# **1** Separating overlapping bat calls with a bi-directional long short-term

### 2 memory network

# 3 Using deep neural network to separate overlapping bat calls

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## 16 Abstract

Acquiring clear and usable audio recordings is critical for acoustic analysis of animal vocalizations. Bioacoustics studies commonly face the problem of overlapping signals, but the issue is often ignored, as there is currently no satisfactory solution. This study presents a bi-directional long short-term memory (BLSTM) network to separate overlapping bat calls and reconstruct waveform audio sounds. The separation

22 quality was evaluated using seven temporal-spectrum parameters. The applicability of 23 this method for bat calls was assessed using six different species. In addition, 24 clustering analysis was conducted with separated echolocation calls from each 25 population. Results showed that all syllables in the overlapping calls were separated 26 with high robustness across species. A comparison between the seven 27 temporal-spectrum parameters showed no significant difference and negligible 28 deviation between the extracted and original calls, indicating high separation quality. 29 Clustering analysis of the separated echolocation calls also produced an accuracy of 30 93.8%, suggesting the reconstructed waveform sounds could be reliably used. These 31 results suggest the proposed technique is a convenient and automated approach for 32 separating overlapping calls using a BLSTM network. This powerful deep neural 33 network approach has the potential to solve complex problems in bioacoustics.

#### 34 Author summary

35 In recent years, the development of recording techniques and devices in animal 36 acoustic experiment and population monitoring has led to a sharp increase in the 37 volume of sound data. However, the collected sound would be overlapped because of 38 the existence of multiple individuals, which laid restrictions on taking full advantage 39 of experiment data. Besides, more convenient and automatic methods are needed to 40 cope with the large datasets in animal acoustics. The echolocation calls and 41 communication calls of bats are variable and often overlapped with each other both in 42 the recordings from field and laboratory, which provides an excellent template for 43 research on animal sound separation. Here, we firstly solved the problem of

44 overlapping calls in bats successfully based on deep neural network. We built a 45 network to separate the overlapping calls of six bat species. All the syllables in 46 overlapping calls were separated and we found no significant difference between the 47 separated syllables with non-overlapping syllables. We also demonstrated an instance 48 of applying our method on species classification. Our study provides a useful and 49 efficient model for sound data processing in acoustic research and the proposed 50 method has the potential to be generalized to other animal species.

51 **Introduction** 

52 The structural identification of vocal units is essential in animal acoustic studies for 53 sound feature analysis, sound emitter recognition, and species identification and 54 monitoring. However, wild animal monitoring, both in the field and in the laboratory, 55 often involves problems caused by the overlapping of different vocal units in time and 56 frequency space, which prevents the components from being suitable for parameter 57 analysis. As a result, the separation of overlapping sounds is an important task in 58 bioacoustic signal processing. However, existing analysis software often struggles to 59 process overlapping calls and previous research on the acoustic identification of 60 animals primarily focuses on extracting target signals from background noise for 61 species classification or population monitoring [1-4]. The process of separating 62 overlapping calls from mixed sounds has received little attention to date and 63 researchers conventionally abandon sounds that overlap in both time and frequency, 64 requiring an extension of the experimental period to obtain sufficient non-overlapping 65 recordings [5, 6]. As such, an effective method for successfully and automatically

separating overlapping calls would be of significant interest and benefit to animalresearchers.

68 Previous studies using deep neural networks have produced promising results for 69 automated sound recognition in complex acoustic environments for animal species 70 recognition and classification [6-8]. However, in this study, we consider the more 71 difficult task of separating different types of syllables from overlapping calls and 72 reconstructing sound waves from these separated signals. Existing techniques used for 73 animal sound separation often require prohibitive quantities of labelled data. For 74 example, multiple-instance machine learning (MIML) algorithms were proposed for 75 use in sound feature extraction and species identification in birds [1]. However, this 76 technique requires a cropped mask of a signal segment (without overlap) in order to 77 extract each syllable.

78 Deep learning networks have been applied to bioacoustic studies but have primarily 79 been used for classification. For instance, convolutional bidirectional recurrent neural 80 networks (CBRNNs) have been used to identify the presence of bird calls in audio 81 samples [4]. Acoustic features were learned by the network (a classifier) and the 82 presence or absence of a bird call was output as an indicator. Convolutional neural 83 networks (CNNs) have been used to predict the presence of a search-phase bat 84 echolocation call in spectrograms. This binary classification problem was used to 85 detect the presence of bats [2]. To our knowledge, the use of deep learning techniques 86 to separate animal calls that overlap in both time and frequency space has yet to be 87 reported.

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88 Multiple studies have been conducted using deep learning-based supervised speech separation with humans. Early systems included shallow models that performed a 89 90 linear transformation of given mixture features during the prediction time interval. 91 This has included Gaussian mixture models [9], support vector machines [10], and 92 non-negative matrix factorization [11]. However, in real-world scenarios, the mapping 93 relationship between mixture signals and sources is typically a nonlinear 94 transformation. Nonlinear models, such as deep neural networks (DNNs), are 95 therefore highly applicable because of their ability to identify nonlinear structures in 96 audio signals [12-14]. Additionally, recurrent neural networks (RNNs) that exhibit the 97 temporal behavior of a time sequence can be trained to predict time-frequency masks 98 for target signals and separate sources from a mixed waveform [15]. Specifically, long 99 short-term memory (LSTM) networks, a variation of RNN models that exhibit strong 100 learning capabilities and simple construction, have been widely used for word and 101 continuous speech recognition [16-18]. By concatenating two separate LSTM 102 networks, bidirectional LSTMs (BLSTMs) can predict each element of a sequence 103 based on past and future context and can naturally account for the temporal dynamics 104 of speech. These models are typically faster and more accurate than standard RNNs in 105 frame-by-frame phoneme classification [19]. In addition, the BLSTM network can 106 compensate for exploding and vanishing gradient issues that can occur during the 107 training of standard RNN models [20]. At present, BLSTMs have achieved 108 state-of-the-art performance for speech recognition [14, 21], natural language 109 processing [22, 23], and speaker-independent speech separation [24]. As such, a

110 BLSTM model was selected in this study for overlapping bat call separation.

111 Echolocating bats have two vocal repertoires, stereotypical echolocation calls for 112 orientation and a variety of communication calls for social activities [25-27]. 113 Recordings from both field and laboratory studies indicate that utterances from 114 individual bats often overlap in both time and frequency, which provides an excellent 115 template for research on overlapping sound separation in animals. The primary 116 objective of this study is to develop a technique for separating two target signals 117 (echolocation and socialization calls) from mixtures of acoustic sounds. Although 118 deep leaning has been employed in the acoustic classification of multiple species, 119 including nonhuman primates [28], birds [4], whales [5], and bats [2, 3], the goal of 120 the present study is distinct from these previous cases in which deep neural networks 121 were primarily used as classifiers.

122 Both overlapping and non-overlapping calls (of both echolocation and 123 communication types) were recorded from each of the collected bat species studied in 124 our previous work. We developed a BLSTM network and used the recorded 125 non-overlapping calls to train the model. Recorded overlapping calls were input to the 126 trained model and separated. Independent sound files were then reconstructed for each 127 separated signal. The correctness of these separated signals was measured by 128 comparing the temporal-spectrum parameters between separated calls and the initially 129 recorded (non-overlapping) calls from each species. Finally, clustering analysis was 130 conducted to classify the bats using separated echolocation calls, which provided a 131 practical application of the proposed technique.

## 132 **Results**

133 The proposed algorithm performed well and achieved high accuracy in separating 134 overlapping calls for each of the six species. The BLSTM model was iteratively 135 trained until the training and validation losses reached a minimum. Loss is a 136 summation of errors made with each sample in the training or validation sets and 137 measures how well the model adapts during optimization. Training loss for this model 138 decreased significantly in the first epoch. The validation loss function tended toward 139 an asymptotic value, indicating the training algorithm had converged (S2 Fig). The 140 BLSTM model converged slightly faster when training with CF bat samples (as 141 opposed to FM samples).

All echolocation and communication calls in the overlapping signals were correctly extracted during the separation procedure, regardless of their pulse duration or energy characteristics (see Table 1 and Fig 1). In addition, low-intensity FM components in echolocation pulses were successfully extracted from three CF bat species (Figs 1d, 1e, and 1f).

147 Table 1. Separation results.

Species	Call type	Number of syllable types	Number of syllables in overlapping calls	Number of overlapping syllables	Number of separated syllables
Rhinolophus	Echolocation	1	14	14	14
ferrumequinum	Communication	4	8	8	8
Vespertilio	Echolocation	1	21	13	13
sinensis	Communication	4	8	8	8
Hipposideros	Echolocation	1	28	19	19
armiger	Communication	6	13	13	13
Myotis	Echolocation	1	54	36	36

macrodactylus	Communication	6	15	15	15
Rhinolophus	Echolocation	1	42	30	30
pusillus	Communication	6	10	10	10
In in	Echolocation	1	26	16	16
Ia io	Communication	4	11	11	11

148

149	Fig 1. Spectrograms from original recordings of overlapping calls and calls separated by
150	the BLSTM network. The first graph represents each line of the original overlapping calls
151	and the second and third graphs show the separated echolocation and communication calls,
152	respectively.
153	
154	A comparison of seven temporal-spectrum parameters from the separated calls
155	and the original recorded non-overlapping calls showed no significant differences (Fig
156	2 and S3 Table). In addition, parameter deviations in separated calls and original
157	non-overlapping calls showed minimal RMSE values for both echolocation and
158	communication signals (Fig 3 and Fig 4). Clustering analysis performed with

separated echolocation calls produced an accuracy of 93.8% across species (Fig 5).

160

Fig 2. Comparisons between the separated and original calls. Two principle
components extracted from seven temporal-spectral parameters were used in the study.
Results for echolocation and communication calls are shown in (A-F) and (G-L),
respectively.

Fig 3. A comparison of deviations for separated and original echolocation calls.
The RMSE value is shown under each plot. The vertical axis represents values for

167 each parameter and the horizontal axis represents the number of syllables measured. 168 The red triangles represent separated calls and the blue dots represent original calls. 169 Abbreviations include duration (duration), Fstart (starting frequency), Fend (ending 170 frequency), Fpeak (peak frequency), Fmin (minimum frequency), Fmax (maximum 171 frequency), and bandw (bandwidth). 172 Fig 4. A comparison of deviations in separated and original communication calls. 173 The RMSE value is shown under each plot. The vertical axes and abbreviations are 174 the same in Fig 3. 175 Fig 5. Clustering analysis for six bat species based on their separated 176 echolocation calls. Overlapping echolocation signals cannot be used for species

- 177 identification until after separation.
- 178

### 179 **Discussion**

The BLSTM network used in the present study achieved high accuracy in 180 181 separating overlapping echolocation and communication calls from bats. The training 182 and validation loss for the model also exhibited fast convergence and high robustness 183 for bat vocalizations. In particular, the separated calls extracted by the proposed 184 algorithm were reconstructed as waveform files with nearly the same quality as the 185 non-overlapping calls, suggesting BLSTM networks to be useful tools for separating 186 signals in future bioacoustic research, such as sound analysis, acoustic identification, 187 species classification, and wild animal monitoring.

188 It was difficult to compare the performance of this algorithm with that of previous

189	studies, primarily because of differences in the experimental procedure. However, a
190	comparison of temporal-spectrum parameters between separated calls and
191	non-overlapping calls was included as an evaluation metric. The seven parameters
192	used in this study are commonly used in bat studies to describe the temporal-spectral
193	features of syllables [26, 29]. Statistical results for this comparison showed no
194	significant differences and small deviations in parameters between separated calls and
195	original recordings, indicating the system was able to separate calls without affecting
196	syllable quality. In addition, clustering analysis conducted with reconstructed
197	echolocation calls was highly accurate (93.8%) for species classification, indicating
198	that calls separated from overlapping signals could be used to synthesize initial data.
199	The BLSTM network exhibited good performance across all six bat species using
200	both narrow and broad time-frequency calls. It also successfully separated different
201	syllable types from both overlapping echolocation and communication calls (Table 1,
202	Fig 1). No species-specific a priori knowledge or particular acoustic sensor was
203	directly encoded into the system, making it generalizable to other animal populations
204	with additional training data. Although the dichotomy between communication and
205	echolocation calls is relatively drastic, the proposed separation system has potential
206	applications for other species, as such mixtures are very common in bats. In the future,
207	more complex emitter-independent separation could be conducted using the proposed
208	system, such as combinations of echolocation or social calls from other animals.
209	While deep learning models generally perform better when provided with more data,
210	training with bat calls requires fewer samples than human speech separation, in which

211	available training sets can exceed hundreds of hours [13]. One possible reason for this
212	may be the high signal-to-noise ratio (SNR) of bat sounds recorded with high-quality
213	ultrasound devices. Previous studies have indicated that a high SNR can improve
214	separation accuracy [30] and our results suggest this model was suitable for use with
215	small, high-quality datasets. Although the sound data in this study were sampled in
216	controlled lab conditions, producing recordings that were essentially free of
217	background noise, acoustic analysis software could potentially optimize the separation
218	further by excluding any background noise that was present in the signal.

219 Future studies will also assess the performance of this network for other animal 220 species. Stereotypical patterns and clearly classifiable syllables have been observed in 221 the vocalizations of birds, non-human primates, whales, dolphins, and several other 222 species [31-33]. Features used in the proposed BLSTM were log spectral magnitudes, 223 which can be acquired from any vocal sound. This could potentially lead to robust 224 software that is not specific to a certain species or task. The model could also be 225 generalized to other animals, though limitations may exist. In addition to the quality 226 and quantity of training samples, hyper-parameters must be tuned in accordance with 227 the data [34, 35].

### 228 Conclusion

A sound separation model was proposed for extracting bat calls, achieving excellent results. This is the first experimental evidence that the BLSTM model is suitable for separating overlapping bioacoustic signals. These results provided a new source for sound data analysis in animal acoustics research, which may contribute to sample sizes and improve efficiency. This study also demonstrates the potential of
deep neural networks for applications to animal vocalization research, including
species classification and speech separation.

#### 236 Materials and Methods

#### 237 Sound recording and data preparation

238 Species selection and sound sources. Echolocation calls from bats are primarily

- composed of constant frequency (CF) components and frequency modulated (FM)
- components. Social calls are composed of CF, FM, and noise-burst (NB) components.
- FM calls have short pulse durations and wide bandwidths. As such, they overlap with
- social calls less in time but more in frequency. In contrast, CF calls have long pulse
- 243 durations and narrow bandwidths. They overlap with social calls more in time but less
- in frequency. In consideration of the varied overlapping patterns found in bat calls, we
- selected both CF bats (Rhinolophus ferrumequinum, Hipposideros armiger, and
- 246 Rhinolophus pusillus) and FM bats (Vespertilio sinensis, Myotis macrodactylus, and
- 247 *Ia io*) to test the separation capabilities of the proposed network, including six
- 248 different species to test method generalizability.

249 Source sound files from *V. sinensis*, *M. macrodactyllus*, *R. ferrumequinum*, *R.* 

- 250 *pusillus*, and *H. armiger* were collected from previous studies in our lab (S1 Table).
- 251 Sound files for *Ia io* were selected from unpublished data as follows. Bats captured
- from the field were housed in a husbandry room with abundant food and fresh water.
- 253 During each sound recording experiment, 4–5 bats were transferred to a temporary
- 254 cage. Sound recordings were collected using the Avisoft UltraSoundGate 116H

255	(Avisoft Bioacoustics, Berlin, Germany) and a condenser ultrasound microphone
256	(CM16/CMPA, Avisoft Bioacoustics). The sampling frequency was set to 375 kHz at
257	16 bits. The recording experiment lasted five days in order to acquire a sufficient
258	number of recordings, beginning at 18:00 and finishing at 6:00 the following morning.
259	S1 Table shows sample numbers and locations for the bats, as well as the total
260	duration of sound files selected for the study. All experimental procedures complied
261	with the ABS/ASAB guidelines for the Use of Animals in Research and were
262	approved by the Committee on the Use and Care of Animals at the Northeast Normal
263	University (approval number: NENU-W-2010–101).
264	Sound analysis. The total duration of recorded sound files (i.e., original recording
265	files) used for each bat species is shown in S1 Table. We employed Avisoft-SASLab
266	Pro (Version 5.2.12, Avisoft Bioacoustics, Berlin, Germany) to identify
267	non-overlapping and overlapping syllables in echolocation and communication calls.
268	These syllables and calls were described and classified following the nomenclature
269	developed by Kanwal, Matsumura (36) and Ma, Kobayasi (37). The recorded
270	non-overlapping calls were used for preparing training files of each call type and the
271	recorded overlapping calls were used for separation.
272	Data preparation. Supervised machine learning algorithms use training samples to
273	"learn" the steps required for completing a task. The training phase in this study
274	involved preparing clear and non-overlapping echolocation and communication calls,
275	selected from original recording sounds. In this process, the BLSTM network learned
276	features found in both call types.

277	Training samples consisted of randomly selected non-overlapping syllables in
278	echolocation and communication calls from each bat species (in the original
279	recordings), with signal-to-noise ratios (SNRs) above -20 dB. The echolocation
280	training files contained 1,300-6,240 pulses and the communication training files
281	contained 780-1,800 syllables (S1 Table). Although the quantity of selected syllables
282	varied between studies, the data was sufficient for model training. Efforts were made
283	to include roughly equivalent quantities of each syllable type. Time intervals between
284	syllables in the training files were consistent with those of the original recordings. The
285	lengths of training files for echolocation and communication calls were the same for
286	each bat species (S1 Table).
287	Model training and call separation
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298 Fig 6. The BLSTM model architecture and workflow graph.

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299	The model was trained using the files for one bat species in each trial.
300	Echolocation and communication call training files were loaded using the librosa
301	(version 0.6.2) Python package. Frames from the two sound files were read and added
302	together to create sound mixtures. Sound features used for training (log spectral
303	magnitudes) were extracted from this mixture. The extraction process was completed
304	using a short-time Fourier transform (STFT) with a Hamming window (length of 512
305	and shift of 256).

306 The mixture from each bat species was then segmented into 100-frame samples, 307 all of which were divided into a training set and a validation set using a ratio of 2:1 308 (see S1 Table for detailed sample quantities). The training set, validation set, and 309 indicator labels were combined and input to the model. The validation set was used to 310 optimize tuning parameters and evaluate call separation performance. Indicator labels 311 were set to 0 or 1, representing the two types of calls in the mixture. Ideal binary 312 masks were used to train the network and gradients were calculated using shuffled 313 mini-batches (batch size of 128) from larger segments.

The output of this model was a set of embeddings that included learned features for both echolocation and communication calls. In this framework, the deep network assigned embedding vectors to each time-frequency bin in the spectrogram. The network then minimized the distance between embeddings dominated by the same call type in each bin while maximizing the distance between embeddings dominated by different call types. The output was then compared with the validation set and indicator labels to calculate loss, which was back propagated from the output to the

321	input through each layer. Model weights and parameters were then updated based on
322	the calculated loss and training was completed after sufficient iteration epochs.
323	Separation stage. In this stage, overlapping echolocation and communication calls
324	were randomly selected from the original recordings to create a sound file of test sets,
325	used for separation. The log spectral magnitudes of the overlapping calls were then
326	extracted, combined into samples, and input to the trained model. The phases of calls
327	extracted from the sound files were also saved for use in sound reconstruction. The
328	trained model then output embeddings for each segment (100 frames) in a process
329	similar to the training stage. Embeddings were clustered using the k-means method
330	from Scikit-learn (Version 0.20.0) to produce time-frequency masks. The number of
331	clusters corresponded to the number of call types in the mixture (2 - echolocation and
332	communication). These masks and the clustering method were then used to determine
333	which parts of each segment in the overlapped calls would be preserved or neglected
334	based on their correspondence to each call type. For example, if the maximum
335	magnitudes were more likely to belong to echolocation calls, the related mask values
336	were set to 1 and the others were set to 0, allowing the echolocation calls to be separated
337	correctly. Finally, output calls were reconstructed using the inverse fast Fourier
338	transform (IFFT) function numpy.fft.ifft in NumPy (Version 1.15.1). The IFFT
339	transformed the magnitude into a wave using phase information saved at the beginning
340	of the separation stage. The model produced two waveform files, each containing one
341	call type. Additional detail concerning the sound separation algorithms can be found in
342	the work of Hershey (2016).

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### 343 Model evaluation

344 The quality of reconstructed echolocation and communication calls was assessed 345 by comparing their temporal-spectrum parameters to the non-overlapping calls 346 selected from the original recording files (excluding training data). Avisoft-SASLab 347 Pro was used for automatic parameter measurements of duration, bandwidth, peak 348 frequency, minimum frequency, maximum frequency, starting frequency, and ending 349 frequency. A t-SNE (t-distributed stochastic neighbor embedding - R3.6.1 package) 350 analysis was adopted for dimensionality reduction. Two dimensions were extracted 351 from these seven parameters for original and separated syllables and compared with 352 one-way ANOVA (aov in R3.6.1) or two-sided Wilcoxon signed-rank tests 353 (wilcox.test in R3.6.1), depending on their fit to a normal Gaussian distribution. The 354 significance level was set to 0.05 for all tests. We adopted the root mean square error 355 (RMSE) to measure and avoid obscuring individual variations between reconstructed 356 and original calls. Clustering analysis was conducted using the reconstructed 357 echolocation calls from the six bat species, to assess whether the separated calls could 358 be further used in species classification.

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- 373 Writing original draft: Kangkang Zhang, Ying Liu.
- 374 Writing review & editing: Ying Liu, Walter Metzner.
- 375 **Competing interests**
- 376 The authors have declared that no competing interests exist.

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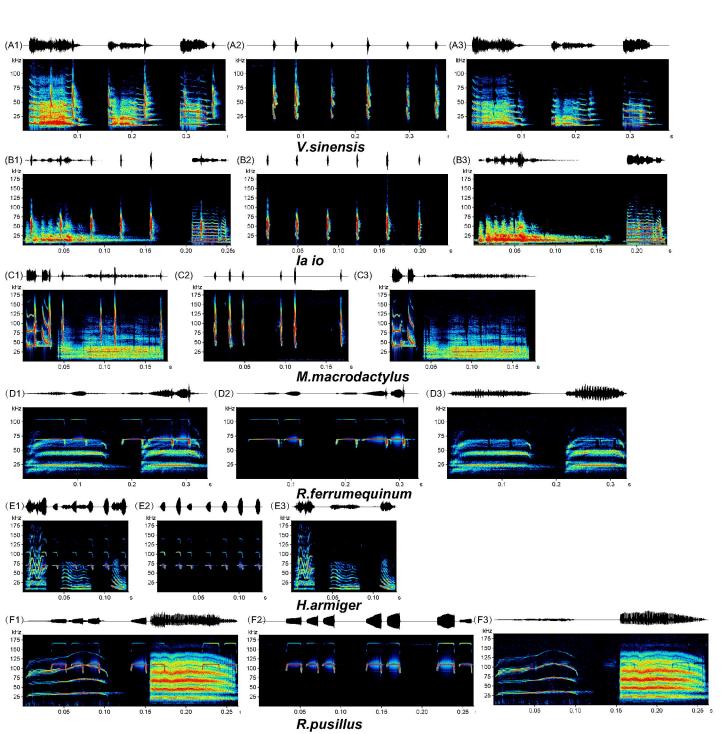
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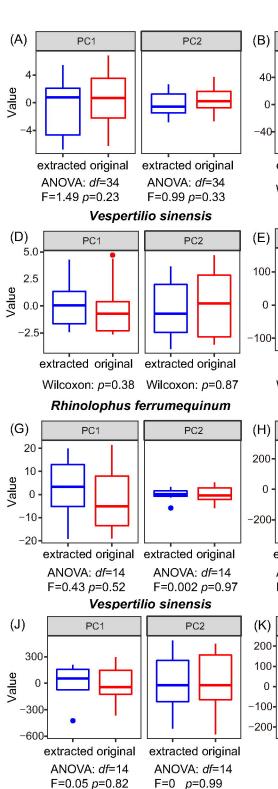
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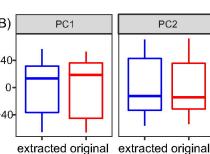
## 477 Supporting information

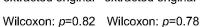
- 478 S1 Table. A summary of calls used for model training.
- 479 S2 Fig. Training loss and validation loss during model training.
- 480 S3 Table. Statistical comparisons of principle components extracted from seven
- 481 parameters. No significant differences were observed between parameters for
- 482 separated and original syllables. A one-way ANOVA was used to test the normal
- 483 distributed data and a two-sided Wilcoxon signed-rank test was used to assess the data
- 484 that did not conform well to a normal distribution.



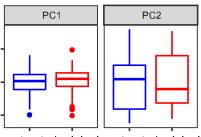


Rhinolophus ferrumequinum



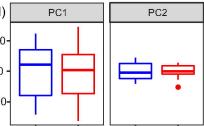






extracted original extracted original Wilcoxon: *p*=0.44 Wilcoxon: *p*=0.67

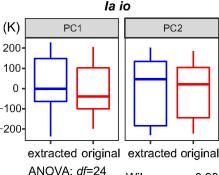
### Hipposideros armiger

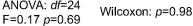


 extracted original
 extracted original

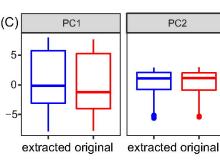
 ANOVA: df=20
 ANOVA: df=20

 F=0.002 p=0.97
 F=0.002 p=0.97



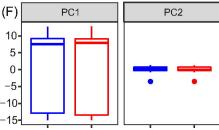






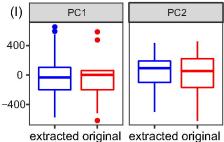
Wilcoxon: p=0.20 Wilcoxon: p=0.94

## Myotis macrodactylus



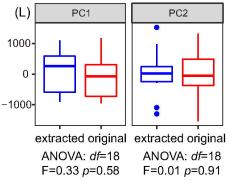
extracted original extracted original Wilcoxon: p=0.81 Wilcoxon: p=0.31

### Rhinolophus pusillus



ANOVA: *df*=28 ANOVA: *df*=28 F=0.05 *p*=0.82 F=0.02 *p*=0.89

Myotis macrodactylus



Rhinolophus pusillus

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