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2 **Spatial variation in black-headed night monkey (*Aotus nigriceps*) vocalizations**

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23 **Acknowledgements**

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25 Manu Learning Center (CREES) staff for hosting us, clearing trails, and providing valuable insight into location and

26 behavior of groups. We are indebted to students and staff from the School for Field Studies who assisted with data

27 collection and logistics. We carried out data collection in accordance with the legal requirements of Peru, and with

28 permission of the Amazon Conservation Association and CREES.

29 **Abstract**

30 Quantitative acoustic analysis has been used to decipher individual differences, population structure, and taxonomic  
31 diversity in numerous primate species. We previously described three distinct call types in wild *Aotus nigriceps*, and  
32 now assess acoustic differences in two of these call types between social groups and spatially distinct populations.  
33 Acoustic parameters for both analyzed call types exhibited significant variability between groups. Similarly,  
34 geographically distant field sites were acoustically distinct from one another. Several groups also used a variation of  
35 a common call: a triplet Ch Ch instead of a duplicate. Other groups made use of ultrasonic frequencies which have  
36 not previously been reported in *Aotus*. Our results suggest that *Aotus nigriceps* exhibits substantial acoustic  
37 variability across sites that could potentially be useful for taxonomic classification, although additional  
38 geographically distant populations still need to be sampled. The possibility of individual signatures also exists and  
39 will require recording vocalizations from known individuals.

40 **Key words:** vocalizations, *Aotus nigriceps*, night monkey, Peru, acoustics

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57 **Introduction**

58 Primates use vocalizations to communicate about the presence of predators (Zuberbühler 2002; Schel et al.  
59 2009), location of food sources (Slocombe and Zuberbühler 2005), nesting behavior, travel intentions and group  
60 cohesion (Boinski 1996), territorial defense (Raemaekers and Raemaekers 1985; Cowlshaw, 1992), mate  
61 assessment and pair bonding (Cowlshaw 1996; Geissmann and Orgeldinger 2000). Primate acoustic signals may  
62 also be used for kin recognition and can convey information on age, sex, body size, and rank (Salmi and  
63 Hammerschmidt 2014). The diversity of these vocal signals makes acoustics a convenient tool to explore differences  
64 among primates at various taxonomic levels.

65 Despite the importance of acoustic communication in primates, evidence of population level differences in  
66 primate vocalizations are relatively limited (Green 1975; Maeda and Masataka, 1987; Mitani et al., 1992; Fischer et  
67 al. 1998; Mitani et al. 1999; Delgado 2007; Wich et al. 2008; de la Torre and Snowdon 2009; Wich et al. 2012).  
68 Acoustic variation between populations can exist for any of several reasons: divergence through cultural drift in  
69 species that learn their vocalizations (i.e. inaccurate copying transmitted vertically or horizontally), genetic drift  
70 following reproductive isolation, or local adaptation in response to sexual selection, habitat transmission properties,  
71 predation pressure, or social selection pressures (Yoktan et al. 2011).

72 Most evidence suggests that non-human primates are not vocal learners; however, several recent studies  
73 have found that primates can learn slight modifications to their vocalizations (Watson et al. 2015; Takahashi et al.  
74 2017). Examining patterns of geographic variation in call structure can provide some evidence for or against the  
75 presence of vocal learning; for example, if there is a sharp acoustic divide between two spatially contiguous areas  
76 that show no evidence of genetic divergence (i.e. vocal dialects), this often suggests the presence of vocal learning.

77 Spatial variation in vocal characteristics within a species could be a valuable tool for addressing a variety of  
78 ecological questions. For example, primate calls can be used to distinguish species, populations, groups, and  
79 individuals (Table 1). If individuals could be distinguished from one another solely using quantitative acoustic  
80 analysis, population size could be estimated by combining acoustic analysis and line transect surveys (Terry et al.  
81 2005; Marques et al. 2013; Kalan et al. 2015). Moreover, variation in specific call characteristics could be used to  
82 infer group membership, or be used for taxonomic classification, supplementing morphometric or genetic data.

83 Night monkeys, *Aotus* spp., are a useful model for investigating patterns of acoustic variation because  
84 nocturnal and forest-dwelling species tend to rely heavily on vocalizations to communicate with one another. We

85 have previously reported on the vocal repertoire of wild *Aotus nigriceps*, describing three calls: the Squeak, Ch Ch,  
86 and Long Trill (Helenbrook et al. 2018). In this study we focus on quantitatively comparing acoustic variation of  
87 two of these calls between groups and distant populations.

88

## 89 **Methods**

90 Eleven *Aotus nigriceps* groups were sampled (Fig.1): eight at the Villa Carmen Biological Station in  
91 Pilcopata, Peru (12°53'39"S, 71°24'16"W), and three at CREES - the Manu Learning Center, on the edge of Manu  
92 National Park (12°47'22"S 71°23'32"W). The two field sites are separated by a low mountain range (~1143m) and  
93 are just over 10 km apart at their nearest borders. Villa Carmen has a long history of development, ecotourism and  
94 agriculture. The groups sampled near the station lived in secondary forest, often dominated by bamboo or cane,  
95 whereas groups sampled at CREES inhabited recovering clear-cut to primary rainforest where bamboo and cane  
96 were largely absent. Research groups of 3-8 observers went into the field from 5:30-7:30am and 5:30-7:30pm for a  
97 total of 28 days at Villa Carmen and nine days at CREES to collect acoustic data, times when *A. nigriceps* groups  
98 are known to be active near their nesting sites. Several recordings also took place during the day as part of a separate  
99 behavioral study.

100 A Zoom H1 Handy Recorder was coupled with a RØDE NTG-2 condenser shotgun microphone and shoe  
101 shockmount on a micro boompole at a distance varying from 2-25m. Digital recordings were made at 48 kHz  
102 sampling frequency with 16 or 24-bit amplitude resolution. Acoustic analysis was conducted using Raven Pro 1.5  
103 sound analysis software (Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, New York). Calls  
104 were digitized and measured spectrographically (DFT size 512, time resolution 3.1 ms, Hann window with 50%  
105 overlap). Twenty-four acoustic parameters were measured for each call (Table 2).

106 Inter-group differences were analyzed using non-parametric Kruskal-Wallis tests coupled with post-hoc  
107 multiple comparisons of mean ranks tests with a Bonferroni correction. A Mann-Whitney *U* test was used to assess  
108 site differences between Villa Carmen and CREES. Stepwise discriminant function analysis was used to explore  
109 acoustic parameters that could be used to classify social groups. For model selection, a stepwise forward method  
110 was used (statistic, Wilk's Lamda) with the criteria  $F_{to\ enter}=3.84$  and  $F_{to\ remove}=2.71$ , and a tolerance level of <0.01  
111 (STATISTICA). This process was repeated for both call types separately. Variables that failed a tolerance test where  
112 there was an almost exact linear relationship with other variables, did not enter the analysis. We used a 10-fold cross

113 validation in which 90% of the calls were randomly chosen to calculate discriminant functions, while 10% was  
114 excluded for testing. Differences between observed and expected frequencies of duplicate versus triplicate Ch Ch  
115 calls was measured using Fisher's Exact Test. All recordings were conducted non-invasively, minimized impact on  
116 behavior, and avoided excessive disturbance, and were therefore deemed exempt from the Institutional Animal Care  
117 and Use Committee approval. All applicable international, national, and/or institutional guidelines for the care and  
118 use of animals were followed.

119

## 120 **Results**

121 Three vocalizations have been described in wild *Aotus nigriceps* populations: Squeak, Ch Ch, and Trill  
122 (Helenbrook et al. 2018). In this study, we analyzed acoustic variability for the two most common calls, the Squeak  
123 (N=1302) and the Ch Ch (N=556; Fig. 2). For Squeaks we only measured the dominant harmonic since it was  
124 consistently found across all sampled groups. The Trill was not used because of its rarity across most groups. At  
125 least ten calls were analyzed from each of seven night monkey groups, ranging in size from 2-5 individuals  
126 (Mean=3.7). The dominant harmonic of the Squeak ranged from a mean minimum frequency of 1591 Hz (Range:  
127 74-3055 Hz; SD 470) to a mean maximum frequency of 2742 Hz (Range: 2010-4443; SD 252). The Ch Ch call  
128 ranged from a mean minimum frequency of 1698 Hz (Range: 44-9092; SD 1182) to a mean maximum frequency of  
129 11636 Hz (Range: 3109-23726 Hz; SD 2538).

130 Acoustic measurements varied significantly between groups (Fig. 3; Table 3). Differentiation between two  
131 or more monkey groups was found for all forty-eight independent vocal characteristics (2 call types x 24  
132 measurements,  $p < 0.001$ ). Discriminant function analysis distinguished among groups for both call types: Squeak  
133 (Wilks' Lambda=0.06,  $F(60,6706)=81.98$ ,  $p < 0.0000$ ) and Ch Ch (Wilks' Lambda=0.06,  $F(48,2592)=42.58$ ,  
134  $p < 0.0000$ ) (Table 4 and Fig. 4). Cumulative significant functions were able to explain 87.4% of variance among  
135 groups using only Squeak calls, and 87.8% of the variance among groups using only Ch Ch calls. Classification  
136 accuracy was similar for both the Squeak (87.4%) and the Ch Ch (76.4%). Duration (90%) and energy parameters  
137 for Squeak and Ch Ch, respectively, provided the greatest discriminatory power at the group level (Table 4).

138 Twelve acoustic parameters were significantly different between Villa Carmen and CREES biological field  
139 stations (Table 6). Discriminant function analysis identified seven Squeak parameters that significantly distinguished

140 locations: low and high frequency, bandwidth, duration (90%), delta time, IQR duration, and max time – the first of  
141 which contributed the greatest discriminatory power; and eight Ch Ch parameters significantly distinguished  
142 locations: low and high frequencies, bandwidth, energy, peak frequency, Q1 frequency, frequency (5%) and center  
143 time– the last of which contributed the greatest discriminatory power. The two sites were found to be significantly  
144 different based on Squeak (Wilks' Lambda=0.77,  $F(13,1281)=29.09$ ;  $p<0.0000$ ) and Ch Ch (Wilks' Lambda=0.40,  
145  $F(13,526)=61.77$ ;  $p<0.0000$ ). Classification accuracy was 93.8% for Squeak (5 out of 13 CREES measurements and  
146 116 out of 116 at Villa Carmen), and 100.0% for Ch Ch. Cumulative significant functions were able to account for  
147 47.7% of variance between locations using the Squeak, and 77.7% using the Ch Ch call.

148 Other differences were observed between groups as well. The Ch Ch was predominately found in a series  
149 of two (“in duplicate”) (88.3% of cases); however, four groups also produced calls in triplicate (i.e. Ch Ch Ch). Out  
150 of 556 total Ch Ch calls, 65 were in triplicate (11.7%), with 2.8% in T2A, 1.3% in A, 50.4% in B, and 2.8% in E.  
151 The distribution of triplicate calls across groups differed significantly from even distribution across groups, with  
152 Group B exhibiting nearly four times as many triplicates as expected ( $p=0.0000$ ). In addition, two groups were  
153 observed using ultrasonic frequencies as part of the Ch Ch call (>20kHz): group C (N=3) at Villa Carmen and T2A  
154 (N=1) at CREES.

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## 156 **Discussion**

157 The majority of acoustic parameters for both calls differed significantly between groups and geographic  
158 locations, though single acoustic parameters alone were not sufficient to predict group membership. Variance of  
159 acoustic parameters overlapped in nearby groups, making absolute classification difficult. However, there was a  
160 consistent pattern whereby calls from the same groups and population tended to cluster together based on similar  
161 acoustic measurements. Population level classification was more accurate, largely driven by acoustic parameters of  
162 the Ch Ch call. Quantitative analysis of acoustic traits may therefore be useful in elucidating group and population  
163 level differences and may provide useful insight into the underlying phylogenetic relationships between groups,  
164 populations and potentially species of *Aotus*. However, additional recordings are needed both at the group and  
165 population levels, preferably with more distant populations included.

166 We were unable to investigate individual acoustic variability because of our inability to pair calls to  
167 specific individuals in a complex environment at night. Based on various other primate studies it is likely that

168 individuals can be differentiated based on vocal signatures (Table 1). However, confirmation of vocal individuality  
169 will require either analysis in captivity or pairing video and audio recordings in wild nesting groups. If recordings  
170 can be attributed to specific individuals, then acoustic analysis could be used to establish whether individual  
171 conspecifics vary predictably in their vocalizations. Establishing the ability to vocally differentiate individuals  
172 would be particularly useful for a nocturnal species such as the black-headed night monkey, allowing researchers to  
173 study group composition solely based on vocal recordings.

174         Aside from differences in acoustic parameters, two other acoustic differences were discovered among  
175 groups. First, a triplet Ch Ch call was found in recordings from groups T2A, A, B, E. Though relatively rare within  
176 the sampled populations (11.7% of cases), over half of these cases were found in Group B. The other groups at Villa  
177 Carmen that used the triplet call are likely of the same population since they are isolated on all but one side and in  
178 relative proximity to group B (<1300m at furthest extent). The prevalence of the triplet call in Group B suggests that  
179 this is not an aberration but rather a consistent modification of a common call. The fact that the triplet call only  
180 occurred in certain groups could reflect any number of possibilities including increased prevalence of a particular  
181 behavioral context, or a vocal innovation (genetic or learned). Alternatively, it is possible that the presence of both  
182 duplicate and triplet Ch Ch calls is the ancestral state and the absence of the triplet call is derived. Either way,  
183 additional sampling of nearby groups – coupled with underlying population genetics analysis - would confirm  
184 whether this is a relatively unique acoustic irregularity which is independent of underlying population structure, or  
185 whether this call variation routinely arises and is widespread. Likewise, being able to obtain calls specific to  
186 individuals through video and audio pairing in nests would allow us to decipher whether all individuals within a  
187 particular group use the triplet call.

188         It is uncertain whether the use of ultrasonic frequencies in night monkeys is rare or whether this is a  
189 common response to environmental pressures such as inter-species competition for lower frequencies or predator  
190 avoidance. Of course, other nocturnal primates (i.e., *Tarsius*, *Galago*, *Microcebus*, *Nycticebus*) and some diurnal  
191 neotropical primates (i.e., *Callithrix* and *Cebuella*) produce calls containing ultrasonic frequencies, though only the  
192 tarsiers produce calls entirely within the ultrasonic range, with the other species always producing dominant  
193 frequencies in the human audible range (Ramsier et al. 2012). In several species, the use of ultrasound appears to be  
194 context specific, often in the presence of predators, including humans (e.g. Rahlfs and Fichtel 2010; Gursky-Doyen  
195 2013).

196 *Aotus* currently consists of eleven described species based on both phenotypic and genotypic evidence.  
197 Night monkey taxonomy has been revised considerably based on differences in karyotypes, morphology, molecular  
198 sequencing, malaria sensitivity, immunological responses, and geographic isolation (Menezes et al. 2010). Despite  
199 this, few specimens from any one study have come from *Aotus nigriceps* despite this species having one of the  
200 largest ranges of any *Aotus* species. Moreover, the current taxonomic classification lumps *A. nigriceps* populations  
201 from areas with considerably different elevations and from areas separated by significant river systems. Thus, the  
202 possibility remains that further evolutionary and conservation management units may exist. Considering the distinct  
203 differences in call types previously described between *Aotus* species and the use of quantitative acoustic sampling to  
204 differentiate many other primate species, we anticipate that further analysis would prove useful in differentiating  
205 population-level or species-level taxonomy.

206 Finally, *Aotus nigriceps* likely produce more than the three described call types since captive *Aotus* species  
207 have exhibited larger vocal repertoires. In captive situations it is easier to record night monkeys at close distances  
208 and calls can be induced in different situations, which could facilitate observation of a wider variety of call types.  
209 We anticipate that with continued sampling these additional call types could also be recorded in the wild.

210

#### 211 **Conflict of Interest**

212 The authors declare that they have no conflict of interest.

213

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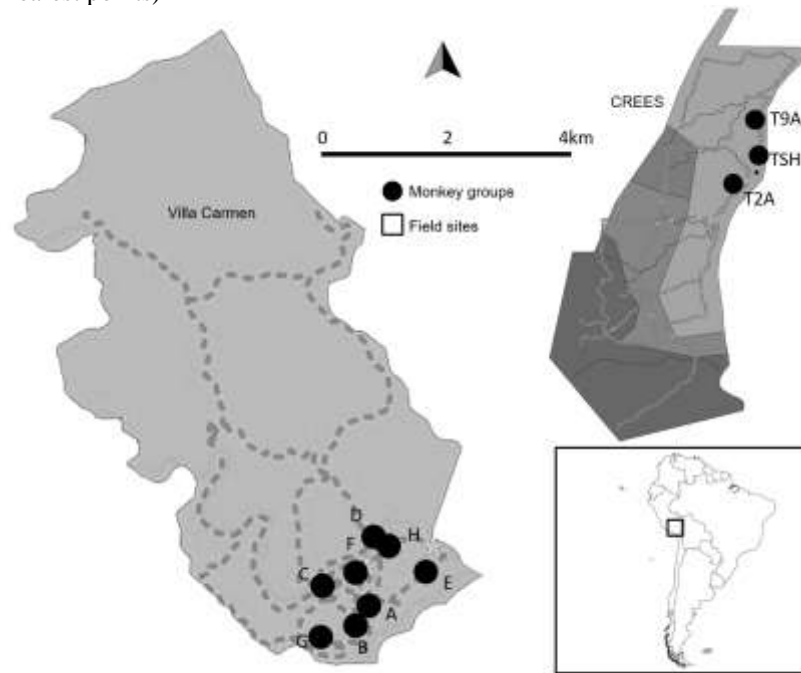


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303 **Fig. 1** Field research conducted at Villa Carmen Biological Station (eight black-headed night monkey groups) and  
304 CREES – Manu Learning Centre (three groups), in southeastern Peru. A mountain ridge separates the two field  
305 stations (10.1km at nearest points)

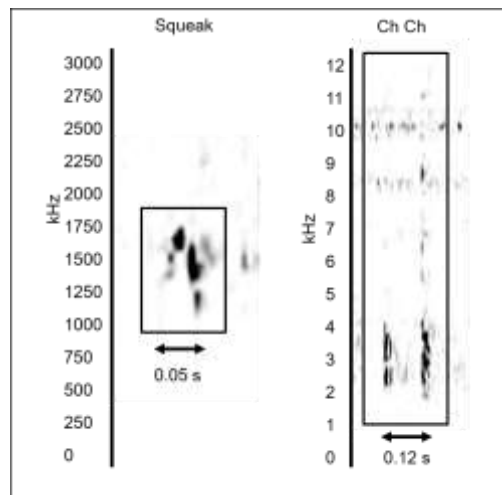


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309 **Fig. 2** Spectrogram representing both calls quantitatively analyzed in this study: the Squeak (left) and the Ch Ch  
310 (right). Both calls have been previously described (Helenbrook et al. 2018). In this example, a duplicate Ch Ch  
311 is depicted. The upper range of the spectrogram is faint, partially a result of minimizing background noise in the 7-8  
312 kHz from insects

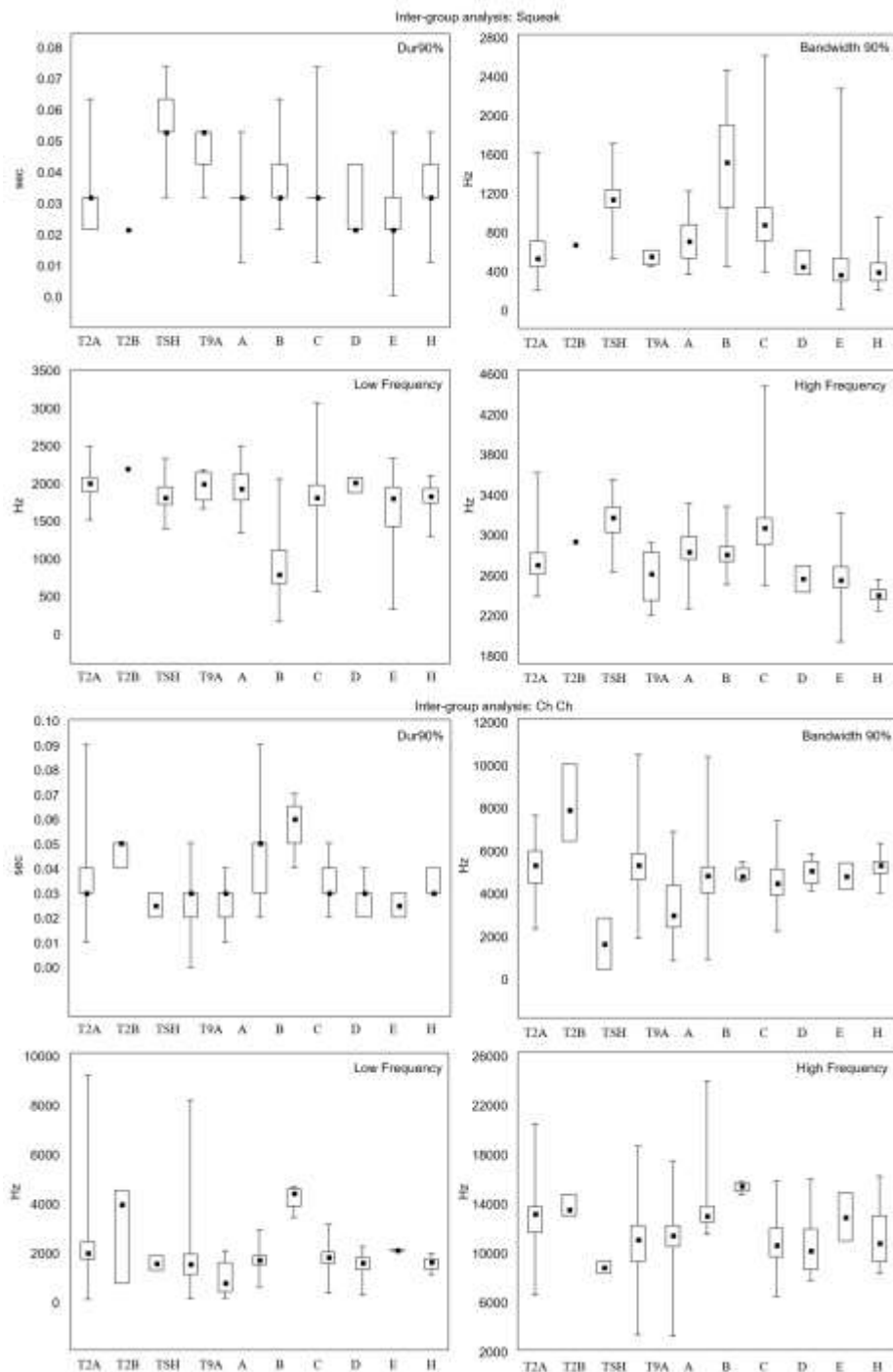


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317 **Fig 3** Boxplot plate of four descriptive acoustic measurements (e.g., duration, maximum frequency, minimum  
318 frequency, and bandwidth) for the Squeak and Ch Ch across all groups. Median represented by solid box, box plot is  
319 25-75% of call variability, and whiskers are minimum and maximum which do not signify significance, rather  
320 distribution of values is depicted. Note that inter quartile ranges are depicted instead of standard error or deviation  
321 because of the non-parametric nature. T2A, TSH, and T9A groups are from CREES while A-H are from Villa  
322 Carmen. Significant differences illustrated in Table 3



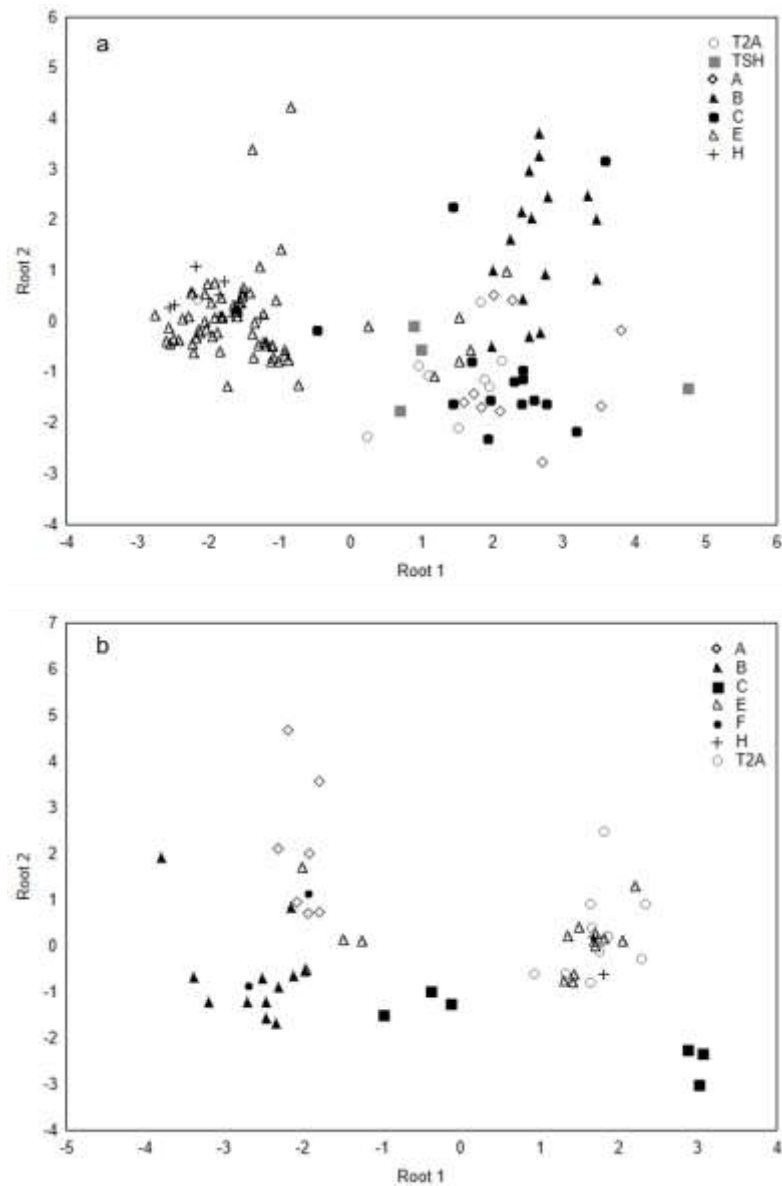
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326 **Fig 4** Canonical score scatterplot. The biplot axes are the first two canonical variables. These define the two  
327 dimensions that provide maximum separation among groups. We used a 10-fold cross validation in which 90% of  
328 the calls were randomly chosen to calculate discriminant functions. Here we present the results of the 10% excluded  
329 for testing

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337 **Table 1** Evidence of primate acoustic variation at various taxonomic levels.

Primate species (N)	Individual	Group	Population	Subspecies	Species	Call description	Study
<i>Cebidae, Callitrichinae</i> (28)	-	-	-	-	Yes	Long call	Garbino 2018
<i>Galago</i> spp. (8)	-	-	-	-	Yes	Loud call	Zimmermann 1990
<i>Galago alleni</i>	-	-	-	-	Yes	Loud call	Ambrose 2003
<i>Galago crassicaudatus, G. garnettii</i>	-	-	-	-	Yes	Loud call	Masters 1991
<i>Galago senegalensis, G. moholi</i>	-	-	-	-	Yes	14 calls	Zimmermann 1988
<i>Gorilla gorilla</i>	Yes	-	-	-	-	8 calls	Salmi et al. 2014
<i>Hylobates muelleri</i>	Yes	-	No	-	-	Great call	Clink et al. 2017
<i>Hylobates muelleri</i>	Yes	-	Yes	-	-	Great call	Clink et al. 2018
<i>Lepilemur</i> spp. (10)	-	-	Yes	-	-	Loud call	Méndez-Cárdenas et al. 2008
<i>Microcebus</i> spp. (3)	-	-	-	-	Yes	Whistle, Purr, Chitter	Hending et al. 2017
<i>Microcebus</i> spp. (3)	-	-	-	-	Yes	Advertisement call	Braune et al. 2008
<i>Microcebus murinus</i>	-	-	Yes	-	-	Trill	Hafen et al. 1998
<i>Tarsius</i> spp. (4)	-	-	-	-	Yes	Loud call (Duet call)	Nietsch 1999
<i>Varecia variegata</i>	-	-	-	Yes	-	Loud call	Macedonia and Taylor 1985

339 **Table 2** Name and description of acoustic parameters measured. Not all parameters were included in discriminant  
 340 function analysis because of redundancy.

Parameters	Description
341 Low frequency (Hz)	The lower frequency bound of the selection
342 High frequency (Hz)	The upper frequency bound of the selection
343 Bandwidth 90%	The difference between the 5% and 95% frequencies
344 Energy (dB)	The total energy within the selection bounds
345 Dur90%	The difference between 5% and 95% times
346 Delta frequency (Hz)	The difference between the upper and lower frequency limits of the selection
347 Peak frequency	The frequency at which max power occurs within the selection
348 Delta time (s)	The difference between the begin and end time for the selection
349 Center frequency (Hz)	The frequency that divides the selection into two frequency intervals of equal energy
350 Q1 frequency (Hz)	The frequency that divides the selection into two frequency intervals containing 25% and
351	75% of the energy in the selection
352 Q3 frequency (Hz)	The frequency that divides the selection into two frequency intervals containing 75% and
353	25% of the energy in the selection
354 Max power	The maximum power in the selection.
355 Frequency 5%	The frequency that divides the selection into two frequency intervals containing 5% and
356	95% of the energy in the selection
357 Frequency 95%	The frequency that divides the selection into two frequency intervals containing 95% and
358	5% of the energy in the selection
359 Center time	The point in time at which the selection is divided into two time intervals of equal energy
360 Q1 time	The point in time that divides the selection into two time intervals containing 25% and
361	75% of the energy in the selection
362 Q3 time	The point in time that divides the selection into two time intervals containing 75% and
363	25% of the energy in the selection
364 IQR duration (s)	The difference between the 1 <sup>st</sup> and 3 <sup>rd</sup> quartile times
365 Time 5%	The point in time that divides the selection into two time intervals containing 5% and 95%
366	of the energy in the selection
367 Time 95%	The point in time that divides the selection into two time intervals containing 95% and 5%
368	of the energy in the selection
369 Max time	The first time in the selection at which a spectrogram point with power equal to max
370	power occurs
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372	Quantitative acoustic characteristics analyzed (Raven).

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374 **Table 3** All *Aotus nigriceps* vocal measurements were found to have significant ( $p < 0.001$ ) inter-group differences.  
 375 Post-hoc analysis was conducted using multiple comparisons of mean ranks with Bonferroni adjustment. All  
 376 associations significant at  $p < 0.05$ .

377	378 Call Measurement	378 Group	378 Significant Associations (p value)
379	<i>Ch Ch</i>		
380	Dur90%	A	C(0.00); D(0.04)
381		B	C(0.00); D(0.01)
382	High frequency	C	E(0.00); F(0.01); T2A(0.00)
383		A	C(0.00); D(0.02); T2A(0.00)
384		B	C(0.00); D(0.04); T2A(0.00)
385		C	E(0.00); F(0.00); H(0.01)
386		D	E(0.01); F(0.01); T9A(0.05)
387		E	T2A(0.00)
388	Low frequency	F	T2A(0.00)
389		A	B(0.00); D(0.03); T2A(0.00)
390		B	C(0.00); D(0.00); E(0.00); T2A(0.00)
391		C	T2A(0.02)
392	Bandwidth 90%	A	B(0.00); E(0.00)
393		B	C(0.00); E(0.00); F(0.00); H(0.00); T2A(0.00); TSH(0.00)
394		E	T2A(0.00)
395		TSH	T9A(0.04)
396			
397	<i>Squeak</i>		
398	Dur90%	A	B(0.00); E(0.01); TSH(0.00)
399		B	C(0.02); E(0.00); T2A(0.00); TSH(0.00)
400		C	E(0.00); TSH(0.00)
401		E	H(0.00); T2A(0.00); TSH(0.00); T9A(0.05)
402		H	TSH(0.00)
403		T2A	TSH(0.00)
404	High frequency	A	C(0.03); E(0.00); H(0.00); TSH(0.02)
405		B	C(0.00); E(0.00); H(0.00); TSH(0.00)
406		C	E(0.00); H(0.00); T2A (0.00)
407		D	TSH(0.04)
408		E	H(0.00); T2A(0.00); TSH(0.00)
409	Low frequency	H	T2A(0.00); TSH(0.00)
410		A	B(0.00); E(0.00)
411		B	C(0.00); D(0.01); E(0.00); H(0.00); T2A(0.00); TSH(0.00); T9A(0.00)
412		C	T2A(0.00)
413		E	T2A(0.00)
414	Bandwidth 90%	H	T2A(0.00)
415		A	B(0.00); E(0.00); H(0.00); TSH(0.01)
416		B	C(0.00); E(0.00); H(0.00); T2A(0.00)
417		C	E(0.00); H(0.00); T2A(0.00)
418		E	T2A(0.00); TSH(0.00)
419	H	T2A(0.01); TSH(0.00)	
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429 **Table 4** Stepwise discriminant function analysis of all non-redundant measured acoustic parameters.

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Call Measurement	Wilks' Lambda	Partial Lambda	p value
432 <i>Squeak</i>			
433 Bandwidth 90%	0.07	0.85	0.00
434 Energy	0.06	0.92	0.00
435 Dur90%	0.07	0.82	0.00
436 Frequency 95%	0.06	0.88	0.00
437 Max power	0.06	0.89	0.00
438 IQR duration	0.06	0.96	0.00
439 Center frequency	0.06	0.98	0.00
440 Q1 frequency	0.06	0.93	0.00
441 IQR bandwidth	0.06	0.90	0.00
442 Max time	0.06	0.88	0.00
443			
444 <i>Ch Ch</i>			
445 Energy	0.17	0.33	0.00
446 Dur90%	0.06	0.92	0.00
447 Bandwidth 90%	0.05	0.94	0.00
448 IQR duration	0.06	0.96	0.00
449 Frequency 95%	0.06	0.89	0.00
450 Center frequency	0.09	0.65	0.00
451 Delta frequency	0.07	0.77	0.00
452 Q1 frequency	0.07	0.79	0.00

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**Table 5** Discriminant function analysis correct classification utilizing 10-fold cross validation at group level.

Squeak alone

Group	% Correct	A	B	C	E	H	T2A	TSH
A	66.7	<b>6</b>	0	3	0	0	0	0
B	75.0	4	<b>12</b>	0	0	0	0	0
C	78.6	0	2	<b>11</b>	1	0	0	0
E	89.9	4	1	0	<b>62</b>	1	1	0
H	75.0	0	0	0	2	<b>6</b>	0	0
T2A	44.4	3	0	0	1	0	<b>4</b>	1
TSH	75.0	0	0	1	0	0	0	<b>3</b>

460 Ch Ch alone

Group	% Correct	A	B	C	E	F	H	T2A
A	100	<b>7</b>	0	0	0	0	0	0
B	83.3	2	<b>10</b>	0	0	0	0	0
C	83.3	0	1	<b>5</b>	0	0	0	0
E	66.7	3	1	0	<b>10</b>	0	0	1
F	0	1	1	0	0	<b>0</b>	0	0
H	0	0	0	0	1	0	<b>0</b>	1
T2A	90.9	0	0	0	1	0	0	<b>10</b>

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465 **Table 6** Mann Whitney results comparing group acoustic parameters between distant locations (~10km). Sample  
 466 size (Ch Ch: CREES 108 and VC 441) and (Squeak: CREES 136 and VC 1150). \*All associations significant at  
 467  $p < 0.05$ .

469	Call Measurement	CREES Mean	VC Mean	U	P-value
470	<i>Squeak</i>				
471	Low Frequency	1896	1557	41532	0.00*
472	High Frequency	2905	2715	48217	0.00*
473	Bandwidth 90%	737	633	57946	0.00*
474	Energy (dB)	75	89	51471	0.00*
475	Dur90%	0.036	0.28	50231	0.00*
476	Peak Frequency	2577	2395	47752	0.00*
477	Delta Time	0.049	0.041	51588	0.00*
478	Center Frequency	2525	2370	40555	0.00*
479	Q1 Frequency	2341	2242	56039	0.00*
480	Q3 Frequency	2687	2477	37523	0.00*
481	Frequency 5%	2079	1951	71783	0.11
482	IQR Duration	0.02	0.015	51031	0.00*
483	Max Time	99	222	47045	0.00*
484					
485	<i>Ch Ch</i>				
486	Low Frequency	2504	1497	12490	0.00*
487	High Frequency	12901	11268	13405	0.00*
488	Bandwidth 90%	4991	4263	16333	0.00*
489	Energy (dB)	112	88	11554	0.00*
490	Dur90%	0.03	0.03	23617	0.89
491	Peak Frequency	5904	5596	20661	0.03*
492	Delta Time	0.04	0.04	23239	0.70
493	Center Frequency	5703	5541	23653	0.91
494	Q1 Frequency	4585	4740	17581	0.00*
495	Q3 Frequency	6810	6235	18448	0.00*
496	Center Time	376	98	8585	0.00*
497	IQR Duration (s)	0.02	0.02	23220	0.68
498	Frequency 5%	3255	3215	20142	0.01*
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