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BIOACOUSTICS OF THE BLACK-HEADED NIGHT MONKEY, *AOTUS NIGRICEPS*

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Introduction

Only three studies have assessed *Aotus* vocalizations (known as either owl or night monkeys). None of these studies have assessed the black-headed night monkey (*A. nigriceps)* found in southeastern Peru, northern Bolivia, and central-western Brazil, nor have they focused on bioacoustics of *Aotus* spp. in the wild. Of these three captive-based studies, 13 different call types have been described in *A. lemurinus* (Andrew 1963; Moynihan 1964) and *A. azarae* (Kantha et al. 2009). Most of these calls are distinct from one another; however, there are some inconsistencies in naming that need to be addressed, as described in Table 1. First, the Trill described by Andrew (1963) has similar structure and bandwidth to the Scream described by Moynihan (1964). Moynihan does describe a Low Trill which is distinct from the Trill in Andrew (1963), and the Low Trill described by Kantha et al. (2009) does not resemble structure, bandwidth, or call duration of the Trill in Andrew (1963) or the Low Trill in Moynihan (1964). Therefore, we suggest that the Trill in Andrew (1963) is structurally distinct from the Low Trill in Moynihan (1964), and the Low Trill in Kantha et al. (2009) appears to be a distinct call from the other two studies. Secondly, one of the representative Squeaks described by Andrew (1963) is very similar to the Gruff Grunt (Moynihan 1964). Each has numerous harmonics, a similar descending frequency across the exact call duration, and a very similar bandwidth. Therefore, the Gruff Grunt should be renamed Squeak, as it was previously described in Andrews (1963). Thirdly, the Squeak described by Moynihan (1964) is structurally similar to the Twitter described by Andrews (1963) and should be renamed. Fourth, neither the Long Scream nor the Short Scream described by Kantha et al. (2009) appear to be similar in structure, bandwidth, or duration to the Scream in Moynihan (1964) and should potentially be renamed. Fifth, the Moan described by Moynihan (1964) is not similar to the Moan in Kantha et al. (2009) in structure, bandwidth, or duration. The low frequency band is structurally similar to a variant Low Trill described by Moyniahn 1964, though the quality of the spectrogram in Kantha et al. (2009) makes it difficult to confirm this possibility. And finally, the Gulp described by Kantha et al. (2009) is similar to the Gulp in Moynihan (1964); however, the bandwidth in the

earlier study is far less than 1 kHz, while the Kantha et al. (2009) study shows an upper frequency of at least 10 kHz. Based on the available data and without further descriptive analysis in Kantha et al. (2009), these should likely be considered distinct calls.

Table 1. Vocal descriptions from all previously published captive and semi-captive Aotus studies. Several similar calls were found in both Moynihan 1964 and Kantha et al. 2009, though both were included because they were found in different species and have different bandwidth and duration.

Species	Call type	Mean Bandwidth (Hz)	Mean Duration (msec)	Description
Aotus lemurinus griseimembra **(a)**	Twitter	2000-8000	40	Descending from 3.5-4 kHz to 2-3 kHz; Long rapid series once every 200 msec. A fundamental frequency (2-4 kHz) with a second harmonic at 5-8* kHz
	Trill	1000-7000*	300	Series of Twitters
Aotus lemurinus griseimembra **(b)**	Squeak	500-5000	100	"Guinea pig squeak" starting at lower frequency (500 Hz) and rising steeply to 4-4 kHz; or, simply protracted lower frequency (500-700 Hz)
	Sharp Call	1000-15000	150-250	Sometimes sharp initial click. Once every 150-250 msec
	Boom	150	1000+	Low soft call by vibration of false vocal cords
	Low Trill	240-500*	600*	Bubbling series of low-pitched notes, rapid but distinguishable series. Varied from 3-12 notes
	Gulp	0-650	100-260	Very common. Moderately loud and rather "liquid" sounding. Uttered in series of 2 to 3 notes. Series repeats rapidly
	Gruff Grunt	100-4000*	100*	Most common. Uttered as singly or in short series of two to five notes. Successive notes uttered at slightly irregular intervals. Longer pauses. Low-pitched, moderately long, and moderately loud. Multiple harmonics
	Squeak	1000-9500*	50*	1-3.5 kHz fundamental frequency, followed by two harmonics ranging from 4-9.5 kHz
	Sneeze Grunt	0-1000*	200*	Sneeze superimposed upon a loud Gruff Grunt. Usually uttered as a single note, though sometimes two in quick succession. Often associated with Gulps, single Low or High Trill, or Moan
	Resonant Grunts	Unknown	Unknown	More complex than Gruff Grunt and rarer. Speed loudness, and pitch varied considerably. Long series of 10-15 notes. Most common series included soft and low notes first half of series (softer than Gruff Grunt), and progressively louder towards middle of series. Towards climax similarly loud, resonating, and rapid. Finally, slowed in tempo, lower and softer
Aotus azarae **(c)**	Moan	220-480*	600*	Brief, soft, moan. Most common call type in captive individuals. Often uttered along with other calls before or after, including Low Trills, Gulps, and Sneeze Grunts
	Scream	2000-8500*	800	High pitched but wavering. Quite prolonged. Multiple harmonics (though an exact number not given)
	Moan	140-300	500	No description given
	Long Scream	2800-5800	260	No description given
	Gulp	1800-5800	54	No description given
	Low Trill	2000-3100	52	No description given
	Short Scream	980-3300	190	No description given
	Sneeze Grunt	1580-3310	50	No description given

(a) Andrew 1963 (2 laboratory animals) and (b) Moynihan 1964 (12 semi-captive animals) originally classified as *A. trivirgatus*; (c) Kantha et al. 2009 (16 laboratory animals). *Estimates based solely on published spectrogram.

Vocalization analysis for owl monkey species is particularly important as it is difficult to differentiate between individuals or determine group dynamics because they are nocturnal. A vocal assessment could potentially be used to differentiate individuals and assess group membership during new group encounters simply based on bioacoustics (Salmi et al. 2014). The objective of this study, consequently, was to add to the *Aotus* bioacoustic literature by sampling several geographically dispersed groups of wild *A. nigriceps* (Fig. 1) in southeastern Peru.

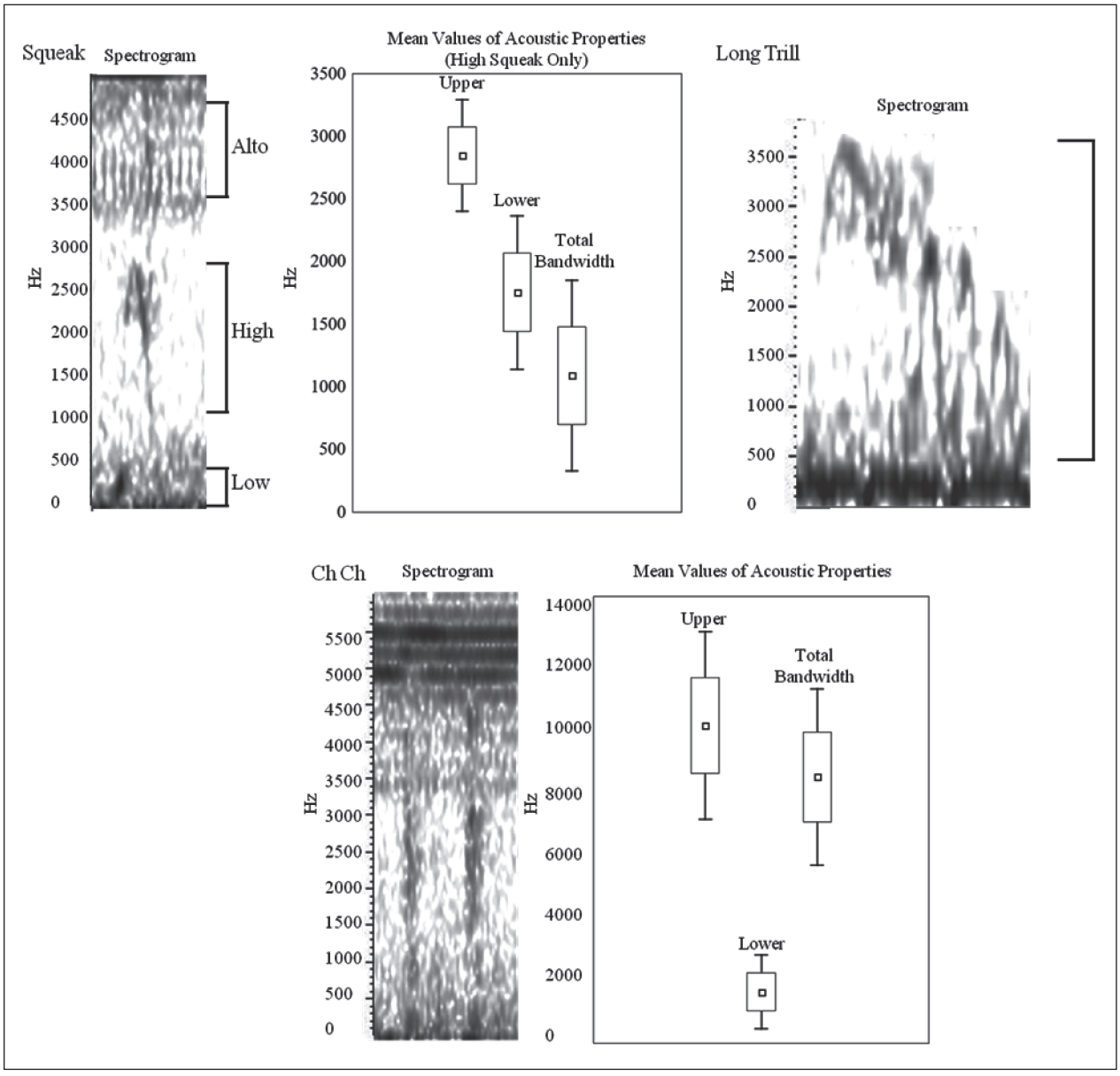

Figure 1. Spectrogram images (left) and frequency distribution of calls (right) for three described calls: Twitter (High), Ch Ch, and Long Trill. Frequency distributions represent upper frequency range (A), lower frequency range (B), and bandwidth (C) for each call. Box and whisker represent mean, 1 standard deviation, and 2 standard deviations. For squeak, only low, high, and alto spectrograms are shown. Ch ch calls shown as common pair.

Methods

Bioacoustic recordings were collected from eleven *A. nigriceps* groups ranging in size from 2-5 individuals. Sampling occurred April-May 2016, October-November 2016, and April 2017, for a total of twenty-eight days at Villa Carmen Biological Station (12°53'39»S, 71°24'16"W) and nine days at Manu Learning Center (CREES) research station (12°47′22″S 71°23′32″W) - both in premontane rainforest. Seven groups were sampled Villa Carmen and four groups at CREES, both located on the edge of Manu National Park buffer zone in the Peruvian Amazon. Villa Carmen has a long history of development, ecotourism and agriculture. Sampled areas were largely dominated by secondary forest. At CREES, groups were sampled in areas of rainforest regeneration. Groups of 2-7 researchers searched for night monkey groups from 5:30-7:30 am and 5:30-7:30 pm, times when *A. nigriceps* groups are known to be active near their nesting sites. Two recordings took place before 5:30 am, when researchers left the field station early to visit more distant night monkey groups. Additional vocalizations were heard during the day, though none were included in this study. A Zoom H1 Handy Recorder was coupled with a RØDE NTG-2 condenser shotgun microphone and shoe shockmount on a micro boompole. Recordings were collected at a sampling frequency of 48 kHz at a distance varying from 2-25 m. Certain groups were sampled more extensively than others, due to logistic feasibility. When feasible, behaviors associated with vocalizations were categorized into three activities: resting, traveling, or feeding. All calls were compared to the three previous

Aotus studies (Andrew 1963; Moynihan 1964; Sri Kantha et al. 2009). Audio recordings were analyzed using the spectral audio program Raven Pro 1.5 Sound Analysis Software (Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, New York). Duration of calls, dominant and fundamental frequencies, high and low frequencies, and total bandwidth were then measured. Amplitude was not analyzed because we could not control for size or age of individuals. All recordings were conducted non-invasively, minimized impact on behavior, and avoided excessive disturbance, and the research was therefore deemed exempt from the Institutional Animal Care and Use Committee approval. All research adheres to the principles for the ethical treatment of primates set forth by Neotropical Primates. Audio files analyzed during the current study are available from the corresponding author on reasonable request through ResearchGate.

Results

Three distinct calls were identified in our study, including the Twitter (N = 165), Ch Ch (N = 122), and Long Trill (N = 3) (Figure 2). In a limited number of cases, we were able to note an associated behavior with calls. Of 46 High Twitter calls, 47.8 % took place while the individual was traveling and 52.1 % of calls took place while resting. Of 19 Ch Ch calls, 36.8 % were associated with travel, and 63.2 % with resting. No behaviors were recorded during the Long Trill.

Figure 2. Photo of the black-headed night monkey, *Aotus nigriceps*. Photo credit: Jessica A. Suarez.

The Twitter was named after the similarly structured Twitter call described by Andrew (1963), though measurable differences exist in number of harmonics and bandwidth (Table 2). The Twitter was found in all but one group and had up to 3 additional harmonics beyond the fundamental frequency (191-553 Hz), ranging from a mean of 1,755-2,847 Hz (High), to 3,666 – 4,140 Hz (Alto), to 3,562 – 4,808 Hz (Ultra). The low fundamental frequency and high dominant harmonic were always present in the Twitter, whereas the Alto and Ultra were only found in single groups at Villa Carmen. The mean bandwidth of the Ch Ch call was 1,611 – 9,961 Hz with a mean duration of 183 msec.

Table 2. Acoustic properties of three calls in wild black-headed night monkeys, *Aotus nigriceps*. The squeak is characterized by a fundamental frequency (low), and dominant frequency (high).

Call Type	Bandwidth (Hz)	Mea Duration (msec)	Description
Twitter			Similar in structure of Twitter described by Andrew (1963), though additional harmonics described here.
Ultra	3562-4808	49	
Alto	3666-4140	20	
High	1755-2847	43	
Low	191-553	21	
Ch Ch	1611-9961	183	Similar to Gulp described by Kantha et al. (2009); however, this name does not correspond with the Gulp described by Moynihan (1964) which has a much lower frequency (<1000 Hz). Therefore, we have renamed to reflect a new call type.
Long Trill	470-3046	170	Similar to original Trill described by Andrew (1963) and the Scream described by Moynihan (1964). There are some structural differences which is why we refer to it as the Long Trill

The Ch Ch call is similar to the Gulp described by Kantha et al. (2009); however, this name does not correspond to the Gulp described originally by Moynihan (1964). Therefore, we have renamed the call as an onomatopoeic description, Ch Ch. The Ch Ch call was recorded as two closely paired repetitive sounds in ten out of eleven groups (84.4 % of measured cases), a Ch Ch Ch (triplet) in four groups (14.8 % of measured cases), and a Ch Ch Ch Ch (quadruplet) in one group (0.01 % of measured cases). Only four groups at Villa Carmen exhibited a triplet Ch Ch, and a group at CREES produced the call in a quadruplet. The Long Trill is named in part after the Trill described originally by Andrew (1963), though structurally there are differences. Namely, in our study the call descends in frequency, whereas there is an oscillation around 3,500-5,000Hz in *A. lemurinus*. The Long Trill is also found primarily between 3,500-5,000HZ, whereas our study had a higher frequency around 3,000HZ. There were several instances of the Long Trill, but only in two groups found at Villa Carmen Biological Station. However, only a single instance had a clear spectrogram that could be used for analysis due to the complexity of the acoustic landscape at that particular frequency.

Discussion

No known studies have assessed the bioacoustics of any wild *Aotus* species. Furthermore, no studies have analyzed *Aotus nigriceps* vocalizations, even in captivity. This study describes three calls in *A. nigriceps* (Twitter, Ch Ch, and Long Trill), though continued field research may reveal additional calls. Thirteen other calls have previously been described in *A. azarae* and *A. lemurinus*, which suggests that the vocal repertoire of *Aotus nigriceps* is likely larger than described here. However, captive settings allow researchers to control for background noise and to elicit calls in response to experimental stimuli, which may increase the number of calls found in captivity compared to a wild setting. Bioacoustic information in wild primates is also likely to be different than those in captivity because of their capacity to adjust calls in an environment filled with background white noise from both biotic and abiotic factors (Brumm et al. 2004). For example, the frequency range of captive *Aotus* species was previously reported to be 140-5,800 Hz; however, some of our calls peaked at 13,612 Hz (e.g., Ch Ch), and all but one call fell within the previously reported range. This could be an adaptation to a much more crowded acoustic environment, where other organisms use a similar bandwidth (Ey and Fischer 2009). We report initial findings of behavioral associations with vocalizations, though caution must be taken in interpreting these results since the majority of behaviors couldn't be documented due to dense foliage or total darkness. Our continued research includes aspects of intra- and inter-group variability, geographic variability, inter-species diversity, and behavioral playback experiments in wild *Aotus nigriceps* populations.

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NEW RECORDS OF THE BLACK TITI MONKEY *(PLECTUROCEBUS CINERASCENS)* (PRIMATES, PITHECIIDAE) IN THE ALTO VALE GUAPORÉ, BORDER REGION BETWEEN BRAZIL AND BOLIVIA

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Introduction

The black titi monkey, previously classified as *Callicebus cinerascens* (Spix, 1823), from molecular evidence is currently included in the genus *Plecturocebus* (Byrne et al., 2016). Has a gray coat over almost all the body with a contrasting brown patch on the back. It feeds on fruits, leaves, insect and seeds. It inhabits dry land environments, Campinarana (forest fragments with poor, rocky, shallow soil and a mix of trees and bamboo), secondary forests and relatively open areas. Its known distribution is still imprecise due to the limited number of records for the species (Veiga et al., 2008). Current knowledge about *Plecturocebus cinerascens* distribution indicates occurrence in the Madeira-Tapajós interfluves (Noronha et al., 2007), with records on the left bank of the Juruena River, the region of the headwaters of the Roosevelt River, the right bank of the Cabixi River, the right bank of the Alto Guaporé River among municipality of Vila Bela da Santíssima Trindade and Pontes e Lacerda, and within the Juruena-Teles Pires interfluves (Sampaio et al., 2012). The western limit may be the municipality of Pimenteiras do Oeste, in the middle of the Vale do Guaporé, in the state of Rondônia, Brazil (Gusmão and Costa, 2014). The species is considered endemic to Brazil.

For this poorly known species (Sampaio et al., 2012) with an imprecise distribution delimitation especially in relation to its southern and southeastern limits (Veiga et al., 2008), new record reports are extremely important. The present study presents four new observations of *P. cinerascens* in the