Acoustic identification of Mexican bats based on taxonomic and ecological constraints on call design

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SUMMARY

1. Monitoring global biodiversity is critical for understanding responses to anthropogenic change, but biodiversity monitoring is often biased away from tropical, megadiverse areas that are experiencing more rapid environmental change. Acoustic surveys are increasingly used to monitor biodiversity change, especially for bats as they are important indicator species and most use sound to detect, localise and classify objects. However, using bat acoustic surveys for monitoring poses several challenges, particularly in mega-diverse regions. Many species lack reference recordings, some species have high call similarity or differ in call detectability, and quantitative classification tools, such as machine learning algorithms, have rarely been applied to data from these areas.
2. Here, we collate a reference call library for bat species that occur in a megadiverse country, Mexico. We use 4,685 search-phase calls from 1,378 individual sequences of 59 bat species to create automatic species identification tools generated by machine learning algorithms (Random Forest). We evaluate the improvement in species-level classification rates gained by using hierarchical classifications, reflecting either taxonomic or ecological constraints (guilds) on call design, and examine how classification rate accuracy changes at different hierarchical levels (family, genus, and guild).
3. Species-level classification of calls had a mean accuracy of 66% and the use of hierarchies improved mean species-level classification accuracy by up to 6% (species within families 72%, species within genera 71.2% and species within guilds 69.1%). Classification accuracy to family, genus and guild-level was 91.7%, 77.8% and 82.5%, respectively.
4. The bioacoustic identification tools we have developed are accurate for rapid biodiversity assessments in a megadiverse region and can also be used effectively to classify species at broader taxonomic or ecological levels. This flexibility increases

their usefulness when there are incomplete species reference recordings and also offers the opportunity to characterise and track changes in bat community structure. Our results show that bat bioacoustic surveys in megadiverse countries have more potential than previously thought to monitor biodiversity changes and can be used to direct further developments of bioacoustic monitoring programs in Mexico.

Keywords: acoustic identification, guild, hierarchical classification, random forest, machine learning, Neotropical, whispering bats.

INTRODUCTION

Effective conservation depends on our ability to define, measure and track ecological communities through time and space (Magurran *et al.* 2010). Although biodiversity monitoring programmes are critical to assess the impact of anthropogenic change, many are biased towards high latitude, temperate countries (Collen *et al.* 2009). Megadiverse countries (e.g., Indonesia, Mexico, Zaire) cover only 34% of the Earth surface, yet they harbour 70% of the world's biodiversity and are undergoing rapid environmental degradation (Mittermeier *et al.* 1997). In spite of the great conservation opportunity these hotspot regions offer, biodiversity monitoring programmes are often lacking, causing considerable knowledge gaps.

Bioacoustic surveys, especially for bats, are increasingly used to survey and monitor biodiversity responses to anthropogenic change (Jones *et al.* 2013; Amorim *et al.* 2014). Echolocating bats use sound to detect, localise and classify objects (Schnitzler *et al.* 2003) making them detectable both remotely and non-invasively. Bats are also ideal biodiversity indicators since they have a wide range of ecological traits, different tolerances to environmental variables and play key roles in ecosystems (Jones *et al.* 2009; Russo & Jones 2015). However, using bat acoustics as a monitoring tool poses several challenges, especially in megadiverse and tropical regions (Walters *et al.* 2013). First, in spite of the growing efforts to create more bat call reference recording libraries, tropical and megadiverse regions have rarely been included in such initiatives. This is compounded by recording method heterogeneity (e.g., full spectrum, frequency division, heterodyne), which makes compiling comprehensive libraries difficult (Walters *et al.* 2013). Such poor and uneven coverage of intra- and inter-specific variation makes Identification of bat calls for these regions challenging

Second, although it is possible to identify many bat species based on their calls, phylogenetic relatedness, ecological similarities, and call plasticity have led to overlapping

100 structures and high call similarity among and within species in some groups (Obrist 1995;
101 Jones & Teeling 2006). For example, species may have similar calls within families and
102 genera (Jung *et al.* 2007, 2014), and ecological guild membership may also reflect foraging
103 and echolocation behaviour (e.g., aerial insectivores, gleaners) (Denzinger & Schnitzler
104 2013). An additional challenge is that bat species differ in detectability of their calls. Aerial
105 insectivores typically produce loud calls of high-intensity and low frequency, whereas
106 'whispering' bats (including many bats in the families Phyllostomidae, Natalidae, and
107 Thyropteridae) often produce low-intensity, high frequency calls (Griffin 1958). However,
108 recent findings suggest that some 'whispering' bat calls are more detectable than previously
109 thought. For example, *Macrophyllum macrophyllum* and *Artibeus jamaicensis* can emit calls
110 as loud as those of many aerial insectivores (Brinkløv *et al.* 2009). *Otonycteris hemprichii*, a
111 passive gleaner, can also operate as an aerial hawker and can adjust its call intensity
112 depending on foraging mode even while flying in the same habitat type (Hackett *et al.* 2014).

113 Third, although acoustic species identification tools for different species are developing
114 rapidly (e.g., European bats Walters *et al.* 2012; birds Stowell & Plumbley 2014), they
115 remain rare for megadiverse regions. The immense amount of data obtained from acoustic
116 monitoring can be daunting and automatic analytical tools are extremely useful in analysing
117 such data (Walters *et al.* 2013). Bat call identification tools have been mainly developed
118 using multivariate statistical techniques such as discriminant function analysis (e.g.,
119 Vaughan *et al.* 1997; Russo & Jones 2002; Avila-Flores & Fenton 2005; MacSwiney *et al.*
120 2008) or machine learning algorithms (e.g., Skowronski & Harris 2006, Walters et al. 2012),
121 the latter generally providing higher species-level classification accuracy (Armitage & Ober
122 2010; Britzke *et al.* 2011; Keen *et al.* 2014). Machine learning algorithms have mostly been
123 applied to classify data at one level of categorisation (e.g., species) and have rarely
124 incorporated hierarchical information to aid classification accuracy (e.g., species within
125 families or orders). Hierarchical classification approaches have been shown to improve
126 general species classification accuracy for European bat calls up to 13% (Parsons & Jones

2000; Walters *et al.* 2012). Assigning taxa to classes within a hierarchy may reduce model complexity and minimise misclassifications outside their hierarchy (Vens *et al.* 2008). However, if an erroneous hierarchy is applied, then classification errors are added cumulatively across different levels, leading to a reduction in classification accuracy.

A hierarchical classification approach may be useful to classify calls to broader classes (e.g., genera, families or guilds) when reference material is missing for species, or where discrimination at species-level is difficult. For example, where there is high call variability within species, or a high overlap of call parameters between species. Although identification to species is most desirable, monitoring the status of the same recognizable signal over time without specific identification may be sufficient in some situations (Redgwell *et al.* 2009; Armitage & Ober 2010). Finding alternatives to species-level studies is needed in megadiverse areas, which usually face considerable financial and data constraints but are a priority for rapid conservation assessments.

Here, we collate a reference call library for bat species that occur in a megadiverse country to create acoustic identification tools using machine learning algorithms. We focus on Mexico because it contains one of the highest number of species in the world and has one of the highest rates of species extinction and habitat loss (Myers *et al.* 2000; Brooks *et al.* 2002). We also evaluate the improvement in species-level classification rates gained by using hierarchical classifications reflecting either taxonomic or ecological constraints on call design. Our results show that accurate bioacoustic identification tools can be developed for rapid biodiversity assessments in megadiverse regions where hierarchies generally improve species-level classifications. These tools can also be used effectively to classify calls at broader levels, so increasing the usefulness of the tool when there are incomplete species reference recordings.

MATERIALS AND METHODS

153 Reference call library

154 We collated reference search-phase echolocation calls for bat species that occur in Mexico
155 through a combination of field work and donated material. Field work was conducted in
156 central and northern Mexico from June 2012 to May 2013 at 35 sites (Fig. 1a). Bats were
157 caught with mist nets and identified to species level using field keys (Reid 1997; Medellín *et*
158 *al.* 2008), before being released. Full spectrum, real time recordings were made from all
159 individuals in the habitat in which they were captured using a Pettersson D1000x detector,
160 sampling rate 500kHz, high pass filter off (Pettersson Elektronik AB, Uppsala, Sweden).
161 Files were saved in WAV format on a flash card. We obtained 907 recordings of 39 species
162 from 6 families (see Table S1 in Supporting Information). Additionally, 1,403 full spectrum
163 recordings of bat calls from 87 species that occur in Mexico were donated by colleagues,
164 giving a total of 2,310 recordings (each recording was assumed to contain one individual call
165 sequence) from 92 species in 8 families (68% of species and 100% of families of bats
166 occurring in Mexico). These recordings were obtained from bats released in different ways
167 using several different real time or time-expanded full spectrum detectors, and in a range of
168 habitats across species' distributions (including localities outside Mexico) (Table S2). The
169 inclusion of call variation in the dataset avoids generating biases for any particular recording
170 situation or method (Walters et al. 2013), and provides the acoustic identification tools with
171 more flexibility and generality (see Walters et al. 2012).

172 Taxonomy followed Simmons (2005), but because of taxonomic changes since 2005 we
173 assume that Natalidae contains only one species, *Natalus stramineus* (López-Wilchis *et al.*
174 2012). Data from *Molossus sinaloae* and the new species *M. alvarezi* (González-Ruiz *et al.*
175 2011) were analysed together as *M. sinaloae* because most of the material was recorded
176 prior to the description of the new species. As some species are hard to identify in the field,
177 we only used the material which were confidently identified. To examine the taxonomic and
178 geographic coverage of the reference call library within Mexico, distribution maps were
179 downloaded from the IUCN mammal assessments (IUCN 2012) and species richness within

each 50 km^2 grid cell was estimated by overlaying and counting how many of those range maps overlap in each grid cell (Hawths Tools, Beyer, 2004). We then calculated the proportion of species both recorded and used in our classifiers from out of those potentially distributed in each cell.

Acoustic Identification Tools

We visually inspected all recorded sequences using the sound analysis software BatSound Pro v.3.31b (Pettersson Elektronik AB, Uppsala Sweden) to remove non search-phase calls. We distinguished search-phase calls from approach-phase and terminal-phase calls as these phase shifts are characterized by a decrease in call duration and interval, and increase in repetition rate (Schnitzler & Kalko 2001). Social calls were distinguished from echolocation calls by their duration, frequency and pattern of change over time, with social calls being more sporadic and often of a lower frequency range (Fenton 2003). In addition, bats were recorded in situations that significantly minimized the presence of social calls and approach and end-phase echolocation calls (e.g., recorded in open spaces upon release). We then automatically extracted and parameterised search-phase calls using the in-built algorithms in Sonobat v.3 (Szewczak 2010) (following methods in Walters *et al.* 2012). For species which used harmonics, we used measurements from the call used as the main harmonic. We measured a total of 21,064 search-phase echolocation calls from 1,692 sequences and 85 species in 8 families, with each sequence assumed to be from a different individual. Material recorded in Mexico contained 16,344 calls, 1,187 sequences from 65 species in 7 families across 91 different localities (Fig. 1a).

We used Random Forest (RF) models (randomForest package, Liaw & Wiener 2002) to train the classifiers, rejecting species that had less than five sequences. RF models consist of a collection or ensemble of decision tree classifiers where each classifier is randomly built using a bootstrapped sample of the training dataset (Breiman 2001). Each classifier is estimated based on probabilities using a selection of the predictor variables (in our case call parameters) that best separate the classes of interest (e.g., species, families) at different

branching splits or nodes in the tree. RF model classifications are then derived from averages of the tree ensembles. RF models possess several advantages over other machine learning algorithms as they are not affected by heteroscedasticity or distributional errors in the data, are not sensitive to outliers or irrelevant variables, can deal with mixed data and missing variables, and are relatively simple to train using reasonable computational resources (Olden *et al.* 2008). We selected 27 of the relevant call parameter variables (following methods in Walters *et al.* 2012) extracted and parameterised by Sonobat (Table S3), and ran a grid search to find the mtry value (optimal number of variables to be randomly sampled at each node). This value was allowed to range from 2-10, in steps of one. Each forest was grown to 2,000 trees and the final mtry value and number of trees were selected for their highest accuracy. The final set of parameters used was 1,000 trees and an mtry value of three. We used the coefficient of the Gini impurity index (used by the RF models to select the most informative variables at nodes during training), as an indicator of call parameter variable importance (Breiman 2001).

We trained four different RF model classifiers: Classifier 1 - Species-level without a hierarchy; Classifier 2 - Species-level within a family hierarchy (see call examples in Fig. S1a-f); Classifier 3 - Species-level within a genus hierarchy (see call examples in Fig. S1g); and Classifier 4 - Species-level within a guild hierarchy, following definitions of guilds from Denzinger & Schnitzler (2013) (see call examples in Fig. S2a-e): Guild 1 represented Open space aerial foragers; Guild 2 - Edge space aerial foragers; Guild 3 - Edge space trawling foragers; Guild 4 - Narrow space flutter detecting foragers; and Guild 5 - Narrow space passive gleaning foragers and Guild 6 - Narrow space passive/active gleaning foragers. Guild 7 - Narrow space active gleaning foragers was not included in the study because of the lack of reference material.

We used five-fold cross-validation to assess the accuracy of all four RF classifiers and assigned the individual calls into the five folds by sequence rather than individual calls (Stathopoulos *et al.* 2014). This procedure ensured that calls from the same individual (i.e.,

sequence) were not used in the same training and testing run of the cross-validation to avoid over-fitting. We set a maximum of 100 calls per species for Classifier 1 and a minimum of 20 calls per species for Classifiers 2, 3 and 4, as a compromise between maximising the number of calls and balancing the datasets, since RF classifiers tend to be biased towards the majority class (species, genus, family or guild with the highest number of training calls) (Chen *et al.* 2004). Only the highest quality calls were selected from each sequence (determined by the signal to noise ratio given by Sonobat), until the selected number of calls was reached. However, for some species with smaller sample sizes, we continued selecting calls from sequences in descending order of quality until we had used all available data or reached the number of calls allowed (Table S4). The number of calls selected per sequence was a compromise between maximising the number of calls and avoiding over-fitting the RF models. Sample sizes after this selection process were 4,685 calls and 1,378 sequences from 8 families, 32 genera, and 59 species that occur in Mexico. See Figure S3 for an outline of the analytical procedure.

As we used recordings from locations from both inside and outside of Mexico, we checked that the variation in call parameters recorded in locations outside of Mexico did not impact species classification accuracy. To investigate this, we compared model accuracy using the four classifiers of two datasets consisting of 47 species recorded from locations inside Mexico and the same species recorded from all locations. We found very little difference in classification accuracy between the two datasets. Classifier 1 had the biggest difference in classification accuracy, albeit with only 1.5% reduction in correct classification rates (67.1% and 65.6% for inside Mexico and for all locations, respectively). We therefore used recordings from outside Mexico to complement species with less than five Mexican sequences. All analyses were performed in R version 3.0.2 (R Development Core Team 2013).

RESULTS

Database coverage

Our collated library of echolocation call recordings covered 69% of the species, 79% of the genera, and 100% of the families occurring in Mexico. Data of high enough quality to build the automatic identification tools covered 43% of the species, 51% of the genera, and 100% of the families (Table S5). There was generally a good representation of species for the identification tools within genera and families (>50%), except for Phyllostomidae, where only 19% of the species were represented. Species coverage was more comprehensive within the central and northern parts of Mexico for both the library and identification tools (Fig. 1b-c).

Acoustic Identification Tools

Overall 16 out of the 27 parameters used to train the models contributed most to all classifiers (based on a score >30 for the Gini Coefficient from the RF models) (Table S6, Fig 2a-d). Although different parameters were important for each hierarchy, the most important overall were Fc Characteristic call frequency (kHz), FCtr Frequency at the center of the call duration (kHz), FLed Frequency of the ledge (kHz), StartF Frequency at the start of a call (kHz), HFreq Highest call frequency (kHz), and FMPwr Frequency of the maximum call amplitude (kHz) (Fig. 2a-d, see Table S3 for further variable definitions).

Overall mean species-level classification accuracies for Mexican bat species varied across the four classifiers between 66.0% (Classifier 1: Species-level without a hierarchy) and 72.0% (Classifier 2: Species-level within a family hierarchy), with Classifiers 3 (Species-level with a genus hierarchy) and 4 (Species-level with a guild hierarchy) having accuracies of 71.2% and 69.1%, respectively (Table 1). Across all classifiers, on average the highest classification accuracies where found for species within families Natalidae (100%), Mormoopidae (94.6%), Thyropteridae (81.5%), and Emballonuridae (77.7%), with the lowest found within Noctilionidae (70.4%), Molossidae (67%), Vespertilionidae (51.5%), and Phyllostomidae (51.4%) (Fig. 3). Phyllostomid species were mostly misclassified with other

289 phyllostomids or with vespertilionids, whereas vespertilionids were commonly misclassified
290 with other vespertilionids or with molossids (Table 1). For the ecological classifier, species
291 within Guild 4 (narrow space flutter detecting foragers) (100%), Guild 3 (edge space trawling
292 foragers) (74.6%), and Guild 1 (open space aerial foragers) (63.8%) had on average the
293 highest classification rates. The lowest average classification rates for species were found
294 within in the gleaners (Guild 5 58.5% and Guild 6 57.7%) Guild 2 (edge space aerial
295 foragers) (54.5%) (Fig. 4).
296
297 Classification accuracy at different hierarchical levels was highest at family-level with a mean
298 of 91.7% across all families (Table 1, Fig. 3), where Natalidae and Mormoopidae had the
299 highest classification accuracies (100% and 97.3%, respectively). Noctilionidae had the
300 lowest classification accuracy (72.8%) and was frequently misclassified as Molossidae (17%
301 of the calls). Genus-level mean classification accuracy was 77.8% across all genera (Table
302 1), *Natalus* (Natalidae) and *Rhynchonycteris* (Emballonuridae) had the highest classification
303 accuracies (100%), and 18 genera had accuracies >80% (Fig. 3). The genus *Myotis* yielded
304 a classification accuracy of 73.8%, with two species over >80% (*Myotis thysanodes* and
305 *Myotis keaysi*) and only 4 with <50%. Genera with the lowest classification accuracies
306 (<50%) were in the Phyllostomidae and Vespertilionidae (Fig. 3). Phyllostomids were mostly
307 misclassified as other phyllostomids, while vespertilionids were misclassified as other
308 vespertilionids and molossids. Mean guild-level classification accuracy was 82.5% across all
309 guilds (Table 1, Fig. 4). Guild 4 (narrow space flutter detecting foragers) had the highest
310 classification accuracy (100%), followed by Guild 6 (88.3%) although 6% of these calls were
311 misclassified with Guild 5. Guild 5 had the worst classification accuracy (68%), and 18% of
312 calls were misclassified as Guild 6 (Fig. 4).
313
314 DISCUSSION

315 We have collated the most extensive bat acoustic library for a megadiverse region (all
316 families and over half of the species occurring in Mexico) and developed the most
317 comprehensive bat acoustic automated species-level classifiers to date. The mean species-
318 level classification accuracy rate of 66-72% (depending on which hierarchy is chosen) is
319 reasonable given the high level of call similarity of the bat species in this area (Walters et al.
320 2013). The species-level classifiers also contain a large variation in accuracy rates, where
321 some species are classified to >80% accuracy (species of Emballonuridae, Mormoopidae,
322 Natalidae and Thyropteridae), with the poorest results overall from species of
323 Vespertilionidae and Phyllostomidae. This suggests that acoustic monitoring may be more
324 feasible focusing on a few species whose calls can be reliably classified.

325 The bat call library and classifiers incorporate both extensive geographic (from 9 countries
326 within the species range of Mexican bats) and intra-specific variation in call types (e.g., the
327 classifiers were trained on the different search-phase echolocation call types found within
328 molossid species, Jung *et al.* 2014). However, the species-level classifiers have a very low
329 coverage of Phyllostomidae and results should be interpreted with caution. It has been
330 traditionally assumed that whispering bats, which include all phyllostomids, echolocate at
331 intensities that were too low for the inclusion of these species in acoustic studies. However,
332 recent field studies of their echolocation behaviour challenged these assumptions about their
333 echolocation characteristics (Brinkløv *et al.* 2009; Hackett *et al.* 2014). Future work should
334 focus on collecting more reference material for the family, to better assess its potential for
335 acoustic monitoring programmes.

336 Our classifiers will be the most accurate in regions where there is a higher coverage of the
337 species present, such as the less species-rich arid and semi-arid regions of Mexico. These
338 ecosystems (e.g., xerophytic scrubland and grasslands) cover at least 40% of the territory
339 (Rzedowski 2006), and together with other North American drylands, support some of the
340 biggest concentrations of mammalian abundance, because bats can form colonies of several
341 millions of individuals (O'Shea & Bogan 2003). These bat populations can provide important

342 ecosystem services such as pollination and control of insect populations (Cleveland *et al.*
343 2006; Munguía-Rosas *et al.* 2009). These important arid and semi-arid environments are
344 increasingly threatened by environmental changes (Villers-Ruiz & Trejo-Vázquez 2003;
345 Rodríguez-Estrella 2007) and future efforts should focus on these arid areas where there are
346 considerable information gaps.
347
348 Our species-level classifier mean accuracy was similar to that of previous studies of bats for
349 species shared with this study (Mexico - MacSwiney *et al.* 2008; Stathopoulos *et al.* 2014;
350 West Indies - Pio *et al.* 2010; United States - Skowronski & Harris 2006; Britzke *et al.* 2011).
351 However, our classification accuracies were slightly lower for some species compared with
352 previous work. This is a consequence of the higher number of classes (species) included in
353 our classifiers compared to all previous studies. Higher numbers of species increases the
354 similarity in the call parameters of several species. For example, we included 26
355 vespertilionids and 8 *Myotis* species, compared to 6 vespertilionids and one *Myotis* in
356 MacSwiney *et al.* (2008) and 9 vespertilionids and two *Myotis* in Stathopoulos *et al.* (2014).
357 Our study nearly triples the number of species used compared to any other quantitative bat
358 call classification study in the Americas or any other megadiverse tropical region in the
359 world. We also included a wide range of ecological, technological and methodological
360 variation in the training dataset, which on one hand increases the classification challenge,
361 but on the other makes the classifiers more robust to real-world recording situations. In spite
362 of the great difference in the number of species used here, we also obtained higher
363 classification accuracies to species level and better mean accuracies than previous studies
364 (e.g., Pio *et al.* 2010; Stathopoulos *et al.* 2014).
365
366 Our use of taxonomic and ecological guild hierarchies improved mean species-level
367 classification rates. By using hierarchical classification approaches the number of final
368 classes is considerably reduced and misclassifications are limited to classes within the
369 respective hierarchy (Vens *et al.* 2008). Mean species-level classification accuracies were

most improved using a family hierarchy, closely followed by genera (72% and 71%, respectively), although not all species improved their accuracies (contrary to other studies - Parsons & Jones 2000; Walters *et al.* 2012). The genus-level hierarchy produced the highest number of species-level classifications with >80% accuracy but for many genera not all species were included in the analysis and genus-level taxonomic names can be subject to rapid changes (Simmons 2005). This may suggest that using a genus-level hierarchy may be more problematic than a family hierarchy, especially with incomplete reference material. For example, accuracy may decrease as more species are included, whereas variation within a family may be already adequately represented. In contrast, classification to genus level may be more helpful to reduce the number of options of possible misclassifications inside the hierarchy and further methods for call identification could then be applied (e.g., visual inspection).

Although we found species-level classification rates within an ecological guild-level hierarchy were worse than species-level classification rates within either taxonomic hierarchy, classification of calls to guild-level performed well and could provide a useful alternative to taxonomic-level classifiers. Gleaners, in particular the speciose family Phyllostomidae, are the most abundant and diverse in bat communities in the Americas, yet poorly represented in acoustic libraries. Our results at family and guild-level suggest that there is a good potential for accurate acoustic identification of gleaners. As more sensitive microphones with better signal/noise ratios become available, the detectability of these species will improve, as will the potential for monitoring them acoustically. Guilds 5 and 6, representing gleaning foragers, were frequently confused with each other, so these should be grouped into one class, since the main difference among them is how they use other non-echolocation cues to forage (Denzinger & Schnitzler 2013).

Acoustic analysis techniques are evolving rapidly and there is a growing tendency to replace classifications based on parameters extraction with those of whole signal analysis. However, applications of these approaches have mainly focused on bird and marine mammal

acoustics (e.g., Ren *et al.* 2009; Damoulas *et al.* 2010) and most bat acoustic classification tasks still represent classifications with a few parameters and further classify them using manual or non-parametric techniques. Such whole signal analyses in bat acoustics are growing (Obrist *et al.* 2004; Skowronski & Harris 2006; Stathopoulos *et al.* 2014) but should be further explored. However, exploration of new approaches requires adequate reference material collected in a systematic way, controlling for variation introduced by the use of different methods, and we strongly encourage further efforts to collect comprehensive reference bat call libraries.

Applications

Standardized identification tools such as these, offer the opportunity for objective and repeatable identifications of monitoring 'units' to identify changes in populations, distributions or community structures through time and space. Furthermore, hierarchical approaches offer the flexibility to adapt the identification tools to the purpose of the study or monitoring programme and the geographic and taxonomic coverage of the reference material available. Although the accuracy reached for some groups might not be sufficient for studies targeting their particular species (e.g., *Myotis* spp.), the hierarchical classifiers can act as filters for large amounts of data. The use of hierarchies considerably reduces the list of species to which an unknown call could belong, thus making detailed inspections and further validations more feasible.

Hierarchical classifications, in particular at family-level, could help reduce the costs of monitoring tropical bat communities, which is crucial due to the limited funding these regions often devote to conservation efforts. Despite the relatively poor classification accuracies to species-level within the guild-level hierarchy, classification to guild-level could be used to rapidly characterize ensemble/environment associations or to track changes in community structure. The hierarchical approach may be improved through the use of regional classifiers which allow the reduction of the number of classes and the improvement of classification

accuracy. However, such an approach should be used with caution as least known species or those with expanding ranges could be ignored.

CONCLUSIONS

Our study shows that there is more potential for bat acoustic monitoring in megadiverse countries than previously considered. Hierarchies considerably reduced the complexity of call identification at different levels and provided sufficient confidence in the classification of unknown calls into higher taxonomic levels and ecological guilds. While the classifiers did not provide high classification accuracies for several species, they did offer the opportunity to have objective and repeatable identification of monitoring 'units' to implement in national acoustic monitoring programmes.

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DATA ACCESSIBILITY

Complete call measurements for each of the 4,685 search-phase calls from 1,378 individual sequences of 59 bat species used to create the classification tools and R scripts have been uploaded to DataDyrad (doi:10.5061/dryad.760r8).

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610

611 **Table 1.** Comparison of classification accuracies of four acoustic classifiers for Mexican bat
612 species (n=59 species). Where Classifier 1 represents species-level without a hierarchy;
613 Classifier 2: species-level within families; Classifier 3: species-level within genera; Classifier
614 4: species-level within guild. Misclassification represents those classes having the most
615 misclassifications with each other for each classifier and level, where Phyllo Phyllostomidae;
616 Vesp Vespertilionidae; Molo Molossidae; and Noct Noctilionidae.

617

Classifier	Level	Mean accuracy %	Accuracy range %	% of classes ≥80% accuracy	% of classes ≤60% accuracy	Misclassifications
1	Species	66	4.2-100	29	41	Species of Phyllo with themselves or Vesp; Vesp with themselves or Molo
2	Species	72	0-100	32	44	Species within families
	Family	91.7	72.8-100	88	0	Noct with Molo
3	Species	71.2	0-100	36	37	Species within genera
	Genus	77.8	0-100	56	16	Phylo with other Phylo and Vesp genera; and Vesp with Phylo and Molo genera
4	Species	69.1	4.5-100	25	44	Species within guilds
	Guild	82.5	68-100	50	0	Guild 5 with Guild 6; Guild 6 with Guild 5

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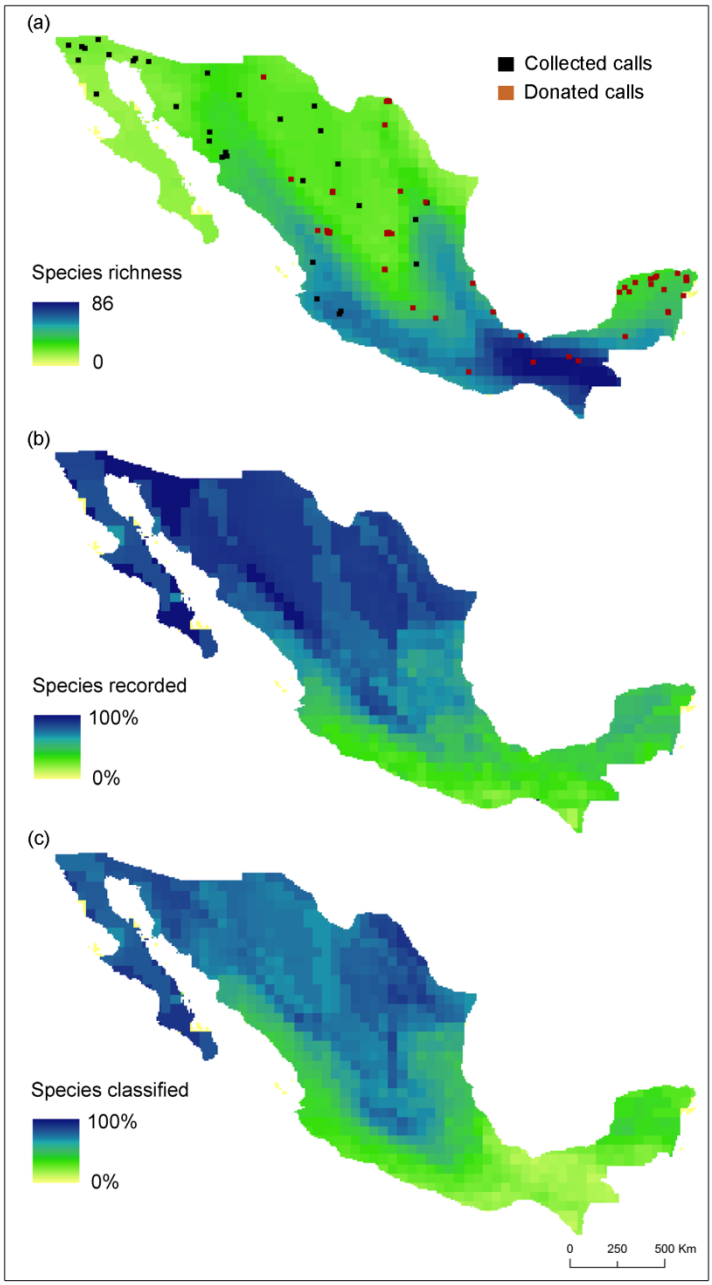

Figure 1. Spatial coverage of the number of species recorded in Mexico using a grid size of 50 km^2, where (a) shows recording locations in solid squares (n = 91) overlaid with bat species richness, (b) proportion of species recorded compared to potential species richness in each grid, and (c) proportion of species used in the classifiers compared to potential species richness in each grid. A gradient of light green to dark blue indicate higher number of species and higher percentages. Black solid squares represent collection sites which were sampled in this study and red solid squares represent collection sites of donated material.

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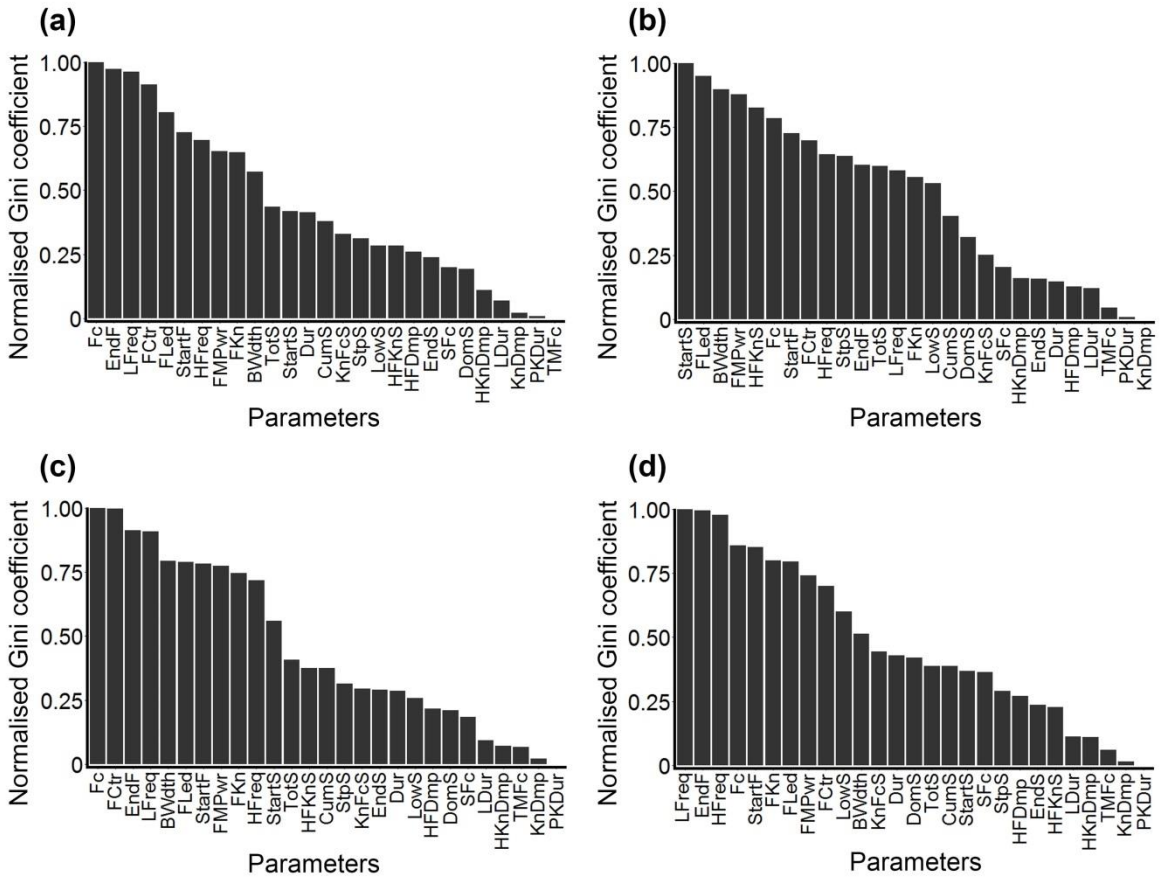

630

631 **Figure. 2.** Echolocation call parameters (n = 27) selected to build each Random Forest
632 classifier ranked by Gini Coefficient where (a) Classifier 1: Species-level without a hierarchy;
633 (b) Classifier 2: Species-level within a family hierarchy; (c) Classifier 3: Species-level within a
634 genus hierarchy; and (d) Classifier 4: Species-level within a guild hierarchy. See Table S3
635 for parameter definitions.

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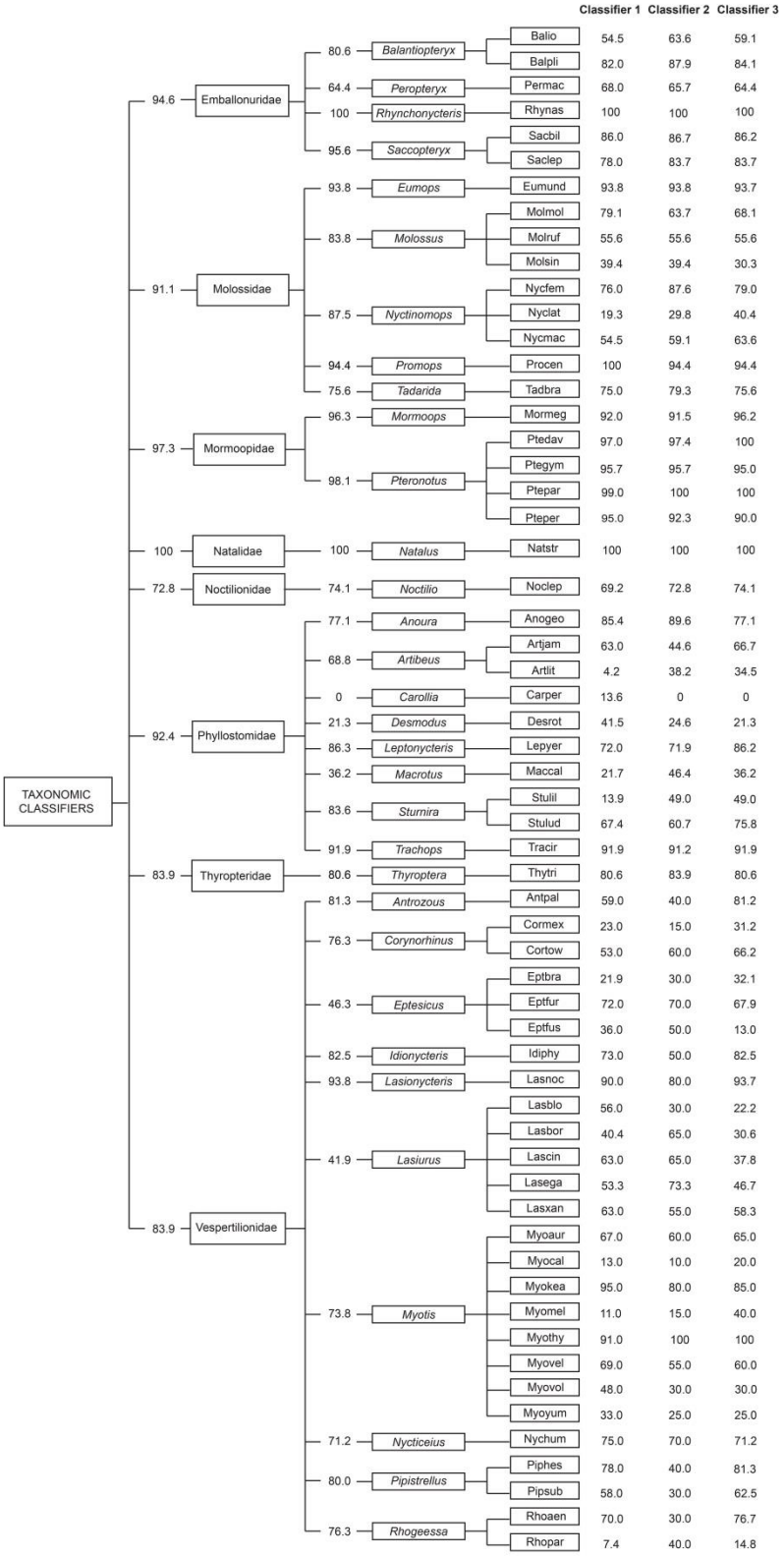
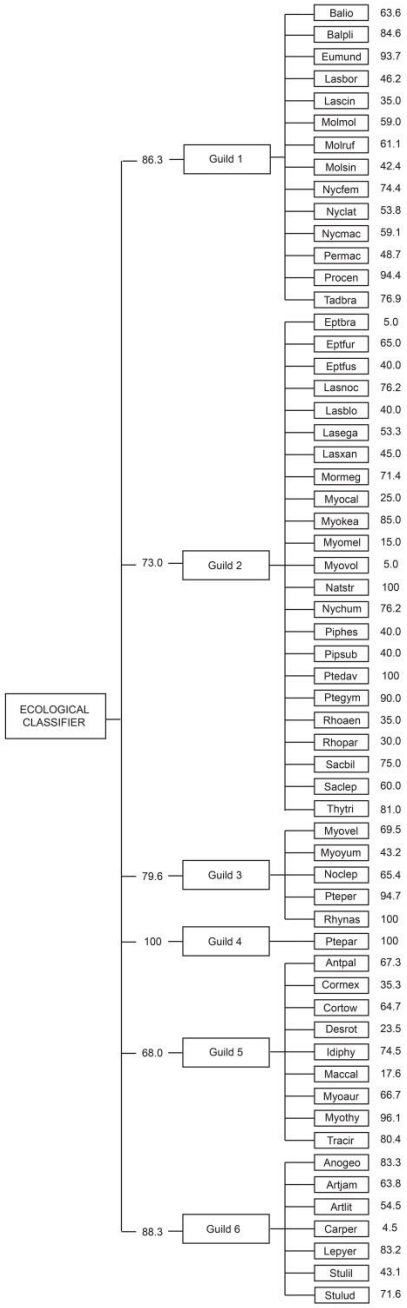


Figure. 3. Random Forest percentage classification accuracies obtained for the taxonomic classifiers (Classifiers 1-3). Species-level accuracies are shown at the end of each branch for Classifier 1, 2 and 3. Classification accuracies per family and genus are shown in the middle of each branch (n = 59 species).



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643 **Figure 4.** Random Forest percentage classification accuracies obtained for the ecological
644 guild classifier (Classifier 4). Species-level accuracies are shown at the end of each branch.
645 Classification accuracies per guild are shown in the middle of each branch (n = 59 species).
646 Guild 1 - Open space aerial foragers; Guild 2 – Edge space aerial foragers; Guild 3 – Edge
647 space trawling foragers; Guild 4 – Narrow space flutter detecting foragers; Guild 5 - Narrow
648 space passive gleaning foragers and Guild 6- Narrow space passive/active gleaning
649 foragers.

650 **Supporting Information**

651 Additional Supporting Information may be found in the online version of this article:

652 **Table S1**. Metadata for the search-phase echolocation calls collected during field work in
653 Mexico from June 2012 to May 2013.

654 **Table S2**. Metadata for the search-phase echolocation calls donated for this study.

655 **Table S3**. Definitions of the 27 call parameters extracted by Sonobat v.3 used for training
656 the Random Forest classifiers.

657 **Table S4**. Number of classes included in each hierarchy of the four classifiers and number of
658 calls used in the training process.

659 **Table S5.** Taxonomic coverage of the bat call library within each family for the number of
660 genera and species recorded / used in the classifiers.

661 **Table S6**. Descriptive statistics (mean and standard deviation) for the 16 most important call
662 parameters ranked by Random Forest Gini Coefficient measured by Sonobat for the 59
663 species.

664 **Figure S1**. Spectrograms showing the inter-specific variability of representative search-
665 phase echolocation calls within taxonomic groups used for the classifiers.

666 **Figure S2**. Spectrograms showing inter-specific variability of representative search-phase
667 echolocation calls within ecological guilds used for the classifiers.

668 **Figure S3**. Schematic representation of the protocol used to build the classifiers.