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PRELIMINARY INVESTIGATION INTO THE DIFFERENCES IN VOCALISATIONS BETWEEN WILD SAKI MONKEYS (*PITHECIA* SPP.)

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Introduction

Information on primate vocalisations can be applied in several ways, including: improving captive welfare, as a census tool for cryptic species, or to investigate the impacts of anthropogenic disturbance on species' behaviour (Delgado and van Shaik, 2000; Konrad and Geissman, 2006; Jacobsen et al., 2010). Vocalisations can be used as a taxonomic tool, and structural differences between calls have been used to compare a wide variety of taxa, including species of gibbon (*Hylobatidae* spp., Ruppell, 2010), marmosets (*Callithrix* spp., Mendes et al., 2009), owls (*Strigidae* spp., Flint et al., 2015), wolves (*Canis* spp., Kershenbaum et al., 2016), and galagos (*Galagidae* spp.,

Svensson et al., 2017). Additionally, differences in vocalisations across taxonomic groups can be used to help determine genetic distances between species or investigate why vocal behaviours evolved (Blumstein and Armitage, 1998; Ord and Garcia-Porta, 2012).

One primate group that recently has been revised taxonomically is the genus *Pithecia*, the saki monkeys, in which five species were previously described: *P. monachus*, *P. aequatorialis*, *P. irrorata*, *P. albicans*, and *P. pithecia* (Hershkovitz, 1987). After a review of morphological data, the group was rearranged into 16 species, including three subspecies elevated to full species and five newly described species (Marsh, 2014). It has been suggested that taxonomic research should utilize several disciplines in combination so as to decrease the risk of inaccurate conclusions (Schlick-Steiner et al., 2010). In the case of the sakis, the use of purely morphological features opens the opportunity for the taxonomic conclusions to be tested by evidence based on genetic structure, behaviour, ecology, or life history, among others.

In the current study, vocalisations produced by wild golden-faced sakis, *Pithecia chrysocephala*, white-faced sakis, *P. pithecia*, and Equatorial sakis, *P. aequatorialis*, were compared to study how calls might differ between closely related species (*P. pithecia* and *P. chrysocephala*), and whether any features were conserved across more evolutionarily separated species (*P. chrysocephala* and *P. pithecia* compared to *P. aequatorialis*). Before the reclassification of *Pithecia* by Marsh (2014), *P. chrysocephala* was considered a subspecies of *P. pithecia*, and so these taxa were expected to display similar vocalisations.

Methods

Study Species

Pithecia chrysocephala, *P. pithecia*, and *P. aequatorialis* all occupy forest habitats, including Amazonian várzea, igapó, and terra firme forests. However, the species occupy different geographical areas (Fig. 1), with *P. chrysocephala* occurring within the Brazilian states of Amazonas, Pará, and Roraima, and *P. pithecia* found in the states of Pará and Amapá, as well as in the countries of Venezuela, Guyana, French Guiana and Suriname (Marsh, 2014). *Pithecia aequatorialis* is present only in central Peru (Marsh and Heymann, 2018).

Data Organisation and Analysis

We collected vocalisation data for *Pithecia chrysocephala* in June–August 2018 in Manaus, Brazil (−3.083, 59.983) (for methodology and vocal repertoire see Muir et al., 2019). We then gathered vocalisation data on two other *Pithecia* species from previous published studies, which we re-analysed and compared across species. For *P. pithecia*, data from Henline's (2007) study conducted in Isla Redonda, Venezuela (7.766, −62.883) was used, and *P. aequatorialis* data came from Keiren's (2012)

study in the Tahuayo River Amazon Research Center, Peru (-4.383, -73.25). While Henline’s (2007) study was conducted before the reclassification of *Pithecia* species, it is clear that this study did not include vocalisation from *P. chrysocephala* as the *in-situ* part of the study was conducted in Venezuela, far from the natural range of this species, but within that *P. pithecia*. Our study and the two previous studies each report between 5 and 12 distinct vocalisations per species (Table 1). One limitation of these studies is that individual sakis could not be distinguished during recordings and so pseudo-replication is a potential issue as it is unknown how much any given individual contributed to the sample.

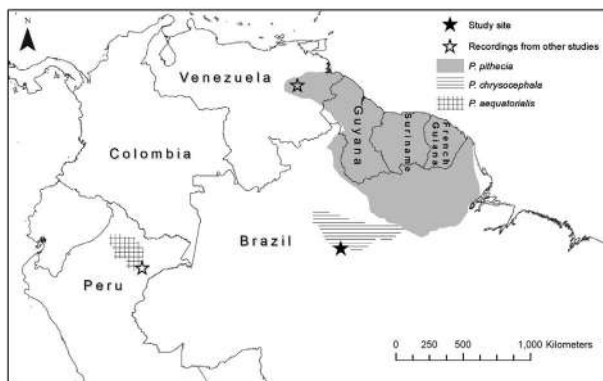


Figure 1. Location of study site and distributions of the studied *Pithecia* species. Map created using spatial data obtained from IUCN (Marsh et al. 2018a; Marsh et al. 2018b; Marsh and Heymann 2018).

In our study calls were initially compared visually by spectrograms from each paper, with similar calls then being statistically compared in terms of acoustic measurements. Similarity in terms of the behavioural context of the calls was also considered. Call measurements of duration and fundamental frequency were taken using the speech analysis program Praat (Boersma, 2001). One sample t-tests were performed using SPSS v.25 to compare call features between species as the raw data was not available for *P. pithecia* and *P. aequatorialis*.

When making comparisons, the two calls within Henline’s (2007) *chucks* group were averaged to allow for a comparison with the graded *chuck* call found in *P. chrysocephala* (Table 1). The *whistle* and *trill* groups were not compared in this manner as the calls within them appeared to differ from those of *P. chrysocephala* when shown as spectrograms. The *soft growl* group was also not compared, due to a small sample size for both *P. pithecia* and *P. chrysocephala*. From Keiran’s (2012) study of *P. aequatorialis*, only the *scream* and *juvenile yip* calls were similar enough on spectrograms to be compared (to the *P. chrysocephala* *throat rattle* and *juvenile cry*, respectively).

Table 1. The complete adult vocal repertoires of *P. chrysocephala*, *P. pithecia*, and *P. aequatorialis* as recorded by Muir et al. (2019), Henline (2007), and Keiran (2012). *P. aequatorialis* calls were not put into groups within their study and so have been grouped according to their similarity to the others based on their description and/or behavioural context.

Group	<i>Pithecia chrysocephala</i>	<i>Pithecia pithecia</i>	<i>Pithecia aequatorialis</i>
Whistles	Whistle (n = 778)	Pee (n = 20)	Bird (n = 23)
		See (n = 6)	Chits (n = 61)
		Chew (n = 8)	Chits/XX (n = 10)
Chucks	Chuck (n = 212)	Chuck (n = 16)	Bark (n = 45)
			Chits with Bark (n = 6)
		Churk (n = 1)	Croak (n = 2)
			Yip (n = 1)
Trills	Trill (n = 253)	Cheeyeep (n = 29)	Trill (n = 5)
		Seeyeep (n = 5)	
		Peeyeep (n = 2)	
		Warbled Trill (n = 8)	
Purrs	Soft Growl (n = 4)	Soft Growl (n = 3)	Growl (n = 17)
Moans and Alarms	Throat Rattle (n = 96)	Throat Rattle (n = 9)	Scream (n = 3)
		Z-trill (n = 3)	
Unknown			Grunt (n = 1)

Results

From spectrograms, *Pithecia chrysocephala* and *P. pithecia* display similar *chuck* calls, while *P. chrysocephala* and *P. aequatorialis* show similar *throat rattle/scream* calls, as well as *juvenile cry/juvenile yip* calls (Fig. 2, Table 2). Additionally, these calls are similarly used in alarm, antagonistic, and parent-offspring contact, respectively.

One-sample t-tests suggested that *Pithecia chrysocephala* and *P. pithecia* differ significantly in the duration of their *chuck* calls, $t(217) = 2.11, p = .036$, and in fundamental frequency, $t(217) = 15.191, p < .001$. This was also suggested for the duration, $t(95) = 21.575, p < .001$, and fundamental frequency, $t(95) = 3.445, p = .001$, of the *throat rattle* call. Comparisons between the *P. chrysocephala* *throat rattle* and *P. aequatorialis* *scream* were also conducted,

with results suggesting that they differ significantly in fundamental frequency, $t(95)=55.401$, $p<.001$, but not in duration, $t(95)=.671$, $p=.504$. The *P. chrysocephala*

juvenile cry and *P. aequatorialis juvenile yip* call differed significantly in duration, $t(107)=15.619$, $p<.001$ and fundamental frequency, $t(107)=35.503$, $p<.001$.

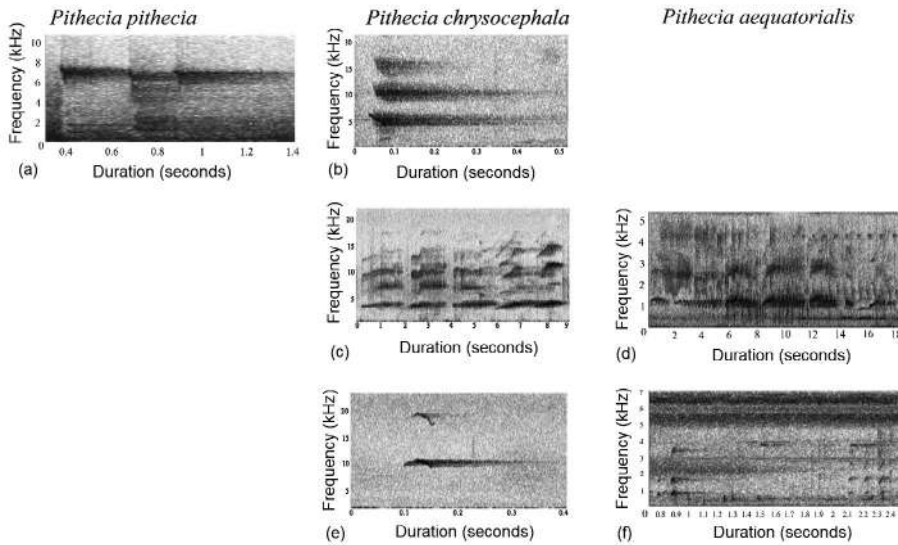


Figure 2. Spectrograms of the vocal repertoires of *Pithecia chrysocephala*, *P. pithecia*, and *P. aequatorialis*, with visually similar calls. Calls include: *P. pithecia* chuck (a), *P. chrysocephala* chuck (b), *P. chrysocephala* throat rattle (c), *P. aequatorialis* scream (d), *P. chrysocephala* juvenile cry (e) and *P. aequatorialis* juvenile yip (f).

Table 2. Structural properties of structurally and contextually similar calls between *Pithecia chrysocephala*, *P. pithecia*, and *P. aequatorialis*.

Species Compared	Calls Compared	Mean Duration in Seconds	Mean Fundamental Frequency in Hz
<i>P. chrysocephala</i>	<i>Chuck</i>	0.352	6307.10
<i>P. pithecia</i>	<i>Chuck</i>	0.335	6507.15
<i>P. chrysocephala</i>	<i>Throat rattle</i>	1.537	3029.84
<i>P. aequatorialis</i>	<i>Scream</i>	1.554	784.38
<i>P. chrysocephala</i>	<i>Juvenile cry</i>	0.419	6486.16
<i>P. aequatorialis</i>	<i>Juvenile yip</i>	0.158	766.73

Discussion

At a glance, structural properties between *Pithecia pithecia* calls in Henline (2007) and those of the current study of *P. chrysocephala* are very similar, with *chucks* being the shortest calls, *throat rattles* the longest, *chucks* and *trills* at the highest frequencies, and *soft growls* at the lowest. However, few calls between these species were sufficiently similar on spectrograms to be compared statistically. All visually similar calls among *P. chrysocephala*, *P. pithecia* and *P. aequatorialis* were found to have differences in their structure in terms of their fundamental frequency.

These preliminary findings add to the morphological evidence presented by Marsh (2014) on the distinctness of *Pithecia* taxa, supporting the classification of *P. chrysocephala* as a separate species from *P. pithecia*, rather than a subspecies. However, as only one population of each

species was studied, the possibility of variations within populations of a single evolutionary unit must be considered before any more substantial claims can be made.

The calls of *Pithecia aequatorialis* appeared notably less similar to *P. chrysocephala* than *P. pithecia*, potentially reflecting the greater evolutionary distance between these species. Phylogenetic differences in call structure across a genus have been previously observed in a number of primate taxa, including sportive lemurs, *Lepilemur* spp., in which species that were less closely related to each other had more distinctly different calls (Mendez-Cárdenas et al., 2008). Similar results have been obtained with titi monkeys, Callicebinae sp., (Ardet et al., 2018). It is also possible that these calls are conserved across the genus, similar to how the startle call is conserved across mouse lemurs, *Microcebus* spp. (Zimmerman, 2012).

Future studies could usefully investigate differences among *Pithecia* populations and examine the influence of habitat differences on vocalisations. It would also be interesting to investigate *Pithecia* vocal behaviour in the context of all pitheciids, as several similarities are evident throughout this group (Bezerra et al., 2017). For example, the *tcho* calls, *whistles*, and *loud screams* of golden-backed uacaris, *Cacajao ouakary*, are similar to the *P. chrysocephala* *chucks*, *whistles*, and *throat rattles* respectively (Bezerra et al., 2010). Likewise, juvenile *B-calls* (an alarm call) of black-fronted titi monkeys, *Callicebus nigrifrons*, are similar to juvenile *P. chrysocephala* *peeps*, as are the juvenile *purrs* of red-bellied titi monkeys, *Plecturocebus moloch*, to juvenile golden faced saki *trews* (Moynihan, 1966; Berthet et al., 2018). Additionally, the *chucks* and *intergroup call/throat rattle* of *Plecturocebus moloch* and *Pithecia chrysocephala* resemble each other (Moynihan, 1966; Caselli et al., 2014). More wide-scale comparisons across all pitheciid species could also be conducted to examine the social and ecological effects on the evolution of their communication, and why such calls appear to be similar. However, a full phylogenetic comparison of *Pithecia* vocalisations is currently limited by a lack of data, and so further studies of *Pithecia* and pitheciid species are recommended. The development of a pitheciid vocalisation database as suggested by Bezerra et al. (2017) would be ideal for this purpose.

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- occurrence, area of occupancy, and conservation status (IUCN, 2019). In times where human-induced deforestation in forest fragments is of major scientific concern (Canale et al., 2012; Dirzo et al., 2014; Galletti et al., 2016) and outbreaks of yellow fever virus are severely impacting populations of wild non-human primates in Brazil's Atlantic Forest (Holzmann et al., 2010; Almeida et al., 2012; Bicca-Marques et al., 2017), the discovery of a group of threatened primate species in a small fragment of Atlantic Forest should be celebrated.

We report here the first record of a group of brown howler monkeys, *Alouatta guariba clamitans*, in São Pedro da Aldeia, Rio de Janeiro, Brazil. *A. guariba* is the primate species with most records and with the largest distribution in the Atlantic Forest (Culot et al., 2019). In Rio de Janeiro state brown howlers inhabit the coastal and the northern regions (Gregorin, 2006), with populations occurring in at least 23 protected areas (Bicca-Marques et al., 2018). The deforestation and fragmentation of the southern and southeastern forests have played an important role in decreasing its current distribution compared to its historical occurrence (Bicca-Marques et al., 2018). It is currently listed as a Vulnerable species on the IUCN Red List of Threatened Species, with ongoing population decline (Jerusalinsky et al., 2020).

Study site

Our study site (22°43'20.02"S, 42°07'25.37"W) is a small patch of lowland seasonal semideciduous forest with 36 ha. It has an elliptical shape, with altitude varying from 7 to 35 m above sea level. Locally known as Ilha dos Macacos (Monkeys' Island), it is connected to other fragments of Atlantic Forest totaling about 418 ha (Fig. 1). The site lies in a swampy plain bordered by two small rivers that drain northward up to the basin's main river, Rio Una. Grassy fields characterize the landscape. The rainy season (December-May) turns most of the plain into wetlands. The forest fragment studied lies in a higher terrain so that waters reach only part of its border. Cattle ranching, eucalyptus forestry and agriculture characterize local land use around the fragment (Bastos, 2020). The local climate is an interface between two Köppen-Geiger climate classes, Aw and BSh (Barbiéri, 1984, 1997), and the precipitation is between 900–1,000 mm per year (Pinto et al., 2011).

Results and discussion

During our survey focused on floristic and phytosociological data collection (Bastos, 2020), we unexpectedly heard howls at the study site. Therefore, we decided to collect *ad libitum* data (Altmann, 1974) on all monkey observations during every visit. We registered howler vocalizations in 10 of 31 visits. The records encompassed two years, from May 2018 to February 2020. The only visualization was in January 2020. We observed an

FIRST RECORD OF BROWN HOWLER MONKEYS IN SÃO PEDRO DA ALDEIA, RIO DE JANEIRO, BRAZIL

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Introduction

The discovery of a threatened species in a new locality provides important information to reassess its extent of