

# **Social behaviour and vocalizations of the tent-roosting**

## **Honduran White Bat**

Ahana Aurora Fernandez<sup>1\*</sup>, Christian Schmidt<sup>2</sup>, Stefanie Schmidt<sup>2</sup>, Bernal Rodríguez-Herrera<sup>3+</sup>, Mirjam Knörnschild<sup>1,4,5+\*</sup>

<sup>1</sup> Museum für Naturkunde - Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany

<sup>2</sup> Institute of Conservation Genomics, University of Ulm, Germany

<sup>3</sup> Escuela de Biología, Centro de Investigación en Biodiversidad y Ecología Tropical, Universidad de Costa Rica, San José, Costa Rica

<sup>4</sup> Smithsonian Tropical Research Institute, Balboa, Panama

<sup>5</sup> Animal Behavior Lab, Freie Universität Berlin, Germany

+ Joint senior authors

\*Corresponding authors: [ahana.fernandez@mfn.berlin](mailto:ahana.fernandez@mfn.berlin), [mirjam.knoernschild@mfn.berlin](mailto:mirjam.knoernschild@mfn.berlin)

Short title: Social behaviour and vocal repertoire of *Ectophylla alba*

## 19 **Abstract**

20 Bats are highly gregarious animals, displaying a large spectrum of social systems with different  
21 organizational structures. One important factor shaping sociality is group stability. To maintain group  
22 cohesion and stability, bats often rely on social vocal communication. The Honduran white bat,  
23 *Ectophylla alba* exhibits an unusual social structure compared to other tent-roosting species. This small  
24 white-furred bat lives in perennial stable mixed-sex groups. Tent construction requires several  
25 individuals and, as the only tent roosting species so far, involves both sexes. The bats' social system  
26 and ecology render this species an interesting candidate to study social behaviour and social vocal  
27 communication. In our study, we investigated the social behaviour and vocalizations of *E. alba* in the  
28 tent by observing two stable groups, including pups, in the wild. We documented 16 different  
29 behaviours, among others, play and fur chewing, a behaviour presumably used for scent-marking.  
30 Moreover, we found 10 distinct social call types in addition to echolocation calls, and, for seven call  
31 types, we were able to identify the corresponding behavioural context. Most of the social call types  
32 were affiliative, including two types of contact calls, maternal directives, pup isolation calls and a call  
33 type related to the fur-chewing behaviour. In sum, this study entails an ethogram and describes the  
34 first vocal repertoire of a tent-roosting phyllostomid bat, providing the basis for further in-depth  
35 studies about the sociality and vocal communication in *E. alba*.

36

37 **Keywords:**

38 *Ectophylla alba*, vocal repertoire, ethogram, play, social communication

## 39 Introduction

40 Bats are social animals exhibiting a large spectrum of social systems with varying degree of complexity.  
41 This includes species living in perennial stable groups [e.g. 1] and species exhibiting social structures  
42 characterized by fission-fusion dynamics [e.g. 2]. One of the factors shaping sociality in bats is social  
43 group stability. Stable group living offers various benefits, including information transfer about food  
44 and roosts, and the evolution of cooperative behaviours such as allo-maternal care, allo-grooming and  
45 food sharing [2]. In bats, social vocal communication is a major factor facilitating group formation and  
46 cohesion thus supporting group stability [1, 3, 4]. A well-studied example, evolved to maintain group  
47 cohesion, is the contact call system in the foliage roosting bat, *Thyroptera tricolor* [5]. This bat species  
48 roosts in furled leaves of *Heliconia* plants, an ephemeral and often sparsely available resource. Roosts  
49 are only inhabitable for one day, hence, *T. tricolor* is daily forced to find and switch to new roosts [6].  
50 Interestingly, despite constant roost switching, *T. tricolor* forms very stable perennial social groups [5].  
51 To maintain group cohesion *T. tricolor* evolved a specialized call-and-response system, including  
52 inquiry calls to locate group members and response calls to recruit group members to the roost [6].  
53 Another foliage roosting species, which exhibits an interesting social system is the phyllostomid bat  
54 *Ectophylla alba* [7]. *Ectophylla alba* is a small (i.e. 6-9g) neotropical phyllostomid bat species which is  
55 endemic to the Caribbean slope of Central America, known to construct and roost in tents [7, 8]. It is  
56 well known for its' characteristic white fur and yellow skin colouration of the ears and nose-leaf, an  
57 adaption primarily evolved for camouflage associated with tent-roosting [9]. However, the yellow skin  
58 colouration, particularly of the nose-leaf, appears to be a sexually dichromatic trait, suggesting a  
59 secondary function as sexually selected signal [10]. The most commonly described mating system in  
60 tent-roosting bats is polygyny [i.e. harem structure composed of one male and several females; 11]. In  
61 contrast, *E. alba* forms mixed-sex groups with an average size of 5-6 individuals [12]. Interestingly,  
62 although genetic relatedness among adult social group members is very low [13], the groups are very  
63 stable over time, switch roosts together, and group members appear to have preferred individuals  
64 with whom they associate while roosting [12]. Furthermore, both sexes are involved in tent

65 construction [8], in contrast, it is commonly supposed that in other tent-roosting species only males  
66 construct tents [7]. Several individuals are involved in tent construction, a process which requires  
67 several nights until finalization [14]. However, it is not yet understood if these individuals all belong to  
68 the same social group.

69 *Ectophylla alba*'s unusual social organization, the high group stability despite the low relatedness and  
70 potential cooperative tent construction render this bat species an interesting candidate to study social  
71 behaviours and vocalizations mediating social interactions. Although the ecology of *E. alba* was  
72 intensively studied during the past decades, only very little is known about its' social behaviour [12]  
73 and information about vocal communication is restricted to a single study, describing one call type  
74 emitted on the wing close to the roost [15]. This study aimed to describe the social behaviours and  
75 vocalizations in the roost to establish an ethogram and a vocal repertoire of *E. alba* based on  
76 observations of wild individuals in their natural habitat.

77

## 78 **Material and methods**

### 79 **Study site and subjects**

80 We monitored two groups of *E. alba* in La Tirimbina Biological Reserve, Heredia Province, in the North-  
81 East of Costa Rica (10°26'N, 83°59'W) from May to July 2010. La Tirimbina Biological Reserve contains  
82 fragments of secondary tropical wet forest and has been the centre of detailed investigations on the  
83 natural history of *E. alba* in the last decade [8, 10, 12, 14, 16-18]. The first *E. alba* group that we  
84 monitored consisted of four individuals (two adult males, one adult lactating female and her non-  
85 volant male pup), the second group (Fig 1 A) of 6-10 adult individuals of both sexes (the core group  
86 consisted of five adult males, one adult lactating female and her non-volant male pup; the sex of the  
87 other three adult bats that joined the core group on and off could not be determined). Females are  
88 polyestrous, they give birth to a single pup in September, respectively April [11]. Pups are born with  
89 fur and become more independent at an age of 3-4 weeks when they start to fly [14]. Bats of the first

90 group could be individually distinguished via colour marks on their fur (Fig 1 B). Therefore, this was our  
91 focal group for behavioural observations and sound recordings; the other group was only occasionally  
92 observed and recorded to complement our data. During the 8-week observation period, both groups  
93 constructed new tents in the vicinity of their old ones and subsequently switched roosts.

94 → Fig 1.

95 **Fig 1. Observation of wild *Ectophylla alba*.** **A:** Picture of the second group roosting in the tent during  
96 the day. Clearly visible is the yellow coloration of the ears of the adult individuals, whereas the pups'  
97 ears are still almost white. **B:** Temporal colour marking of the fur to distinguish individual bats.

## 98 Behavioural Observations

99 We conducted behavioural observations in the bats' tents using a digital video camera with night-shot  
100 function (Sony Handycam DCR-SR32) and two infra-red lights (Sony HVL-IRM). The video equipment  
101 was placed directly underneath the tent and did not seem to disturb the bats. The video camera was  
102 connected via a 5 m cable to a video walkman (Sony DV-D900E) which allowed us to store the video  
103 recordings on mini-DV tapes (Sony DVM60PR3; 1.5 h run-time) and watch the video footage in real-  
104 time without disturbing the bats. This set-up permitted synchronous behavioural observations and  
105 sound recordings (see below for details). Video footage was analysed using the VLC Media Player  
106 (v1.0.5, VideoLan, France).

107 Since *E. alba* roosts cryptically during the day [7], we assumed that most social interactions would take  
108 place at dawn and dusk when bats are returning to or leaving the roost, or during the night when bats  
109 return to their tent [12]. To test this, we collected behavioural data over 22 hours by monitoring the  
110 first group for 3h periods spread equally over several days and nights covering the period from 9:47  
111 am until 8:03 am of the next day. After defining the periods with the highest activity, we restricted our  
112 behavioural observations and sound recordings to these periods using *ad libitum* sampling [19]. Data  
113 was collected every night if the weather permitted it. During heavy rain, no data was collected.

114 Each social behaviour type was considered either a state or an event [19] and was listed in an  
115 ethogram. States were defined as behaviours with a minimum duration of ten seconds, including  
116 behaviours during which the same motor actions were performed repeatedly (e.g., wing fluttering).  
117 Events were instantaneous and singular (e.g. wing stretching) and occurred during a state. For each of  
118 the adult individuals in the first group (n=3) the 22 hours of observation time was split into two  
119 biological meaningful periods: the night-period, which included the time from leaving the tent at dusk  
120 to returning at dawn, and the day-period, which included the time from returning to the tent at dawn  
121 to leaving at dusk. Subsequently, we calculated the duration of each state (in seconds) and converted  
122 the durations into percentages to be able to compare these between individuals and day- respectively  
123 night-periods. The pup was not yet weaned and not foraging on his own; therefore, we decided not to  
124 split the 22-hour observation period for the pup.

## 125 **Sound Recordings**

126 We used a high-quality ultrasonic recording setup (500 kHz sampling rate and 16-bit depth resolution)  
127 consisting of an ultrasonic microphone (Avisoft USG 116Hm with condenser microphone CM16;  
128 frequency range, 1-200 kHz) connected to a laptop computer (JVC, MP-XP741DE) running the software  
129 Avisoft-Recorder v4.2 (R. Specht, Avisoft Bioacoustics, Berlin, Germany). The behavioural context of  
130 each vocalization type in the vocal repertoire was assessed with simultaneous behavioural  
131 observations and video recordings. Sound and video recordings were synchronized with a bat detector  
132 (Pettersson D980, Pettersson Elektronik, Uppsala, Sweden) that was set on frequency division mode  
133 and connected to the audio channel of the video camera.

134 On one occasion, the video recordings contained two previously unknown vocalization types which  
135 were only recorded with the camcorder's built-in microphone and not with the high-quality ultrasonic  
136 recording setup. Therefore, these two vocalization types were excluded from our acoustic analyses but  
137 we discuss the behavioural context in the results and corresponding spectrograms can be found in the  
138 supplements.

## 139 **Acoustical Analyses**

140 Prior to acoustic analyses, vocalizations were visually classified into distinct social call types (i.e., social  
141 refers to calls other than echolocation calls) based on spectro-temporal features in the spectrograms;  
142 the different behavioural contexts in which vocalizations occurred were annotated based on  
143 behavioural observations of bats in the roost. Subsequent acoustic analyses were conducted to  
144 characterize the different social call types and assess their acoustic distinctiveness. We used Avisoft-  
145 SASLab Pro (v5.0, R. Specht, Berlin, Germany) for acoustic analyses. Only calls with good signal-to-noise  
146 ratio that were not overlapped by other calls or background noise were selected for acoustic  
147 measurements (116 in total; 5-41 per vocalization type). All calls were multiharmonic and some had  
148 an undulating structure (more than one frequency modulation). Since different harmonics were  
149 emphasized (i.e., had the largest amplitude) in different social call types, we used the strongest  
150 harmonic for measurements; we thus selected harmonics that contributed most to the acoustic  
151 impression of different social call types. We determined the start and end of calls manually based on  
152 the oscillogram. Subsequent measurements were taken from spectrograms created with a Hamming  
153 window with 512-point fast Fourier transform and 93.75 per cent overlap (frequency resolution: 977  
154 Hz; temporal resolution: 0.064 ms). For all calls, we measured one temporal parameter (duration) and  
155 three spectral parameters (peak frequency at the start, middle and end of each call). Thus, we used  
156 four parameters per call to assess the acoustic distinctiveness of different vocalization types.  
157 Additionally, we further characterized undulating calls by measuring the peak frequency (pf) at every  
158 local maximum, minimum and inflexion point; values were subsequently averaged per call (mean  
159 maximum pf, mean minimum pf, mean inflexion point pf).

## 160 **Statistical analysis**

161 We simultaneously included all four acoustic parameters in a discriminant function analysis (DFA), all  
162 of which were checked for multicollinearity. We used an 'n-1' cross-validation procedure which  
163 classified each call based on discriminant functions established with all social call types other than the  
164 one being classified. Prior probabilities were adjusted to unequal group sizes. All statistical tests were  
165 performed with SPSS (v.22, SPSS Inc., Chicago, IL, U.S.A.)

166

## 167 **Results**

### 168 **Social behaviours**

169 We observed six behavioural states and ten different behavioural events occurring in the tent-roost  
170 (Table 1). Most behaviours were observed during both the day- and night-period. During the 22-hour  
171 observation period, the states "fur chewing" and "playing" were only observed during the night-  
172 period, whereas the event "change position" was only noted during the day-period. However, outside  
173 of the 22-hour observation period, "change position" was also observed during the night.

174 Adult bats exhibited two main activity peaks during the 22 hours; one before sunset and one around  
175 sunrise. This coincides with the time at which adult bats leave, respectively, return from foraging at  
176 night. The activity peaks were characterized by increased auto-grooming, wing stretching and frequent  
177 position changing in the roost. The analysis of the 22-hour observing period revealed that during the  
178 day-period, the adult bats spent the majority of time resting (male 1: 91.9%, male 2: 96.7%, female:  
179 77.4% of the time). A short amount of the time they spent auto-grooming (male 1: 8.1%, male 2: 1.5%,  
180 female: 1.5% of the time) and, in the case of the female, nursing (21.1%). The female nursed the pup  
181 three times before leaving for foraging at sunset and twice in the early morning after returning to the  
182 tent at sunrise. The longest nursing duration was observed at 6 am when the female nursed her pup  
183 for one hour and 21 minutes.



184 **Table 1. Ethogram describing behaviours of *E. alba* observed in the tent.**

State or Event	Behaviour	Description	Estimated frequency of occurrence	Performing sex	Age
S	Resting	Roosting motionless, often with concealed faces (not to be equated with sleeping)	Very common	Both sexes	all
S	Scanning	Echolocating with twitching ears and nose-leaf, often directed at the ground below the roost	Very common	Both sexes	All
S	Self-Grooming	Tending to coat and wings with tongue and hind feet	Very common	Both sexes	All
S	Fur chewing	Conspicuous, prolonged licking and/or gentle biting the fur of conspecifics (of both males and females and pups) between the shoulder blades and coating it with saliva	Rare	Only males	Adults
S	Allo-Grooming	Mother grooming her non-volant pup	Common	Only females	Adults
S	Nursing	Mother breastfeeding her non-volant pup	Common	Only females	Adults
S	Licking	Soliciting maternal care by licking the corners of her mouth or her belly; often followed by nursing	Common	Both sexes	Pups
S	Twitching	Maternal signal for the pup to release the teat	Common	Only females	Adults
S	Shaking	Rapid whole-body muscle contractions, presumably for thermoregulation	Rare	Both sexes	Pups
S	Playing	Conspicuous, prolonged and seemingly playful engagement with a torn piece of leaf in the roost	Rare	Both sexes	pups
S	Startle posture	Raising of half-opened wings high above head and back, followed by covering face with raised half-open wings	Rare	Both sexes	Pups
S	Flight practice	Rapid wing fluttering while clinging to the roost surface with hind feet	Common	Both sexes	Pups
E	Yawning	Exposing gum and teeth (when resting, cleaning or nursing)	Common	Both sexes	All
E	Changing position	Climbing to a different roosting position within the day-roost (when resting)	Very common	Both sexes	All
E	Wing stretching	Stretching of one wing (when resting, cleaning or nursing)	Very common	Both sexes	All
E	Hitting	Aggressively hitting conspecifics with partly outstretched wing (when resting)	Rare	Both sexes	Adults
E	Urinating/Defecating	Urinating/Defecating by arching back; never when in body contact with conspecifics (when cleaning)	Rare	Both sexes	All

185

186 During the night-period, adult bats spent most of the time foraging (i.e., were absent; male 1: 96.5%,  
187 male 2: 95.2 %, female: 95.9% of the time). The female visited the tent twice during the night to nurse  
188 her pup (3.6%) and spent some time self-grooming (0.5%). One of the males visited the tent only once,  
189 whereas the second male visited the tent five times and stayed for a short period. During their visits,  
190 males were resting (male 1: 0.3%; male 2: 0.8%) and self-grooming (male 1: 1.08%, male 2: 2.5%).  
191 Furthermore, they engaged in fur chewing (male 1: 2.14%, male 2: 1.4%).

192 The pups' main activities (22h-period was not split in day- and night-period) constituted of resting  
193 (63.7%), nursing (12.8%), auto-grooming (8.5%) and changing position in the tent (0.9%). Although  
194 changing position was usually considered an event, in this case, it was defined as a state because the  
195 pup was continuously changing his position in response to being gently bitten by an adult male (i.e.,  
196 male fur chewing behaviour). At night, the pup was absent for short periods of time (14.1%). At this  
197 stage in ontogeny, the pup engaged in first flight attempts. However, compared to the other group  
198 members, the pup was the least time away from the tent. Furthermore, the pup occasionally engaged  
199 in a behaviour which was defined as play (Table 1). Note that some states were not observed during  
200 this continuous 22-hour recording, therefore, they are not included in the calculation of time-budgets  
201 but are nevertheless described in the ethogram. Two unusual and rare behaviours are subsequently  
202 described in greater detail; namely "fur chewing" and "play".

203 "Fur chewing" in males: After manoeuvring behind the back of a roosting group member, males were  
204 observed licking and gently biting the fur between the shoulder blades for a prolonged time (up to 13  
205 minutes, Fig 2). While chewing the fur, males sometimes simultaneously were trembling their folded  
206 wings. After chewing, the bitten individual showed a visible patch of wet fur from the saliva. In most  
207 cases, males were chewing fur on the back of a female, but it was also observed that males chewed on  
208 the back of each other. In one occasion, a male that returned to the roost performed this behaviour  
209 on the pup who was roosting alone in the tent (for about 6 minutes, see video S1). The individual being  
210 bitten remained mostly calm, sometimes started self-grooming, wing stretching and changing the

211 position, with the fur chewing individual firmly clinging on. Eventually, the individual being bitten (if it  
212 was not the pup) also engaged in biting/licking another group member.

213 “Play” in pups: While being alone in the tent, the pup started to investigate a torn piece of the roost  
214 leaf (see video S2, Fig 2). First, the pup started sniffing the leaf piece, and soon after used both  
215 thumbs/claws and wrists to grasp the leaf piece. Once grasped, the pup started chewing on the piece.  
216 The pup chewed on the leaf piece for a few seconds, stopped and started scanning. This behavioural  
217 sequence was repeated several times. Sometimes, the pup also started cleaning, wing stretching or  
218 moving around after chewing on the leaf piece. At one time, the pup was observed to inspect the  
219 modified midrib of the tent (i.e. the part of the leaf which is modified during tent construction to  
220 collapse the leaf next to the cut to achieve the typical shape of *E. alba* tents, see [8]. Afterwards, the  
221 pup turned back to the leaf piece and started chewing again, while simultaneously using the wrists and  
222 claws grasping and holding on to it. Chewing could get quite vigorous, and eventually, the pup started  
223 to bend the leaf piece to some extent.

224 → Fig 2.

225 **Fig 2. Social behaviours of *E. alba*.** **A:** Fur chewing behaviour. The male (M) is chewing/licking the back  
226 of the pup (P). **B:** Nursing. The pup (P) is attached to the nipple of the female (F). **C:** Licking. The pup  
227 (P) is licking the mouth of the female (F) to solicit nursing. **D:** Twitching. The female (F) is shaking the  
228 pup (P) off after nursing. **E:** Play. The pup is playing with a torn leaf piece inside the tent. **F:** Startle  
229 response. The pup (P) shows the typical wing posture when frightened, in this case by a grasshopper  
230 that has wandered into the tent. For detailed descriptions of the behaviours see Table 1.

## 231 Social vocalizations

232 *E. alba* produced ten distinct social call types in addition to echolocation calls, and for most call types  
233 the behavioural context in which they were uttered could be defined. The social call types SC9 and  
234 SC10 were not included in the statistical analysis because they were only recorded once with the  
235 microphone of the camcorder (Fig S1). The visual classification of eight social call types was confirmed  
236 by the classification success of the cross-validated DFA (88.8% of all call types were classified correctly,

237 Table 2, Fig 3B). The acoustic parameter that contributed most to the distinction of social call types  
238 was peak frequency in the centre of the call, peak frequency at the start and the end of the call  
239 contributed moderately to the call distinction, whereas duration only played a minor role. A  
240 conspicuous feature of the social vocalizations in *E. alba* is the suppression of the fundamental  
241 frequency and the lower harmonics in some of the call types (SC1, SC2; SC5-SC7, Table 3).

242 → **Fig 3.**

243 **Fig 3. The eight social call types of *E. alba*.** **A:** Spectrograms depicting the eight social call types (SC1-SC8) and  
244 two echolocation calls (EC) of *E. alba*. The spectrograms correspond to the natural appearance of those call types;  
245 i.e., suppression of the lower harmonics in types SC2, SC5, SC6, SC7. Social call types SC9 and SC10 are depicted  
246 in the supporting information (S\_Fig 1). Information about acoustic parameter measurements is depicted in Table  
247 S1. Echolocation calls are shown for comparative reasons. The context in which the social call types were emitted  
248 is described in Table 2. Spectrograms were created using a 1024-point FFT and a Hamming window with 87.5 %  
249 overlap. **B:** The spacing of the eight social call types of *E. alba* in a two-dimensional signal space defined by the  
250 first 2 discriminant functions. Each social call type is represented by a distinct symbol, small black symbols  
251 represent centroids (i.e., the canonical mean of all calls per type). Note that EC are not included in the DFA.

252

253 The DFA included acoustic measurements obtained from the harmonics that contributed most to the  
254 acoustic impression of different social call types. As a control, we calculated a second DFA including  
255 acoustic measurements of the fundamental frequency which corroborated the results of the first DFA  
256 (supporting information).

257

258 **Table 2. Classification success in per cent (%) of the cross-validated discriminant function analysis.**

Social calls	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	No. of calls
SC1	95.0 (18)	0.0	0.0	0.0	5.0 (2)	0.0	0.0	0.0	20
SC2	0.0	62.5 (5)	0.0	0.0	12.5 (1)	0.0	25.0 (2)	0.0	8
SC3	0.0	0.0	100 (12)	0.0	0.0	0.0	0.0	0.0	12
SC4	0.0	0.0	0.0	100 (15)	0.0	0.0	0.0	0.0	15
SC5	20.0 (1)	20.0 (1)	0.0	0.0	60.0 (3)	0.0	0.0	0.0	5
SC6	0.0	4.9 (2)	0.0	0.0	0.0	92.7 (38)	0.0	2.4 (1)	41
SC7	0.0	33.3 (3)	0.0	0.0	0.0	0.0	66.7 (6)	0.0	9
SC8	0.0	0.0	0.0	0.0	0.0	16.7 (1)	0.0	83.3 (5)	6

259 Classification table showing how many call types were correctly classified. The number in each box indicates the  
 260 correct classification of each correct call type (row) assigned to each predicted call type (column), i.e. each row  
 261 sums up to 100 %. Numbers in brackets depict the number of calls (total call number: N=116). The total number  
 262 of measured calls per type is indicated to the right of the matrix.

263

264 **Table 3. Acoustic parameters of the eight social call types.**

Social calls	N	Duration [ms]	Peak freq. start [kHz]	Peak freq. centre [kHz]	Peak freq. end [kHz]	Measured harmonic
SC1	20	5.01 ± 1.2	116.6 ± 13.0	79.8 ± 7.8	57.6 ± 6.2	3d
SC2	8	9.6 ± 4.8	94.3 ± 4.7	83.5 ± 4.2	80.2 ± 5.8	10th
SC3	12	50.5 ± 6.5	21.0 ± 0.6	20.4 ± 1.3	20.5 ± 1.6	1st
SC4	15	24.4 ± 6.5	41.3 ± 2.6	39.7 ± 1.5	40.0 ± 2.3	2nd
SC5	5	7.1 ± 2.4	99.5 ± 19.2	72.3 ± 5.9	65.9 ± 3.9	3th
SC6	41	49.1 ± 20.4	86.3 ± 8.8	84.3 ± 6.3	86.3 ± 8.3	8th
SC7	9	9.3 ± 2.8	93.6 ± 11.7	91.6 ± 10.2	93.1 ± 13.8	4th
SC8	6	18.9 ± 4.5	83.0 ± 9.1	87.7 ± 11.2	60.6 ± 11.9	3th

265 The table depicts mean and standard deviation for each social call type averaged over all calls measured per  
 266 type (column 2). The frequency parameters were measured in the harmonic which contained the most energy  
 267 (column 7).

268

269 Furthermore, for seven out of ten social call types the behavioural context in which they were uttered  
 270 was elucidated (Table 4). Three social call types were uttered in an affiliative context, namely SC2, SC3,

271 SC5 (Fig 3). Two social call types were uttered in the context of mother-pup interactions, namely SC4  
 272 and SC6 (Fig 3). In agonistic context, two social call types were uttered (see supplements), namely SC9  
 273 and SC10. Echolocation calls were uttered during flight and during alert behaviour in the roost (i.e.,  
 274 scanning, see Table 1). Most social call types were uttered singly and with a single exception (SC7)  
 275 monosyllabic (Table 4).

276 **Table 4. Behavioural context of the social call types.**

Social call	Production mode	Syllable type	Behavioural context	Frequency of occurrence (ranked)	Sex	Age
SC1	In series	Monosyllabic	Unknown	1	Both sexes	All
SC2: Grooming/biting call	Singly	Monosyllabic	Emitted while male gently bites or grooms the neck of a female	2	Males Females?	Adults
SC3: Contact call 1	Singly	Monosyllabic	Emitted on the wing by individuals flying in the vicinity of the tent (both empty and occupied tent), and emitted while leaving the tent	3	Unknown	Adults
SC4: Maternal directive	Singly	Monosyllabic	Emission immediately followed by nursing	2	Females	Adults
SC5: Contact call 2	Singly	Monosyllabic	Emitted on the wing by individual flying in the vicinity of the tent; and emitted while leaving the tent	4	Unknown	Adults
SC6: Isolation call	Singly	Monosyllabic	Solicitation of nursing	2	Both sexes	Pups
SC7	Singly	Multisyllabic	Unknown	5	Unknown	Unknown
SC8	Singly	Monosyllabic	Unknown	2	Unknown	Unknown
SC9	In series	Monosyllabic	Aggressive/Distress	6	Both sexes	Unknown
SC10: Screech	In series	Monosyllabic	Aggressive/Distress	6	Both sexes	Adults

277 The frequency of occurrence corresponds to the number of recording sessions during which the social call type  
 278 was recorded: 1 being the most frequent type, 6 the less frequent type. Sex denotes the sex of the emitter of a  
 279 given social call type. Age denotes which age group (pup/adult) uttered the social call type.

## 280 Discussion

281 This study provides the first description of the behavioural ethogram and vocal repertoire of *E. alba* in  
282 the roost. We identified 16 different behaviours, including two particularly interesting ones; the “fur  
283 chewing” behaviour performed by adult males, and, the description of a pup behaviour, which very  
284 likely meets the criteria defining play in animals. The vocal repertoire is constituted of 10 distinct social  
285 call types, and for seven call types, the behavioural context was determined.

286 The temporal occurrence of social behaviours shows clear differences between day and night periods.  
287 During the day, aside from the two main activity peaks at dusk and dawn, the bats were almost  
288 exclusively resting. Roosting quietly during the day could be owned to avoid alerting day predators,  
289 such as primates [20, 21] but could also be a method to save energy. At night, as expected, adult  
290 individuals spent most of their time foraging but paid short visits to their tent. During her visits at night,  
291 the female was regularly nursing her pup. Former research also showed that, during their visits at  
292 night, females spent a considerable amount of time nursing and/or grooming the pup, especially during  
293 early ontogeny before pups became more independent [12]. Because pups are born almost furless,  
294 maternal care is especially crucial during the first days after birth, probably for reasons of  
295 thermoregulation. Adult males mostly engaged in self-grooming during their roost visits at night.  
296 Furthermore, the most interesting behaviours, “fur chewing” performed by adult males and “play” in  
297 pups, were both only observed at night. As described in the results, “fur chewing” was performed by  
298 males only. Our observations conform to the description of the behaviour in a former study, by  
299 Rodríguez-Herrera and colleagues [12]. A new observation in our study was that adult males perform  
300 “fur chewing” not only on adult males and females but also on pups. From the behavioural response  
301 of the bitten individuals, both adult and pup, it seemed that “fur chewing” did not cause any pain (see  
302 also Rodríguez-Herrera et al. 2019) [12]. At most it was possibly perceived as irritating (e.g., for a while,  
303 the pup who was being bitten tried to shake off/get away from the male, see video S1) as the bitten  
304 individual hardly showed any serious attempts of escape or strong resistance.

305 It remains speculative if “fur chewing” is a behaviour for scent-marking group members or if it is a form  
306 of allo-grooming. Allo-grooming is mainly observed in stable social groups (e.g. harems, maternity  
307 colonies) with varying degree of relatedness [2]. Besides strengthening of social relationships, allo-  
308 grooming is also exchanged for other social benefits [1, 2, 22, 23]. So far, allo-grooming among adult  
309 individuals (not including mother-pup grooming) was described for a few bat species only [2, 24].  
310 However, in contrast to other species where allo-grooming was observed [e.g. 1, 22, 23], *E. alba* seems  
311 to restrict grooming to a very specific spot on the back, not including other body parts [this study and  
312 12]. Furthermore, after “fur chewing”, a visible wet spot remained on the back of the receiver (video  
313 S1). This is reminiscent of the scent-marking behaviour of group members in *Noctilio leporinus* and  
314 *Cynopterus sphinx* [25, 26]. *Noctilio leporinus* females, who form stable perennial groups, rub their  
315 heads on other females’ heads and backs to scent-mark them [25]. In *C. sphinx*, individuals form so-  
316 called grooming clusters, where individuals hold on to each other while distributing saliva on body  
317 parts of group members [26]. In both species, scent-marking was associated with group member  
318 recognition. In *E. alba*, “fur chewing” could have a similar function; group recognition through scent.  
319 *Ectophylla alba* forms very stable social groups [12], although the genetic relationship between adult  
320 individuals is almost zero [13]. It is known that groups switch together to new roosts, which are usually  
321 close to the currently occupied tent within a small area [17]. Tent construction is costly; time spent  
322 manipulating a leaf cannot be invested in foraging, and construction requires several nights [14].  
323 Furthermore, *E. alba* has specific requirements to its roosting microhabitat [20], probably limiting the  
324 availability of potential roosting areas and, therefore, increasing the value of suitable places. A scent  
325 signature could assist the identification and recognition of social group members joining a roost. This  
326 would also explain why males perform this behaviour not only on females but also on pups.  
327 Nevertheless, besides scent-marking “fur chewing” could also strengthen social bonds between  
328 individuals, as observed in other species [2]. It remains to be investigated which function(s) the  
329 observed “fur chewing” plays in *E. alba*.



330 A behaviour characterized as play in animals is defined as (i) a non-fully functional behaviour, (ii) being  
331 spontaneous and voluntary, (iii) different from a formal performance of functional behaviour, e.g.  
332 exaggerated or incomplete, (iv) repeatedly performed during a period of an individual's life, and, (v)  
333 performed only when the animal is free from stress [27]. There are several hypotheses about the  
334 function of play in young animals [e.g. 28], and despite opposing views regarding certain aspects, most  
335 agree that one of the main functions of play is to refine one's motor skills [29]. Play behaviour is  
336 grouped into three categories; social play, locomotor play and object play [29]. Studies about play in  
337 animals are very scarce, and in bats, it has only been observed in a few occasions. Social play was  
338 described in *Pteropus giganteus*, where young individuals engage in play-fight and wrestling, first with  
339 their mothers, later among subadult individuals [30]. Similar play fighting was also described for other  
340 Pteropodidae species [31]. Young vampire bats engage both in object- and social play, the latter  
341 involving mounting, wrestling and chasing [32]. The pups' handling of the torn leaf piece, we observed  
342 for the first time in this study, meets the criteria of object play behaviour. The pups' object play might  
343 be a precursor to later actual tent construction behaviour. Adult tent construction involves biting and  
344 puncturing the leaf using the teeth, and further extension of these holes by claws until the leaf  
345 collapses next to the cut [8]. By grasping a part of the leaf with the thumbs and repeatedly pulling it  
346 up, down and inward the leaf bends downward forming the final shape of the tent [8]. Several motor  
347 patterns of adult tent construction are found in the pup's object play; the chewing of the leaf, the  
348 grasping of a leaf piece with the thumbs and wrists and finally the bending and moving of the leaf piece  
349 (but instead of using the thumbs the pup used both, thumb and mouth, see video S2). The pups'  
350 behaviour is reminiscent of motor patterns used by adults in tent construction, but not yet fully  
351 functional (criteria i & iii). The pup was free from stress, voluntarily engaging with the leaf piece  
352 (criteria ii & iv). Moreover, this behaviour was observed several times during different nights (v).  
353 However, our sample size is restricted to observations of a single pup. The observed behaviour could  
354 also be explained by curiosity towards an unexpected object (torn leaf piece) present in the roost.

355 The repertoire size of *E. alba* described here is within the size range of the vocal repertoire sizes of  
356 other phyllostomid bats (*Glossophaga soricina* [n=15 social call types, 33], *Glossophaga commissarisi*  
357 [n=8 social call types, 33], *Carollia perspicillata* [n=10 social call types, 34] and *Phyllostomus discolor*  
358 [n=12 social call types, 35]). Our description of the vocal repertoire is incomplete, because we did not  
359 observe courtship behaviour. Nevertheless, the repertoire described here most likely includes the  
360 majority of the social call types (outside of the mating context). Most of the social call types were  
361 affiliative (SC2-SC6), and although the exact context of call types SC1, SC7-SC8 was not elucidated, they  
362 were uttered in neutral, non-aggressive situations (Table 4). This corresponds to the observed  
363 behaviours; aggressive/agonistic behaviour like “wing hitting” was only rarely observed (Table 1). The  
364 only aggressive/distress vocalizations (Fig S1) were recorded during an incident when a mosquito stung  
365 a bat in a sensitive spot on his back that was temporarily hairless because of a telemetry tag that had  
366 recently fallen off. Similarly, a study from Rodríguez and colleagues never noticed agonistic behaviour  
367 in the group they observed [12]. So far, it is still unknown whether males exhibit aggressive behaviour  
368 in the mating context, and, for example jointly defend a tent or their roosting area.

369 The pup isolation call of *E. alba* is different from the isolation calls of other phyllostomid bats because  
370 its fundamental frequency and the lower harmonics are suppressed (Fig. 3, Table 3). In other  
371 phyllostomids studied so far, most of the sound energy of pup isolation calls is located in the  
372 fundamental frequency [P. hastatus, P. discolor, G. soricina, C. perspicillata 36, 37-39]. This particular  
373 spectral characteristic of *E. alba* isolation calls might be an adaption to its roosting ecology. The tents  
374 offer less protection from predation compared to the roosting sites of the other species, due to their  
375 resistance and stability (i.e. leaf versus cave or tree hole) and probably also due to their location (in  
376 the understory, less than 2m above ground) [14]. Restricting the isolation calls’ sound energy to a  
377 narrow high-frequency band could create a communication channel for *E. alba* that is circumventing  
378 the hearing range of some predators; for instance, it is known that small primates successfully predate  
379 tent roosting bats [21]. The isolation call of *E. alba* also differs in its duration from the isolation calls of

380 the other species. Why these isolation calls have such a long duration and whether this is possibly  
381 related to the intensity of solicitations for nursing could be investigated in further studies.

382 The social call types SC3 and SC5 were uttered on the wing, in the vicinity to the roost, and while  
383 leaving the tent. The call type SC3 is similar to the social call described in the study of Gillam and  
384 colleagues [15]. They recorded this specific social call in the vicinity of tent roosts and once before  
385 entering the roost [15] which corroborates our observations. Following Gillam and colleagues, we  
386 hypothesise that these social call types serve as contact calls. They are not used to attract and recruit  
387 group members for roosting, a function that contact calls recorded in other foliage-roosting bats have  
388 [6, 15]. They might serve for the coordination of group formation; however, the purpose of group  
389 formation in the vicinity to the tent is completely speculative at this point. Besides the potential  
390 function of group formation, these calls might additionally signal roosting area occupancy to other  
391 social groups roosting in the vicinity. In both scenarios, group member recognition is crucial; therefore,  
392 in future studies, it would be interesting to investigate if the social call types SC3 and SC5 encode a  
393 vocal group signature and if they elicit phonotaxis in receivers.

394 The affiliative social call type SC2 was uttered before "fur chewing", always by the active (i.e., fur  
395 chewing) and never by the passive (i.e., individual whose fur was chewed) bat. We never recorded a  
396 vocal response of the passive individual. To our knowledge, there is no other study describing a social  
397 call associated with scent-marking and/or allo-grooming. This social call might be an appeasement  
398 vocalization to signal non-aggressive intention towards the passive individual. Further, it might  
399 simultaneously strengthen dyadic relationships between individuals. *E. alba* seem to prefer to  
400 associate with particular group members within the roost [12], and this call might encode an individual  
401 signature facilitating social interactions. However, it is not known if "fur chewing" occurs more  
402 frequently between particular group members.

403 Overall, our research contributes to the growing number of studies on social behaviour and vocal  
404 repertoire descriptions in phyllostomid bats. Our results allowed us to raise new questions and  
405 formulate hypotheses about particular social behaviours and social call types which can be tested in

406 future observational and experimental studies. With our study, we hope to initiate further studies  
407 about social behaviour not only in *E. alba* but other bat species, and especially encourage further  
408 studies describing vocal repertoires of bats to assess the communicative capacity of this speciose  
409 taxon.

410

## 411 **Acknowledgements**

412 We would like to thank the entire team of the Tirimbina Biological Reserve for their support and  
413 providing excellent research conditions. Simon P. Ripperger helped to improve this manuscript with  
414 well-conceived comments.

415

## 416 **References**

- 417 1. Wilkinson GS, Boughman JW. Social calls coordinate foraging in greater spear-nosed bats.  
418 *Animal Behaviour*. 1998;55(2):337-50.
- 419 2. Kerth G. Causes and consequences of sociality in bats. *Bioscience*. 2008;58(8):737-46.
- 420 3. Gillam E, Fenton MB. Roles of acoustic social communication in the lives of bats. *Bat*  
421 *bioacoustics*: Springer; 2016. p. 117-39.
- 422 4. Chaverri G, Ancillotto L, Russo D. Social communication in bats. *Biological Reviews*.  
423 2018;93(4):1938-54.
- 424 5. Chaverri G, Gillam EH, Vohnhof MJ. Social calls used by a leaf-roosting bat to signal location.  
425 *Biol Letters*. 2010;6(4):441-4.
- 426 6. Chaverri G, Gillam EH, Kunz TH. A call-and-response system facilitates group cohesion among  
427 disc-winged bats. *Behavioral Ecology*. 2012;24(2):481-7.
- 428 7. Rodríguez B, Medellín RA, Timm RM. Murciélagos neotropicales que acampan en hojas:  
429 *Editorial Inbio*; 2007.

- 430 8. Rodríguez-Herrera B, Medellín RA, Gamba-Ríos M. Tent building by female *Ectophylla alba*  
431 (Chiroptera: Phyllostomidae) in Costa Rica. *Acta Chiropterologica*. 2006;8(2):557-60.
- 432 9. Galván I, Garrido-Fernández J, Ríos J, Pérez-Gálvez A, Rodríguez-Herrera B, Negro JJ. Tropical  
433 bat as mammalian model for skin carotenoid metabolism. *Proceedings of the National*  
434 *Academy of Sciences*. 2016;113(39):10932-7.
- 435 10. Rodríguez-Herrera B, Rodríguez P, Watson W, McCracken GF, Medellín RA, Galván I. Sexual  
436 dichromatism and condition-dependence in the skin of a bat. *J Mammal*. 2019;100(2):299-  
437 307.
- 438 11. Chaverri G, Kunz TH. Ecological determinants of social systems: perspectives on the  
439 functional role of roosting ecology in the social behavior of tent-roosting bats. *Advances in*  
440 *the Study of Behavior*. 42: Elsevier; 2010. p. 275-318.
- 441 12. Rodríguez-Herrera B, Arroyo-Cabrales J, Medellín RA. Hanging out in tents: social structure,  
442 group stability, male behavior, and their implications for the mating system of *Ectophylla*  
443 *alba* (Chiroptera: Phyllostomidae). *Mammal Research*. 2019;64(1):11-7.
- 444 13. Gutiérrez EG, Vivas-Toro I, Carmona-Ruíz D, Villalobos-Chaves D, Rodríguez-Herrera B,  
445 Real-Monroy MD, et al. Socio-spatial organization reveals paternity and low kinship in the  
446 Honduran white bat (*Ectophylla alba* Allen, 1892) in Costa Rica. *Integrative Zoology*.
- 447 14. Rodríguez-Herrera B, Ceballos G, Medellín RA. Ecological aspects of the tent building process  
448 by *Ectophylla alba* (Chiroptera: Phyllostomidae). *Acta Chiropterologica*. 2011;13(2):365-72.
- 449 15. Gillam EH, Chaverri G, Montero K, Sagot M. Social calls produced within and near the roost in  
450 two species of tent-making bats, *Dermanura watsoni* and *Ectophylla alba*. *PLoS One*.  
451 2013;8(4):e61731.
- 452 16. Rodríguez-Herrera B, Viquez-R L, Cordero-Schmidt E, Sandoval JM, Rodríguez-Durán A.  
453 Energetics of tent roosting in bats: the case of *Ectophylla alba* and *Uroderma bilobatum*  
454 (Chiroptera: Phyllostomidae). *J Mammal*. 2016;97(1):246-52.

- 455 17. Villalobos-Chaves D, Spínola-Parallada M, Heer K, Kalko EK, Rodríguez-Herrera B. Implications  
456 of a specialized diet for the foraging behavior of the Honduran white bat, *Ectophylla alba*  
457 (Chiroptera: Phyllostomidae). *J Mammal*. 2017;98(4):1193-201.
- 458 18. Ripperger SP, Rehse S, Wacker S, Kalko EK, Schulz S, Rodriguez-Herrera B, et al. Nocturnal  
459 scent in a 'bird-fig': a cue to attract bats as additional dispersers? *PLoS one*.  
460 2019;14(8):e0220461.
- 461 19. Altmann J. Observational study of behavior: sampling methods. *Behaviour*. 1974;49(3):227-  
462 66.
- 463 20. Rodríguez-Herrera B, Medellín RA, Gamba-Rios M. Roosting requirements of white tent-  
464 making bat *Ectophylla alba* (Chiroptera: Phyllostomidae). *Acta Chiropterologica*.  
465 2008;10(1):89-95.
- 466 21. Boinski S, Timm RM. Predation by squirrel monkeys and double-toothed kites on tent-making  
467 bats. *American Journal of Primatology*. 1985;9(2):121-7.
- 468 22. Kerth G, Almasi B, Ribí N, Thiel D, Lüpold S. Social interactions among wild female Bechstein's  
469 bats (*Myotis bechsteinii*) living in a maternity colony. *Acta Ethologica*. 2003;5(2):107-14.
- 470 23. Carter G, Leffer L. Social grooming in bats: are vampire bats exceptional? *PLoS One*.  
471 2015;10(10):e0138430.
- 472 24. Kershenbaum A, Blumstein DT, Roch MA, Akçay Ç, Backus G, Bee MA, et al. Acoustic  
473 sequences in non-human animals: a tutorial review and prospectus. *Biological Reviews*.  
474 2016;91(1):13-52.
- 475 25. Brooke AP. Social organization and foraging behaviour of the fishing bat, *Noctilio leporinus*  
476 (Chiroptera: Noctilionidae). *Ethology*. 1997;103(5):421-36.
- 477 26. Rathinakumar A, Cantor M, Senthilkumar K, Vimal P, Kaliraj P, Marimuthu G. Social grooming  
478 among Indian short-nosed fruit bats. *Behaviour*. 2017;154(1):37-63.
- 479 27. Burghardt GM. *The genesis of animal play: Testing the limits*: Mit Press; 2005.

- 480 28. Spinka M, Newberry RC, Bekoff M. Mammalian play: training for the unexpected. The  
481 Quarterly review of biology. 2001;76(2):141-68.
- 482 29. Bekoff M. Social play behavior. Bioscience. 1984;34(4):228-33.
- 483 30. Neuweiler G. Verhaltensbeobachtungen an einer indischen Flughundkolonie (*Pteropus g.*  
484 *giganteus* Brunn). 1. Zeitschrift für Tierpsychologie. 1969;26(2):166-99.
- 485 31. Nelson JE. Behaviour of Australian pteropodidae (Megacheroptera). Animal Behaviour.  
486 1965;13(4):544-57.
- 487 32. Park S-R. Observation on the Behavioral Development of the Common Vampire Bat  
488 *Desmodus rotundus*—Play Behaviors. Journal of the Mammalogical Society of Japan.  
489 1990;15(1):25-32.
- 490 33. Knörnschild M, Glöckner V, Von Helversen O. The vocal repertoire of two sympatric species  
491 of nectar-feeding bats (*Glossophaga soricina* and *G. commissarisi*). Acta Chiropterologica.  
492 2010;12(1):205-15.
- 493 34. Knörnschild M, Kalko EKV, Feifel M. Male courtship displays and vocal communication in the  
494 polygynous bat *Carollia perspicillata*. Behaviour. 2014;151(6):781-98.
- 495 35. Lattenkamp EZ, Shields SM, Schutte M, Richter J, Linnenschmidt M, Vernes SC, et al. The  
496 vocal repertoire of pale spear-nosed bats in a social roosting context. Frontiers in Ecology and  
497 Evolution. 2019;7:116.
- 498 36. Engler S, Rose A, Knörnschild M. Isolation call ontogeny in bat pups (*Glossophaga soricina*).  
499 Behaviour. 2017.
- 500 37. Esser KH, Schmidt U. Mother-Infant Communication in the Lesser Spear-Nosed Bat  
501 *Phyllostomus-Discolor* (Chiroptera, Phyllostomidae) - Evidence for Acoustic Learning.  
502 Ethology. 1989;82(2):156-68.
- 503 38. Knörnschild M, Feifel M, Kalko EKV. Mother-offspring recognition in the bat *Carollia*  
504 *perspicillata*. Animal Behaviour. 2013;86(5):941-8.

505 39. Bohn KM, Wilkinson GS, Moss CF. Discrimination of infant isolation calls by female greater  
506 spear-nosed bats, *Phyllostomus hastatus*. *Animal Behaviour*. 2007;73:423-32.

507

## 508 **Supporting information**

509 **S1 Video. Male fur chewing.** This video captures the pup and an adult male together in the roost at  
510 night (the other four individuals of this social group are absent). Directly after landing in the tent, the  
511 male briefly smells the pup and directs himself behind the pup. Immediately, he starts biting/chewing  
512 the pup's fur. The biting is accompanied by wing trembling. The pup seems irritated, trying to move  
513 around. At some point, it attempts to stretch its wing. After a few seconds, the male starts cleaning  
514 himself. At the end of the video the pup turns around and a wet part can be spotted on its back.

515 **S2 Video. Pup play behaviour.**

516 This video captures the pup alone in the roost at night. The detailed description of the pup's behaviour  
517 can be found in the results section of the study.

518 **S1 Fig. Spectrograms of social call types SC9 and SC10.**

519 Spectrograms depicting the social call types SC9 and SC10 of *E. alba* **(A)**. Both social call types were  
520 emitted by an adult male in response to a mosquito sting on a hairless spot on the back of the bat. The  
521 vocalizations were accompanied by agonistic behaviour, including wing flapping and hitting  
522 conspecifics with folded wings. Both social call types were emitted in series and both were  
523 concatenated to a vocal sequence **(C)**. Both social call types were recorded only with the camcorder's  
524 built-in microphone and not with the high-quality ultrasonic recording setup. This is the reason why  
525 the recordings are clipped at 20 kHz. Spectrograms were created using a 512-point FFT and a Hamming  
526 window with 75% overlap.

527



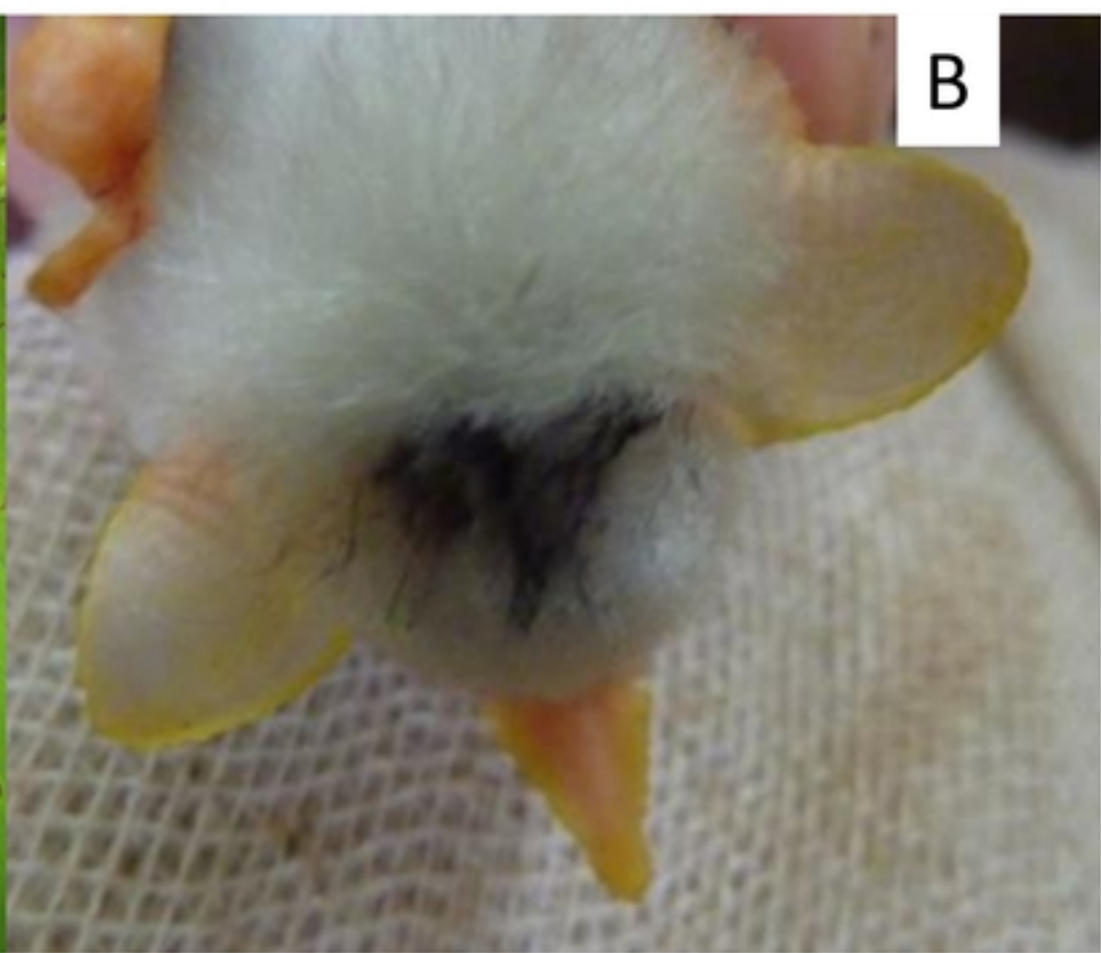


Fig 1

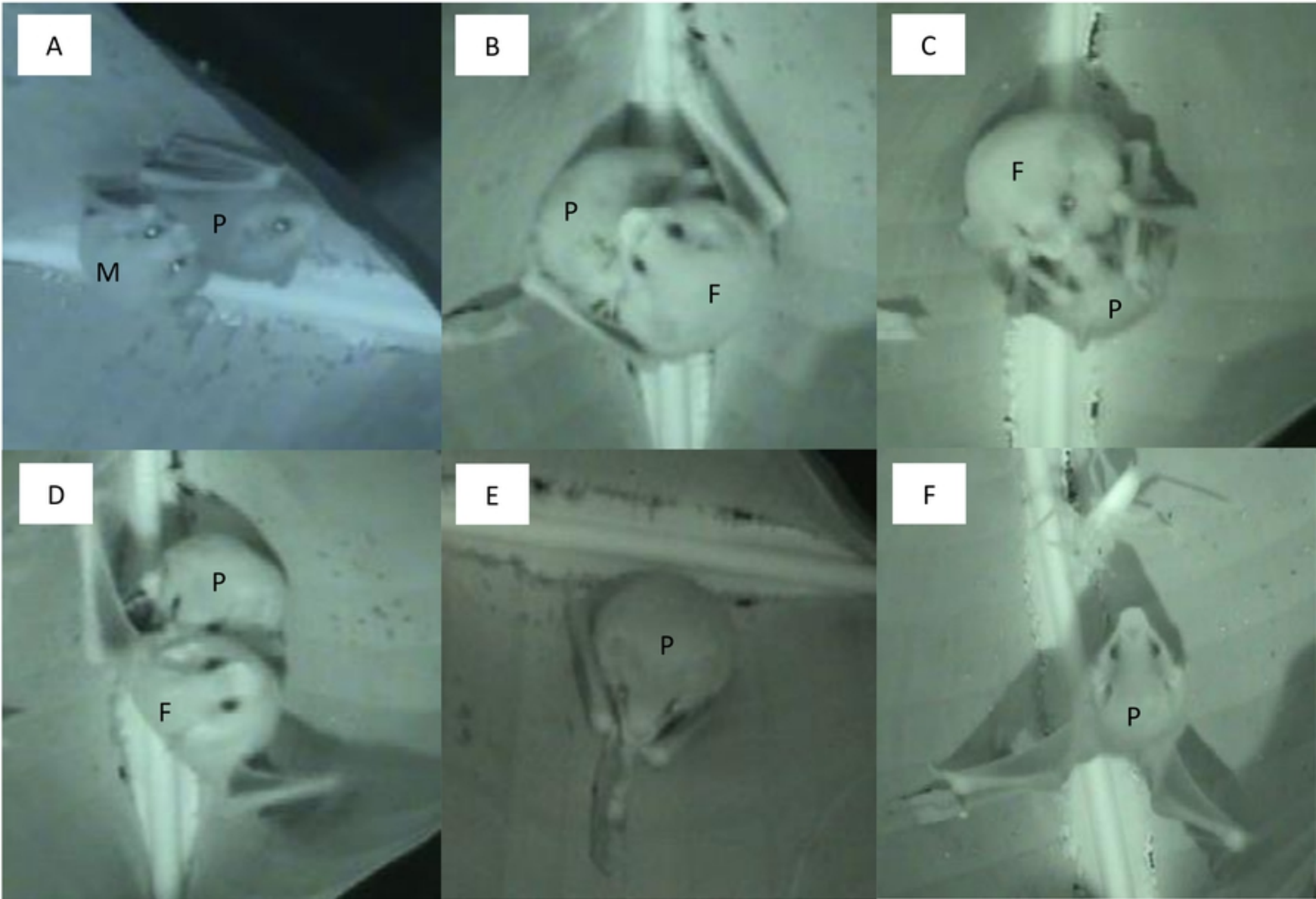


Fig 2

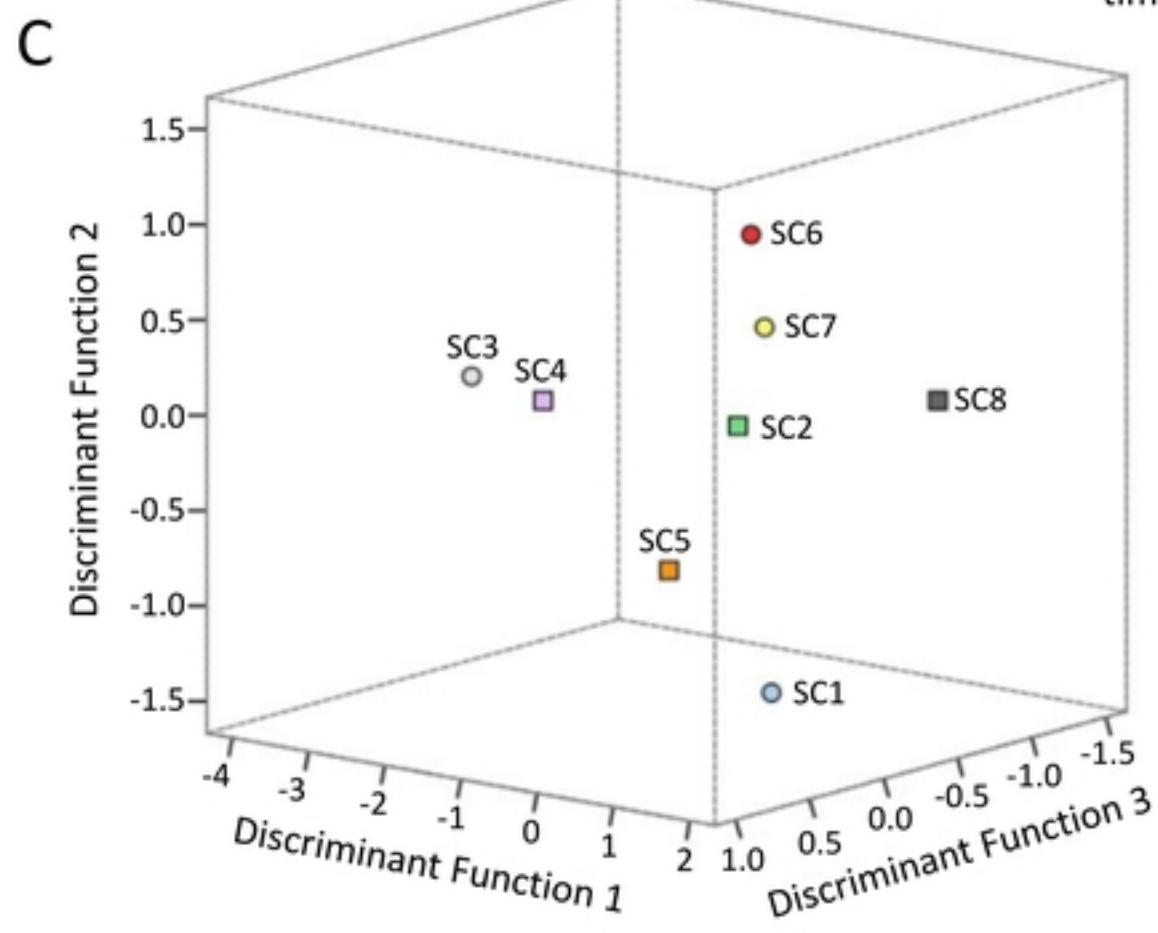
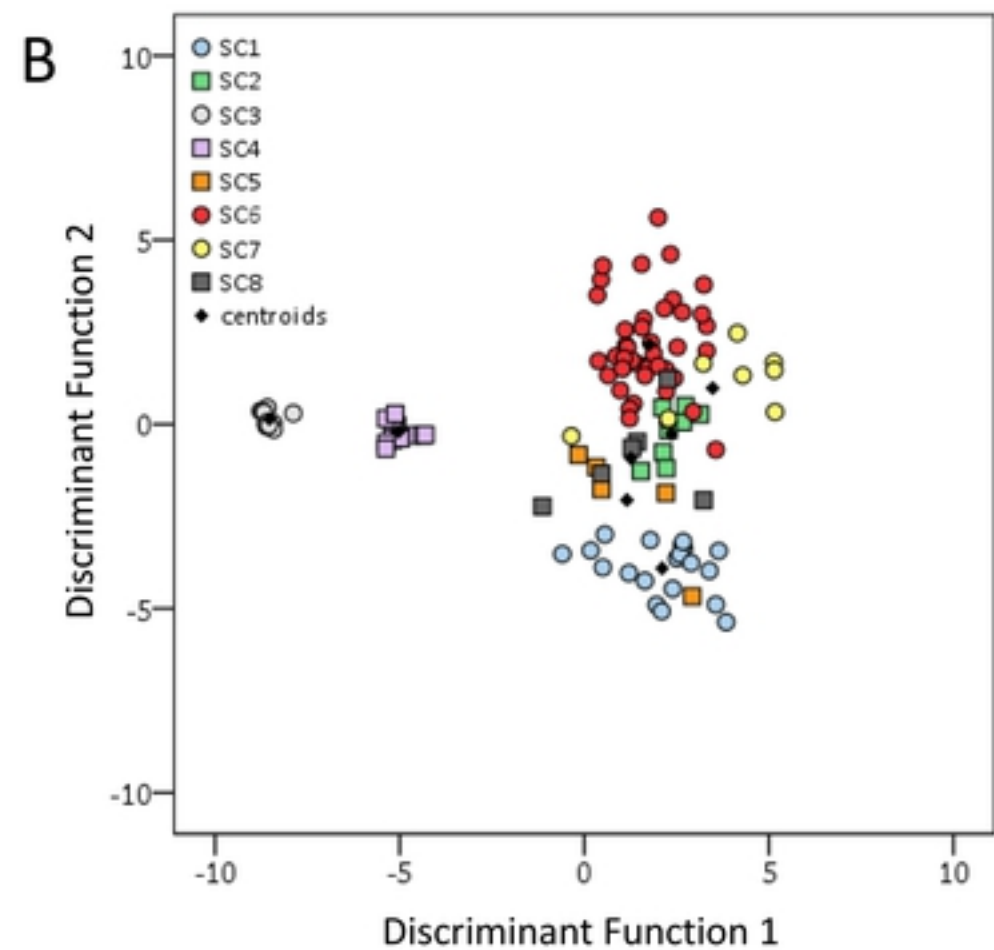
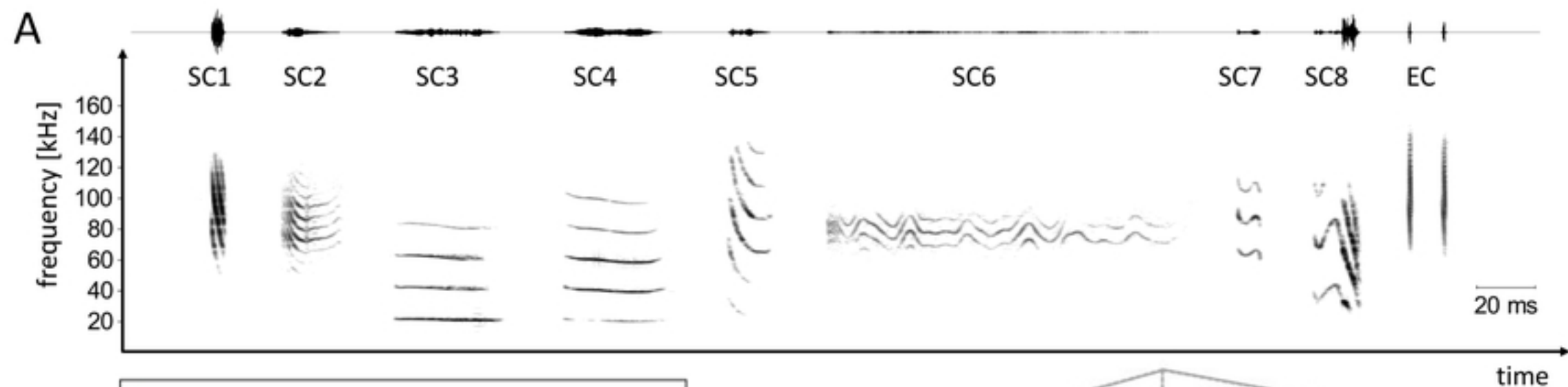


Fig 3