

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

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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 classifiers 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.

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20 Abstract

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24 also depends on the ease to monitor and detect demographic trends in their populations. The long
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27 analysis of sounds emitted by organisms has been considered useful to gain insight into species-
28 specific biotic and abiotic interactions, which can further be applied to conservation. Besides
29 manual identifications of bat calls, a number of automated species identification programs using
30 regional call classifiers have been introduced into the market as an efficient tool in monitoring of
31 bat populations. Most of these programs have not been validated using field data. This study
32 evaluates the reliability of two automated softwares, SonoChiro and Kaleidoscope Pro, in
33 comparison to manual identifications of field data collected from the Neotropical region. There
34 was low agreement between the two automated methods at the species level, fair agreement at
35 the genus level and moderate agreement at the family level. There was also a significant
36 difference between the proportions of correctly identified calls of the two-automated software at
37 the species level identifications. Major challenges for using automated identification software
38 include the need for comprehensive call libraries of the regions under scope; major opportunities,
39 on the other hand, include the widespread possibility to monitor spatiotemporal patterns of bat
40 activity. Overall, there are serious gaps that preclude a widespread application of automated
41 programs ecological and conservation studies of bats, but it has the potential to serve as an
42 effective tool.

43 **Keywords:** Bioacoustics; Chiroptera; Kaleidoscope; SonoChiro.

44 Introduction

45 Most bat species produce ultrasound for orientation, navigation and hunting prey (Adams
46 and Pedersen 2013). Bats emit a signal (pulse) of a certain frequency and then perceive the
47 reflected signal (echo) which returns after hitting a target or surrounding objects in the
48 environment (Schnitzler and Kalko 2001; Fenton 2003; Adams and Pedersen 2013). These
49 ultrasounds produced by bats are known as echolocation calls and have co-evolved over time
50 depending on various ecological and physical factors (Murray et al. 2001; Obrist et al. 2007).
51 When hunting for prey, bat echolocation calls are characterized by three phases: search phase,
52 approximation phase and terminal buzz phase (Murray et al. 2001). Echolocating bats use tonal
53 signals with structured changes in frequency over time ranging between 8 and 200kHz (Fenton
54 2003; Adams and Pedersen 2013). Bats also produce social calls when mating, foraging, and
55 during distress, aggression and mother-offspring interactions (Wilkinson and Boughman 1998;
56 Fenton 2003; Budenz et al. 2009; Furmankiewicz et al. 2011). Echolocation and social calls are
57 species- specific and, in some cases, even colony-specific (Fenton 2003).

58 Biologists characterize bat calls using parameters of the pulse such as frequency
59 modulation (FM), harmonic level, duration (D or t), inter-pulse interval (IPI), frequency of
60 maximum energy (FME), maximum frequency (F_{\max}), minimum frequency (F_{\min}) and bandwidth
61 ($BW = F_{\max} - F_{\min}$) (**Figure 1**). This is used to identify the calls to species level.

62 Bats are nocturnal mammals, difficult to catch and sensitive to anthropogenic intrusion
63 which make them difficult to account for only using traditional capturing methods with mist nets
64 or harp traps (MacSwiney et al. 2009; Russo and Voigt 2016). Acoustic monitoring has emerged
65 as a non-invasive, time-efficient method which can be used to study spatiotemporal patterns of
66 bat diversity and activity (Russo and Voigt 2016; Silva et al. 2017; Stathopoulos et al. 2017) and

67 is not limited by inaccessible environments or bad weather conditions (Skalak et al. 2012;
68 Marques et al. 2016). Acoustic monitoring has helped researchers gain knowledge about bat
69 behavior, habitat preferences, foraging strategies, distribution, abundance, population trends and
70 about species that are difficult to capture (Miller and Degn 1981; Fenton et al. 1987; Vaughan et
71 al. 1997; Verboom et al. 1999; Marques et al. 2016; Stathopoulos et al. 2017).

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

87 The Neotropics show a very high diversity of bats with numerous gaps in knowledge
88 about their ecology, behavior, acoustic classification and conservation status (Zortéa and Alho
89 2008; Adams and Pedersen 2013). Bats of this region, as well as other regions, are under threat

90 due to changes caused by anthropogenic activities such as alteration of land-use, invasive
91 species, air, water and noise pollution (Mendes and De Marco 2017). Therefore, the need for
92 efficient and accurate species identification methods for larger areas has rapidly escalated and
93 resulted in the availability of many automated software in the market. SonoChiro and
94 Kaleidoscope are two such programs that have been used in previous studies for automated
95 species identification with region specific call classifiers and careful speculation (Slough et al.
96 2014; Michaelsen 2016; Toffoli 2016). Even though, the producers of the software insist that the
97 accuracy rates are high, researchers are aware of the inaccuracies and use manual identifications
98 for certain species most of them have never actually been tested on field data (Russo and Voigt
99 2016). Lemen et al. (2015) used unidentified field data to compare the performance of 4
100 automated programs and found an average pair-wised agreement of 40%. More recently a study
101 in Sweden showed poor performance of classifiers used by Kaleidoscope Pro and SonoChiro
102 because the identifications were not reliable (Rydell et al. 2017).

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

110 The aim of this study is to evaluate the reliability of two automated programs (SonoChiro
111 and Kaleidoscope Pro) that are widely used for automated identifications, for Neotropical bat
112 species. The agreement between the two automated and manual identifications for the same

113 dataset was predicted to be low at species and genus level identification but not at the family
114 level. Using the manual identifications as absolute true species, the second hypothesis was that
115 there would be a difference in the proportion of correctly identified between the two-automated
116 software. SonoChiro was predicted to perform better than Kaleidoscope because SonoChiro can
117 give group (family and genera) and species level identifications separately while Kaleidoscope
118 uses only species classifiers (Rydell et al. 2017).

119 **Materials and methods**

120 *Field Collection*

121 Our study species included eight out of nine families of Chiroptera found in Brazil,
122 namely Emballonuridae, Furipteridae, Molossidae, Mormoopidae, Natalidae, Noctilionidae,
123 Thyropteridae and Vespertilionidae. In Brazil, these families cover a total of 93 species (Arias-
124 Aguilar et al. *submitted*), of at least 178 occurring in Brazil (Nogueira et al. 2014). The
125 recordings were collected at two sites at 10 different sampling points at the National Park of
126 Brasília in Federal district of Brasília, which is situated in the center of the Brazilian Cerrado.
127 The Cerrado is composed of woodlands, savannas, grasslands and dry forests and forms the
128 second largest biome of Brazil (Klink & Machado 2005). The recording was made over two
129 periods, August and September 2016, which correspond to the middle and the end of the dry
130 season respectively. The SM2 Bat detector (Wildlife Acoustics, U.S.A;
131 www.wildlifeacoustics.com) was used to record bat calls at the sites, without using any filter for
132 the ambient noise. The data used for this paper was secondary data collected under the license
133 number #27719-13 issued by the ICMBIO, which is the institution that grants permits to work in
134 protected areas.

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

140 ***Automated identification of recordings***

141 For the automated identification, the 3,465 15-second duration files were analyzed using
142 SonoChiro v.3.0 (Biotope, France www.biotope.fr) and Kaleidoscope Pro 3.14B (Wildlife
143 Acoustics, U.S.A; www.wildlifeacoustics.com). The settings used were: for SonoChiro - type of
144 recorder (SM2 Bat), region (Amazonian basin), time expansion (x1), maximum call duration
145 (0.5), sensitivity (7), for Kaleidoscope Pro – filter noise files (keep noise files), signal of interest
146 (8-120kHz, 2-500ms, minimum two calls), classifiers (Neotropical bats), (0 Neutral sensitivity).
147 The sensitivity scale of SonoChiro ranges from 10 to 0 and that of Kaleidoscope is +1 to -1.
148 They are calculated differently but essentially range between giving results for low quality pulses
149 (more sensitive) and only high-quality pulses (more accurate). The output generated by the two
150 automated programs is expected to show group and species level identifications. The
151 identifications that may not be attempted result in “parasi” (SonoChiro), “no ID” or “Noise”
152 (Kaleidoscope Pro).

153 ***Manual identification of recordings***

154 The identifications were made manually on 44% of the recordings used for automated
155 identifications (1506 WAVE files) using Avisoft SASLab Pro (Specht 2004). The spectrogram
156 for each recording was created using the following parameters: FFT length (1024), frame size
157 (100%), Overlap (87.5%) and Hamming window. The parameters determine the frequency and

158 time resolution of the pulse or sequence in the spectrogram. Frequencies below 10kHz were
159 filtered out using noise filter for better identification. The recordings attempted to be manually
160 identified required at least three clear pulses and any overlapping pulses were discarded to avoid
161 any bias. The parameters that were observed and tabulated to identify the calls up to species level
162 were: i) average call duration of at least three pulses; ii) number of harmonics and maximum
163 energy harmonic; iii) number of call types; iv) pulse structure (FM, CF or qCF); v) frequency of
164 maximum intensity (FME); vi) maximum frequency (Fmax); vii) minimum frequency (Fmin);
165 viii) bandwidth (BW); and ix) inter-pulse interval (IPI) (**Figure 1**). Some additional parameters
166 were measured when required, such as initial frequency (F_{initial}), end frequency (F_{end}) and
167 individual parameters of different call types. The identification was done using an Illustrated
168 identification key to the calls of Brazilian bats (Arias-Aguilar et al. *submitted*).

169 *Statistical analysis*

170 The data compiled for statistical analysis included family, genus and species level
171 identifications for the automated programs (SonoChiro and Kaleidoscope Pro) and manual
172 identifications. The agreement between the three sets of identifications for each of the levels
173 (family, genus and species) was tested using the inter-rater reliability Fleiss's kappa statistic
174 (Dunn 1992). Further, the manual identifications were assumed as true identifications and the
175 number of correctly identified recordings were recorded for each of the automated software.
176 Overall difference in proportion of correctly identified files at each level (species, genus and
177 family) between the two automated programs was computed using Chi-squared tests. True
178 positives, false positives, true negatives and false negatives for each species were calculated for
179 SonoChiro and Kaleidoscope Pro. True positives of each software were all the identifications of
180 a species matched with manual identifications. False positives were those where the presence of

181 species was identified incorrectly by the software while false negatives were those where the
182 species was present but not perceived by the software. True negatives were calculated by
183 accounting for all the recordings where other species were identified.

184 **Results**

185 A total of 643 and 274 WAVE files were not identified by the automated programs and
186 manually by an expert, respectively. Therefore, these were removed, and the remaining 602
187 WAVE files were used for the further analyses.

188 *Agreement between two automated and manual identifications*

189 Following Dunn (1992) agreement level described as Poor if $\kappa < 0.00$, Slight if $0.00 \leq \kappa \leq$
190 0.20 , Fair if $0.21 \leq \kappa \leq 0.40$, Moderate if $0.41 \leq \kappa \leq 0.60$, Substantial if $0.61 \leq \kappa \leq 0.80$ and
191 Almost perfect $\kappa > 0.80$, the Fleiss's kappa statistic value showed that there was low agreement
192 between the three sets of identifications at the species level ($\kappa=0.145$), fair agreement at the
193 genus level ($\kappa=0.326$) and moderate agreement at the family level ($\kappa=0.456$). The total number
194 of recordings that were agreed on at the species, genus and family level was 23, 89 and 285
195 WAVE files respectively (**Figure 2**).

196 *Comparison of the proportion of correctly identified files*

197 There was a significant difference between the proportion of correctly identified recordings by
198 two automated programs at the species level ($X^2 = 280.54$, $df=1$, $p < 0.05$) and family level ($X^2 =$
199 20.917 , $df=1$, $p < 0.05$) (**Figure 3**). The percentage of correctly identified species by SonoChiro
200 and Kaleidoscope Pro was 5%. At the family level, 77% of the recordings were correctly
201 identified by SonoChiro and 65% was correctly identified by Kaleidoscope Pro. There was no
202 significant difference between the proportions of correctly identified files by the two automated

203 programs at the genus level ($X^2 = 1.608$, $df=1$, $p > 0.05$). The percentage of correctly identified
204 genera was 48% for SonoChiro and 52% for Kaleidoscope Pro.

205 *Correctly and misidentified species by automated software*

206 In Table 1 is shown the number of true positives, false positives, true negative and false
207 negatives calculated for each species manually identified from the 602 WAVE files: *Eptesicus*
208 *brasiliensis*, *Eptesicus fernalis*, *Lasiurus blossevilli*, *Lasiurus ega*, *Molossos currentium*,
209 *Molossus*, *Molossops temminckii*, *Myotis lavalis*, *Myotis nigricans*, *Myotis riparius*, *Peropteryx*
210 *leucoptera/paldioptera*, *Peropteryx macrotis*, *Promops nasutus* and *Pteronotus parnellii*. The
211 genera *Cynomops*, *Eumops*, *Nyctinomops* and *Tadarida* could not be manually identified to the
212 species level. The species of genera *Myotis* and *Peropteryx* had no true positives for
213 Kaleidoscope Pro but SonoChiro identified two out of five *Myotis riparius* and the only
214 *Peropteryx macrotis* call correctly. *Eptesicus brasiliensis*, *Molossus currentium*, *Promops*
215 *nasutus* and *Pteronotus parnellii* were misidentified by both programs. *Lasiurus ega* calls were
216 identified correctly by Kaleidoscope Pro but not by SonoChiro in the two instances it was
217 present. Most *Eptesicus fernalis* calls were identified correctly by SonoChiro (9 out of 10) and
218 Kaleidoscope (7 out of 10) but they had 148 and 18 false positives respectively. Almost 88% of
219 *Lasiurus blossevilli* calls were identified correctly by Kaleidoscope but none by SonoChiro.
220 Species of Molossidae, *Molossus* and *Molossops temminckii*, were identified correctly 80.5% and
221 84% of the time respectively. On the other hand, SonoChiro misidentified 80% *Molossus* and all
222 *Molossops temminckii* calls.

223 **Discussion**

224 The low agreement between the three different methods, two automated and one manual,
225 for species identification raises a concern about the reliability of automated species identification

226 for bat monitoring and studies in the neotropics. Bats, unlike birds and other echolocating
227 animals, alter certain parameters of their calls depending on their interaction with the
228 environment or other species (Jones 1997; Kalko and Handley 2001; Chaverri et al. 2010). This
229 would make it difficult to distinguish between individuals in species-rich areas, such as the
230 Neotropical region, where certain bats species might occupy similar niches and hence would
231 have overlaps in call structures.

232 *Classification methods*

233 Lemen et al. (2015) suggested that the low levels of agreement between software could
234 be because of recordings collected with different recording devices but in our study the call
235 database was the same and recorded using the same bat detector. This discrepancy could be
236 attributed to the difference in sensitivity scale, classification method and the classifiers used by
237 each of the methods. The sensitivity setting in the software allows researchers to manipulate the
238 detectability of a call in the recording i.e. high sensitivity setting would detect even low-quality
239 pulses and low sensitivity setting would detect only high quality, clear pulses. Even though both
240 the software were set at similar sensitivity, SonoChiro can detect and classify more calls
241 compared to Kaleidoscope Pro. In the presence of more than one species in one recording,
242 SonoChiro has the ability to identify up to three species while Kaleidoscope identifies only what
243 it perceives as the dominant call in the recording. Also, considering classification methods,
244 SonoChiro detects any calls present on the recording and then classifies them using Random
245 Forest classification method, which in this case uses active learning/ negative labelling (Bas et al.
246 2013). This method is supposed to have a powerful confidence index and can spot obvious errors
247 in calls from diverse sources (Beard 2007; Cutler et al. 2007). On the other hand, the
248 classification method of Kaleidoscope Pro uses error rates calculated from the confusion

249 matrices of specific regional classifiers to determine the most likely distribution of the different
250 species. The error rates for confusion matrices from different geographic regions and habitat
251 types might be different leading to misidentifications (Agranat 2012). To reduce the
252 misidentification rates, SonoChiro computes confidence levels for group and species level
253 identification while Kaleidoscope can give possible alternative identifications for the data; both
254 retrieve unknown classifications. Previously used automated identification methods were not
255 able to provide confidence levels, alternative and unknown classifications; the lack of these
256 variables might result in higher levels of misidentifications and has been criticized (Adams et al.
257 2010).

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

265 *Intraspecific variation and interspecific overlap*

266 Although, SonoChiro showed discrepancies when compared to manual identification,
267 there was a gradual improvement from species to genus to family level identifications.
268 Kaleidoscope could correctly identify more species than SonoChiro, but it only gives species
269 level identification with no confidence indices. Therefore, SonoChiro might be at a better
270 advantage as it is able to identify certain individuals at least up to the genus level. This
271 information can be useful to survey and monitor specific focal genera (Rydell et al. 2017). At the

272 species level, there were some species correctly identified by one or the other software but only
273 *Eptesicus fernalis* and some *Molossus* calls were correctly identified by both. *Eptesicus fernalis*
274 was often misidentified as *Lasiurus blossevilli* probably because the two species have similar call
275 structures and frequency ranges. The main difference noted while manually identifying these
276 species is the transition of the downward frequency modulation (FM_d) to quasi constant
277 frequency (qCF), that is highly marked by a sharp edge in *E. fernalis* as compared to a curved
278 one for *L. blossevilli* (Arias-Aguilar et al. *submitted*). The species of the genus *Myotis* were
279 mostly misidentified by both software programs. Previous studies using automated
280 identifications also refer problems when distinguishing *Myotis* species; in fact, this genus, while
281 highly specious and widespread worldwide, tends to show very similar call designs level and
282 suggest that *Myotis* species tend to have very similar call designs and frequency ranges, probably
283 due to phylogenetic constraints (Parsons and Jones 2000; Rydell et al. 2017) and, eventually due
284 to ecological convergence. *Myotis lavalii* was only recently described as a separate species from
285 *Myotis nigricans* complex and a possible sympatry of these species has been suggested
286 (Moratelli and Wilson 2013). SonoChiro was able to identify the genera *Peropteryx* and
287 *Pteronotus* correctly almost 100% of the time but at species level it failed to do so. Species of
288 these genera as well share call design and frequency ranges; therefore, we suggest that the call
289 parameters considered for species level identification might be too similar for the software to
290 classify. On the contrary, Kaleidoscope misidentified all the calls of the genera *Peropteryx* as
291 *Centronycteris* and *Pteronotus* as *Noctilio*, possibly because of interspecific overlaps amongst
292 these species. The genera *Peropteryx* and *Centronycteris* are from the family Emballonuridae
293 and have similar call structure with qCF component (Jung et al. 2007). Similarly, genera
294 *Pteronotus* and *Noctilio* have similar call structure with CF -FM component but are from

295 different families (Suga 1990).

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

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

322 *Classifiers used by automated software*

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

341 are regionally or quantitatively limited (Adams et al. 2010), they should not be used as the only
342 source of identification in monitoring and surveying of bats until this barrier is overcome.

343 *The choice of relevant call parameters for species identification*

344 Call structure and harmonics are usually enough for information about the family and
345 often also genus. However, species identification implies measurements of additional
346 parameters, ideally measured in several calls or pulses (Adams et al. 2010; Adams and Pedersen
347 2013). For example, the differentiation between *Peropteryx* species is based on FME. However,
348 because FME intervals slightly overlap between species, FME measurements may often not be
349 enough for species discrimination. Walters et al. (2012) established a continental scale tool for
350 acoustic identification of European bats using 12 different parameters to characterize frequency
351 and time course of the call and this tool was tested to give robust classifications. Still, it was
352 unable to give reliable identifications in several occasions. This means that more parameters may
353 be necessary for discriminating species with very similar calls.

354 *Application Framework*

355 Considering the limitations of automated acoustic software, we provide an application
356 framework, which can potentially be used to gain more information about species of bats in
357 ecology and conservation field. **Figure 4** represents a schematic diagram of a possible
358 application framework for automated bioacoustics software. The challenges that exist in applying
359 acoustics to monitor biodiversity are the need for robust identifications to species level and the
360 ability of acoustic surveys to provide reliable information about population trends (Walters et al.
361 2012; Adams and Pedersen 2013; Frick 2013). Ecological and conservational studies are
362 complementary to an extent because information produced by the first would benefit the latter
363 field and vice-versa.

364 Currently, automated identification programs are capable of providing preliminary
365 information to focus research efforts in a certain area. Further improvements can be achieved by
366 accounting for the intraspecific variability and interspecific overlap of bat calls (Russo and Voigt
367 2016). Using acoustic filters to extract more specific call parameters could also prove beneficial
368 to differentiate at the species level (Clement et al. 2014). Other important aspects to consider
369 before automated species identification is applied to the data collected, in particular the
370 standardization of sampling methods, the implementation of statistically powerful sampling
371 designs, and systematic and long-term sampling (Sampaio et al. 2003; Skalak et al. 2012; Adams
372 and Pedersen 2013).

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

383 *Species richness and composition*

384 Studying the assemblage of bats in an area requires information about individual species
385 to calculate species richness and to determine species composition (Briones-Salas et al. 2013;
386 Mendes et al. 2014). Both the automated programs give species level identification. To calculate

387 species richness, the number of species identified by the software might be sufficient; even if
388 some species are misidentified, if there is a certain level of certainty that what is interpreted as
389 two different species are indeed so, richness estimates may be reasonably accurate. For species
390 composition, on the other hand, the identifications must be accurate. In this case, it would be
391 better to use the highest level of sensitivity in the program which will retrieve results only for
392 only high-quality pulses. Further confirmation, using supervised identifications of a certain
393 percentage of randomly chosen calls, might be required before using this information.

394 *Density, abundance and activity*

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

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

404 The number of feeding buzzes has been used as a proxy of foraging activity (Miller 2001;
405 MacSwiney et al. 2009), may be especially relevant for determining foraging habitats and thus
406 help in spatially prioritization for bat conservation. The presence of social calls has been
407 considered an indication of a nearby roost (Chaverri et al. 2010; Furmankiewicz et al. 2011) or
408 swarming sites (Furmankiewicz et al. 2013). Data retrieved from the automated software may

409 provide information on specific behavioural patterns like mating, mother-infant interactions and
410 territoriality.

411 *Conservations implications*

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

421 *Final Considerations*

422 There are still several gaps in the concept of applying automated identification programs
423 for bat monitoring projects, but they have some important immediate applications and a great
424 potential for improvement. Acoustic surveys are gradually becoming one of the main methods
425 for monitoring and surveying bats globally considering that, in some situations, they account for
426 more species than traditional monitoring methods, and are non-invasive, which is an important
427 consideration when working with more sensitive species. Also, and perhaps more importantly,
428 passive acoustic monitoring presents a high value-for-money ratio, retrieving an immense
429 volume of information with low cost and human effort. The problem is exactly the immense
430 volume of data retrieved by this method; only by using automated software we will be able to
431 deal with terabytes of acoustic information. Technological advances might soon be able to

432 optimize automated identification programs and classifiers to make it an extremely powerful tool
433 in ecology and conservation. This also means that researchers across the world should contribute
434 with high-quality calls for the development of local and regional classifiers. The development of
435 freeware, for example under the R environment, should be promoted. Indeed, more people use
436 freeware, users may be willing and able to adapt or fix the program (for example by adding calls
437 to existing libraries or by improving classification methods), and other developers may learn
438 from the program, or base new work on it. The warbleR package (Araya-Salas and Smith-
439 Vidaurre 2016) which presently only aims at streamlining the analysis of animal acoustic signals,
440 may be a good starting point. In the meantime, it is important to carry out validation tests for the
441 classifiers in the available software before using them to test hypotheses or take management
442 decisions.

443 ***Conclusion***

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

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

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Figure 1

Typical spectrogram view of the echolocation call of Pteronotus parnelli

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 (F_{max}), minimum frequency (F_{min}), frequency of maximum energy (FME), time duration (t), inter-pulse interval (IPI) and harmonics (HF, H2, H3, H4).

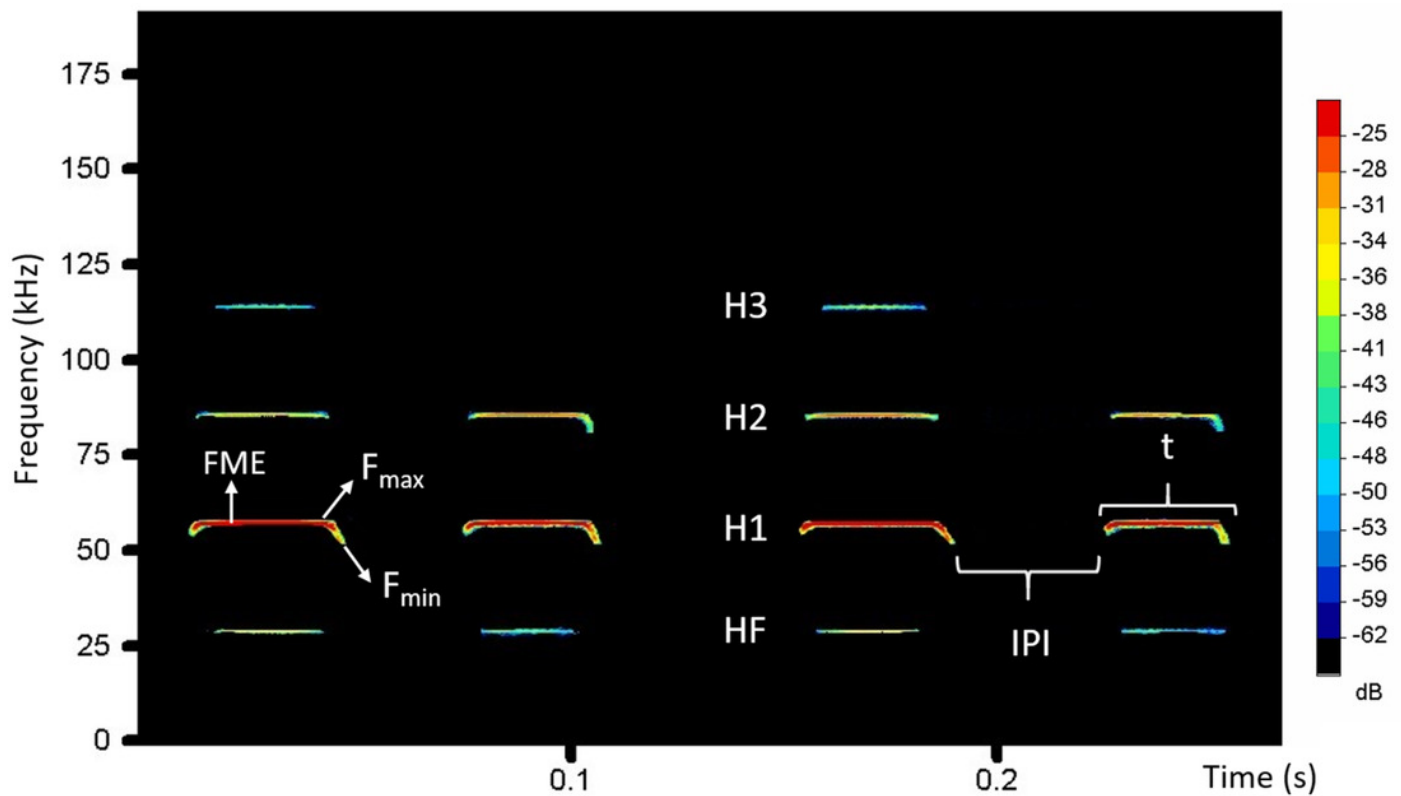


Figure 2

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

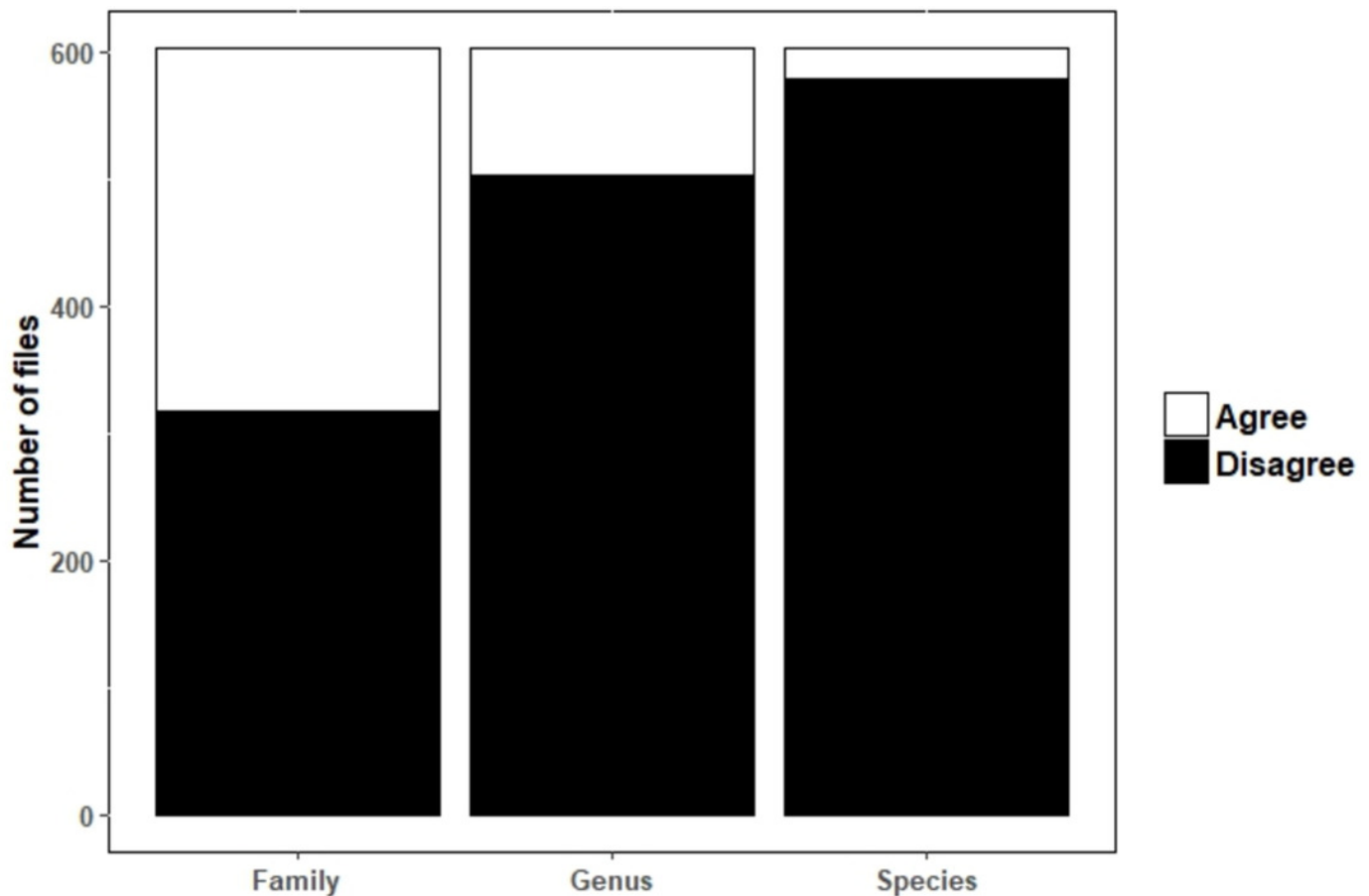


Figure 3

Stacked bar chart indicating the proportion of correctly identified files for each software.

For Kaleidoscope, species = 48%, genus = 52%, family = 65% and for SonoChiro, species= 5%, genus=48% and family=77%. The y-axis shows the number of files and the x-axis is the two-automated software

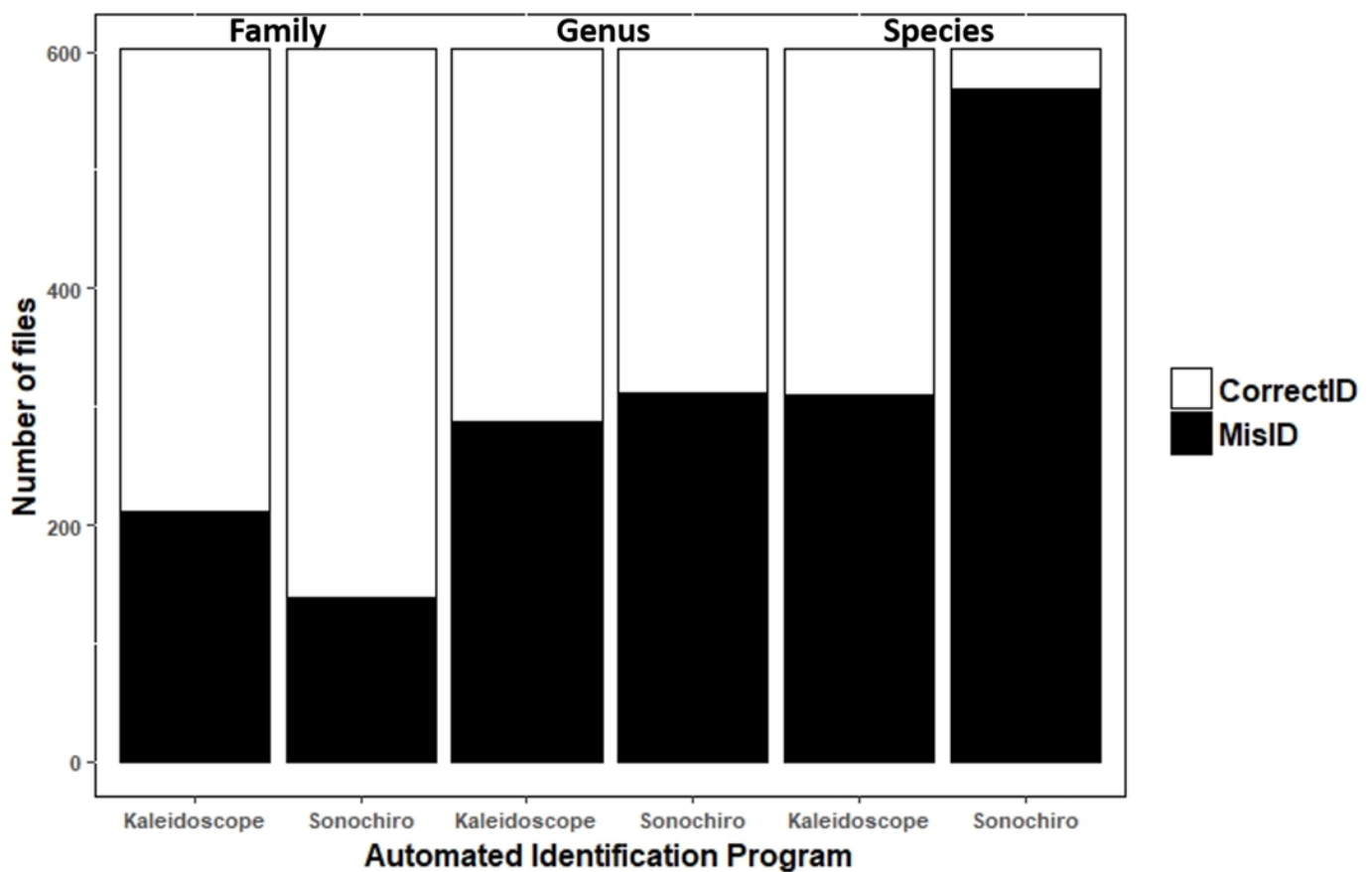


Figure 4

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

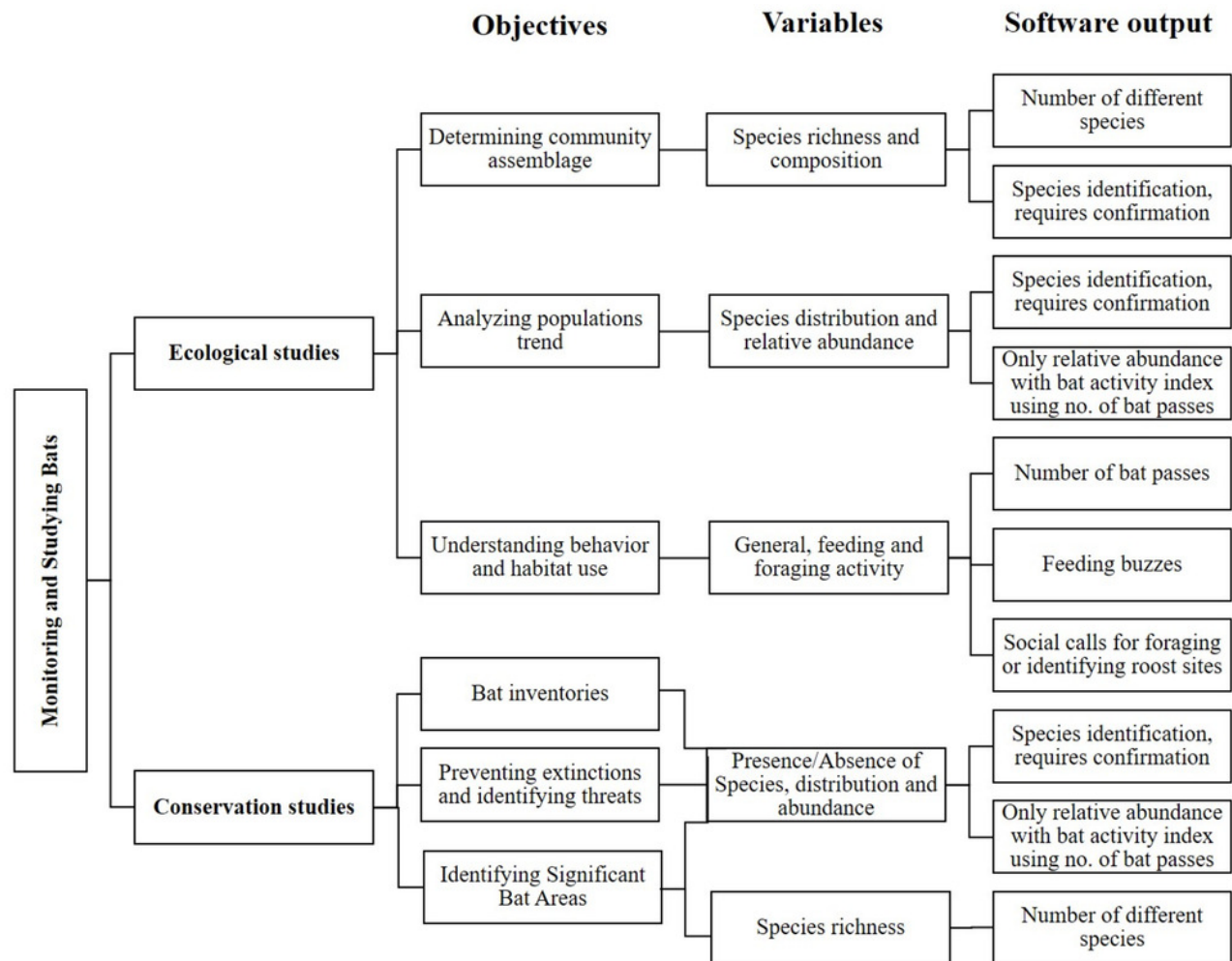


Table 1 (on next page)

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

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

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