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Vocal repertoire of free-ranging black howler monkeys' (*Alouatta pigra*): call types, contexts and sex-related contributions

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ABSTRACT

Alouatta species utter the most powerful primate vocalizations in the Neotropics and are well-known for their loud and long-lasting male howling bouts. However, the diversity of acoustic structures used in these howling bouts, as well as in non-howling contexts, and the relative contribution of the different group members to the entire vocal repertoire, needed to be explored further. This report provides the first detailed description of the vocal repertoire of black howler monkeys (*Alouatta pigra*), focusing on acoustic structures and contexts of emission of both loud and soft calls as well as on the contribution rate of males and females to the different call types. Three free-ranging social groups of black howler monkeys living in Palenque National Park, Mexico were monitored. We identified twelve acoustically discriminable call types, eight described

previously and four described here for the first time. A few call types were systematically emitted either isolated or during howling bouts, but most of them could be heard in both calling contexts. Three call types were emitted only by females and two only by males. Adult males' call rates (for the seven shared call types) were higher than those of females but only when considering calls emitted within howling bouts. Our contextual analysis enabled us to divide call types into potential functional categories, according to their degree of contribution, to intra-group vs. inter-group interactions and to neutral-positive vs. negative situations. We then discussed how socio-ecological factors, notably sex differences in social behaviors, may explain the variability found in the vocal repertoire of this species and compared our findings with the literature on other primate species.

Keywords: New World monkeys, vocal repertoire, vocal communication, acoustic structure, sex-differences.

INTRODUCTION

A detailed quantitative description of a species' vocal repertoire is a prerequisite for an in-depth understanding of its communication abilities, particularly for animals living in visually-closed habitats who mainly rely on calls to communicate [Marler, 1977]. Vocal repertoires typically include several acoustically and contextually

distinguishable call types. The presence of easily distinguishable call types is more typical for forest-dwelling species than for species living in open habitats with fewer constraints on sound propagation [Gautier & Gautier, 1977; Fischer et al., 2001]. While most forest-dwelling monkeys have thus developed a discrete vocal repertoire, composed of stereotyped call types that limit the risk of possible confusion, savannah primates, living in more open habitats, possess a more graded vocal repertoire with several transient acoustic structures in addition to the main call types [Gautier & Gautier, 1977; Fischer et al., 2001]. However, this is not true for all forest-dwelling primates, as some have been shown to have a graded repertoire [Marler, 1972]. Additionally, even in species presenting a discrete repertoire, some call types appear much more varied than others depending on their function [contact calls are more varied than alarm calls - Bouchet et al., 2013, and general alert calls are more varied than predator-specific alarm calls - Keenan et al., 2013]. Modification of certain acoustic parameters within a given call type can be used to signal a more detailed level of information [e.g. internal state of caller, type or degree of external danger, individual or social identity: Marler et al., 1992].

Habitat type is not the only factor determining the size and the structure of vocal repertoires, notably of primates. A species' social structure and organization are other key determining factors. First, species with more complex social structures possess more complex vocal repertoires [McComb & Semple, 2005]. Second, a caller's social status impacts both call structure and call rate [Roush & Snowdon, 1999]. In particular,

there is evidence of large inter-individual variations among callers of different ages and sexes. For example in guenons, groups are composed of one adult male, who are socio-spatially isolated and strongly involved in activities related to group protection and travelling, and several adult females, who form the social core of the group. Interestingly, while the vocal behaviors of immature male and female guenons are similar, those of adults differ significantly between sexes [e.g. Bouchet et al., 2012]. However, in species forming multi-male groups, where males interact more frequently with all group members, authors found much less difference in the vocal repertoires of male and female adults [e.g. Lemasson et al., 2013].

Sex differences in vocal patterns can range from the use of sex-specific vocalizations to more subtle differences when members of both sexes use the same calls at different rates or in different contexts [Dubreuil et al., 2015]. One often-cited example of a sex-related call type is the loud and long vocalizations of many territorial forest-dwelling primate species [Mitani & Stuht, 1998]. Loud calls are typically emitted by males and function in regulating inter-group spacing, protecting home range borders [Gautier & Gautier, 1977], defending food resources [Wich & Nunn, 2002], maintaining group cohesion [Cheney et al., 1996] and attracting mates [Delgado, 2006]. However, some authors report that females also emit long and loud calls either on a regular basis [Zimmermann, 1995; Van Belle, 2015] or on rare occasions [Ouattara et al., 2009a].

Alouatta species utter the most powerful (i.e. long and loud) primate vocalizations in the Neotropics [da Cunha et al., 2015]. When it comes to investigate

vocal repertoires, howler monkeys are interesting subjects for several reasons. First, the fact that they live in a forest habitat and are intolerant to neighboring groups favors intra- and inter-group vocal communication [da Cunha et al., 2015]. Second, they can form uni-male groups, as well as more complex social groups including two or three adult males, with clear differences in social activities [Di Fiore et al., 2011]. Hence, the varied social structure in this genus offers an interesting perspective to explore the level of sex-specificity in the vocal repertoire. Third, as the inflatable throat sacs of male howler monkeys are much larger than those of females [Dunn et al, 2015], their abilities in producing particular acoustic structures potentially differ [Schön, 1971]. Fourth, they produce their calls within long-lasting howling bouts, as well as isolated outside howling events, and the relative contribution of the different call types to both vocal events have received little attention [Baldwin & Baldwin, 1976; Drubbel & Gautier, 1993; Whitehead, 1995].

A large literature can be found when it comes to the flexibility of loud call usage (i.e. variations in call rates and contexts of emission) particularly in male howler monkeys. Howling bouts could thus serve a broad range of functions such as group socio-spatial cohesion, competition with neighboring groups over resources (space/food) or mates, attraction of females and infanticide avoidance [see review in Kitchen et al., 2015]. Most studies have suggested that one key function of this collective vocal behavior is to regulate inter-group spacing and to announce the occupancy of an area [Van Belle et al., 2013a]. Da Cunha & Byrne, [2006] found in A.

caraya that playing back loud calls from stranger males inside the home range of the tested group triggered the emission of more roars than when the speakers were located at the border, hence not simulating an invasion. Briseño-Jaramillo et al., [2015] found something similar using a violation-of-attempt playback paradigm with *A. pigra*. The voice of different neighboring males were played back either from a congruent (in the appropriate neighboring home range) or from an incongruent (in a home range on the opposite side) location. Incongruent situations triggered a stronger response from tested subjects (e.g. faster approach, more vocal responses). The idea that males defend resources is clearly supported in several studies, but it remains unclear what kind of resources are primarily defended [Kitchen et al., 2015]. For example, Holzmann et al., [2012] found that the frequency of howling bouts was not related to the abundance of high-quality food, to the spatial position in the home range, to the spatial distribution of feeding resources nor to the presence of infants in the group. Even sister species appeared to differ in their call usage. Howling bouts of *A. pigra* presented acoustics characteristics reflecting higher intergroup competition (longer bouts and faster call rates) while *A. palliata* presented acoustics characteristics reflecting higher intragroup competition (shorter bouts and slower call rates) [Bergman et al., 2016]. At last, males show variations in their initiation and participation rates to howling bouts according to their age, social experience and social position in the group as well as number of offspring sired [Kitchen, 2004; Van Belle et al., 2008; 2014a].

In terms of call usage, however, few studies have focused on females or in immature group members as well as in non-howling vocal events and soft calling, even if we know that those individuals join howling bouts [Kitchen, 2006; Holzmann et al., 2012; Van Belle, 2015]. Females are said to reserve their participation to contexts where cooperation with the central male was needed [Kitchen, 2006]. A recent study interested in numerical superiority influences and intergroup dominance relationships on individual participation during intergroup encounters suggested that both sexes responded similarly in deciding to participate in collective group defense [Van Belle & Scarry, 2015]. Moreover, apart from flexibility in call usage, few studies focused on the acoustic diversity of howler monkeys' vocal repertoire, even in adult males and even during howling bouts. Most descriptions available concern the two most frequent male loud calls [i.e. variants of roars and barks: Whitehead, 1995; Kitchen, 2000; Bergman et al., 2016].

Flexibility can also be found at the production (i.e. acoustic structure) level. Several studies identified context-dependent loud call acoustic variants. Whitehead, (1985) discriminated in *A. palliata* two roar types ("roar variants" and "full roars"), the former emitted during dawn choruses or mild/distant group interactions and the latter during close group encounters. Similarly, in *A. guariba*, close group encounters triggered more roars than barks [Chiarello, 1995]. Additionally, Drubbel and Gautier (1993) discriminated "short" and "long roars" in *A. macconnelli*, the former being produced during short-range interactions and the latter during nighttime choruses. At

last, Van Belle and Scarry [2015] found in *A. pigra* longer vocal displays when the neighbor group was composed by equal number of resident males than when the number of neighboring males was higher. Nevertheless, acoustic descriptions of call types (especially long distance calls such as roars and barks) have been published for only 5 of the 14 species of this genus [*Alouatta palliata* - Baldwin & Baldwin, 1976; Whitehead, 1987; *A. palliata aequatorialis*, *A. palliata mexicana* and *A. seniculus* - Whitehead, 1995; Bergman et al., 2016; *A. macconnelli* - Drubbel & Gautier, 1993; *A. pigra* - Bergman et al., 2016, *A. belzebul* and *A. guariba* - Whitehead, 1995].

Hence, despite the recent breakthroughs in howler monkeys' vocal usage, much is still needed to be learned in terms of acoustic diversity and flexibility of call usage. The aim of this study was to investigate in detail black howler monkeys' (*Alouatta pigra*) vocal repertoire (acoustic structure and context of emission), taking into account loud and soft calls, intra- and inter-group contexts of calling, adults and subadults, and males and females. Although the contextual and temporal patterns of male black howler monkeys' loud calls have been described previously [e.g. Kitchen, 2004, Kitchen et al., 2004; Kitchen et al., 2015; Van Belle et al., 2013a] here we wanted to focus on the acoustic diversity of sounds emitted by this species and on the relative contributions of group members to the entire vocal repertoire. We aimed to: a) identify the different call types and evaluate their acoustic distinctiveness (using male calls), b) assess the variations of callers' contributions to the emissions of call types by comparing age and

sex classes and by analyzing two calling patterns (within and outside howling events), and c) evaluate variations of the general context of emission of all call types.

METHODS

Study sites and groups

We observed three free-ranging social groups (Balam, Motiepa, Pakal) of black howler monkeys (*Alouatta pigra*). The study groups live in Palenque National Park (17°27'51" N, 99°01'30" W). The park covers 1771 ha and includes 597 ha of primary tropical rain forest, 300 ha of regenerating forest and 874 ha of pasture land [Díaz Gallegos, 1996]. Estrada et al. (2002) reported the presence of 19 groups of howler monkeys in this park. The three study groups had known adjacent home ranges and were all surrounded by neighbors [Van Belle et al., 2012; Briseño-Jaramillo et al., 2015]. Each group was followed for 29 days between February 2012 and July 2012 (Total contact hours: Balam 285 hrs, Motiepa 295 hrs, Pakal 291hrs). All group members were known individually from former studies and were well habituated to human presence [Van Belle & Estrada, 2008; Van Belle et al., 2014a; Briseño-Jaramillo et al. 2015]. The three groups Balam/Motiepa/Pakal were composed of 2/2/3 adult males, 2/1/3 adult females, 1/1/0 subadult males, 0/0/1 subadult females, and 1/3/0 juveniles respectively.

Our research complied with the protocols of the Animal Care Committee of Universidad Nacional Autónoma de México and adhered to the legal requirements of México. Our protocols were approved by the “Dirección General de Vida Silvestre (SEMARNAT), permit # SGPA/DGVS/00692/08”. This research adhered to the American Society of Primatologists principles for the ethical treatment of primates.

Acoustic recordings

The groups were followed continuously from 06:00 to 18h00 by MBJ. A directional microphone (SONY ECM-672) and a tie microphone (EUROPSONIC ECM 104) connected to a digital audio recorder (MARANTZ PMD661) were used for recordings (Sample rate 44.1 kHz, resolution 16 bit, WAV format). The first track was used to record monkey calls and the second track was used to record comments on callers' identities and contexts. All recordings were made at comparable distances (i.e. 20 to 30 meters).

Calls were recorded with two sampling methods:

- 1) *All occurrence* sampling was used to record calls emitted within howling bouts.

Howling bouts are long vocal sequences typically combining several call types emitted continuously by one or several group members [Kitchen, 2004]. We defined a “howling bout” as a series of calls emitted in a row during at least one minute with no

inter-call silence longer than ten minutes (according to Holzmann et al 2012; Bergman et al. 2016). Each time a given howling bout started (at least once or twice a day), we commented on caller identity of each call. Given the scarcity of these vocal events, when a howling bout started, we immediately stopped any other sampling activity to concentrate on it. During howling bouts, the experimenter (MBJ), with the help of two field assistants, kept track of the different group members (with each observer monitoring the individuals nearest to him/her) and tried to identify as many individual callers as possible (assistants raised their hands when their focal subject was calling). Given the fact that the number of individuals per group was limited, especially in regards to the number of individuals calling at the same time, this could be done relatively easily. When two individuals overlapped their calls and produced different call types (which occurred rarely), it was still possible to comment on the identity of each caller, one call type usually starting a bit before the other. We discarded the call units to which no caller could be definitively assigned (i.e. less than 1%) from our analysis.

2) *Focal sampling* was used to record calls emitted outside howling bouts. A focal sample lasted 30 min. All adults and subadults were observed in a random order (Average number of focal samples per individual per group and total focal duration per group: Pakal 73.3 +/- 10.2 – 256.5 total hours, Motiepa 128.7 +/- 2.9 – 257.5 total hours, Balam 104.2 +/- 2.7 – 260.5 total hours, see Table 1). Only the calls emitted by the focal subject were recorded at that time.

Call classification

Based on pilot analyses, MBJ pre-ascribed all recorded calls to stereotypic call types [i.e. call heard at least two times and produced by at least two individuals – Lemasson & Hausberger, 2011] by auditory and visual inspections of spectrograms, using AvisoftSAS Lab Pro software (Berlin, Germany). Call types were defined in adequation with the partial vocal repertoires previously published on any *Alouatta* species [e.g. Collias & Southwick, 1952; Baldwin & Baldwin, 1976; Kitchen, 2000; da Cunha & Jalles-Filho, 2007]. A subset of the calls (N=330) were then used to test how reliable human observers discriminated the different call types by ear [as in Ouattara et al., 2009b]. A Master student in Animal Behavior (naive to the study species) and MBJ listened to these 330 calls in a random order and named them blindly and independently. We assessed inter-rater agreements with Cohen's kappa tests: 1) agreement between the pre-classification and the second blind scoring by MBJ, and 2) agreement between the two experimenters. Experimenters presented high intra-rating (same rater) and inter-rater agreements.

Acoustic analyses

As the lower intensity and overlapping of females' calls constrained acoustic measurements, only adult male calls were analyzed. We semi-randomly selected a sample of 60 call examples for each pre-classified call type (Table 2), so that they were

emitted by a maximum of different male callers (i.e. three to six males according to the call type). Spectrograms were drawn with a Fast Fourier Transformation (FFT), the Hann window type, and a time window of 256 points. We evaluated the same 8 representative acoustic parameters as in Briseño Jaramillo et al. (2015) (see definitions in Table 3, adapted from Kitchen 2000 and Ey et al. 2007): lowest frequency (Low Freq, Hz), highest frequency (High Freq, Hz), 1st and 3rd quartile frequency (Q1freq and Q3freq, Hz), aggregate of entropy (Aggr Entropy, Hz), total power (Tot power, Hz), 90% bandwidth (BW 90%, Hz), total duration (s).

The following statistical treatment was run to validate, by acoustic measurements, our call pre-classification. Normality of each acoustic parameter and each call type measured was confirmed by Shapiro tests ($p > 0.05$ in all cases). We confirmed, by inspecting box plots visually, that the variances of our parameters were sufficiently homogeneous. We transformed the 8 acoustic variables into a set of non-correlated components using Principal Component Analyses (PCA). We confirmed by inspecting Henry line the normality of the components [Armatte, 1995]. By Shapiro test, we also confirmed statistically that the variance of the residuals were normal ($p > 0.05$ in all cases) and by Bartlett test we verified that the variances of each component were of the same magnitude ($p > 0.05$ in all cases). We then ran a MANOVA using these components to assess if the calls did differ acoustically from one another. In order to estimate the reliability of this discrimination and to consider potential pseudoreplication bias, we conducted a permuted discriminant function analysis (pDFA-cross-validated)

using a macro written by Mundry & Sommer (2007). We also ran an additional pDFA analysis excluding Total power because this acoustic parameter is subject to debate in the literature, given its high sensitivity to some external parameters such as recording distance, vegetation structure, and relative orientation of the caller and microphone.

Variations of callers' contributions to the different call types

We calculated the following rates for each social group to estimate the contributions of (sub) adult males and females to the different call types:

- a) *Howling call rate (number of calls per hour per individual)*: for each individual in a given age-sex class and for each call type, we divided the number of calls emitted during all recorded howling bouts by the total duration of these howling bouts; then, individual rates were averaged to have a single rate for each age-sex class;
- b) *Isolated call rate (number of calls per hour per individual)*: for each individual in a given age-sex class and for each call type, we divided the number of calls emitted outside a howling bout by the total corresponding focal duration; then, individual rates were averaged to have a single rate for each age-sex class.

We ran nonparametric Mann-Whitney tests (for unpaired data) to compare call rates of the different age-sex classes.

Contextual observations and analyses

First, to assess variations in the general context of emission of call types, we recorded, using *scan sampling* every 15 minutes, for each observation day, the

following variables: a) type of ***Audience neighbor***: absence “neighbor absent”, or presence of a neighboring group “neighbor present” (a neighbor was considered being present when spotted at a maximum distance of 50m from the focal group); b) ***Home range position***: a) “Peripheral area” (area of range overlap with an adjacent group) and b) “Not peripheral area” (area of range not overlap with an adjacent group). These groups have been studied for several years and home ranges are well known (e.g. Van Belle et al., 2013a, Brisen-Jaramillo et al., 2015). At the time of scans, we tracked our position with regards to published maps of home ranges (Van Belle et al., 2013a) (at the beginning of the study, GPS readings were used to confirm our scoring but later on, with experience, we must acknowledged that we only based our scoring on various visual landmarks and on our own knowledge of the field area); c) ***Group spatial cohesion***: dispersion of group members: not dispersed or dispersed (when more than 50% of the group members were distributed over an area above 25m², as in Candiotti et al., 2012). Each call recorded (all age-sex class callers) was then linked with the contextual variables observed during the immediately-preceding scan sample.

Second, to assess variations of the immediate context of emission of call types, we extracted the following variables at the time of each calling event (all age-sex class callers) from our 30 min - focal samples a) ***Individual activity***: callers’ activities were recorded as either “neutral-positive behavior” (i.e. gazing, approaching or following a group member, playing socially, foraging, feeding or resting) or “negative behavior” (i.e. adopting a vigilant posture while staring in the direction of a conspecific, chasing,

pushing or withdrawing from a conspecific, scent marking, body shaking), and b) ***Post-separation reunion***: we recorded whether the caller, after being separated (more than 100 meters away) from the other group members, moved (“yes” or “no”) towards them. To evaluate the call types, contribution to calling contexts were estimated by binomial tests comparing the observed proportions of each binary variable (Neighbor absent VS present; Peripheral vs Not peripheral; Group spatial cohesion: Dispersed VS Not dispersed; Individual activity: Neutral-positive VS Negative behavior; Post-separation reunion: Yes VS No). To run binomial tests, we did not use an expected distribution of 0.5 but we estimated, separately for each binary variable, the expected distribution by using the time budget observed extracted from the total number of scans sampled during the study (independently from any vocal emission). To avoid contextual dependence between calls emitted during the same howling bout, we selected randomly only one call per call type per howling bout (N=464 calls in all).

Results

Classification of call types

Twelve call types were pre-classified and given a 2-letter name code (selected phonetically) based on the acoustic criteria given in Table 4 (see Figure 1-3 for spectrogram examples). Call types can be distinguished from one another by their intensity, duration and frequency patterns, as well as their production mechanisms (mouth open or closed). All call types could be emitted alone (i.e. single exemplar), and

all call types except RH (modulated roar) and CU (coo) could also be emitted in series (i.e. repetition). Apart from AB (barked grunt), AH (soft scream), RH (modulated roar) and CU (coo), the 8 other call types have been described previously for a related howler monkey species. The reliability for human observers to distinguish different call types by ear was confirmed as the pre-classification presented high intra-rating (same rater) and inter-rater agreements (Cohen's kappa: 0.95 and 0.63 respectively).

This pre-classification was validated by acoustic measurements of male calls. This was possible for 9 of the 12 call types mentioned above as the other three calls were not emitted by males. The first four components (Table 5) explained 91% of the total variability in the 540 calls included in this analysis (60 calls x 9 call types). A MANOVA performed with these components confirmed that call types can be discriminated significantly by their acoustics characteristics ($F=1948.8$, $df = 446.34$, Wilk's $\lambda = 0.0004$, $P < 0.001$).

The permuted Discriminant Function Analyses (pDFA) confirmed the acoustic distinctions between call types. The percentage of correct classification of our call types was higher than expected by chance (calls had 11% chances to be randomly well classified) as 85.8% of the calls were correctly assigned to their call type (Fig. 4).

Classification errors were distributed as follow: MU (moo) – HN (grunt) 1/120; WA (soft bark) – RU (soft roar) 1/120; WA (bark) – WU (soft bark) 19/120; WU (soft bark) – RU (soft and long roar) 9/120. The acoustic parameters that were correlated the best with components 1 and 2 were respectively 90% bandwidth (BW 90%, Hz) and total

duration (s). The additional pDFA ran without the Total Power measure showed a similarly high score of classification (86%).

Variations of callers' contributions to the different call types

We identified two broad calling patterns: isolated call utterances by single callers and long howling bouts by one or several synchronized callers (Table 6 and 7). Females participated in 59 of the 86 howling bouts sampled.

All call types were not found in both contexts. CY (scream) calls were found only outside howling bouts, whereas CU (coo), RH (modulated roar), RU (incipient roar) and WU (soft bark) calls were found only during these sequences (Tables 6 and 7). Also, call rates within howling bouts was call type – dependent, and the same was true for isolated call rates. Indeed, RO (roar) and WA (bark) calls were the most frequent call types emitted during sequences whereas HN (grunt), MU (moo) and AH (soft scream) were the most frequent calls emitted isolated (Tables 6 and 7).

Although most call types were emitted by both males and females, CU (coo), CY (scream) and AW (metallic cackling call) were emitted only by (adult and subadult) females, while RH (modulated roar) and MU (moo) were emitted only by (adult and subadult) males (Tables 6 and 7).

In addition, when focusing on the seven shared call types and on the contributions to howling bouts, we found that males and females did not vocalize with equivalent rates.

Indeed, adult males had, on average, much higher (701.8 ± 993 calls per hour per subject, all call types combined) call rates than adult females (115.9 ± 165.9 calls per hour per subject) (Mann-Whitney test $U = 41$; $P = 0.037$; $N_1 = N_2 = 7$ call types) but also than (male and female) subadults (123 ± 213.7 calls per hour per subject) (Mann-Whitney test $U = 41$; $P = 0.03$; $N_1 = N_2 = 7$), whereas adult females' and subadults' call rates did not differ significantly (Mann-Whitney $U = 27$; test $P = 0.8$; $N_1 = N_2 = 7$). This was not found when looking at the contributions to isolated calling, with adult males (0.33 ± 0.53 calls per hour per subject) showing equivalent call rates to adult females (0.11 ± 0.22 calls per hour per subject) (Mann-Whitney test $U = 18$; $P = 0.4$; $N_1 = N_2 = 7$) and to (male and female) subadults (0.01 ± 0.03 calls per hour per subject) (Mann-Whitney test $U = 37$; $P = 0.1$; $N_1 = N_2 = 7$), the two latter showing also equivalent call rates (Mann-Whitney test $U = 33$; $P = 0.2$; $N_1 = N_2 = 7$).

Now, when running the same comparison at the call type level, we found that adult males had higher loud call rates during howling bouts than adult females for five of the seven shared call types ($N_1 = 6$ males, $N_2 = 5$ females, AB: $U = 42$, $P = 0.03$; AH: $U = 39$, $P = 0.01$; HN: $U = 41$, $P = 0.005$; RO: $U = 41$, $P = 0.002$; WA: $U = 38$, $P = 0.01$; RU: $U = 34$, $P = 0.07$; WU: $U = 1.4$, $P = 0.27$).

Variations of emission contexts of call types

Most of the call types were not emitted exclusively in a particular context but in a broad range of situations. Nevertheless, we described a non-random pattern of

association between most of the call types and several of the contexts sampled. Three contextual patterns were associated with different degrees of the social value related to intra- or inter-group interactions (Table 8). First, some call types appeared involved in intra-group socio-spatial cohesion. Typically, HN (grunt) and MU (moo) calls were emitted preferentially when the group was alone (i.e. with no neighbors) and when the caller was engaged in a neutral-positive activity. Whereas HN (grunt) was mainly emitted when a group was dispersed, MU (moo) was preferentially emitted when the group was not at the periphery of the home range, and during post-separation reunions. Second, one call type (CU – coo) appeared to be involved in intra-group conflicts. Indeed, this call type was emitted principally when the group was at not peripheral home range position and when the caller was interacting agonistically with another group member. Third, all the other call types were emitted preferentially at the periphery of the home range and were most frequently associated with negative behavior. Some of these call types were associated with group dispersion (AB (barked grunt), AH (soft scream), CY (scream) and RO (roar)), and some were emitted preferentially when a neighboring group was around (AH (soft scream), RH (modulated roar) and RU (incipient roar)). Only WU (soft bark) calls were emitted similarly in all contexts.

Discussion

Our analyses of the vocalizations of three free-ranging groups of black howler monkeys showed, at least in males, a relatively discrete vocal repertoire. Here, we were able to discriminate up to twelve call types (among which, nine could be acoustically investigated). We identified four call types never described before. Calls could be single utterances from a given caller or could be part of a long howling bout composed of several concatenated calls from several callers. Call types contributed heterogeneously to both calling patterns. Moreover, 7/12 of the repertoire was emitted by members of both sexes and five calls were found to be emitted by one sex only during our 6-month study. Nevertheless, call rates differed with caller identity and calling context, with adult males contributing more to howling bouts than adult females and subadults, while all group members emitted comparable isolated call rates. Our contextual analysis partly confirmed the relevance of our acoustic classification as we showed that calls were associated non-randomly with contextual variables. We could divide call types according to their contribution to intra-group *vs.* inter-group interactions and to neutral-positive *vs.* negative situations. However, we must clearly acknowledge at this stage that our study duration was short and that the vocal repertoire presented here should be considered with caution. Longer surveys are necessary to confirm the total number of call types, as well as the sex-specificity of each call type. For example, while MU calls were found only emitted by males during our study, we have heard on two very rare

occasions females producing this call type since then. MU call was also reported in females in other *Alouatta* species [da Cunha & Byrne 2013].

Further investigations are needed to really confirm the vocal repertoire size of this species. Indeed, we must acknowledge that our repertoire of 12 calls is based on a relatively short study period and is lacking in acoustic measurements on females. Acoustic measurements are difficult to conduct on females because they typically call at low rates, with low intensity and with frequent overlap with other individual calls. Nevertheless, it is interesting to note that the size of the vocal repertoire found here is comparable to the one of another howler monkey species (*A. caraya*: 11 call types) that forms similar sized groups [Calegaro-Marques & Bicca-Marques, 1995], but is half that of another howler monkey species (*A. palliatta*: 22 call types) who routinely live in larger groups [Baldwin & Baldwin, 1976]. Moreover, black howler monkeys, like several other forest-dwelling monkey species, emit two categories of calls (loud and soft) and possess a relatively discrete vocal repertoire [Zuberbühler et al., 1997; Gamba & Giacoma, 2007]. Degree of loudness (due to the flexibility in the usage of the air sacs) seems to play an important role in the definition of the different call types (soft vs. loud). However, even when excluding the intensity-related acoustic measure, the discrimination of the different call types was still possible.

Our study allowed us to identify four call types not previously described (i.e. AH (soft scream), AB (barked grunt), CU (coo) and RH (modulated roar)). Whether these are really new call types or were included by previous researchers into broader acoustic

categories requires more comparative investigations. Since spectrographic illustrations and acoustic descriptions are not systematically provided in several previous studies, comparisons of call classification is difficult. Also, the fact that all call types were systematically emitted by at least two of our study groups confirms that they were not idiosyncratic structures. However, why all three groups did not produce all calls remains an open question. This may be due to our sampling effort, as these calls were generally rare, but could also be related to socio-ecological inter-group variations.

Isolated calls have been far less studied than calls emitted during howling bouts in howler monkeys. Interestingly, we found that these calls were not necessarily rare in black howler monkeys, but this concerned typically soft calls which may be more important than loud calls in the communication at the intragroup level. Our results are consistent with the idea suggested by da Cunha et al. [2015] who showed that loud calls were more frequent than soft calls during howling bouts and vice versa for isolated calling. However, loud and soft calls were not exclusive to any of these calling patterns. Indeed, the majority of the call types (7/12) were found during both isolated calling and collective howling, supporting the idea that they may all have their own function.

We demonstrated sex-dependent variations of callers' contributions to the vocal repertoire. We found that three howler monkey call types seemed specific to females and two to males. This questions the social role of members of both sexes in the group. The fact that CU (coo) and CY (scream) seems female-specific and are involved in dealing with intra-group conflicts, probably reflects the active role played by females in

the regulation of intra-group social networks. Resident females present strong bonds [Van Belle et al., 2011] and play an active social role as, for example, the variation in social relationships among resident males is related to their ability to establish strong relationships with females [Van Belle et al., 2008]. The fact that RH (modulated roar) is male-specific and is also emitting more frequently in areas where group encounters typically occur supports the idea that males play a defensive social role [Sekulic, 1982], as found in many other species [Kitchen & Beehner, 2007].

However, the fact that 7 call types, including most loud calls, were shared by both sexes, is interesting because it might mean that females are capable of emit loud calls like males but decide not to do it as often during howling bouts. Of course, acoustic comparisons of male and female calls are now needed to notably confirm the sharing of these call types. Those call types are emitted more frequently by adult males than by adult females and subadults when looking at contributions to howling bouts, and as frequently when looking at isolated calling. In *Alouatta* species, males are strongly investing their energy in group defense, mate attraction, social bonding and conflict resolution; vocal signals seem to play an active role in all of those activities [Holzmann et al., 2012; da Cunha et al., 2015]. High call rates in male howler monkeys may thus be explained by sex differences in reproductive strategies and social activity. The fact that males are sometimes considered as the “silent sex” in other species has been related to the point that generally males migrate while females remain in their natal group, forming the social core of the group with long-lasting bonds [Lemasson 2011]. In

howler monkeys, both sexes can migrate [Crockett & Pope, 1988; Clarke et al., 1998], however genetic studies have revealed that black howler females are generally closely related and seem to form matriline, suggesting that males migrate much more than females [Van Belle et al., 2014b].

The social and vocal role of females needs further investigation to be clarified. A former study on the same Palenque National Park population found that females contributed to more than 50% of the howling bouts [Van Belle et al., 2015]. This is confirmed here with a participation to 69% of the howling bouts sampled. This may appear high but when looking at the call rates within howling bouts, females are indeed much less active than males. According to several authors, females reserve their vocal participation when a situation is particularly critical or tense (competition with another group, change in group composition, or change in dominance hierarchy) [Kitchen, 2006; Oliveira, 2002; Miranda et al., 2004]. We noted that females called as often as males outside howling events. So, even if both sexes migrate, females seem to play an essential role in group socio-spatial cohesion. Indeed, females in that species were found to maintain close bonds with several group members and to lead collective group movements [Van Belle et al., 2013b].

Finally, our contextual analysis confirmed that although most calls are produced in all sampled contexts, non-random variations occurred. We found different degrees of implication of calls in intra-group socio-spatial cohesion and in intra- and inter-group conflicts. At this stage only hypothetical call functions can be raised. One call had an

unambiguous affiliative context of emission, i.e. MU (moo) calls exclusively emitted during post-separation reunions. This supports the idea that this call can be categorized as a “reunion-contact call” (“moo” call) [Byrne, 2000; da Cunha & Byrne, 2009; da Cunha et al., 2015]. HN (grunt) appears as a “cohesion-contact call” as it was the second call associated with positive interactions and situations where individuals feed and rest not far from each other. This is supported by previous reports showing that they are often emitted during social play [Carpenter, 1934]. Another call type has a clear function, i.e. CU (coo) calls associated with intra-group conflicts. Hence, this call may function as a “threat call”. Several call types play a possible role in regulating intra-group cohesion (AB (barked grunt), AH (soft scream) and CY (Stress screams)) while others seem to function more in regulating inter-group spacing or conflict (AW (metallic cackling notes), RH (modulated roar), RO (roar), RU (incipient roar), WA (bark), the latter having been considered as a call frequently involved in conflicts in *A. guariba* [Oliveira, 2002]. Understanding the functional differences between these latter calls requires more detailed contextual information (notably with regards to intra-group space occupation) on a larger number of groups, other sampling periods (to control for seasonal variations), as well as playback experiments.

Ultimately, this study contributes to a better knowledge of the vocal repertoire of a Central American primate species and encourages a larger debate on the functions of howler monkeys’ vocal communication. This study also shows that the vocal differences between male and female are more related to differences in social roles,

individual motivation and vocal strategies than to morpho-anatomical constraints. Hence, this study opens new lines of empirical and theoretical research concerning coevolution between primates' social structures or organizations and vocal repertoires or usage notably related to sex differences.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Short calls

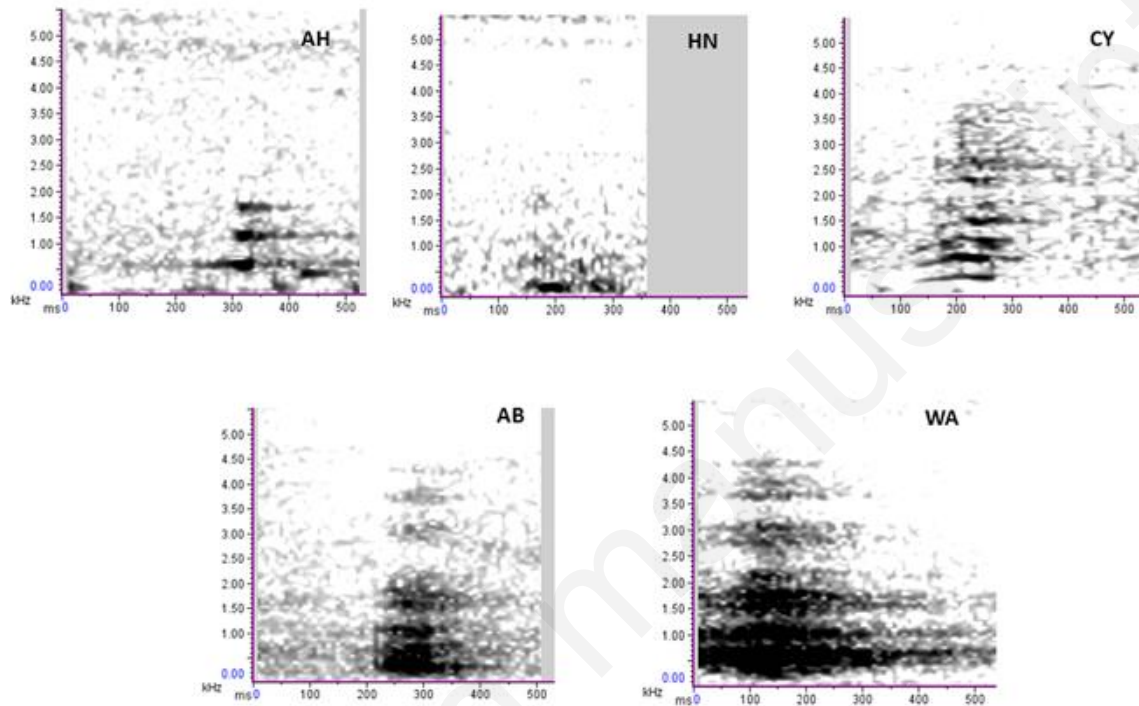


Figure 1. Spectrograms of short call types (X axis: duration in millisecond; Y axis: frequency in kHz). Call type names: AH (soft scream); HN (grunt); CY (stress scream); AB (barked grunt); WA (bark).

Intermediary calls

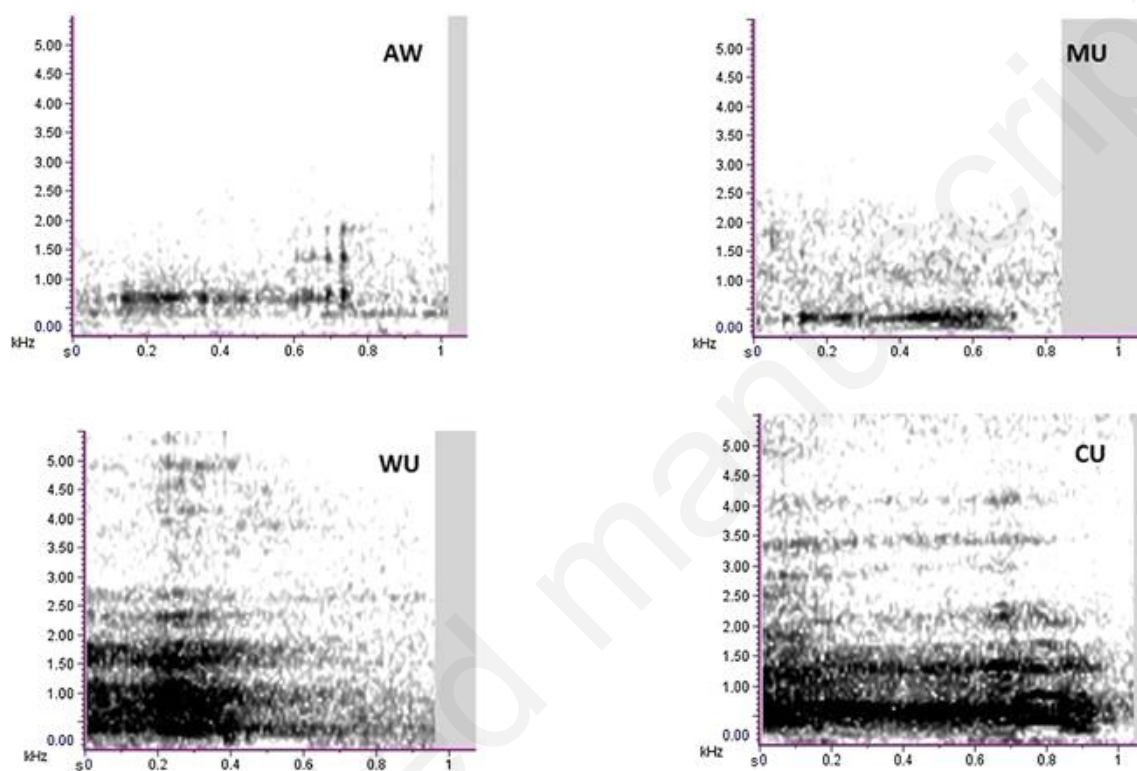


Figure 2. Spectrograms of intermediary call types (X axis: duration in second; Y axis: frequency in kHz). Call type names: AW (Metallic cackling notes); MU (moo); WU (incipient bark); CU (coo).

Long calls

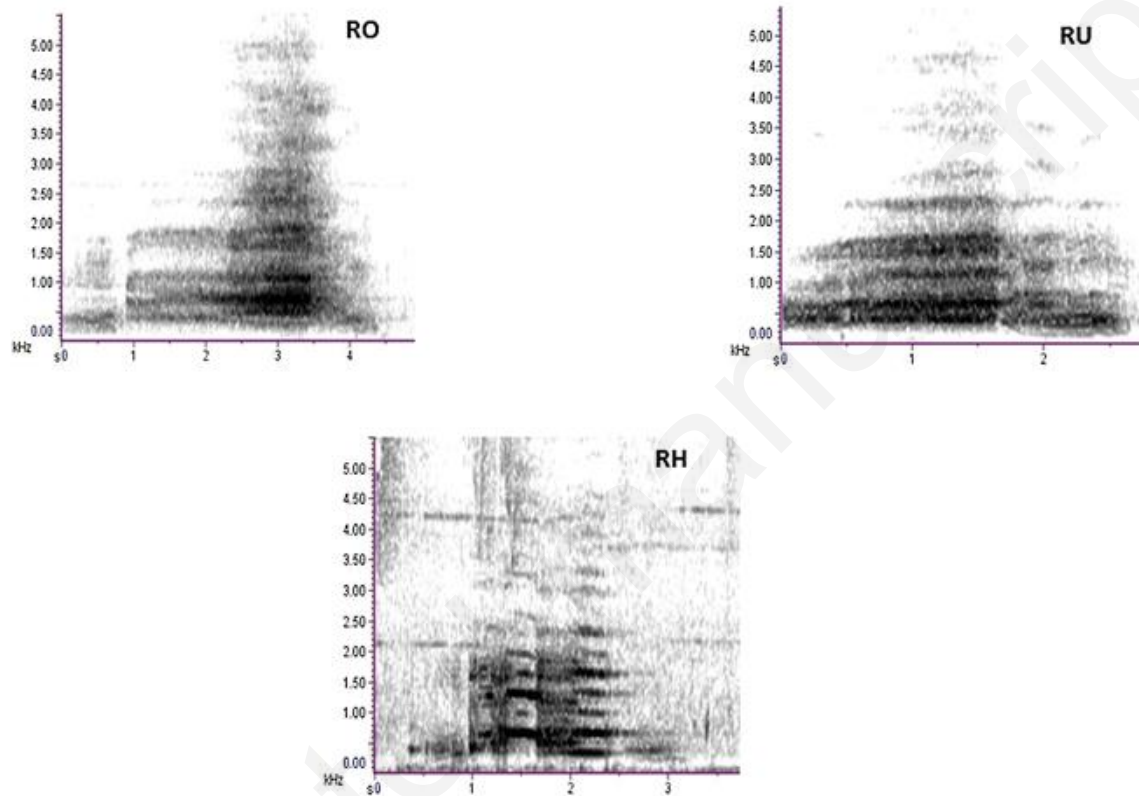


Figure 3. Spectrograms of long call types (X axis: duration in second; Y axis: frequency in kHz). Call type names: RO (roar); RU (incipient roar); RH (modulated roar).

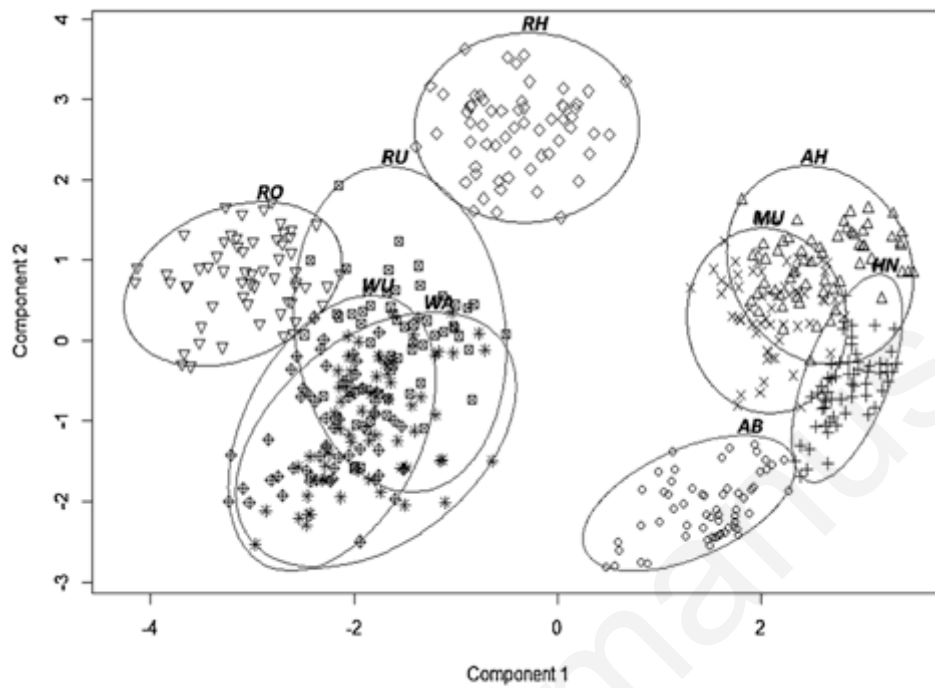


Figure 4. Clustering of the nine call types based on the pDFA analysis.

Table 1. Total number of calls recorded and sampling efforts for each study group and each group member (adult males: GZ, KR, HG, JP, RC, SB and KN; adult females: TE, LZ, ML, ES, PR, PU; subadult males: MI and DO; subadult female: EM).

	BALAM					MOTIEPA				PAKAL						
	GZ	KR	TE	LZ	MI	HG	JP	ML	DO	RC	SB	KN	ES	PR	PU	EM
Total focal duration per individual (hour)	53	53.5	50	51.5	52.5	65	64	66	62.5	37.5	42	27	42	36	34.5	37.5
Number of isolated calls recorded per individual	31	44	22	28	0	54	87	0	18	102	54	9	22	18	12	0
Number of calls recorded within howling bouts per individual	5957	5634	658	625	635	8699	4743	1547	333	10019	4253	1475	789	761	902	2935
Number of howling bouts recorded per group			29				22						35			

Table 2. Number of calls per male and per call type used for the acoustic analysis

Males									
		GZ	HG	JP	KN	KR	RC	SB	TOTAL
Call type	MU	0	8	19	0	0	33	0	60
	HN	15	0	15	0	0	15	15	60
	AB	6	21	20	0	0	13	0	60
	AH	13	0	0	0	15	16	16	60
	RH	0	0	5	22	23	10	0	60
	RO	10	10	10	0	10	10	10	60
	RU	10	10	10	0	10	10	10	60
	WA	10	10	10	0	10	10	10	60
	WU	10	10	10	0	10	10	10	60

Table 3. Acoustic measurement definitions

Acoustic parameter (abbreviation, unit)	Definition
Lowest frequency (Low Freq, Hz)	The lower frequency bound of the call.
Highest frequency (High Freq, Hz)	The upper frequency bound of the call.
1st Quartile Frequency (Q1fre, Hz)	The frequency that divides the call into two frequency intervals containing respectively 25% and 75% of the energy distribution.
3rd Quartile Frequency (Q3fre, Hz)	The frequency that divides the call into two frequency intervals containing respectively 75% and 25% of the energy distribution.
Aggregate of entropy (Aggr Entropy, Hz)	The degree of disorder (i.e. noisiness) in the call.
Total power (Tot power, Hz)	The averaged value of the spectrogram's power spectral density, i.e. the sum of the energy values of all time-frequency bins (i.e. pixels) divided by the number of bins in the spectrogram.
90% bandwidth (BW 90%, Hz)	Amplitude between the frequencies measured at 5 and 95% of the energy distribution.
Total Duration (s)	The temporal difference between the beginning and the end of the call.

Table 4. Acoustic description of the call types

Call type	Intensity	Duration	Frequency pattern	Mouth closed	"Given name". Previous description and species (references)
MU	soft	intermediary	Very low-pitched	X	"Moo" <i>Alouatta caraya</i> (da Cunha & Jalles-Filho, 2007)
AW			Atonal		"Metallic cackling notes" <i>A. palliata</i> (Collias & Southwick, 1952)
HN		short	Atonal	X	"Grunt" <i>A. palliata</i> (Collias & Southwick, 1952, Baldwin & Baldwin, 1976)
AB			Atonal		"Barked grunt"
AH			Tonal, high-pitched, narrow frequency bandwidth		"Soft scream"
CY			Tonal, high-pitched, loud frequency bandwidth		"Stress screams" <i>A. caraya</i> (Calegari-Marques & Bicca-Marques, 1995)
RH	loud	long	Partially tonal, frequency modulated		"Modulated roar"
RO			Atonal, increasing frequency bandwidth		"Roar" <i>A. palliata</i> (Baldwin & Baldwin, 1976), <i>A. pigra</i> (Kitchen, 2000)
RU		very long	Atonal, decreasing frequency bandwidth		"Incipient roar" <i>A. palliata</i> (Baldwin & Baldwin, 1976)
CU		intermediary	Partially atonal, frequency modulated		"Coo"
WA		short	Atonal, high-pitched		"Bark" <i>A. palliata</i> (Baldwin & Baldwin, 1976), <i>A. pigra</i> (Kitchen, 2000)
WU			Atonal, low-pitched		"Incipient barks" <i>A. palliata</i> (Baldwin & Baldwin, 1976)

Table 5. The eight acoustic parameters (mean and standard deviation) for each call type (N= 60 calls / type, see Table 3 for definitions of acoustic measurements). PC1-4: Contribution of the different acoustic parameters to the Principal Component Analysis

	Call types				
	MU	HN	AB	AH	RH
LowFreq (Hz)	129±28	121±16	97±13	261±51	301±53
HighFreq (Hz)	451±72	715±132	1342±193	684±165	2111±430
Q1freq (Hz)	303±57	178±22	232±36	310±49	569±26
Q3freq (Hz)	353±45	309±60	582±69	416±108	724±94
AggrEntropy (Hz)	3±0.5	3±0.6	5±0.5	3±0.7	3±0.4
Total Power (Hz)	76±4.8	66±3	75±6	65±3	87±5
BW90% (Hz)	194±53	432±106	847±145	163±46	707±155
Total duration (s)	0.7±0.1	0.09±0.02	0.16±0.02	0.04±0.01	1.39±0.33
	Call types				
	RO	RU	WA	WU	
LowFreq (Hz)	209±39	227±37	184±41	210±38	
HighFreq (Hz)	4381±636	2457±377	3560±547	3102±569	
Q1freq (Hz)	605±50	482±67	459±71	477±54	
Q3freq (Hz)	865±104	776±126	851±166	1002±94	
AggrEntropy (Hz)	4±0.4	4±0.4	4±0.4	4±0.5	
Total Power (Hz)	87±5	83±5	81±5	83±3	
BW90% (Hz)	1260±148	1249±126	1319±142	1329±110	
Total duration (s)	2.84±0.55	1.11±0.42	0.3±0.05	0.33±0.08	
	Principal Component Analysis				
	PC1	PC2	PC3	PC4	
LowFreq (Hz)	0.1710	0.4853	0.7433	-0.0356	
HighFreq (Hz)	0.4585	-0.0420	-0.0479	-0.2071	
Q1freq (Hz)	0.4158	0.3017	0.0730	-0.0075	
Q3freq (Hz)	0.3145	-0.4270	0.1333	-0.5634	
AggrEntropy (Hz)	0.2186	-0.5374	0.1353	0.4861	
AverPower (Hz)	0.3992	0.1579	-0.1519	0.6030	
BW90% (Hz)	0.4312	-0.2450	0.0976	-0.0119	
Total duration (s)	0.3093	0.3402	-0.6092	-0.1957	

Table 6. Isolated call rates (#calls per hour) and Standard Error (SE) for each age-sex class (F: adult females; M: adult males; SAM: subadult males; SAF: subadult female) given per study group (Motiepa, Balam and Pakal) and for all groups combined. Call types not listed below were not found in this calling context.

	Balam			Motiepa			Pakal			All groups combined	
	M (N=2)	F (N=2)	SAM (N=1)	M (N=2)	F (N=1)	SAM (N=1)	M (N=3)	F (N=3)	SAF (N=1)	Adult males	Adult females
MU	0.1 ± 0.04	0	0	0.28 ± 0.02	0	0.19	0.6 ± 0.58	0	0	0.33 ± 0.25	0
AW	0	0	0	0	0	0	0	0.09 ± 0.03	0	0	0.03 ± 0.05
HN	0.18 ± 0.2	0.3 ± 0.02	0	0.33 ± 0.37	0	0.08	0.8 ± 0.57	0.3 ± 0.06	0	0.44 ± 0.32	0.2 ± 0.17
AB	0.02 ± 0	0	0	0	0	0	0.03 ± 0.03	0.03 ± 0.01	0	0.02 ± 0.02	0.01 ± 0.02
AH	0.36 ± 0.32	0.14 ± 0.001	0	0.48 ± 0.02	0	0	0	0	0	0.28 ± 0.25	0.05 ± 0.08
CY	0	0.06 ± 0.05	0	0	0	0	0	0.03 ± 0.01	0	0	0.03 ± 0.03
RO	0	0	0	0	0	0	0.03 ± 0.01	0	0	0.01 ± 0.02	0
WA	0.05 ± 0.01	0	0	0	0	0.02	0.03 ± 0.03	0.03 ± 0.02	0	0.03 ± 0.03	0.01 ± 0.02

Table 7. Howling call rates (calls per hour) and Standard Error (SE) for each age-sex class (F: adult females; M: adult males. SAF: subadult females; SAM: subadult males) given per study group (Motiepa, Balam and Pakal) and for all groups combined.

	Balam			Motiepa			Pakal			All groups combined	
	M (N=2)	F (N=2)	SAM (N=1)	M (N=2)	F (N=1)	SAM (N=1)	M (N=3)	F (N=3)	SAF (N=1)	Adult males	Adult females
MU	0	0	0	8.83 ± 0.45	0	0.63	0.065 ± 0.11	0	0	2.97±5.08	0
AW	0	0.14 ± 0.2	0	0	0	0	0	2.77 ± 1.48	1.38	0	0.97±1.56
HN	74.51 ± 88.73	0 ± 0	0	81.86 ± 5.58	0	1.89	30.97 ± 25.27	3.89 ± 3.33	0.99	62.45±27.51	1.3±2.25
AB	10.08 ± 5.55	0	0	33.12 ± 21.86	0	0.63	9.49 ± 4.25	0.4 ± 0	0	17.56±13.48	0.13±0.23
AH	14.29 ± 0.4	1.96 ± 0	0	5.36 ± 6.25	0	0	34.19 ± 36.65	0.4 ± 0.5	2.37	17.95±14.76	0.79±1.04
RH	11.34 ± 14.86	0 ± 0	0	0	0	0	5 ± 5.62	0	0	5.45±5.69	0
RO	276.61 ± 8.52	85.43 ± 5.15	86.27	481.23 ± 334	264.35	60.88	337.29 ± 175.63	92.4 ± 40.8	409.88	365.04±105.1	147.4±101.36
RU	155.7 ± 30.1	77.31 ± 0.79	91.04	72.4 ± 17.6	54.26	15.77	73.18 ± 9.49	52.6 ± 21.7	155.73	152.8±78.2	61.38±13.82
CU	0	5.04 ± 1.19	0	0	8.2	0	0	2.17 ± 0.71	0.2	0	5.14±3.02
WA	965.41 ± 18.42	9.8 ± 0.79	0.56	1294.16 ± 579.29	55.52	23.97	540.97 ± 630.66	6.86 ± 8.71	9.49	933.51±377.61	57.39±84.99
WU	115.41 ± 19.01	0	0	142.9 ± 14.7	5.99	1.26	6.23 ± 3.51	0	0	88.18±72.29	2±3.46

Table 8. Association of call types (number of calls) and context (see methods for definitions)

Call type	<i>Audience:</i> Neighbor absent (0.61) / Neighbor present (0.39)	<i>Home range position:</i> Not peripheral (0.59) / Peripheral (0.41)	<i>Group spatial cohesion:</i> Not dispersed (0.57) / Dispersed (0.43)	<i>Individual activity:</i> Neutral-positive (0.63) / Negative behavior (0.37)	<i>Post-separation reunion:</i> Yes (0.22) / No (0.78)	Number of calls sampled
MU	1 / 13 *	12 / 2 *	5 / 9	14 / 0 **	14 / 0 ***	14
AW	4 / 4	1 / 7 *	3 / 5	0 / 8 ***	0 / 8	8
HN	19 / 58 **	34 / 43 **	29 / 48 ***	56 / 21 *	9 / 68 *	77
AB	17 / 16	13 / 20 *	11 / 22 **	8 / 25 ***	9 / 24	33
AH	19 / 15 *	7 / 27 ***	11 / 23 **	7 / 27 ***	7 / 27	34
CY	2 / 4	1 / 5 *	0 / 6 **	0 / 6 **	0 / 6	6
RH	14 / 8 *	5 / 17 ***	14 / 8	0 / 22 ***	0 / 22 **	22
RO	44 / 50	30 / 64 ***	45 / 49 *	15 / 79 ***	12 / 82 *	94
RU	40 / 30 **	18 / 52 ***	36 / 34	10 / 60 ***	6 / 64 **	70
CU	3 / 14	5 / 12 *	8 / 9	3 / 14 ***	2 / 15	17
WA	37 / 41	29 / 49 ***	42 / 36	14 / 64 ***	12 / 66	78
WU	4 / 7	7 / 4	4 / 7	4 / 7	2 / 9	11

The daily distribution for each binary variable (based on all scans sampled) is indicated with proportions in brackets in the first line. Binomial tests: *P ≤ 0.05, **P ≤ 0.01, ***P < 0.001; all other comparisons: P > 0.05.