Comparative Analysis of the Vocal Repertoires of the Indri (Indri indri) and the Diademed Sifaka (Propithecus diadema)

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Abstract:	Strepsirrhine vocalisations are extraordinarily diverse and cross-species comparisons are needed to explore how this variability evolved. We contributed to the investigation of primate acoustic diversity by comparing the vocal repertoire of two sympatric lemur species, Propithecus diadema and Indri indri. These diurnal species belong to the same taxonomic family and have similar activity patterns but different social structures. These features make them excellent candidates for an investigation of the phylogenetic, environmental, and social influence on primate vocal behaviour. We recorded 3 P. diadema groups in 2014 and 2016. From 1872 recordings we selected and assigned 3814 calls to 9 a priori call types, on the basis of their acoustic structure. We implemented a reproducible technique performing an acoustic feature extraction

relying on frequency bins, t-SNE data reduction, and a hard-clustering analysis. We first quantified the vocal repertoire of P. diadema, finding consistent results for the 9 putatively identified call types. When comparing this repertoire with a previously published repertoire of I. indri, we found highly species-specific repertoires, with only 2% of the calls misclassified by species identity. The loud calls of the two species were very distinct, while the low-frequency calls were more similar. Our results pinpoint the role of phylogenetic history, social and environmental features on the evolution of communicative systems and contribute to a deeper understanding of the evolutionary roots of primate vocal differentiation. We conclude by arguing that standardized and reproducible techniques, like the one we employed, allow robust comparisons and should be prioritized in the future.

Comparative analysis of the vocal repertoires of the indri (*Indri indri*) and the diademed sifaka (*Propithecus diadema*)

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Supporting information

Information on group size and composition, sampling days, overall observation time (ESM Table SI) and a comparison between the vocal types identified in our study and the alleged homologous call types in congeneric species (ESM Table SII) are available online.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

MG formulated the idea; DV, CDG, VT, AA, FC, AM, LM collected the data; OF, MG, DV, AA, FC conducted acoustic analyses; DV and MG performed statistical analyses; DV and TR implemented data visualization; MG, DV, AA, CDG wrote the manuscript; all authors provided editorial advice.

1 **Comparative analysis of the vocal repertoires of the indri (*Indri indri*) and the**

2 **diademed sifaka (*Propithecus diadema*)**

3

4

5 ## Abstract

6 Strepsirrhine vocalisations are extraordinarily diverse and cross-species comparisons are needed to

7 explore how this variability evolved. We contributed to the investigation of primate acoustic diversity

8 by comparing the vocal repertoire of two sympatric lemur species, *Propithecus diadema* and *Indri indri.*

9 These diurnal species belong to the same taxonomic family and have similar activity patterns but

10 different social structures. These features make them excellent candidates for an investigation of the

11 phylogenetic, environmental, and social influence on primate vocal behaviour. We recorded 3 *P. diadema*

12 groups in 2014 and 2016. From 1872 recordings we selected and assigned 3814 calls to 9 *a priori* call

13 types, on the basis of their acoustic structure. We implemented a reproducible technique performing an

14 acoustic feature extraction relying on frequency bins, t-SNE data reduction, and a hard-clustering

15 analysis. We first quantified the vocal repertoire of *P. diadema*, finding consistent results for the 9

16 putatively identified call types. When comparing this repertoire with a previously published repertoire

17 of *I. indri*, we found highly species-specific repertoires, with only 2% of the calls misclassified by

18 species identity. The loud calls of the two species were very distinct, while the low-frequency calls were

19 more similar. Our results pinpoint the role of phylogenetic history, social and environmental features on

20 the evolution of communicative systems and contribute to a deeper understanding of the evolutionary

21 roots of primate vocal differentiation. We conclude by arguing that standardized and reproducible

22 techniques, like the one we employed, allow robust comparisons and should be prioritized in the future.

23

24 **Keywords** vocal repertoire - primates - clustering - phylogeny - sensory drive - social complexity

Introduction

Acoustic signals play various roles in mate choice, resource defence, and species recognition in a broad range of taxa (Wilkins et al. 2013), including lemurs (Rakotonirina et al. 2016). Divergence in acoustic traits mediates discrimination within and between species, and has been proposed to play a role in speciation and evolution (Wilkins et al. 2013; Zimmermann 2016). This is particularly true for sympatric cryptic species, in which species-specific vocal signals and recognition systems are involved in driving reproductive isolation. For instance, recent research showed this mechanism in species of the genera *Microcebus* (Braune et al. 2008) and *Phaner* (Forbanka 2020). The complexity of mammalian vocal communication has been studied to understand possible factors determining convergent evolutionary patterns (Charlton and Reby 2016) and species-specific differences (Gamba et al. 2015) and three main evolutionary frameworks have been proposed for the diversification of communication systems and vocal flexibility (Schuster et al. 2012).

First, the Phylogenetic Hypothesis suggests that phylogeny determines the vocal repertoire of a species (Ord and Garcia-Porta 2012), implying that closely-related members of a taxonomic group will have very similar signals (Zimmermann 2017). This hypothesis is supported by studies indicating concordance between vocal and genetic diversity across *Nomascus* species (Thinh et al. 2011). However, there is no evidence indicating a relationship between vocal behaviour and phylogeny across lemurs (Zimmermann 2017; Hending et al. 2020), including the Indriidae family (Ramanankirahina et al. 2016).

Second, the Social Complexity Hypothesis posits that the evolution of vocal communication and that of social life are related (Pollard and Blumstein 2012; Bouchet et al. 2013), such that a more complex social system requires more subtle communicative abilities to mediate interactions among group members (Freeberg et al. 2012). Under this hypothesis, the diversity in the communicative signals of a species is related either to a stable and egalitarian social structure (Mitani 1996) or to group size (McComb and Semple 2005, Kappeler 2019, Peckre et al. 2019). For instance, social structure and social organisation reflect the vocal repertoire complexity in *Cercopithecus neglectus*, *Cercopithecus campbelli*, and *Cercocebus torquatus* (Bouchet et al. 2013).

Third, and finally, the Sensory Drive Hypothesis (Endler 1992) suggests that signals, sensory systems, and microhabitat choice coevolve, with signal evolution being driven by environmental conditions, including predation (Zimmermann 2017). This hypothesis is supported by the acoustic windows occupied by *Microcebus*

spp., *Mirza* spp., and *Cheirogaleus* spp. (Zimmermann 2018) which use high frequency and ultrasonic components. The latter are rare among primates and appear to have evolved to cope with the social and ecological needs of a dispersed social network (Zimmermann 2018). The acoustic frequency window is likely a balance between being conspicuous to conspecifics while remaining cryptic for predators (Zimmermann 2018).

Although many lemurs live in smaller groups than other primates (Kappeler and Heymann 1996) some lemur species live in large groups. Such groups may require sophisticated intelligence (social intelligence; Dunbar 1996) and signals to modulate the relationships among group members (Oda 2008, Matsuzawa 2008). For instance, the gregarious *Lemur catta* has a repertoire of 22 call types (Macedonia 1993) while other species, like *Eulemur rufifrons* and *Propithecus verreauxi*, show referential-like calling (Fichtel and Kappeler 2002). Call types and use also differ with sex in *Eulemur coronatus* (Gamba and Giacoma 200), *Mirza zaza* (Seiler et al. 2019), and *Lepilemur edwardsi* (Rasoloharijaona et al. 2006). Hence, lemur vocal diversity may provide useful information on the selective pressures that may have played a role in the evolution of vocal communication (Oda 2008).

Among lemurs, *Indri indri* is the only species that sings (Giacoma et al. 2010; Baker-Médard et al. 2013; Torti et al. 2013, 2017; De Gregorio et al. 2019). Recent studies showed that indri's song possesses a rhythmic structure (Gamba et al. 2016; De Gregorio et al. 2019, De Gregorio et al. 2021a), conforms to the linguistic laws of brevity (Valente et al. 2021), shows an ontogenetic development (De Gregorio et al. 2021b), and a sex-dimorphic phrase organization (Zanoli et al. 2020). This species shows a rich vocal repertoire, including distinct alarm calls for terrestrial and aerial predators (Maretti et al. 2010) and several call types mediating intra-group dynamics (Valente et al. 2019). In contrast, information on the vocal communication of *Propithecus diadema* is limited to qualitative accounts examining the role of vocal behaviour in contact seeking (Petter and Charles-Dominique 1979), and anti-predatory behaviour (Petter and Charles-Dominique 1979; Macedonia and Stanger 1994; Oda and Masataka 1996; Wright 1998; Fichtel and Kappeler 2002, 2011; Patel and Owren 2012; Fichtel 2014). All *Propithecus* species have call types with comparable structures and functions (Petter and Charles-Dominique 1979; Macedonia and Stanger 1994; Oda and Masataka 1996; Wright 1998; Fichtel and Kappeler 2002, 2011; Patel and Owren 2012; Fichtel 2014; Online Resource 2). An exception is the zzuss, a call type only occurring in the repertoire of *P. diadema*, *P. candidus, P. perrieri,* and

81 *P. edwardsi* (Patel and Owren 2012; Anania et al. 2018; Wright 1998; Macedonia and Stanger 1994). The four

82 western *Propithecus* species (*P. verreauxi*, *P. coquereli*, *P. coronatus*, *P. deckenii*) and *P. tattersalli* do have

83 a call type serving similar functions to the zzuss (terrestrial predator alarming and group coordination, Patel

84 and Owren 2012) but with a different acoustic structure (Petter and Charles-Dominique 1979; Macedonia and

85 Stanger 1994; Oda and Masataka 1996; Fichtel 2014). Within the genus, the most investigated call types are

86 the alarm calls of *P. verreaux*i and *P. coquereli* (Fichtel and Kappeler 2014), and the zzuss of *P. candidus*

87 (Patel and Owren 2012). The latter represents the only quantitative description of a call type of eastern

88 *Propithecus* species.

89 To understand the extent to which the vocal systems of two strepsirrhine species differ, we compared

90 the calls of two sympatric and similar-sized lemur species, *Indri indri* and *Propithecus diadema*, belonging to

91 the same taxonomic family (Indriidae), both inhabiting the same rainforest environment and having diurnal

92 habits (Geissmann and Mutschler 2006). These species are the largest extant lemurs, and their estimated

93 pairwise divergence time ranges between 18 (Kistler et al. 2015; Masters et al. 2013; Federman et al. 2016)

94 and 29-36 MYA (Roos et al. 2004; Fritz et al. 2009; Antonelli et al. 2017; Fabre et al. 2009). *Propithecus*

95 *diadema* lives in multimale/multifemale groups of 2 to 8 individuals (Powzyk 1997; Irwin 2008; Weir 2014),

96 while *I. indri* groups range from 2 to 5 individuals (Glessner and Britt 2005; Torti et al. 2017; Torti et. al 2018;

97 Bonadonna et al. 2020), usually comprising a monogamous reproductive pair and their offspring (Bonadonna

98 et al. 2019). Thanks to these features, they are suitable subjects to investigate the effect of the phylogenetic,

99 environmental, and social influence on their vocal behaviour. Moreover, *Indri indri* and *P. diadema* emit calls

100 in similar contexts. Both species vocalise in the presence of terrestrial disturbance (disturbance call in *P.*

101 *diadema*, wheezing grunt and kiss-wheeze in *I. indri*; Macedonia and Stanger 1994) or aerial predators (roaring

102 vocalisations: Macedonia and Stanger 1994; Powzyk 1997). Calls are also used to coordinate group

103 movements during foraging or displacing activities (Petter and Charles-Dominique 1979; Macedonia and

104 Stanger 1994).

105 We compared the number of distinct call types and their spectro-temporal structure, in the light of the

106 Phylogenetic Hypothesis, Sensory Drive Hypothesis, and Social Complexity Hypothesis. *Indri indri* and *P.*

107 *diadema* belong to the same taxonomic family, so the Phylogenetic Hypothesis predicts that their repertoires

108 should be more similar to one another than to those of more distantly-related species. We also predict the vocal

4

109 repertoires of the two species will be similar to one another, based on the Sensory Drive Hypothesis. Lastly,

110 we tested two versions of the Social Complexity Hypothesis. Frist, if vocal repertoire size is positively related

111 to group size (McComb and Semple 2005), we predict that *P. diadema*, which lives in larger groups, will have

112 a larger repertoire than *I. indri*, which lives in smaller groups. Conversely, if vocal diversification is driven by

113 a stable and egalitarian social structure (Mitani 1996) we predict a larger repertoire in pair-living, monogamous

114 *I. indri*, than in the more despotic *P. diadema,* with its multimale/multifemale groups.

115

Methods

Data collection

118 We conducted the study in four forest sites: Analamazaotra Special Reserve (Madagascar National Parks, 18°

119 56' S - 48° 25' E), Andasibe-Mantadia National Park (Madagascar National Parks, 18° 28' S - 48° 28' E),

120 Mitsinjo Forest Station (Association Mitsinjo, 18° 56' S - 48° 24' E), and Maromizaha Protected Area (Groupe

121 d'Étude et de Recherche sur les Primates de Madagascar, 18° 56' 49" S – 48° 27' 33" E).

122 We collected vocalisations of *I. indri* by sampling 18 habituated groups between 2005 and 2018.

123 Group size ranged from two to six individuals (mean ± SD = 4.2 ± 1.2). We collected vocalisations of *P.*

124 *diadema* by sampling three habituated groups in 2014 and 2016. Group size ranged from eight to ten

125 individuals (mean ± SD = 8.8 ± 0.8). Further information on data collection (groups size and composition,

126 sampling days, and overall observation time) can be found in Online Resource 1. For both species, we followed

127 a focal group for one to five consecutive days, observing animals at a distance ranging from 0.5 to 20 m. We

128 identified individuals using morphological criteria such as fur patterns and other natural marks. Both species

129 are diurnal and their activity pattern is concentrated during the first half of the day (Pollock 1975; Petter and

130 Charles-Dominique 1979). *Indri indri* vocal emissions are concentrated in the early morning (Geissmann and

131 Mutschler 2006). *Propithecus diadema* calls can be emitted anytime throughout the day but are more common

132 early in the morning and at the beginning of the afternoon (Petter and Charles-Dominique 1979). We, therefore,

133 monitored the groups daily, from 06:00 h until their activities started to decrease (usually around 14:00 h),

134 using focal animal sampling to collect data (Altmann 1974). Occasionally, we also collected audio and video

135 recordings of individuals' utterances using *ad libitum* sampling (Altmann 1974). We recorded spontaneous

136 vocalisations using a Sennheiser ME66 or a Sennheiser ME67 shotgun directional microphone (frequency

137 response range of both microphones: 40-20000 Hz ± 2.5 dB) connected to a solid-state digital audio recorder,

138 a Sound Devices 702 (frequency response range: 10-40000 Hz +0.1/-0.5 dB), or a Tascam DR- 100 MKII

139 (frequency response range: 20-20000 Hz +1/-3 dB). We set the recorders at a sampling rate of 44.1 kHz and

140 an amplitude resolution of 16 or 24 bit. We recorded signals emitted from individuals at 15 to 20 m depending

141 on signal intensity, weather conditions, and canopy thickness. We made recordings with the microphone facing

142 the caller or in the direction of the whole group. We did not deliberately manipulate or modify the animals'

143 behaviour and recorded only spontaneous vocal emissions.

144

145   ***Acoustical and statistical analyses***

146 We visually inspected all recordings using Praat 6.0.28 (Boersma and Weenink, University of Amsterdam).

147 For *P. diadema*, we acquired 8946 calls from 1872 initial recordings, of which we chose 3814 calls for acoustic

148 analyses. We selected high-quality vocal emissions (higher intensity and lower background noise) and

149 discarded noisy and overlapping calls (multiple individuals and different species) and vocalisations uttered by

150 infants. We discarded calls where the signal to noise ratio was lower than 12 dB, that were acoustically

151 distorted, or that overlapped with other sounds (Gamba et al. 2015).

152   Vocal emissions can include sequences of repeated temporally close calls. We considered two

153 emissions as distinct calls when they were separated by at least 0.025 s. This threshold is recognized in humans

154 and non-human animals, including primates, as a natural psychophysical boundary representing the minimum

155 time interval needed by the auditory system to differentiate between two distinct acoustic signals (Kuhl and

156 Padden 1983; Liberman 1991). In the field, we noticed that different call types can be emitted sequentially

157 (e.g., the mmm is often uttered after a roar chorus; AA *pers. obs.*). Within the recordings, we found that the

158 most conspicuous association concerned zzuss and tsk. We, therefore, measured the mean duration of the silent

159 interval between these two call types across 145 recorded sequences. We normalized each sound file using a

160 scale to peak function in Praat (Comazzi et al. 2016) and assigned it to nine a priori classes based on audio-

161 visual evaluation (Lemasson et al. 2014). Some call types are described in studies of rainforest *Propithecus*

162 (Petter and Charles-Dominique 1979; Macedonia and Stanger 1994; Wright 1998; Powzyk 1997; Patel and

163 Owren 2012). We chose the names zzuss (n = 400), roar (n = 176), and grunt (n = 145) to ensure consistency

164 with the literature (Macedonia and Stanger 1994; Wright 1998; Patel and Owren 2012). We labelled new call

types according to the sound quality (chatter-squeal, n = 317; soft grunt, n = 221), the hypothesized function (lost call, n = 193), or with onomatopoeic terms (hum, n = 1927; mmm, n = 246; tsk, n = 189). For each call type, we measured duration, mean, minimum, and maximum fundamental frequency. We also considered the range of emission and phonatory mechanisms.

We employed the methodology shown in Valente et al. (2019) and used a custom-made script in Praat to extract spectral coefficients for each call: we measured the total duration of a sound and divided it into ten equal portions. Then, considering a frequency range from 50 to 22000 Hz, representing the frequency spectrum covered by the calls in our sample, we split each portion into frequency bands (or bins) of 500 Hz each (e.g., 50–500 Hz, 501–1000 Hz), then extracted the energy value of each bin (through the function 'Get band energy' in Praat). The resultant dataset included the duration and 220 frequency parameters for each call. We used the Rtsne package (Krijthe 2015) in R (R Core Team 2021) to embed the dataset into a bi-dimensional plan through a t-distributed stochastic neighbour embedding (Van der Maaten and Hinton 2008) with a Barnes-Hut implementation, initializing the algorithm with perplexity = 40 and theta = 0.5. We then submitted the reduced dataset, containing two features, to a clustering procedure, using a k-means algorithm (MacQueen 1967).

Lastly, we investigated whether the two species shared some call types and assessed the difference among the two vocal repertoires. For the comparison, we used a dataset of 3360 calls used to quantify *I. indri*'s repertoire (Valente et al. 2019), containing 10 call types: clacson, hum, grunt, kiss, long tonal call, roar, short tonal call, songbit, wheeze, and wheezing grunt. Valente and colleagues used the same acoustic approach (extraction of duration and spectral coefficients of the calls, Valente et al. 2019), which allowed us to combine the features of all calls of both species into a single dataset. We first reduced the combined data through a t-SNE based compression, then submitted the compressed dataset to a k-means clustering algorithm (MacQueen 1967). We used t-SNE to visualize data.

Ethical note

We conducted observational research without manipulating animals, with permission of the Malagasy Ministry of Environment and Forests, Research permits: 2005 [N°197/MINENV.EF/SG/DGEF/DPB/SCBLF/ RECH], 2006 [N°172/06/MINE NV.EF/SG/DGEF/DPB/SCBLF], 2007 [N°0220/07/MINENV.EF/SG/ DGEF/DPSAP/SSE], 2008 [N°258/08/MEFT/SG/DGEF/DSAP/SSE], 2009[N°243/09/MEF/SG/DGF/

193 DCB.SAP/SLRSE], 2010 [N°118/10/MEF/SG/DGF/DCB.SAP/SCBSE, N°293/10/MEF/SG/DGF/DCB.

194 SAP/SCB], 2011 [N° 274/11/MEF/SG/ DGF/DCB.SAP/SCB], 2012 [N°245/12/MEF/SG/DGF/DCB.SAP/

195 SCB], 2014 [N°066/14/MEF/SG/DGF/DCB.SAP/SCB], 2015 [N°180/15/MEEMF/SG/DGF/DAPT/SCBT],

196 2016 [N°98/16/MEEMF/SG/DGF/DAPT/SCB.Re, N°217/16/MEEMF/SG/DGF/DSAP/SCB.Re], 2017

197 [N°73/17/MEEF/SG/DGF/DSAP/SCB.RE], 2018 [N°91/18/MEEF/SG/DGF/DSAP/SCB.Re]. We declare

198 the data collection procedure conforming to the national legislation and international regulation concerning

199 animal welfare.

200

201 **Results**

202 *t-SNE mapping: Propithecus diadema calls*

203 The algorithm identified eight clouds of points, where each point represents a call and each cloud might

204 represent a cluster (Van der Maaten and Hinton 2008), so we imposed k = 8 for k-means clustering (Fig. 1c).

205 The eight different clusters were mostly consistent with the putative identification of calls and with their

206 acoustic structure (Table 1). Clusters 3, 5, 6, 7, and 8 included one vocal type each: zzuss, chatter-squeal, soft

207 grunt, lost call, and roar, respectively (Fig. 1a, 1c). Conversely, both Clusters 2 and 4 mainly included hum

208 (94% and 84%) and mmm (6% and 16%, Fig. 1b). Grunt and tsk were grouped in Cluster 1 (Fig. 1b, 1c).

209 Analysis of a subsample of 145 zzuss-tsk sequences showed that when these two calls are uttered sequentially,

210 the mean duration of the pause between them is $0.62 \pm$ SD 0.11 s.

8

211
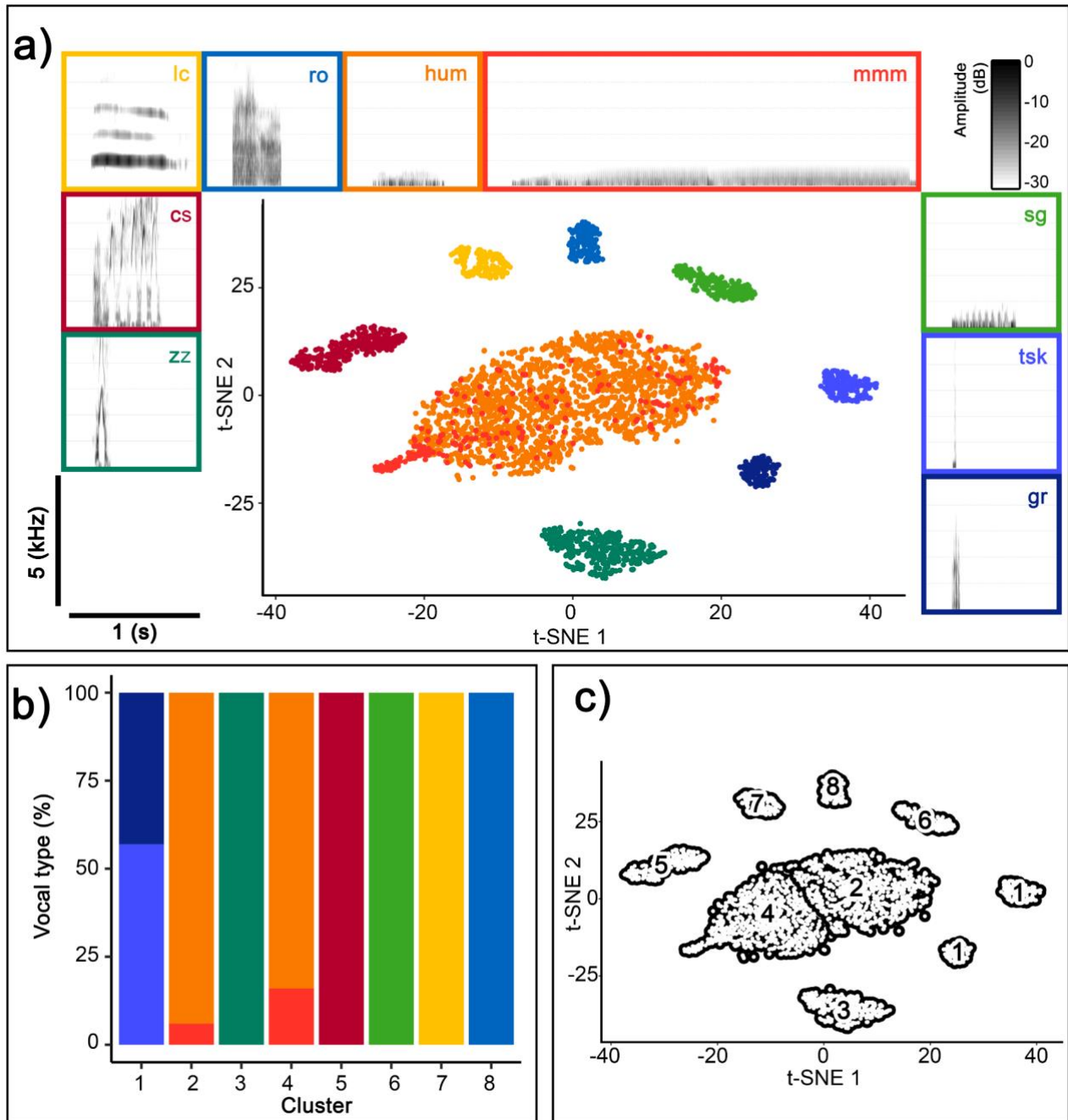
Fig. 1 Representation of *P. diadema* calls (recorded in Maromizaha Protected Area in 2014 and 2016) on a bi-dimensional plan obtained by initializing a t-SNE algorithm with perplexity = 40 and theta = 0.5. a) Visualization of t-SNE mapping combined with a priori identification of call types (cs = chatter-squeal, gr = grunt, hum = hum, lc = lost call, mmm = mmm, ro = roar, sg = soft grunt, tsk = tsk, zz = zzuss). We generated spectrograms (Hanning window, 512 samples, overlap = 64, zero-padding = 16) using the R package *Seewave* (Sueur et al. 2008). b) The distribution of vocal types within the clusters. Colours follow those in panel a. c)

218   Results of k-means clustering on the bi-dimensional vector produced using t-SNE. Numbers indicate clusters

219   (i.e., 1 = Cluster 1).

220 **Table 1** Definition of call types emitted by *Propithecus diadema* recorded in Maromizaha Protected Area in 2014 and 2016.

Call Type	Description	Duration (s)	Mean f0 (Hz)	Max f0 (Hz)	Min f0 (Hz)	Range	Phonatory mechanism
Chatter-squeal	High-amplitude, high-pitched tonal call. Usually uttered in sequences of short signals.	0.38 ± 0.375	2098.61 ± 530.71	2849.51 ± 1466.29	1287.75 ± 1069.53	Long	Semi-open mouth
Grunt	Medium-pitched short pulses. Usually emitted in sequences.	0.06 ± 0.02	1408.98 ± 61.16	1506.98 ± 567.83	1341.28 ± 518.09	Short	Open mouth
Hum	Low-pitched tonal call.	0.40 ± 0.09	281.80 ± 152.08	562.85 ± 771.06	150.15 ± 197.47	Short	Closed mouth
Lost Call	Medium-pitched, medium-amplitude tonal call.	0.71 ± 0.30	927.80 ± 58.65	1014.80 ± 264.00	800.73 ± 148.42	Long	Slightly-open mouth
Mmm	Low-pitched tonal call, normally with low modulation. Often emitted in sequence or in overlap between individuals. Frequently emitted after a roar call.	2.55 ± 0.94	392.91 ± 329.20	1704.57 ± 1603.97	115.73 ± 128.69	Short	Closed mouth, often emitted with the head turning on a transverse plane
Roar	Medium-pitched broadband calls, emitted in choral sequences of short utterances.	0.15 ± 0.08	1058.12 ± 66.23	1141.69 ± 654.49	992.02 ± 639.07	Long	Open mouth
Soft grunt	Low-pitched, low-amplitude short pulses of broadband noise.	0.31 ± 0.15	457.47 ± 172.48	848.21 ± 990.91	313.51 ± 185.10	Short	Closed mouth
Tsk	Low amplitude short clicks. It follows a zzuss or a zzuss-tsk sequence.	0.03 ± 0.02				Short	Semi-open mouth
Zzuss	High-amplitude, high-pitched, harmonic short call. It can be followed by tsk within 1s (mean = 0.62 ± SD 0.11 s).	0.18 ± 0.21	2054.39 ± 474.34	2623.31 ± 969.57	1430.15 ± 948.57	Long	Closed mouth

221 Acoustic parameters (duration, mean, maximum, and minimum fundamental frequency are expressed as mean ± standard deviation). We evaluated the range of

222 emission (short vs. long) based on the call amplitude and the possible occurrence of counter-calling within or between groups.

t-SNE mapping: calls of Propithecus diadema and Indri indri

224 The algorithm identified 16 clouds of points, so we chose k = 16 for k-means clustering. The 16 clusters were

225 partially consistent with the putative identification of calls. Clusters 1, 2, 8, 12, and 16 each included a single

226 call type: soft grunt, chatter-squeal, roar, grunt, zzuss, respectively (all belonging to *P. diadema*'s vocal

227 repertoire, Fig. 2a, 2c). Clusters 6, 9, and 11 included *I. indri*'s clacson, wheezing grunt, and songbit,

228 respectively. Clusters 3, 4, 5, and 7 included mostly hum (95%, 74%, 92%, 96%) and a smaller percentage of

229 mmm, both emitted by *P. diadema* (5%, 26%, 8%, 4% respectively, Fig. 2b). Cluster 10 grouped *I. indri*'s

230 grunt and hum (73% and 27%, Fig. 2b) while Cluster 14 grouped indri's kiss and wheeze (66% and 34%, Fig.

231 2b). Cluster 13 grouped *P. diadema's* lost call (64%) with *I. indri's* roar, long tonal call, and grunt (21, 10%,

232 and 5%, respectively). Cluster 15 included mainly *P. diadema's* tsk (81%) (Fig. 2b).

12

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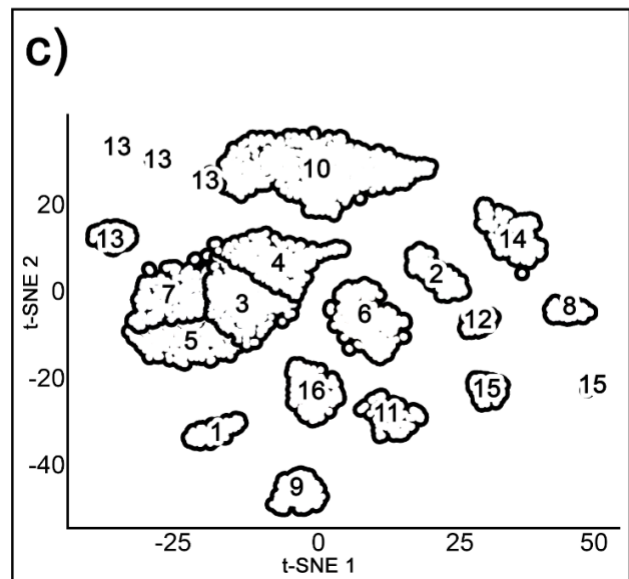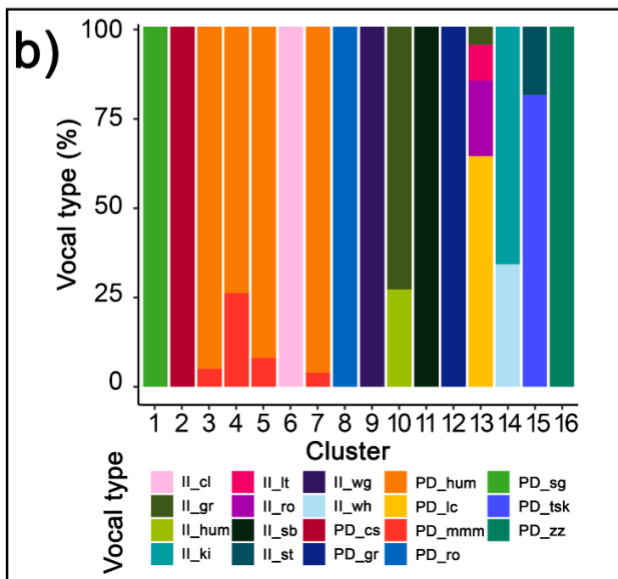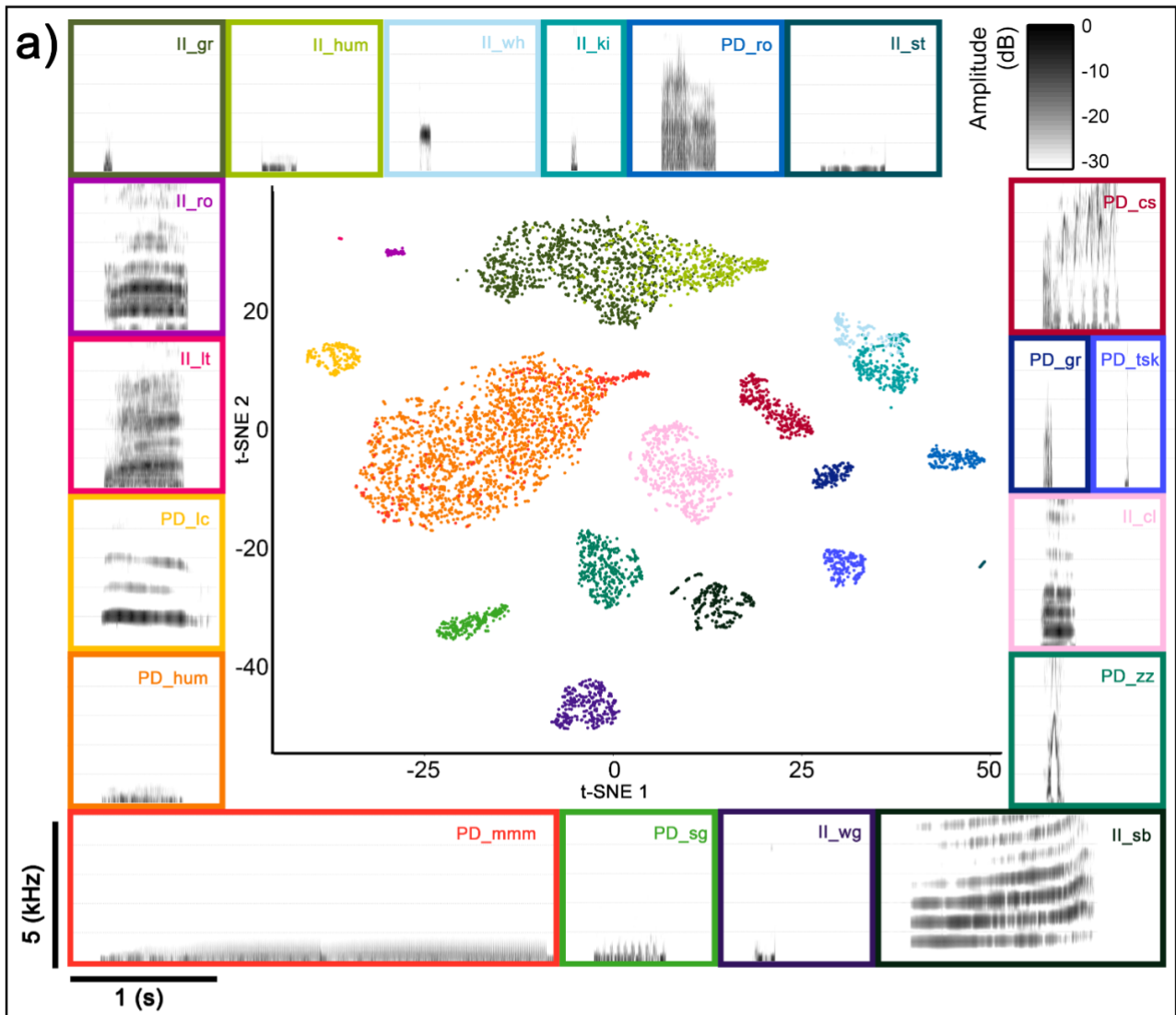
Fig. 2 Representation of *P. diadema* and *I. indri* calls on a bi-dimensional plan obtained by initializing a t-SNE algorithm with perplexity = 40 and theta = 0.5. a) Visualization of the t-SNE mapping combined with

236   the a priori identification of call types (II = *I. indri*; cl = clacson, gr = grunt, hum = hum, lt = long tonal call,

237   ki =kiss, ro = roar, sb = songbit, st = short tonal call, wg = wheezing grunt, wh = wheeze, PD = *P. diadema*;

238   cs = chatter-squeal, gr = grunt, hum = hum, lc = lost call, mmm = mmm, ro = roar, sg = soft grunt, tsk = tsk,

239   zz = zzuss). We recorded calls of *I. indri* in four forest sites (Analamazaotra Special Reserve, Andasibe-

240   Mantadia National Park, Mitsinjo Forest Station, and Maromizaha Protected Area) from 2005 to 2018, and

241   calls of *P. diadema* in Maromizaha Protected Area in 2014 and 2016. We generated spectrograms (Hanning

242   window, 512 samples, overlap = 64, zero-padding = 16) using the R package *Seewave* (Sueur et al. 2008). b)

243   The distribution of the call types within the clusters. Colours follow those in the panel a. c) Results of the k-

244   means clustering performed on the bi-dimensional vector produced using the t-SNE. Numbers indicate the

245   relative clusters (i.e., 1 = Cluster 1).

246

247   Discussion

248   Our cluster analysis of the vocal repertoire of *P. diadema* highlighted the presence of eight clusters,

249   mostly consistent with the a priori identification of the calls, with only a few call types grouping together.

250   Based on acoustic and spectrographic analysis, we identified nine distinct call types. Five clusters showed

251   homogenous grouping of as many call types: lost call, chatter-squeal, soft grunt, zzuss, and roar. Two of the

252   remaining clusters showed a mixture of hum and mmm (94% and 6% in one case, 84% and 16% in the other),

253   possibly indicating some gradation between the two (Wadewitz et al. 2015). The last cluster also grouped two

254   call types: tsk and grunt. Given the results, we estimated the vocal repertoire of *P. diadema* to consist of nine

255   call types, with some showing a graded structure (tsk and grunt, and mmm and hum, in particular). We used

256   this estimate in our comparisons.

257   Comparison between the vocal repertoire of *P. diadema* and that of *I. indri* showed that loud calls of

258   both species possess distinctive features, while some low-frequency calls resulted grouped together, meaning

259   that these call types are characterized by similar spectro-temporal features. We identified eight homogeneous

260   groups. Five (chatter squeal, both grunt and soft grunt, roar, and zzuss) were *P. diadema*'s most distinctive

261   calls. Three (clacson, wheezing grunt, and songbit) were *I. indri* calls. This analysis suggested four clusters

262   mainly consisting of *P. diadema*'s low-pitched calls, like hum and mmm (the latter in smaller percentages). It

263   also confirmed the gradedness between these two call types found in the singles-species analyses. Two other

14

264   clusters (10 and 14) grouped mostly *I. indri'*s low- (grunt and hum; 61%) and medium-pitched calls (wheeze

265   and kiss; 66%). This result is in line with previous analyses of lemur low-pitched calls, in which the grunt,

266   click, grunted hoot, hoot, snort, and long grunt of *Eulemur* ssp. (Gamba and Giacoma 2005, 2007; Gamba et

267   al. 2012; Pflüger and Fichtel 2012; Nadhurou et al. 2015) showed little differentiation compared to alarm calls

268   or high-pitched calls. Interestingly, two other clusters included *P. diadema*'s tsk (81%) and *I. indri*'s short tonal

269   call (19%) as well as *P. diadema*'s lost call (64%) and *I. indri*'s roar and long tonal call (21%; 10%). These

270   clusters grouped voiceless calls (e.g., tsk) and calls with a more broadband structure (e.g., roar, both long and

271   short tonal call). This finding shows how feature extraction can be useful to characterize resonance frequencies

272   of lemur calls, agreeing with earlier evidence (Gamba et al. 2015).

273   *P. diadema*'s roar and *I. indri*'s clacson were among the most distinctive call types. *P. diadema*'s roar

274   is emitted in presence of raptors across congeneric species (Petter and Charles-Dominique 1979; Macedonia

275   and Stanger 1994; Wright 1998; Fichtel and Kappeler 2002), and *I. indri*'s clacson also mediates anti-predatory

276   behaviour and is given in presence of terrestrial predators (Macedonia and Stanger 1994; Maretti et al. 2010).

277   We also found both species' loud calls to be unambiguous (for instance, *I. indri*'s songbit and *P. diadema*'s

278   chatter-squeal and zzuss). Two studies have addressed the role of species-specific signalling in lemurs (Braune

279   et al. 2008, Rakotonirina et al. 2016), with conflicting results. Support for species recognition driven by

280   advertisement calls has been found in *Microcebus* spp. (Braune et al. 2008) while acoustic signalling seems

281   not to be involved in species recognition across *Eulemur* species (Rakotonirina et al. 2016). A mechanism

282   similar to that demonstrated in *Microcebus* spp. (Braune et al. 2008) could allow *I. indri* and *P. diadema* to

283   distinguish among hetero- and conspecifics at distance, in an environment where the acoustic channel is more

284   effective than the visual one (Waser and Brown 1986). The stereotypy we found in the loud calls of our subject

285   species is partly in line with the Sensory Drive (Endler 1992) and the Acoustic Adaptation Hypotheses, both

286   of which state that vocal signals are adapted to the environment in which they are emitted (Morton 1975;

287   Endler 1992). The acoustic structure of vocal signals, and in particular that of those used for long-distance

288   communication, is expected to be optimized to ensure sound propagation. This is especially true in closed

289   habitats, where higher vegetation density represents a greater surface for reverberation and absorption than in

290   open habitats (Waser and Brown 1986). However, our results do not fully support the Sensory Drive

291   Hypothesis, since only a small portion of *P. diadema*'s vocal repertoire (tsk and lost call) clustered with *I.*

15

indri calls. A study of *Microcebus murinus*, *M. ravelobensis*, *M. berthae*, and *M. lehilahytsara* also did not

support the Sensory Drive Hypothesis, suggesting that predatory pressures may be more relevant in shaping

vocal communication than differences in habitat structure (Zimmermann 2016).

Our findings only partially supported our predictions based on the Social Complexity Hypothesis (McComb

and Semple 2005; Bouchet et al. 2013). The hypothesis predicts that the species living in a larger group -

namely *P. diadema* - would have a bigger repertoire size; McComb and Semple 2005). We found no support

this prediction and *P. diadema*'s repertoire consisted of a smaller number of different call types than that of *I.*

indri (10, Valente et al. 2019). Moreover, a repertoire including nine call types, with an average group size of

five individuals (Irwin 2008), conflicts with the group size - vocal repertoire size paradigm. At least two other

primate species with comparable group size (*Saguinus fuscicollis*: 5.9 individuals, *Leontopithecus rosalia*: 5.8

individuals) have a vocal repertoire of 16 call types (McComb and Semple 2005). However, the Social

Complexity Hypothesis also predicts that the species living in an egalitarian social structure - like *I. indri* -

require a more sophisticated communicative system, in terms of the number of different call types in their

repertoire, i.e. the repertoire size; Mitani 1996). Our results, indicating a smaller repertoire in *P. diadema*, are

in line with this second prediction and with studies on other lemur species. For example, the same deviation

from the paradigm group size - vocal repertoire size has been shown in *E. rubriventer* (with an average group

size of three individuals and a repertoire of 14 call types; Gamba et al. 2015) and *I. indri* (with a group size

ranging from four to six individuals and a repertoire of ten call types; Pollock et al. 1975; Valente et al. 2019).

In terms of vocal repertoire size, *P. diadema* is more similar to *I. indri* than to other more

phylogenetically distant species, such as *L. catta* (22 call types; Macedonia 1993) and the sympatric *Varecia*

variegata (16 call types; Pereira et al. 1988; Gamba et al. 2003). Furthermore, the repertoire size in *P. diadema*

is in line with the variation displayed within the Indriidae family (three to ten, Zimmermann 2017) and in

particular with that of two other *Propithecus* species, with a repertoire of six (*P. verreauxi*, Zimmermann 2017)

and 10 call types (*P. candidus,* Patel and Owren 2012). Nonetheless, in contrast with the Phylogenetic

Hypothesis, besides their size, the vocal repertoires of *I. indri* and *P. diadema* differed from each other. This

is not surprising, given that the last common ancestor of the two species lived at least 18 MYA (Kistler et al.

2015; Masters et al. 2013; Federman et al. 2016) and that closely-related Indriidae species show acoustic

differences (*P. deckenii* and *P. coronatus*: Fichtel 2014). Moreover, across lemurs, there is no pattern of vocal

320   similarity based on phylogenetic proximity (Bergey and Patel 2008; Gamba et al. 2015; Zimmermann 2017;

321   Hending et al. 2020). This lack of correlation also applies to the Indriidae family (Ramanankirahina et al.

322   2016). Despite the phylogenetic relatedness, closely-related species exhibiting the same social pattern but

323   different activity mode (diurnal vs nocturnal, respectively), also differ in the complexity of vocal signalling (*I.*

324   *indri* and *Avahi occidentalis*, Ramanankirahina et al. 2016).

325   Interestingly, *P. diadema* had the same vocal repertoire size as *Daubentonia madagascariensis*

326   (studied in captivity; Stanger and Macedonia 1994), which is a solitary nocturnal species (Sterling and

327   McCreless 2006). According to some authors, *D. madagascariensis* descended from the most basal divergence

328   from all other lemur taxa (Delpero et al. 2006), while recent evidence suggests it descended from independent

329   colonization of Madagascar (Gunnell et al. 2018). Thus, considering the phylogenetic history, common

330   ancestry of the vocal behaviour of these species is unlikely.

331   Some of the comparisons we make rely on studies employing analogous methods (i.e., *Eulemur* spp.,

332   Gamba et al. 2015). However, other vocal repertoire estimates rely on different approaches (i.e., Stanger and

333   Macedonia 1994; McComb and Semple 2005). Thus our comparisons should be taken with caution: different

334   methodologies used to measure repertoires lead to very different results and the lack of common acoustic and

335   statistical approaches undermines cross-taxa comparisons (Peckre et al. 2019).

336   The use of computationally accessible and powerful methods opens new perspectives in the study of

337   acoustic signals (Sainburg et al. 2020). The t-SNE embedding allowed efficient analysis of the vocal repertoire

338   of *P. diadema,* in line with findings on other animal species (mammals: *Mus musculus*, *Megaptera*

339   *novaeangliae*, *Pteronura brasiliensis*, *Macaca mulatta*; birds: *Taeniopygia guttata*; Sainburg et al. 2020). The

340   t-SNE also allowed us to compare the calls of *P. diadema* with those of another diurnal species in the Indriidae

341   family, *I. indri* (Valente et al. 2019). In line with studies using unsupervised clustering in the quantitative

342   analysis of animal vocalisations (Gamba et al. 2015; Riondato et al. 2017), we found that the extraction of

343   linear frequency bins revealed a remarkable potential for grouping calls based on their spectrographic

344   similarity, comparable to clusters obtained using dynamic time warping–generated dissimilarity indices.

345   The standardized technique we employed in this study allowed us to reduce the need for a priori human

346   input and to overcome potential limitations due to human perceptual bias (Sainburg et al. 2020). We do not

347   neglect the importance of previous work, but argue that standardized and reproducible techniques (for

348   alternatives see Gamba et al. 2015, where the authors employed a combination of Dynamic Time Warping and

349   clustering algorithms, or Sainburg et al. 2020, where the authors compared the efficiency of data reduction

350   algorithms across multiple datasets) should be prioritized in the future.

351

352   **Conclusions**

353   Our study supports previous findings on lemurs: it is likely that Indriidae vocal diversity has been

354   shaped by a combination of social and environmental characteristics, and phylogenetic history

355   (Ramanankirahina et al. 2016). Further research could investigate synapomorphies and autapomorphies in the

356   vocal repertoires of the Indriidae family. For instance, some call types, such as the roar and the lost call (the

357   first emitted in the anti-aerial predator context, the other used to regulate the group cohesion are comparable

358   in structure and functions across *Propithecus* species (Online Resource 2). On the other hand, the main

359   terrestrial disturbance call differs structurally between two groups of *Propithecus* species, one consisting of

360   the species producing the zzuss (*P. diadema*, *P. candidus, P. perrieri,* and *P. edwardsi;* Patel and Owren 2012;

361   Anania et al. 2018; Wright 1998; Macedonia and Stanger 1994) and the other including the species emitting

362   the tchi-fak (*P. verreauxi*, *P. coquereli*, *P. coronatus*, *P. deckenii*, and *P. tattersalli*- representing the so-called

363   western species, evolutionarily split from eastern species; Pastorini et al. 2001; Mayor et al. 2004; Rumpler et

364   al. 2004 but see Herrera and Davalos 2016). The acoustic divergence between zzuss and tchi-fak does not

365   completely follow the current spatial proximity of these species' distributions, or the type of environment (dry

366   forest, rainforest, transitional forest). Furthermore, acoustic differences in the loud calls of closely-related

367   species living in the same environment have been demonstrated (*P. deckenii* and *P. coronatus*; Fichtel 2014).

368   A comparison among *Propithecus* species could highlight which factors (genetic, anatomical, social,

369   ecological, or biogeographical) have been important in the evolution of vocal signals and provide us with clues

370   about why some acoustic structures have been conserved and others have changed in the divergence of species.

371

372   **Data availability**

373   The dataset is available from the corresponding author on reasonable request.

374

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