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 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.

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2   **region?**

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20   **Abstract**

21   Bat populations are known to be affected by anthropogenic activities because bats are an

22   extremely diverse group occupying almost all available niches in terrestrial environment. Hence,

23   bats are considered bioindicators to monitor changes in the environment, but their value as such

24   also depends on the ease to monitor and detect demographic trends in their populations. The

25   long-term interest of researchers in the acoustic of bats results from the fact that it is a non-

26   invasive, time-efficient method to monitor spatiotemporal patterns of bat diversity and

27   activity.The analysis of sounds emitted by organisms has been considered useful to gain insight

28   into species-specific biotic and abiotic interactions, which can further be applied to conservation.

29   Besides manual identifications of bat calls, some automated species identification programs

30   using regional call classifiers have been introduced into the market as an effective tool in the

31   monitoring of bat populations. Most of these programs have not been validated using field data.

32   This study evaluates the reliability of two automated software, SonoChiro, and Kaleidoscope

33   Pro, in comparison to manual identifications of field data collected from the Neotropical region.

34   There was low agreement between the two automated methods at the species level, fair

35   agreement at the genus level and moderate agreement at the family level. There was also a

36   significant difference between the proportions of correctly identified calls of the two-automated

37   software at the species level identifications. Major challenges for using automated identification

38   software include the need for comprehensive call libraries of the regions under scope; significant

39   opportunities, on the other hand, include the widespread possibility to monitor spatiotemporal

40   patterns of bat activity. Overall, there are serious gaps that preclude a widespread application of

41   automated programs ecological and conservation studies of bats, but it has the potential to serve

42   as a useful tool.

43   **Keywords:** Bioacoustics; Brazil; Cerrado; 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 it 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 adverse 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 areas, are under threat due

90   to changes caused by anthropogenic activities such as alteration of land-use, invasive species, air,

91   water and noise pollution (Aguiar et al. 2016; Mendes and De Marco 2017). Therefore, the need

92   for efficient and accurate species identification methods for more extensive areas has rapidly

93   escalated and resulted in the availability of much automated software in the market. SonoChiro

94   and 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-wise agreement of 40%. More recently a study in

101   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 the

104   performance of the available Neotropical software and their respective classifiers has not been

105   validated previously. The challenge of using automated identification for Neotropical species is

106   that there is a lot of evidence showing inter and intraspecific variability of bat calls due to high

107   species richness (Jones et al. 1992; Jones 1997; Barclay et al. 1999; Murray et al. 2001; Pfalzer

108   and Kusch 2003; Broders et al. 2004; Russ et al. 2004; Jung et al. 2007; López-Baucells et al.

109   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, of at

124   least 178 occurring in Brazil (Nogueira et al. 2014). The recordings were collected at two sites at

125   10 different sampling points at the National Park of Brasília in the city of Brasilia, Federal

126   District, which is situated in the core area of the Brazilian Cerrado. The Cerrado is composed of

127   woodlands, savannas, grasslands and dry forests and forms the second largest biome of Brazil

128   (Klink & Machado 2005) and 40% of its mammals are bats (Aguiar et al. 2016). The recording

129   was made over two periods, August and September 2016, which correspond to the middle and

130   the end of the dry 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 SISBIO/ICMBIO, which is the institution that grants permits to

134   work in 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_{intial}), 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*).

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 (X2 = 1.608, df =1, p >0.05). The percentage of correctly identified

204   genera was 48% for SonoChiro and 52% for Kaleidoscope Pro.

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 furinalis, Lasiurus blossevillii, Lasiurus ega, Molossus currentius,*

209   *Molossus Molossus, Molossops temminckii, Myotis lavali, Myotis nigricans, Myotis riparius,*

210   *Peropteryx leucoptera/palidoptera, Peropteryx macrotis, Promops nasutus* and *Pteronotus*

211   *parnellii*. The genera *Cynomops, Eumops, Nyctinomops* and *Tadarida* could not be manually

212   identified to the species level. The species of genera *Myotis* and *Peropteryx* had no true

213   positives for Kaleidoscope Pro but SonoChiro identified two out of five *Myotis riparius* and the

214   only *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 furinalis 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 blossevillii* 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.

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 furinalis* and some *Molossus* calls were correctly identified by both. *Eptesicus furinalis*

274   was often misidentified as *Lasiurus blossevilliii* probably because the two species have similar

275   call 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. furinalis* as compared to a curved

278   one for *L. blossevillii* (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 lavali* 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.

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 Neotropical

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.

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.

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 studies if the variability of bat calls and more parameters are included in the

446   classifiers (Russo and Voigt 2016). The erroneous classification of species can result in

447   inaccurate distribution mapping of species or selection of incorrect areas to protect. The current

448   programs available in the market have not been tested on field data; relying on species

449   identifications made by these programs for management decision-making may thus have

450   negative conservation consequences. As of now, automated programs can and should be used to

451   make a preliminary round of identification, while files with low confidence values should

452   undergo manual confirmation, in what is called supervised automated identification. A

453   combination of different automated programs used with caution might be able to give a

454 reasonable level of accuracy but does not solve the need for efficient automated software to

455 sample large data sets quickly.

456   The moderate performance of the two automated programs, namely SonoChiro and

457 Kaleidoscope Pro, in identifying bats from the Brasília National Park should not disregard the

458 ability of these programs to be used as essential tool in field of acoustics, ecology and

459 conservation. Currently, Kaleidoscope Pro can be used to filter sound files containing bat calls

460 and SonoChiro can be used to make identifications for most families and several genera.

461 Incorporation of classifiers containing highly variable bat calls from species of different regions

462 and better filters for extracting more specific call parameters can result in a powerful automated

463 tool to make rapid species identifications.

464 **Acknowledgements**

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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 (Fmax), minimum frequency (Fmin), frequency of maximum energy (FME), time duration (t), inter-pulse interval (IPI) and harmonics (HF, H2, H3, H4).
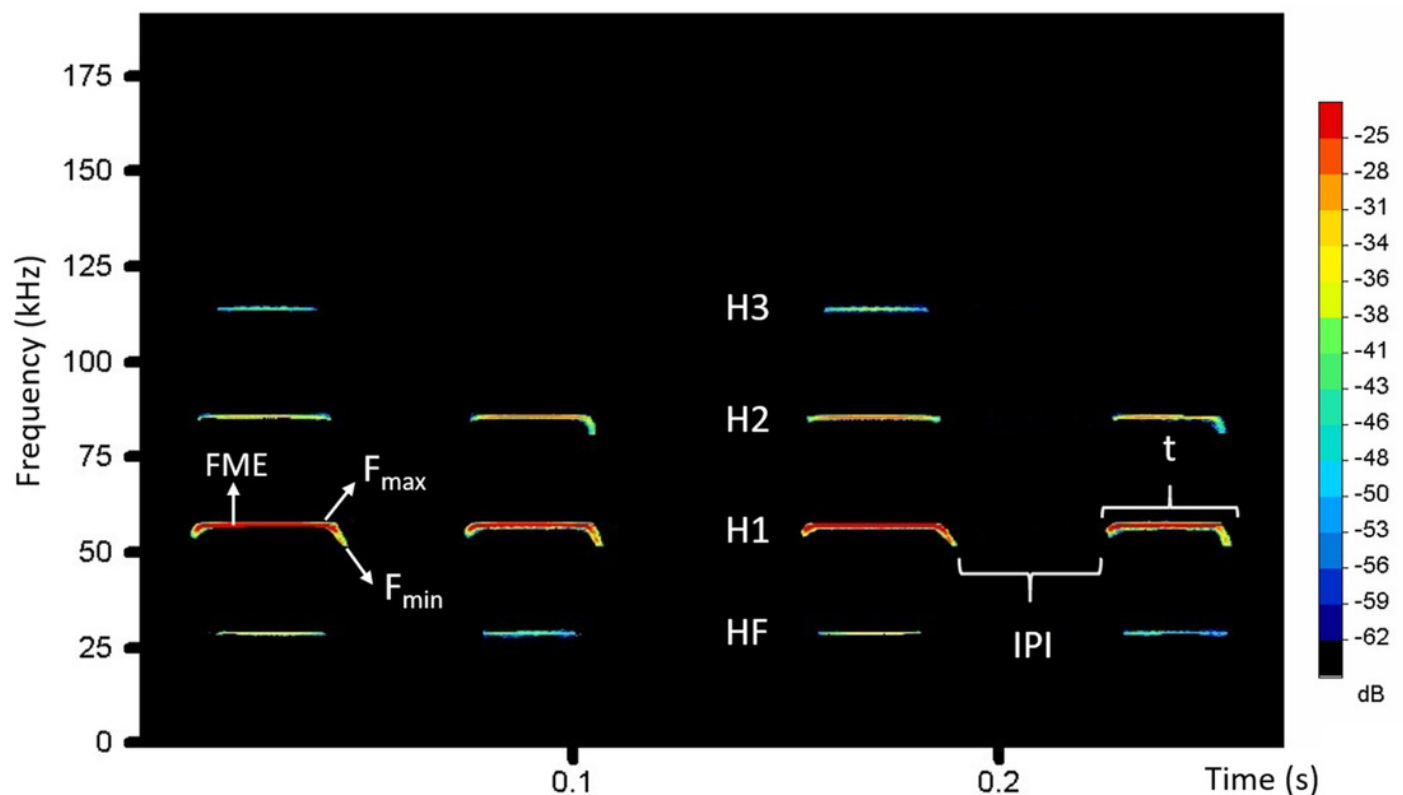
Figure 2

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.
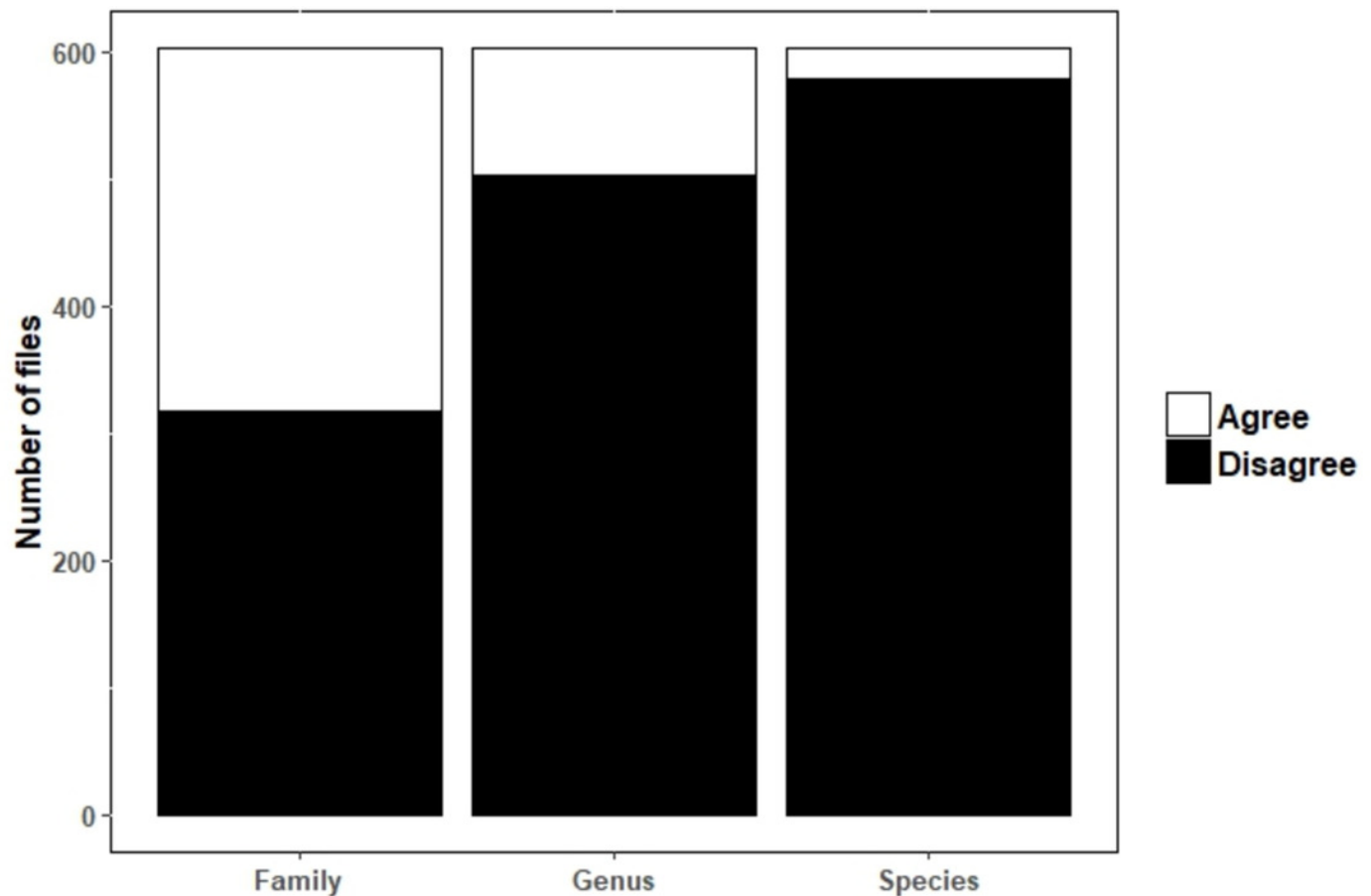
Figure 3

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
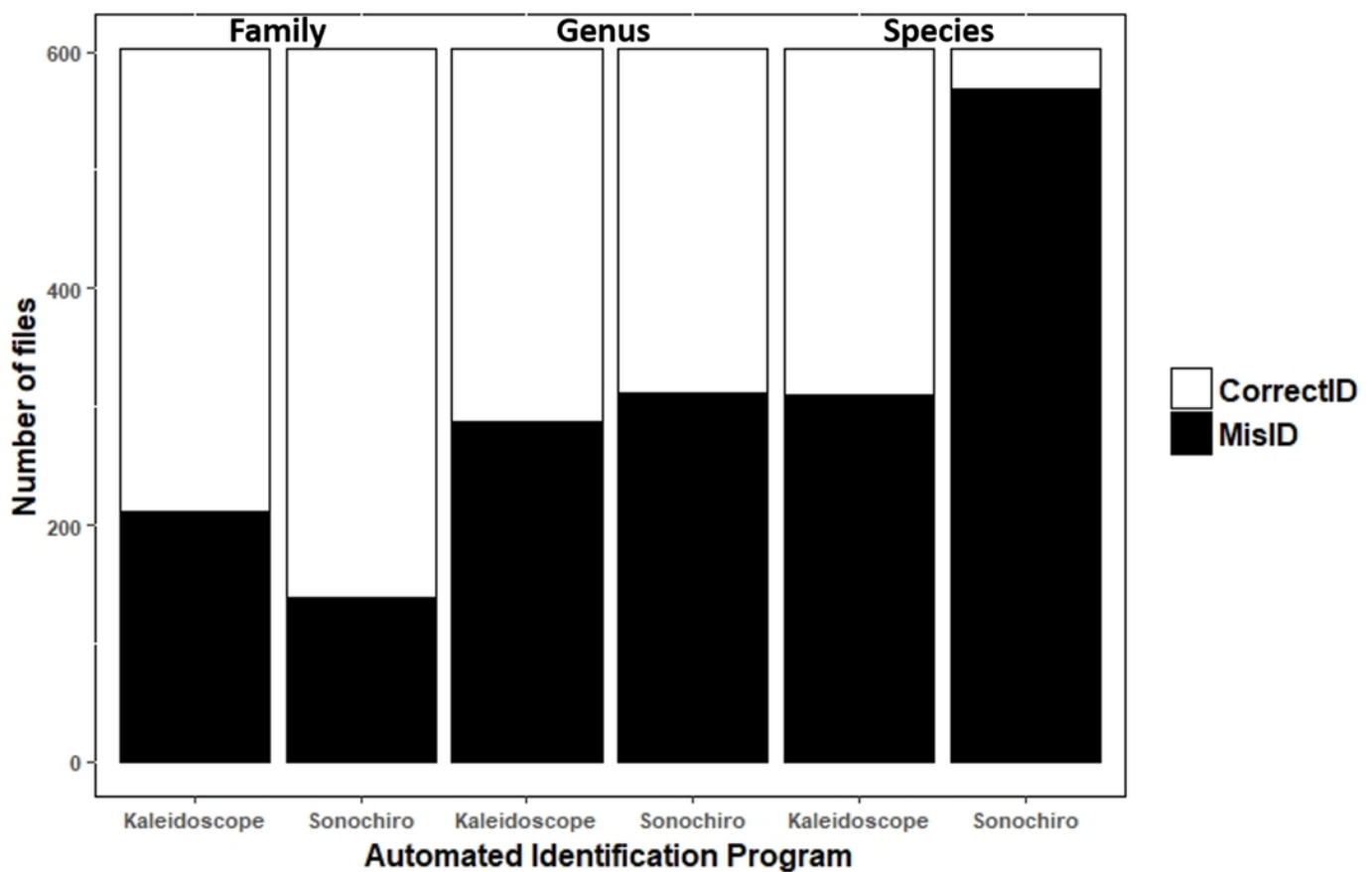
Figure 4

An application framework to use automated acoustic identification software in ecological and conservation studies of bats.
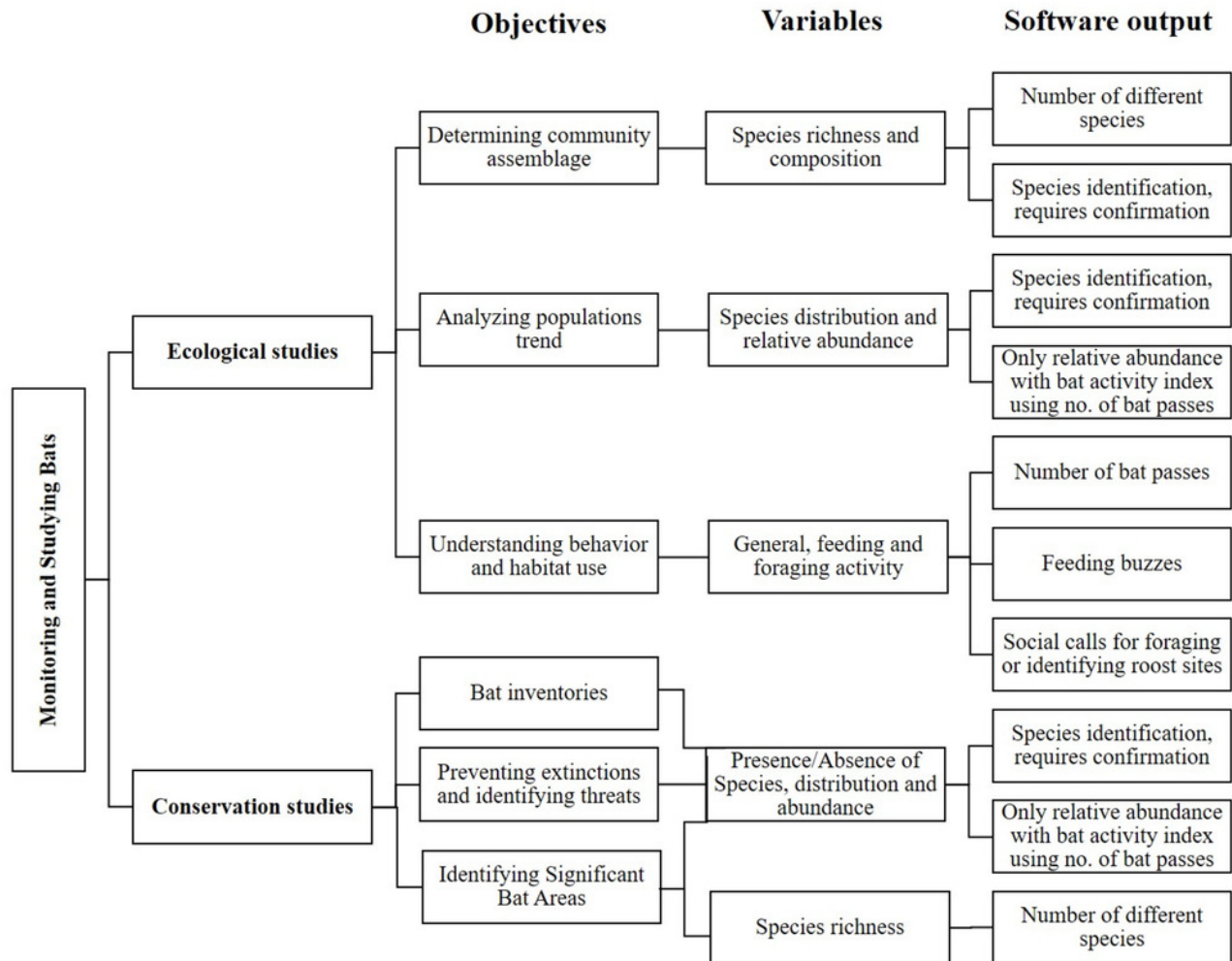
Table 1(on next page)

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
Cynomops sp.	31	0	0	0	0	571	571	31	31
Eptesicus brasiliensis	4	0	0	0	0	598	598	4	4
Eptesicus furinalis	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/palidoptera	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 parnellii	25	0	0	0	0	577	577	25	25
Eumops/Nyctinomops/ Tadarida sp.	97	0	0	0	0	505	505	97	97