



**Manchester
Metropolitan
University**

Bettridge, CM and Kenworthy, SP and Butynski, TM and De Jong, YA and De Kort, SR (2019) Vocal Repertoire and Intraspecific Variation within Two Loud Calls of the Small-Eared Greater Galago (*Otolemur garnettii*) in Tanzania and Kenya. *Folia Primatologica: international journal of primatology*, 90 (5). pp. 319-335. ISSN 0015-5713

Downloaded from: <https://e-space.mmu.ac.uk/625005/>

Version: Accepted Version

Publisher: Karger Publishers

DOI: <https://doi.org/10.1159/000500260>

Please cite the published version

<https://e-space.mmu.ac.uk>

1 Vocal repertoire and intraspecific variation within long-distance calls of the small-eared
2 greater galago (*Otolemur garnettii*) in Tanzania and Kenya

3
4 Caroline M. Bettridge^a, Simon P. Kenworthy^a, Thomas M. Butynski^b, Yvonne A. de Jong^b,
5 and Selvino R. de Kort^a

6
7 ^a Division of Biology & Conservation Ecology, John Dalton East, Manchester Metropolitan
8 University, Manchester, United Kingdom.

9 ^b Eastern Africa Primate Diversity and Conservation Program, P. O. Box 149, Nanyuki, Kenya.

10
11
12 Running head: Loud calls of *Otolemur garnettii*

13
14 Word count: 4793

15
16
17 Corresponding author: Caroline Bettridge, Division of Biology & Conservation Ecology, John Dalton
18 East, Manchester Metropolitan University, Manchester, UK, M1 5GD, email:
19 c.bettridge@mmu.ac.uk. Tel: 0161 247 1198.

20
21
22 Key words: Advertisement call; Alarm call; Bioacoustics; Bushbaby; Communication;
23 Nocturnal primates; Population differences; Vocalisations

24
25

26 **Abstract**

27

28 Vocal repertoires and call structure can provide insights into the behaviour and evolution of
29 species, as well as aid in taxonomic classification. Nocturnal primates have large vocal
30 repertoires. This suggests that acoustic communication plays an important role in their life
31 histories. Little is known about the behavioural context or the intraspecific variation of their
32 vocalisations. We used autonomous recording units (ARUs) and manual recorders to
33 investigate the vocal behaviour of the small-eared greater galago (*Otolemur garnettii*) in
34 Kenya and Tanzania. We describe the vocal repertoire and temporal calling patterns of two
35 subspecies; *O. g. panganiensis* and *O. g. kikuyuensis*. We found considerable intraspecific
36 structural differences in the long-distance calls. These are congruent with the current
37 subspecies classification. The differences in vocalisations between populations are not
38 consistent with the 'Acoustic Adaptation Hypothesis', rather they are likely a result of
39 geographic variation due to isolation caused by vegetational barriers in southern Kenya.

40

41

42 **Introduction**

43

44 Detailed knowledge of vocal repertoires may provide insight into the evolution of
45 communication systems [Maynard Smith and Harper, 2003]. The 'Social Complexity
46 Hypothesis for Communication' [Bradbury and Vehrencamp, 2001; Freeberg et al., 2012;
47 Fischer et al., 2017] proposes that species living in more complex social environments have
48 evolved more complex vocal communication systems. The idea that social complexity drives
49 vocal complexity derives support from the positive relationship in non-human primates
50 between vocal repertoire size and group size, and with time spent grooming [McComb and
51 Semple, 2005]. For example, red-capped mangabeys (*Cercocebus torquatus*), which live in
52 large and relatively despotic multimale-multifemale groups with frequent interactions, have a
53 more complex vocal repertoire compared to De Brazza's monkeys (*Cercopithecus neglectus*),
54 which live in small family groups [Bouchet et al., 2013].

55

56 Knowledge of particular types of vocalisations can contribute to our understanding of
57 behaviour, evolution and taxonomy. Spectral parameters of calls reflect requirements for
58 transmission in different habitats, as well as constraints imposed by body size [Masters,
59 1991]. The 'Acoustic Adaptation Hypothesis' relates acoustic variables to habitat features
60 (e.g. density of vegetation) [Ey and Fischer, 2009], yet primate vocalisations are particularly
61 resilient to modification by the immediate environment [Doyle, 1978]. Within a species'

62 vocal repertoire, close-range vocalisations are predicted to be more variable (i.e. less distinct)
63 than long-range vocalisations (often referred to as ‘loud calls’ or ‘advertisement calls’)
64 [Marler, 1967]. Among primates, loud calls are indicators of taxonomic delineation and many
65 African monkeys can be readily distinguished on the basis of their loud calls [Struhsaker,
66 1970; Waser, 1982; Jones et al., 2005].

67

68 Intraspecific geographic variation in calls can result from differences in habitat, mechanisms
69 of social learning, and genetic isolation. Geographic variation in calls is common in
70 songbirds, who learn their vocalisations from conspecifics. This variation may be the result of
71 inaccurate copying [Slater, 1989; Podos and Warren, 2007]. Less is known about geographic
72 variation in primate vocalisations, although orangutans (*Pongo* spp.) [Delgado, 2007] and
73 savanna monkeys (*Chlorocebus* spp.) [Price et al., 2014] show differences in temporal and
74 spectral call structure among populations. Intraspecific differences in call structure also occur
75 among populations of robust chimpanzees (*Pan troglodytes*) [Mitani et al., 1999; Crockford
76 et al., 2004], silvery gibbons (*Hylobates moloch*) [Dallmann and Geissmann, 2001]), and
77 pygmy marmosets (*Callithrix pygmaea*) [De La Torre and Snowdon, 2009]. In strepsirhines,
78 comparison of the spectral parameters of the loud call is the most useful diagnostic tool for
79 subspecies identification [Zimmermann, 2012].

80

81 Non-human primates are historically described as non-learners with respect to the
82 development of their vocal system. Evidence is accumulating, however, that acoustic
83 plasticity is present in non-human primates and that it consists of subtle acoustic changes on
84 top of innately determined call structures [Roian Egnor and Hauser, 2004]. This implies that
85 spectral and temporal parameters in the vocalisations of primates could differ among
86 populations as a result of social learning, adaptation to local habitats, or drift.

87

88 Vocalisations can convey information about the caller’s identity [Owren et al., 1997;
89 Herbinger et al., 2009], size [Reby et al., 2005], physical condition [Benítez et al., 2016],
90 motivation [Silk et al., 2000; Manser, 2001], and about external events such as predator
91 presence or type [Seyfarth et al., 1980; Zuberbühler, 2001]. Some call types, such as those
92 indicating presence of a predator, are predicted to be less variable than those more strongly
93 influenced by sexual selection, and containing information about the caller’s identity and/or
94 quality [Butynski et al., 1992; Irwin et al., 2008]. Geographic variation in vocal behavior has

95 been described in a wide range of species, but generally not in alarm calls [Zuberbuhler
96 2009].

97

98 Galagos (Galagidae) have large, diverse, species-specific vocal repertoires. Their
99 vocalisations have contributed to species delineation (e.g. southern lesser galago (*Galago*
100 *moholi*), northern lesser galago (*G. senegalensis*), Somali galago (*G. gallarum*), Angolan
101 dwarf galago (*Galagoides kumbirensis*) [Zimmermann et al., 1988; Zimmermann, 1995;
102 Butynski and De Jong, 2004, Svensson et al., 2017]. There has been no attempt, however, to
103 explore variation in call structure among populations of the same species of *Otolemur*.

104

105 The small-eared greater galago (*Otolemur garnettii*) is the second largest galago species with
106 a mean adult body weight ca. 770 g [Olsen and Nash, 2002]. This species is endemic to
107 eastern Africa where it occurs in forest and woodland. There are currently four recognized
108 subspecies [Grubb et al., 2003; Harcourt and Perkin, 2013; De Jong and Butynski, 2018; De
109 Jong et al., in press]: Kikuyu small-eared galago (*O. g. kikuyuensis*) in the Kenya Highlands
110 east of the Eastern Rift Valley (e.g. Nairobi, Ngong Hills, Aberdare Range, Mount Kenya,
111 Mathews Range); Pangani small-eared galago (*O. g. panganiensis*) in extreme central
112 southern Kenya (Loita Hills, Tavetta), southwards through eastern Tanzania (Mount
113 Kilimanjaro, Lake Manyara, Eastern Arc Mountains, Southern Highlands) to extreme
114 northern Mozambique (Ruvuma River); Zanzibar small-eared galago (*O. g. garnettii*) is
115 restricted to three Tanzanian islands in the Indian Ocean (Zanzibar, Pemba, Mafia); white-
116 tailed small-eared galago (*O. g. lasiotis*) in extreme northeastern Tanzania (Tanga)
117 northwards along the coastal plain of Kenya to the Juba River in southern Somalia (Fig. 1)
118 [De Jong and Butynski 2009, 2018; Harcourt and Perkin, 2013; De Jong et al., in press].

119

120 [[[Figure 1. here

121

122 Kessler et al. [2015] compared temporal and frequency parameters of what they referred to
123 as *O. garnettii* ‘bark calls’ from eight captive individuals. They described barks as individual
124 call units that are emitted either singly or as part of a longer, more complex, call series. They
125 equated the call series to ‘trailing calls’ [Bearder et al., 1995] and ‘long calls’ [Becker et al.,
126 2003]. The long calls identified by Becker et al. [2003] do not, however, follow the same
127 structure as the trailing calls described by Bearder et al. [1995]. In addition, the calls analysed
128 by Kessler et al. [2015] were triggered by disturbance. They may not be equivalent to the call

129 units that comprise spontaneous trailing calls, but rather are more similar to alarm calls. They
130 found what they described as ‘moderate’ evidence for individual difference but no evidence
131 for difference based on sex.

132

133 Here we provide an overview of the vocal repertoire of wild populations of *O. garnettii* and
134 compare alarm calls and trailing calls (both referred to as ‘loud calls’ in many publications)
135 of *O. g. panganiensis* and *O. g. kikuyuensis*.

136

137 **Methods**

138

139 *Study Sites*

140 We recorded vocalisations of *O. g. panganiensis* at two sites in northern Tanzania one
141 encompassing narrow strips of riverine woodland and the other a banana plantation, and
142 vocalisations of *O. g. kikuyuensis* from four sites in central Kenya, which include mid
143 montane forest and riverine or non-riverine woodland (Fig.1; Table 1). These sites represent a
144 range of habitats and populations.

145

146 [Table 1 here.

147

148 *Recordings of Vocalisations*

149 We recorded calls using one of two methods. First, in Kwa Kuchinja and Aberdare NP, we
150 placed one or three autonomous recording units (ARUs, Wildlife Acoustics SM2) at sites
151 where *O. garnettii* were known to occur. We placed the ARUs >800 m apart and set them to
152 record continuously from 10 min before sunset until 10 min after sunrise. Recordings were
153 made in stereo at a sampling frequency of 44.1 kHz and 16 bits and saved in wav format. At
154 Mweka, Meru FR, Meru NP and Masinga Reservoir, we manually recorded calls using a
155 Marantz PMD660 recorder and Sennheiser ME67 (Mweka) or ME66 (all other sites)
156 microphone, with a sampling frequency of 44.1 kHz.

157

158 *Acoustic Analyses*

159 Using ARUs, we collected 12 hours and 20 minutes of audio data during one night in
160 Aberdare NP, and 169 hours of audio data across 16 nights in Kwa Kuchinja. We used Syrinx
161 software (J. Burt, Seattle, WA, USA) to visually identify and extract calls. Any recording that
162 looked like a potential *O. garnettii* call was confirmed by ear. We then categorised

163 vocalisations based on temporal parameters, repetition rate, and frequency and amplitude
164 modulations, and matched them where possible to the equivalent call in previous studies. We
165 calculated the call rate as the number of calls/hour.

166

167 For structural analysis, we concentrated on the two most common call types; ‘trailing calls’
168 (most likely advertisement calls) and ‘clustered squawk’ (most likely an alarm call, possibly
169 the same as the ‘loud calls’ or ‘barks’ of Becker et al. [2003]). We used Avisoft SASlab pro
170 version 5.2 (Avisoft Bioacoustics, Berlin, Germany) to measure seven temporal and spectral
171 parameters of a smaller sample of good quality trailing calls and clustered squawks. Trailing
172 calls (Fig. 2) start with a rather flat unit (or note) followed by a number of frequency
173 modulated units and increases in level towards the end of the call. Clustered squawks (Fig. 2)
174 start with the loudest unit and subsequent units have a lower level. For each call we
175 measured: (a) call duration from the start of the first unit to the end of the last unit (sec); (b)
176 unit duration for all units in a call (sec); (c) interunit duration between all units in a call (sec);
177 (d) number of units in a call; (e) peak harmonic (the harmonic with the highest amplitude); (f)
178 minimum and maximum peak frequencies of the fundamental harmonic (Hz), measured from
179 the unit within a call with the lowest peak, and the unit with the highest peak frequency in
180 their fundamental harmonic, respectively. We compared these call parameters between *O. g.*
181 *kikuyuensis* and *O. g. panganiensis*.

182

183 [[[Fig. 2 here.

184

185 We conducted all statistical analyses in R [R Core Team 2013]. For each call we calculated
186 mean unit duration, mean interunit duration, and mode peak harmonic. Peak harmonic
187 reflects the harmonic that is most likely to contain the frequency with the highest energy.
188 Since the number of harmonics is not a continuous variable we used the mode. Subsequently,
189 we tested all parameters for normality using the Shapiro-Wilks’ Test. None of the variables
190 for trailing calls were normally distributed (all p values <0.003). For clustered squawks, only
191 mean unit duration, mean interunit duration, and minimum peak frequency of the
192 fundamental frequency were normally distributed (all other p values <0.006).

193

194 Call parameters were compared using the nrmv package available at [http://CRAN.R-](http://CRAN.R-project.org/package=nrmv)
195 [project.org/package=nrmv](http://CRAN.R-project.org/package=nrmv). This package performs nonparametric multivariate analysis of
196 variance (MANOVA) tests based on the inference methodology described by Bathke et al.

197 [2008]. It allows multiple analysis of variance-like nonparametric tests in situations where
198 the classical, parametric multiple analysis of variance is not applicable (e.g. small or
199 unequal sample sizes, no normal distribution) [Ellis et al. 2017]. Nonparametric ‘relative
200 effects’ are provided as effect estimators. ‘Relative effects’ measure the probability that a
201 value obtained from one experimental group is larger than a value randomly chosen from the
202 whole dataset. In addition to these global hypothesis tests, the package provides a more
203 detailed comparison of which variables or factor levels contribute to any significant
204 differences.

205

206

207 **Results**

208 *Vocal Repertoire*

209

210 The vocal repertoire recorded at Kwa Kuchinja and Aberdare NP, along with temporal
211 measures and the likely equivalent calls from previous studies, is described in Table 2. The
212 audio files for each call type are presented on www.wildsolutions.nl/garnettii. Spectral
213 measures and samples sizes of the high-quality calls analysed are provided in Table 3. The
214 two most frequently recorded call types are the trailing call and clustered squawk; both are
215 given throughout the night (Fig. 3). Trailing calls start to be produced after 19:00 h and
216 continue until shortly after 06:00 h. Trailing calls elicited a responding trailing call from at
217 least one other individual in 47.6% of cases. There is little variation in the temporal
218 distribution of trailing calls, although they increase slightly during 04:00-06:00 h. There are
219 few clustered squawks before 19:00 h or after 06:00 h, whereas their occurrence increases
220 slightly during 00:00-05:00 h.

221

222 [[[Table 2 here

223

224 It is not possible to determine the number of individuals recorded by this study. However,
225 based on overlapping calls, distance among callers, and knowledge of the population at the
226 study sites, we conservatively estimate a minimum of three individuals at Aberdare NP, nine
227 at Kwa Kuchinja, two at Mweka and Meru NP, three at Meru FR, and one at Masinga
228 Reservoir.

229

230 [[[Table 3 and fig. 3 here.

231

232 *Subspecific Differences*

233 We analysed a total of 31 clustered squawks and 258 trailing calls for subspecific differences.
234 Due to differences in sampling effort and methods, we had a greater number of *O. g.*
235 *panganiensis* calls (clustered squawk: Kwa Kuchinja N=20, Mweka N=2; trailing call: Kwa
236 Kuchinja N= 218; Mweka N=4) than *O. g. kikuyuensis* calls (clustered squawk: Aberdare NP
237 N=5, Meru FR N=3, Masinga N=1; trailing call: Aberdare NP N=18, Meru FR N=12, Meru
238 NP N=6).

239

240 Trailing calls differed significantly between the two subspecies (Fig. 2); ANOVA-type test
241 value $F_{3,96,490.56} = 21.61$, $p < 0.001$. Relative effects (Table 4) show that for the variables
242 interunit duration, minimum fundamental peak, and mode peak harmonic, the probability that
243 a randomly chosen call from *O. g. kikuyuensis* exhibited a higher value than a randomly
244 chosen call from the full data set was 83%, 79% and 61%, respectively. The probability that a
245 randomly chosen trailing call from *O. g. panganiensis* exhibited a higher value than a
246 randomly chosen call from the full data set was 74% for number of units and 68% for call
247 duration. Posthoc tests for individual variables showed that subspecies differed significantly
248 in: number of units ($F_{1,123.98} = 37.57$; $p < 0.001$), interunit duration ($F_{1,123.98} = 51.95$; $p < 0.001$),
249 call duration ($F_{1,123.98} = 18.43$; $p < 0.001$), mode peak harmonic ($F_{1,123.98} = 6.58$; $p = 0.01$), and
250 minimum peak frequency of the fundamental harmonic ($F_{1,123.98} = 53.28$; $p < 0.001$; Fig. 4).

251

252 [[[Table 4 here.

253 Figure 4 here.

254

255 Clustered squawks also differed between the two subspecies (Fig.2); ANOVA-type test value
256 $F_{3,50,81.21} = 3.145$, $p = 0.023$. Relative effects (Table 4) show that for the variables mode peak
257 harmonic and interunit duration the probability that a randomly chosen clustered squawk
258 from *O. g. kikuyuensis* exhibited a higher value than a randomly chosen clustered squawk
259 from the full data set was 77% and 61%, respectively. The probability that a randomly chosen
260 clustered squawk from *O. g. panganiensis* exhibited a higher value than a randomly chosen
261 clustered squawk from the full data set was 74% for number of units, 72% for call duration,
262 and 81% for maximum peak frequency of the fundamental harmonic. Posthoc tests for
263 individual variables show that subspecies differed significantly in number of units ($F_{1,23.17}$

264 =4.58; $p=0.04$), mode peak harmonic ($F_{1,23,17}=7.89$; $p = 0.01$), and maximum peak frequency
265 of the fundamental harmonic ($F_{1,23,17} = 7.11$; $p=0.014$; Fig. 5).

266 [[[Fig 5 here.

267

268 **Discussion**

269

270 This study is the first to present an overview of the range of *O. garnettii* calls produced by
271 wild populations in Tanzania and Kenya, as well as the temporal distribution of the two most
272 common calls, clustered squawks and trailing calls. We show that *O. g. panganiensis* and *O.*
273 *g. kikuyuensis* differ in the structure of these two calls, with trailing calls differing in more
274 parameters than clustered squawks.

275

276 We describe 11 call types for *O. garnettii*. The currently described vocal repertoires of
277 nocturnal primates ranges from two in Calabar angwantibo (*Arctocebus calabarensis*)
278 [Charles-Dominique, 1977]) to 18 in northern lesser bushbaby (*Galago senegalensis*)
279 [Zimmermann, 1985]. In contrast, for diurnal primates the size of the vocal repertoire ranges
280 from six in the common woolly monkey (*Lagothrix lagotricha*) [Casamitjana, 2002] to 38 in
281 bonobos (*Pan paniscus*) [Bermejo and Omedes, 1999].

282

283 The graded call system [Marler, 1977] of *O. garnettii* makes it challenging to separate some
284 call types. Our estimate of 11 call types is, therefore, conservative. Some call types were
285 recorded more frequently than others. This is partly a reflection of the recording method as
286 ARUs do not record low volume calls unless the caller is close to the recorder. This implies
287 that close contact calls are underrepresented relative to the louder advertisement and alarm
288 calls. Since we did not record at least three call types previously reported from captive *O.*
289 *garnettii* at close proximity [Becker et al. 2003], or in response to artificial stimuli [Kessler et
290 al., 2015], it is likely that the vocal repertoire presented here can be expanded by at least
291 three call types. Nonetheless, among nocturnal primates, only *G. senegalensis*, with 18 call
292 types, is known to have more call types than *O. garnettii*.

293

294 Vocal repertoire size has been linked to social complexity in primates [McComb and Semple
295 2005]. Like many other nocturnal primates, *O. garnettii* has a dispersed social system where
296 females, but not males, have over-lapping home ranges [Nash and Harcourt, 1986]. These
297 authors state that adults usually sleep alone whereas at Kwa Kuchinja adults frequently sleep

298 in pairs or even groups of up to four (C. Bettridge, unpublished data). It is possible that
299 variation in the social organization of *O. garnettii* is influenced by habitat, and specifically
300 the availability of suitable vegetation [Nash and Harcourt, 1986]. The relatively large vocal
301 repertoire of *O. garnettii* suggests that individuals which sleep or forage alone remain in
302 regular contact with conspecifics [Charles-Dominique, 1978].

303

304 There is little temporal variation in the occurrence of trailing calls and clustered squawks;
305 both are produced throughout the night between 19:00 h and 06:00 h. Loud calls are common
306 across a wide range of primate taxa and are species-specific acoustic signals that carry over
307 long distances [Gautier and Gautier, 1977; Hohmann and Fruth, 1995; Wich and Nunn, 2002;
308 Delgado, 2006]. They are thought to advertise the location of the caller and to provide other
309 information such as caller identity, sex or quality. Most nocturnal prosimians use loud,
310 repeated, distinct calls for distant communication [Zimmermann, 1990].

311

312 It is most likely that the trailing call of *O. garnettii* functions as an advertisement call.
313 Additional evidence for this is that 50% of all trailing calls triggered a responding trailing call
314 from conspecifics (Table 2). The slight increase in the occurrence of trailing calls during
315 04.00–06.00 h may be related to maintaining social cohesiveness prior to reaching sleeping
316 sites, as in the golden brown mouse lemur (*Microcebus ravelobensis*) [Braune et al., 2005].
317 The function of clustered squawks is less clear, although they have been described as distress
318 or alarm calls [Honest, 1996; Becker et al., 2003].

319

320 In this study we identified variation in the acoustic structure of the trailing call and clustered
321 squawk between *O. g. panganiensis* and *O. g. kikuyuensis*. The trailing call of *O. g.*
322 *panganiensis* is of lower frequency than that of *O. g. kikuyuensis*. In contrast, the clustered
323 squawk of *O. g. panganiensis* is of higher frequency compared to *O. g. kikuyuensis*.

324

325 It has been proposed that in closed habitats vocalisations will be longer with a lower
326 repetition rate and lower frequencies as an adaptation to the habitat-specific transmission
327 requirements (see Ey and Fischer [2009] for a review). *Otolemur garnettii* lives in forests,
328 forest-agriculture mosaics, and woodlands. The majority of *O. g. panganiensis* calls used in
329 this study were obtained from a narrow strip of riverine woodland that is surrounded by
330 savanna. The vegetation in this habitat is less dense than in the forests where the *O. g.*
331 *kikuyuensis* calls were recorded. It seems unlikely, therefore, that the lower frequency of the

332 trailing call of *O. g. panganiensis* is related to habitat structure. Furthermore, if habitat-
333 specific transmission requirements select for spectral structure of calls, then we would expect
334 both the trailing call and the clustered squawk to differ in the same direction in the two
335 subspecies. We conclude, therefore, that the differences observed in this study are likely the
336 result of lack of gene-flow (isolation), rather than an adaptation to local sound transmission
337 characteristics.

338

339 *Otolemur g. panganiensis* appears to be separated from *O. g. kikuyuensis* by a geographical
340 gap of ca. 45 km between Nairobi and Lake Magadi that includes the Taru Desert and
341 Eastern Rift Valley (Fig. 1). The floor of the Eastern Rift Valley at Lake Magadi is low (600
342 m asl), wide (>32 km), dry (mean annual rainfall ca. 400 mm), hot (mean maximum
343 temperature ca. 35 °C [Bennun and Njoroge, 1999]), and lacks perennial rivers [Butynski and
344 De Jong, 2018]. This gap lies within the Northern *Acacia-Commiphora* Bushlands and
345 Thickets Ecoregion [Olsen et al., 2001]. The habitats in this arid region are unsuitable for *O.*
346 *garnettii* and, serve as a barrier between *O. g. panganiensis* and *O. g. kikuyuensis*.

347 The shortest distance between the two *O. g. panganiensis* recording sites and the four *O. g.*
348 *kikuyuensis* recording sites is ca. 280 km (between Mweka and Masinga Reservoir). This area
349 is largely comprised of the Taru Desert which also lies within the Northern *Acacia-*
350 *Commiphora* Bushlands and Thickets Ecoregion. The Taru Desert includes the Amboseli
351 Basin and Tsavo West NP, extends westward towards Lake Magadi and northward to the
352 Athi River near Nairobi (Fig. 1). The Taru Desert is ca. 1100–1500 m asl, has a mean annual
353 rainfall of ca. 300 mm, and holds no perennial rivers [Butynski and De Jong, 2018], and,
354 therefore, does not have habitats suitable for *O. garnettii*. The Taru Desert separates three
355 subspecies of *O. garnettii*: *panganiensis*, *kikuyuensis*, *lasiotis*.

356

357 The Eastern Rift Valley and Taru Desert are also barriers for other forest-dependent primates;
358 Mount Kilimanjaro guereza (*Colobus caudatus*), Mount Kenya guereza (*Colobus guereza*
359 *kikuyuensis*), Mau Forest guereza (*Colobus guereza matschiei*) [Butynski and De Jong,
360 2018]; Zanzibar Sykes's monkey (*Cercopithecus mitis albogularis*), Kolb's monkey
361 (*Cercopithecus mitis kolbi*). In addition, they represent the northern limit for miombo silver
362 galago (*Otolemur crassicaudatus monteiri*) [De Jong and Butynski 2018].

363

364 Identifying the degree of intraspecific variation in calls is important if differences in calls are
365 to be used in taxonomic assessments or to determine which calls and call parameters relate to

366 transmission requirements in their habitat rather than to genetic divergence. Comparing our
367 results for the trailing call of *O. g. panganiensis* and *O. g. kikuyuensis* with those obtained by
368 Masters [1991] for *O. g. lasiotis* and large-eared greater galago *Otolemur crassicaudatus*,
369 shows some interesting patterns. Parameters for the trailing call for the three subspecies of *O.*
370 *garnettii* differ from those of *O. crassicaudatus* — particularly the temporal features. This is
371 congruent with the current taxonomy (Table 5).

372

373 The trailing call also differs among the three subspecies of *O. garnettii*; call length and
374 number of call units are more similar between *O. g. kikuyuensis* and *O. g. lasiotis* than
375 between *O. g. kikuyuensis* and *O. g. panganiensis* or between *O. g. lasiotis* and *O. g.*
376 *panganiensis* (Table 5). Some of these spectral differences might be attributed to different
377 measurement techniques used by Masters (1991) and this study. We cannot, however,
378 exclude the possibility that the differences are genuine, and could support further taxonomic
379 revision.

380

381 Using ARUs to record the calls means we do not know the number of calling individuals, nor
382 the sex and age of the individuals in our sample. This is counteracted, to some extent, by the
383 use of multiple ARUs at Kwa Kuchinja, by placing ARUs in different locations on different
384 nights, and by the inclusion of calls from multiple, distant populations of each subspecies.
385 Evidence from Kessler et al. [2015] suggests that *O. garnettii* calls cannot be discriminated
386 by sex, so the differences in our results are unlikely to be due to sex differences in our
387 sample. Age is also unlikely to be a major factor, as in mammals, alarm calls in particular
388 undergo minor modification during the individual's development. Alarm calls in young
389 individuals appear almost adult-like on first expression, suggesting that there is little role for
390 vocal learning in the production of alarm calls [Blumstein and Munos, 2005; Hollén and
391 Radford, 2009].

392

393 Most of the calls used in this study were recorded over a short time. Although some
394 recordings were obtained from both subspecies at the same time of year, we do not have
395 enough information about the study populations to know how they might relate to mating
396 season. More data on the behavioural context of calls, perhaps obtained using playbacks to
397 elicit responses, would shed light on the function both of the trailing call and clustered
398 squawks.

399

400

401 **Conclusion**

402 The trailing call and clustered squawk (both long-distance calls) of *O. garnettii*, differ
403 between *O. g. panganiensis* and *O. g. kikuyuensis*. This difference appears to be the result of
404 a lack of gene-flow across habitats in southern Kenya that are unsuitable for *O. garnettii*.
405 These differences confirm putative classification based on morphological traits of these
406 populations into subspecies [Grubb et al., 2003; Harcourt and Perkin, 2013]. ARUs are useful
407 tools for behavioural and taxonomic studies of galagos and other nocturnal primates.

408

409 **Acknowledgements**

410 Data in Tanzania were collected by students at the College of African Wildlife Management,
411 Mweka. We are grateful to the College of African Wildlife Management for permission to
412 record calls on their property, and logistical support in the field, and to TAWIRI for granting
413 permission to record galago calls (Reference: AD.C.12 Vol.11.68.2014). We thank Amie
414 Wheeldon for providing recordings from the Aberdare NP, and Kenya Wildlife Service and
415 NACOSTI for permission to record calls at the four sites in Kenya
416 (NACOSTI/P/18/5151/21938). Andrew Wolfenden and Roisin Stanbrook kindly helped set-
417 up and monitor the ARUs. Finally, we are grateful to the two anonymous reviewers whose
418 thoughtful comments greatly improved the manuscript.

419

420 **Statement of Ethics**

421 The authors have no ethical conflicts to disclose.

422

423 **Disclosure Statement**

424 The authors have no conflicts of interest to declare.

425

426 **Author contributions**

427 CB and SdK formulated the original idea and designed the study. CB, SdK, TB & YDJ
428 collected acoustic recordings in the field. SPK and SdK conducted acoustic analysis. CB and
429 SPK carried out statistical analysis. CB took the lead in writing the manuscript. All authors
430 provided critical feedback and contributed to the writing of the manuscript.

431

432 **References**

- 433
- 434 Bathke AC, Harrar SW, Madden LV (2008). How to compare small multivariate samples
435 using nonparametric tests. *Computational Statistics and Data Analysis* 52: 4951–4965.
- 436 Bearder SK, Honess PE, Ambrose L (1995). Species diversity among galagos with special
437 reference to mate recognition. In *Creatures of the Dark* (Alterman L, Doyle GA, Izard MK,
438 eds.), pp. 331–352. Boston, Springer.
- 439 Becker ML, Buder EH, Ward JP (2003). Spectrographic description of vocalizations in
440 captive *Otolemur garnettii*. *International Journal of Primatology* 24: 415–446.
- 441 Benítez ME, le Roux A, Fischer J, Beehner JC, Bergman TJ (2016). Acoustic and temporal
442 variation in gelada (*Theropithecus gelada*) loud calls advertise male quality. *International*
443 *Journal of Primatology* 37: 568–585.
- 444 Bennun L, Njoroge P (1999). *Important Bird Areas in Kenya*. East African Natural History
445 Society, Nairobi, Kenya.
- 446 Bermejo M, Omedes A (1999). Preliminary vocal repertoire and vocal communication of
447 wild bonobos (*Pan paniscus*) at Lilungu (Democratic Republic of Congo). *Folia*
448 *Primatologica* 70: 328–357.
- 449 Blumstein DT, Munos O (2005). Individual, age and sex-specific information is contained in
450 yellow-bellied marmot alarm calls. *Animal Behaviour* 69: 353–361.
- 451 Bouchet H, Blois-heulin C, Lemasson A (2013). Social complexity parallels vocal
452 complexity: a comparison of three non-human primate species. *Frontiers in Psychology* 4: 1–
453 15.
- 454 Bradbury JW, Vehrencamp SL (2001). *Principles of Animal Communication* (2nd ed.).
455 Sunderland, MA: Sinauer Associates.
- 456 Braune P, Schmidt S, Zimmermann E (2005). Spacing and group coordination in a nocturnal
457 primate, the golden brown mouse lemur (*Microcebus ravelobensis*): the role of olfactory and
458 acoustic signals. *Behavioral Ecology and Sociobiology* 58: 587–596.
- 459 Butynski TM, Chapman CA, Chapman LJ, Weary DM (1992). Use of male blue monkey

460
461 pyow calls for long-term individual identification. *American Journal of Primatology* 28:
462
463 183–189.
464
465 Butynski TM, De Jong YA (2004). Natural history of the Somali lesser galago (*Galago*
466 *gallarum*). *Journal of East African Natural History* 93: 23–38.
467
468 Butynski TM, De Jong YA (2018). Geographic range, taxonomy, and conservation of the
469 Mount Kilimanjaro guereza colobus monkey (Primates: Cercopithecidae: *Colobus*). *Hystrix*
470 29: 81–85.
471
472 Casamitjana J (2002). The vocal repertoire of the woolly monkey *Lagothrix lagothricha*.
473 *Bioacoustics* 13: 1–19.
474
475 Charles-Dominique P (1977). *Ecology and Behaviour of Nocturnal Primates*. New York
476 Columbia University Press.
477
478 Charles-Dominique P (1978). Solitary and gregarious prosimians: Evolution of social structure
479 in primates. In *Recent Advances in Primatology Vol. 3*. (Chivers DJ, Joysey KA, eds.). pp.
480 139–149. London, Academic Press.
481
482 Crockford C, Herbinger I, Vigilant L, Boesch C (2004). Wild chimpanzees produce group-
483 specific calls: a case for vocal learning? *Ethology* 110: 221–243.
484
485 Dallmann R, Geissman T (2001). Different levels of variability in the female song of wild
486 silvery gibbons (*Hylobates moloch*). *Behaviour* 138: 629–648.
487
488 De Jong YA, Butynski TM (2009). *Primate biogeography, diversity, taxonomy and*
489 *conservation of the coastal forests of Kenya*. Unpublished report for the Critical Ecosystem
490 Partnership Fund, Washington, DC. 142 pp. Available at: www.wildsolutions.nl.

491 De Jong YA, Butynski TM (2018). *Primates of East Africa Pocket Identification Guide*.
492 Global Wildlife Conservation Tropical Pocket Guide Series, Austin, Texas. Available at:
493 <http://www.wildsolutions.nl/primates-east-africa-pocket-identification-guide/>.
494
495 De Jong YA, Butynski TM, Perkin AW, Svensson MS (In press). *Otolemur garnettii*. *The*
496 *IUCN Red List of Threatened Species 2018*. IUCN Species Survival Commission, IUCN,
497 Gland, Switzerland.
498
499 De La Torre S, Snowden C (2009). Dialects in pygmy marmosets? Population variation in
500 call structure. *American Journal of Primatology* 71: 333–342.
501
502 Delgado RA (2006) Sexual selection in the loud calls of male primates: signal content and
503 function. *International Journal of Primatology* 27: 5–25.
504
505 Delgado RA (2007). Geographic variation in the long calls of male orangutans (*Pongo* spp.).
506 *Ethology* 113: 487–498.
507
508 Doyle GA, (1978) Discussion of behavioural factors in prosimian evolution. In *Recent*
509 *Advances in Primatology Vol. 3*. (Chivers DJ, Joysey KA, eds.). pp. 151–157. London,
510 Academic Press.
511
512 Ey E, Fischer J (2009). The 'Acoustic Adaptation Hypothesis' - a review of the evidence from
513 birds, anurans and mammals. *Bioacoustics* 19: 21–48.
514
515 Fischer J, Wadewitz P, Hammerschmidt K (2017). Structural variability and communicative
516 complexity in acoustic communication. *Animal Behaviour* 134: 229–237.
517
518 Freeberg TM, Dunbar RIM, Ord TJ (2012). Social complexity as a proximate and ultimate
519 factor in communicative complexity. *Philosophical Transactions of the Royal Society B:*
520 *Biological Sciences* 367: 1785–1801.
521
522 Gautier JP, Gautier A (1977). Communication in Old World monkeys. In *How Animals*
523 *Communicate* (Sebeok TA, ed.), pp. 890–964. Bloomington, Indiana University Press.

524 Grubb P, Butynski TM, Oates JF, Bearder SK, Disotell TR, Groves CP, Struhsaker TT
525 (2003). Assessment of the diversity of African primates. *International Journal of*
526 *Primateology* 24: 1301–1357.

527

528 Harcourt CS, Perkin AW (2013). *Otolemur garnettii* small-eared greater galago. In *Mammals*
529 *of Africa*. Volume II: *Primates*. (Butynski TM, Kingdon J, Kalina J, eds.), pp. 413–416.
530 Bloomsbury, London.

531 Herbinger I, Papworth S, Boesch C, Zuberbühler K (2009). Vocal, gestural and locomotor
532 responses of wild chimpanzees to familiar and unfamiliar intruders: a playback study. *Animal*
533 *Behaviour* 78: 1389–1396.

534 Hohmann G, Fruth B (1995). Long-distance calls in great apes: sex differences and social
535 correlates. In *Current Topics in Primate Vocal Communication* (Zimmerman E, Newman JD,
536 Jürgens U, eds.), pp. 161–184. New York, Plenum.

537 Hollén LI, Radford AN (2009). The development of alarm call behaviour in mammals and
538 birds. *Animal Behaviour* 78: 791–800.

539 Honess PE (1996). *Speciation among galagos (Primates, Galagidae) in Tanzanian forests*.
540 PhD thesis. Oxford Brookes University, Oxford, UK.

541 Jones T, Ehardt C, Butynski TM, Davenport TRB, Mpunga NE, Machaga SJ, De Luca DW
542 (2005). The highland mangabey *Lophocebus kipunji*: a new species of African monkey.
543 *Science* 308: 1161–1164.

544 Kessler SE, Scheumann M, Hanbury DB, Nash LT, Zimmermann E, Watson SL (2015).
545 Screams in the night: Pilot study reveals moderate evidence for individual differences in
546 lorisoid vocalizations. *International Journal of Primatology* 36: 666–678. doi:
547 10.1007/s10764-015-9847-z.

548 Manser MB (2001). The acoustic structure of surcates' alarm calls varies with predator type
549 and the level of response urgency. *Proceedings of the Royal Society B: Biological Sciences*
550 268: 2315–2324.

551 Marler P (1967). Animal communication signals. *Science* 157: 769–774.

552 Marler P (1977). The structure of animal communication sounds. In *Recognition of Complex*
553 *Acoustic Signals* (Bullock TH, ed), pp. 17–35, Berlin, Springer Verlag.

554 Masters JC (1991). Loud calls of *Galago crassicaudatus* and *G. garnettii* and their relation
555 to habitat structure. *Primates* 32: 153–167.

556 McComb K, Semple S (2005). Coevolution of vocal communication and sociality in
557 primates. *Biology Letters* 1: 381–385.

558 Mitani JC, Hunley KL, Murdoch ME (1999). Geographic variation in the calls of wild
559 chimpanzees: a reassessment. *American Journal of Primatology* 47: 133–151.

560 Nash LT, Harcourt CS (1986) Social organization of galagos in Kenyan coastal forests II:
561 *Galago garnettii*. *American Journal of Primatology* 10: 357–369.

562 Olsen DM, Dinerstein E, Wikramanayake ED, Burgess ND, Powell GVN, Underwood EC,
563 D'Amic JA, Itoua I, Strand HE, Morrison JC, Loucks CJ, Allnutt TF, Ricketts TH, Kura Y,
564 Lamoreux JF, Wettengel WW, Hedao P, Kassem KR (2001). Terrestrial ecoregions of
565 the world. *BioScience* 51: 933–938.

566 Olsen TR, Nash LT (2002) Galago (Galagonidae) body measurements and museum
567 collections data. *African Primates* 6: 50–53.

568 Owren MJ, Seyfarth RM, Cheney DL (1997). The acoustic features of vowel-like grunt calls
569 in chacma baboons (*Papio cynocephalus ursinus*). *Journal of the Acoustical Society of*
570 *America* 101: 2951–2963.

571 Podos J, Warren PS (2007). The evolution of geographic variation in birdsong. *Advances in*
572 *the Study of Behavior* 37: 403–457.

573 Price T, Ndiaye O, Hammerschmidt K, Fischer J (2014). Limited geographic variation in the
574 acoustic structure of and responses to adult male alarm barks of African green monkeys.
575 *Behavioral Ecology and Sociobiology* 68: 815–825.

576 Roian Egnor SE, Hauser MD (2004). A paradox in the evolution of primate vocal learning.
577 *Trends in Neuroscience* 27: 649–654.

578 Reby D, McComb K, Cargnelutti B, Darwin C, Fitch WT, Clutton-Brock TH (2005). Red
579 deer stags use formants as assessment cues during intrasexual agonistic interactions.
580 *Proceedings of the Royal Society B: Biological Sciences* 272: 941–947.

581 Seyfarth RM, Cheney DL, Marler PM (1980). Vervet monkey alarm calls: semantic
582 communication in a free- ranging primate. *Animal Behaviour* 28: 1070–1094.

583 Silk JB, Kaldor E, Boyd R (2000). Cheap talk when interests conflict. *Animal Behaviour* 59:
584 423–432.

585 Struhsaker TT (1970). Phylogenetic implications of some vocalizations
586 of *Cercopithecus* monkeys. In *Old World Monkeys: Evolution, Systematics and Behaviour*,
587 (Napier JR, Napier PH eds.), pp 365–444. New York Academic Press.

588

589 Svensson MS, Bersacola E, Mills MSL, Munds RA, Nijman V, Perkin A, Masters JC,
590 Couette S, Nekaris KAI, Bearder SK (2017). A giant among dwarfs: a new species of galago
591 (Primates: Galagidae) from Angola. *American Journal of Physical Anthropology* 163: 30–43.

592 Waser PM (1982). The evolution of male loud calls among mangabeys and baboons. In
593 *Primate Communication* (Snowdon T, Brown CH, Peterson MR, eds.), pp. 117–144.
594 Cambridge, UK, Cambridge University Press.

595 Wich SA, Nunn CL (2002). Do male long-distance calls function in mate defense? A
596 comparative study of long-distance calls in primates. *Behavioral Ecology and Sociobiology*
597 52: 474–484.

598 Zimmermann E (1985). The vocal repertoire of the adult Senegal bushbaby (*Galago*
599 *senegalensis senegalensis*). *Behaviour* 94: 212–233.

600 Zimmermann E (1990). Differentiation of vocalizations in bush-babies (Galagidae,
601 Prosimian, Primates) and the significance for assessing phylogenetic relationships. *Zeitschrift*
602 *für Zoologische Systematik und Evolutionsforschung* 28: 217–239.

603 Zimmermann E (1995). Loudcalls in nocturnal prosimians: structure, evolution, and ontogeny.
604 In *Current Topics in Primate Vocal Communication* (Zimmermann, E, Newman JD, Jürgens
605 U, eds.), pp. 47–72. New York, Plenum.

606 Zimmermann E (2012). Primate serenades: call variation, species diversity, and adaptation in
607 nocturnal Strepsirhines. In *Leaping Ahead. Developments in Primatology: Progress and*
608 *Prospects*. (Masters JC, Gamba M, Génin F, eds.), pp. 287–295. New York, Springer New
609 York.

610 Zimmermann E, Bearder SK, Doyle GA, Andersson AB (1988). Variations in vocal patterns
611 of Senegal and South African lesser bushbabies and their implications for taxonomic
612 relationships. *Folia Primatologica* 51: 87–105.

613 Zuberbühler K (2001). Predator-specific alarm calls in Campbell's monkeys, *Cercopithecus*
614 *campbelli*. *Behavioral Ecology and Sociobiology* 50: 414–422.

615

Table headings and figure legends

Table 1. The six sites at which recordings of the loud calls of small-eared greater galago (*Otolemur garnettii*) were obtained.

Table 2. Vocal repertoire of small-eared greater galago (*Otolemur garnettii*) showing temporal descriptive measures and equivalent calls from previous studies. Missing values are due to insufficient quality of recording.

Table 3: Frequency parameters for seven small-eared greater galago (*Otolemur garnettii*) calls.

Table 4. Relative effects of subspecies on acoustic parameters of small-eared greater galago (*Otolemur garnettii*) trailing calls and clustered squawks based on a nonparametric multivariate analysis of variance [Bathke et al. 2008]. Values range between 0-1 and indicate the chance that a randomly chosen call from a subspecies exhibits a higher value than a randomly chosen call from the full data set. Durations were measured in seconds; and frequencies in Hz.

Table 5: Species and population differences in trailing call parameters for large-eared greater galago (*Otolemur crassicaudatus*), white-tailed small-eared galago (*Otolemur garnettii lasiotis*), Pangani small-eared galago (*O. g. panganiensis*), and Kikuyu small-eared galago (*O. g. kikuyuensis*). The parameters include: N = total of number of calls included in analysis, LF(Hz) = mean lowest frequency in the fundamental harmonic, PF= the mean frequency with the highest amplitude in the call. Data for *O. crassicaudatus* and *O. g. lasiotis* from Masters [1991]).

Fig. 1: Geographic range of Pangani small-eared galago (*Otolemur garnettii panganiensis*) and Kikuyu small-eared galago (*Otolemur garnettii kikuyuensis*) in northern Tanzania and southern and central Kenya, with the recording locations depicted. Map based on De Jong & Butynski [2018] and De Jong et al. [in press].

Fig. 2: Cluster squawk (left panel) and a trailing call (right panel) of (top) Kikuyu small-eared galago (*Otolemur garnettii kikuyensis*) and (bottom) Pangani small-eared galago (*Otolemur garnettii panganiensis*). Within each panel, there is a spectrum (left), spectrogram (right), and waveform (top). The bottom right panel shows six temporal and spectral parameters measured for analyses: a) call duration (seconds); b) unit duration (seconds); c) inter-unit duration (seconds); d) harmonic with peak frequency (Hz); e) maximum peak frequency of fundamental harmonic (Hz); f) minimum peak frequency of fundamental harmonic (Hz). Parameter d) is also indicated on the top right panel to highlight difference between populations for that parameter. Spectrogram settings: FFT size = 1023; Hamming Window, temporal resolution = 8ms; frequency resolution = 8Hz.

Fig. 3: Temporal distribution of the trailing call and clustered squawk of the small-eared greater galago (*Otolemur garnettii*). The boxes represent the interquartile range (IQR); the heavy line is the median; and the whiskers represent 1.5xIQR; black dots are outliers.

Fig. 4: Trailing call of Pangani small-eared galago (*Otolemur garnettii panganiensis*) and Kikuyu small-eared galago (*Otolemur garnettii kikuyensis*): a) call duration (seconds); b) number of units in call; c) mean inter-unit duration (seconds); d) peak harmonic number; e) minimum peak frequency of fundamental harmonic (Hz). The boxes represent the interquartile range (IQR); heavy line is the median; whiskers represent 1.5xIQR; open circles are outliers.

Fig. 5: Clustered squawks of Pangani small-eared galago (*Otolemur garnettii panganiensis*) and Kikuyu small-eared galago (*Otolemur garnettii kikuyensis*): a) number of units in call; b) peak harmonic number; c) maximum peak frequency of the fundamental harmonic (Hz). The boxes represent the interquartile range (IQR); heavy line is the median; whiskers represent 1.5xIQR; open circles are outliers.