Conservation International's five year strategic plan towards bat conservation, Significant Bat Areas (SBA) are areas harbouring threatened species, high diversity and mega populations of bats (Bat Conservation International 2013). As referred in the previous sections, automated software may be useful to generate preliminary information regarding such areas by accounting for species richness, by detecting habitats with higher levels of bat activity, or even by detecting rare or unknown sonotypes, thus suggesting the presence of cryptic bat diversity. Information on social calls and feeding buzzes retrieved by SonoChiro can also aid in detecting roosting, foraging and mating sites, which would be of utmost importance for bat management and conservation.

Final Considerations

There are still several gaps in the concept of applying automated identification programs for bat monitoring projects but they definitely have some important immediate applications and a great potential for improvement. Acoustic surveys are gradually becoming one of the main methods for monitoring and surveying bats globally considering that, in some situations, they account for more species than traditional monitoring methods, and are non-invasive, which is an important consideration when working with more sensitive species. Also, and perhaps more importantly, passive acoustic monitoring presents a high value-for-money ratio, retrieving an immense volume of information with low cost and human effort. The problem is exactly the immense volume of data retrieved by this method; only by using automated software we will be able to deal with terabytes of acoustic information. Technological advances might soon be able to optimize automated identification programs and classifiers to make it an extremely powerful tool in ecology and conservation. This also means that researchers all across the world should contribute with high-quality calls for the development of local and regional classifiers. The development of freeware, for example under the R environment, should be promoted. Indeed, more people use freeware, users may be willing and able to adapt or fix the program (for example by adding calls to existing libraries or by improving classification methods), and other developers may learn from the program, or base new work on it. The warbleR package (Araya-Salas and Smith-Vidaurre 2016) which presently only aims at streamlining the analysis of animal acoustic signals, may be a good starting point. In the meantime, it is important to carry out validation tests for the classifiers in the available software before using them to test hypotheses or take management decisions.

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