

1 **The use of ultrasonic communication to maintain social cohesion**
2 **in the Javan slow loris (*Nycticebus javanicus*)**

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Abstract

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Only a handful of primate taxa use ultrasonic vocalisations (those ≥ 20 kHz) to communicate. The extent and uses of ultrasonic communication remain poorly understood, potentially ranging from echolocation, advertisement of reproductive status and resource availability, social cohesion, to predator avoidance. Here, using active acoustics whereby the study subjects were observed throughout their activity period, we describe the first purely ultrasonic call from a strepsirrhine primate (family Lorisidae), recorded in a completely wild setting, and hypothesise about its function. We identified one type of ultrasonic call, the doublet click, from 14 Javan slow lorises (*Nycticebus javanicus*) produced by males and females of juvenile, subadult and adult ages within their social groups ($n=791$, $\bar{x}=46.0$ kHz). We ran Quadratic Discriminant Function Analysis finding dominant frequency and doublet click duration as the key parameters for identifying individuals' sex and age. Significantly more vocalisations were produced during affiliative social behaviour suggesting that the call serves a social cohesion function. Considering the range of other cryptic behaviours including slow and silent locomotion, and the high degree of territoriality associated with venomous attacks with conspecifics, the call may also serve as a safety strategy, allowing family members to monitor their territories from other slow lorises and to communicate cryptically whilst avoiding predators.

41 Introduction

42 Vocalisations with sonic (20 Hz – 20 kHz) fundamental and dominant frequencies, containing
43 harmonics reaching into the ultrasonic range, are relatively common in mammals. Yet only a
44 few species are known to emit pure ultrasonic vocalisations, occurring across a range of
45 orders (Carnivora, Cetacea, Chiroptera, Rodentia, Primates) [Gould et al., 1964; Blumberg,
46 1992; Wilson and Hare, 2004]. In general among animals, ultrasonic vocalisations have four
47 primary functions: echolocation, advertisement of reproductive status and resource
48 availability, social cohesion, and predator avoidance (crypsis) [Gould et al., 1964; Arch and
49 Narins, 2008; Coleman, 2009; Ramsier et al., 2012b].

50

51 Among Primates, smaller taxa predominantly use calls with ultrasonic components including
52 *Callithrix*, *Carlito*, *Cebuella*, *Cheirogaleus*, *Galago*, and *Microcebus*, with only two genera
53 larger than 1 kg known to use such calls -- *Nycticebus* and *Prolemur*. Only two species of
54 primates, both haplorhines, are reported to produce vocalisations purely in the ultrasonic
55 range: the Philippine tarsier (*Carlito syrichta*) and the spectral tarsier (*Tarsius tarsier*)
56 [Braune et al., 2005; Ramsier et al., 2012a; Gursky-Doyen, 2013; Gursky, 2015]. With the
57 exception of Gursky [2015], all previous studies of ultrasonic communication in these
58 primates have been conducted in captive or semi-captive settings. The function of ultrasonic
59 calls in primates remains an area of debate.

60

61 Slow lorises (*Nycticebus* spp.) are cryptic nocturnal primates, for which calls with ultrasonic
62 components have been identified in captivity. Slow lorises emit a range of sonic calls in
63 territorial or threatening situations, or during juvenile contact, all of which have ultrasonic
64 components. Captive infant Bengal slow lorises (*N. bengalensis* – the largest of the slow loris
65 species, reaching up to 2 kg) were previously documented emitting potentially pure
66 ultrasonic calls during times of danger (visual isolation from parent and human handling)
67 and while infants “explored” their environments [Zimmermann, 1981, 1985]. No
68 spectrograms or descriptions were produced for these calls. In wild studies of *N. pygmaeus*,
69 *N. coucang*, *N. menagensis* and *N. javanicus*, long distance calls, in particular sharp
70 monosyllabic whistles, are rarely heard by observers (Nekaris, unpublished data). Yet loris
71 behaviours, including the rapid pin-pointing of individuals beyond visual contact as well as
72 the assemblage of multiple animals at a sleep site without any obvious gathering cues,

73 suggest the presence of calls inaudible to human observers [Nekaris, 2003; Nekaris et al.,
74 2006].

75

76 *Nycticebus javanicus* is a nocturnal arboreal primate, with adults weighing on average 905 g.
77 They live mainly in uni-male, uni-female social groups with home-ranges of approximately
78 4.5-10 ha, and use venom to defend these territories [Nekaris, 2014]. For animals with
79 shorter distances between the ears, such as *N. javanicus*, information from the longer
80 wavelength of low frequency sounds is not picked up as readily as higher frequency ranges
81 [Heffner and Heffner, 1992; Popper and Fay, 1997]. The small body size of slow lorises may
82 have made ultrasonic contact calls evolutionarily favourable for communicating one's
83 location to conspecifics [Ramsier et al., 2012b]. Additionally, animals that live in an
84 environment with signal interference from broadband, low-frequency sounds—such as
85 forest insects and rain— may gain a significant increase in the signal propagation and clarity
86 of their calls by calling in a comparatively noise-free ultrasonic frequency range. This
87 increase in propagation and clarity may offset some of the attenuation issues associated
88 with high-frequency sounds. Although the hearing ability of *N. javanicus* remains unknown,
89 captive animals, subsequently identified as *N. bengalensis*, reached sensitivities of 45.3 kHz
90 [Ramsier and Dominy, 2010]. This hearing ability may also help slow lorises to detect
91 ultrasonic call-emitting insects on darker nights [Ramsier et al., 2015]. These factors point to
92 the high potential for presence of pure ultrasonic calls in wild Javan slow lorises.

93

94 Here, we present the first recordings and full description of a purely ultrasonic call made by
95 a strepsirrhine primate, the nocturnal Javan slow loris (*Nycticebus javanicus*). We define the
96 acoustic and temporal structure of the pure ultrasonic call, and examine its behavioural
97 context. We tested the potential function of the call, including echolocation, resource
98 advertisement, and social group cohesion, and as an anti-predator strategy. In particular, we
99 hypothesised an echolocation function if the call was used most often in contexts of feeding
100 on fast-moving prey; resource advertisement if it was used most often in contexts of finding
101 clumped resources such as gum or flowers; social cohesion if it was used most often in
102 social meetings with other animals during the night or for gathering at sleep sites; or as an
103 anti-predator strategy if it was emitted in the presence of potential predators, including
104 humans during capture for health checks.

105 Methods

106

107 We conducted the research near Cipaganti, Garut Regency, West Java (S7°6'6 - 7°7'0 & E
108 107°46'0 - 107°46'50). The habitat consists of agricultural fields interspersed with bamboo
109 and tree plantations and forest patches [Rode-Margono et al., 2014]. We conducted the
110 study on a population of *N. javanicus* that has been continuously monitored since 2012. We
111 collected vocal data on 14 radio-collared individuals from December 2015 until May 2016.
112 We located the individuals using radio-collars weighing 17 g, a Sika receiver with the
113 function to scan group members' positions continuously, and a Yagi-Flex antenna (Biotrack,
114 UK). We observed focal individuals between 1700 h and 0500 h, using Clulite head-torches
115 fitted with red-filters. As animals were habituated, the average follow distance was
116 approximately 5 m from the focal animal, but calls were often heard and recorded from
117 animals at distances greater than this, and we recorded distance to the caller whenever we
118 could with a measuring tape or a laser range finder (Bushnell Yardage Pro).

119

120 We used five-minute focal instantaneous sampling to collect general behaviour about slow
121 lorises following a standard ethogram of the long-term project [Rode-Margono et al., 2014;
122 Poindexter and Nekaris 2017]. The main behaviours include solitary inactivity, alert, travel,
123 forage, feed, aggressive and affiliative social behaviour. We defined 'affiliative social
124 behaviour' as animals following or leading each other, allogrooming, carrying an infant,
125 entering a sleep site, and being in social proximity (<10 m) to other lorises, including resting
126 in proximity [Rode-Margono et al., 2014]. Whenever we detected a vocalisation, we
127 collected continuous data on the behaviours occurring during the calling bout [Altmann,
128 1974; Rode-Margono et al., 2014]. We also recorded vocalisations during the capture
129 procedure to change animals' radio collars and conduct health checks. All animals in this
130 study had known life history data, including sex, age, and social partners. Six of these
131 animals were part of a social pair where both adults of the pair wore radio collars, and had
132 1-2 dependent young at the time of this study. Except for animals with unknown birth dates
133 that were already adult as of 2012 when the study began, we knew the actual age of each
134 animal and calculated their age class as follows [with slight modification from Poindexter
135 and Nekaris 2017]. Infants (1 day to 12 weeks old) were parked, moved only a few trees
136 from the parking site, were largely dependent on the mother for milk, or were frequently

137 carried by family members. Juveniles (5 months to 11 months old) increase their home
138 range size, decrease dependence on the mother for milk, but retain a long fluffy coat, and
139 are occasionally carried by family members. Subadults (~12 months to ~ 20 months) begin
140 forays outside the home range, increase their home size, no longer suckle, and largely lose
141 the long fluffy hair of younger animals. They engage in social interactions, and may carry
142 siblings, but are no longer carried themselves. As Javan slow lorises are extremely territorial,
143 no other slow lorises were present in their ranges. Exceptions included non-temporal
144 overlap with neighbours and dispersing individuals that briefly traversed the ranges of
145 settled animals. The subpanel for Ethics in Animal Research of Oxford Brookes University
146 approved the methods used in this project.

147

148 We recorded the vocalisations using a Wildlife Acoustics Echo Meter 3+ (EM3+) and SMX-UT
149 ultrasonic external microphone fitted with a directional horn (WildlifeAcoustics.com).

150 During all-night follows, one tracker detected the animal and its social partners, one
151 researcher collected data, and one researcher continuously pointed the microphone at the
152 focal animal. The calls were sorted every following day to search for the signature of a
153 possible ultrasonic call. Once we determined the presence of an ultrasonic call, we verified
154 it in the field by pointing the Echo Meter directly at a focal animal, clarified the call was
155 coming from the animal and/or its partner(s), and thereafter could identify the call
156 whenever it was being made. We set the bit-depth to 16-bit and the sampling rate to 256
157 kHz, allowing for calls up to 128kHz to be recorded [Diniz et al., 2002]. Real Time Expansion
158 with an audio frequency division of 10 (RTE 10) allowed continuous monitoring and
159 recording of all frequencies within sample rate. Heterodyne monitoring during vocalisations
160 allowed for higher quality in-field analysis, without compromising the recording output. We
161 standardised gain at +36 dB. Every time a vocalisation was detected, we noted distance to
162 the animal, and behaviour. We noted caller's distances to social partners by checking their
163 radio signal of group members, or visually spotting the partner(s), and measuring the
164 distance with a measuring tape or a laser range finder. Maximum propagation distance was
165 calculated by detecting the distance to the individual making a series of calls from the
166 location of the recorder holding the Echo Meter.

167

168 Data Analyses

169 After converting our output into .WAV files, we generated spectrograms for all recordings
170 using Raven Pro 64-bit [Program, 2014]. After testing these for autocorrelation with a
171 Pearson's correlation test, we only included five in our analysis (Table 1). We used the
172 following Raven Pro settings for the analysis: brightness = 50; contrast = 95; spectrogram
173 window size (FFT size) = 2046; Hann spectrogram slice view = 256 samples (3dB Filter
174 Bandwidth of 1438 Hz); and greyscale map. We matched each call to the behavioural
175 context by looking at the exact times and dates of the recordings.

176

177 Using a Kolmogorov-Smirnov test we found that data were not normally distributed. We
178 created an expected occurrence of vocalisations (based on the behavioural activity budget
179 from 14 focal individuals, $n = 1225$), and then ran a Pearson's chi-squared test to determine
180 whether the expected occurrences were different from the observed occurrences of
181 vocalisations ($n = 293$). We used quadratic discriminant function analysis (QDFA) with cross
182 validation to ascertain if certain acoustic and temporal variables could be used to distinguish
183 different age-classes (adult, sub-adult, and infant) and different sexes [Kessler et al., 2012].
184 We ran a follow up MANOVA test to ascertain whether the variables selected via the QDFA
185 differed significantly between sex and age classes. We only used calls from individuals of
186 known age- and sex-class for the analysis ($n_{\text{calls}} = 89$; $n_{\text{individuals}} = 13$). We performed all
187 statistical analyses using the statistical software R (version 3.5.1) [R Development Core
188 Team 2018], with the "MASS" package [Ripley et al., 2018], "klaR" package [Roever et al.,
189 2018], and "rrcov" package [Todorov, 2018] installed.

190

191 Results

192 Description of the Doublet Click Call

193

194 We recorded one type of ultrasonic vocalisation ($n = 791$) in 552 hours of behavioural
195 observations. The ultrasonic call of *N. javanicus* is emitted purely in the ultrasonic frequency
196 range ($x = 46.00$ kHz; bandwidth: 22.66 - 62.02 kHz). The call is emitted by both sexes and we
197 recorded it for juveniles, subadults and adults in a social group. The call can be emitted by a
198 single individual or by up to four individuals that appear to call in response to one another.
199 We heard pairs, trios and quartets of animals making counter-calls. We could record the

200 distance of the recorder to the slow loris caller 532 times. The distance ranged from 3 m to
201 131 m ($\bar{x}=20.12$ m \pm SD 29.9). Amplitude was not dependent on the distance of the recorder
202 ($n = 532$ Pearson's $\chi^2 = -0.04$, $df = 1$, $p = 0.494$).

203

204 Calls were composed of a variable number of paired pulses, hereafter termed 'doublet
205 clicks' (Fig. 1, Table 1). Each call was composed of between 1 and 2141 doublet clicks. A new
206 call was deemed to have started if the inter-doublet click duration exceeded twice the
207 average inter-doublet click duration (> 116 ms). A single bout consisted of 10.15 ± 50.0 [$\bar{x} \pm$
208 SD] calls, with the longest recorded bout consisting of 443 calls made by four different
209 individuals in a single social group. *Nycticebus javanicus* produced an average of 6.66 ± 2.08
210 bouts per active period (between 1730h and 0600h), with calls being produced at any time
211 during this active period. We found no significant difference in call rate across the active
212 period.

213

214 Behavioural Context of Doublet Click Call

215

216 Of 791 calls recorded, we obtained behavioural context and caller identity for 532 (Fig. 2).
217 The occurrence of vocalisations significantly differed between behaviours ($n = 532$ Pearson's
218 $\chi^2 = 293.7$, $df = 7$, $p < 0.001$). The doublet click call was emitted most commonly during
219 affiliative social behaviour (359 of 532 calls, or 67.5% of calls), while animals followed or led
220 each other or entered a sleep site. The second most common associated behaviour was
221 foraging, during which 16.2% (86 of 532 calls) of calls were emitted. All other behaviours
222 made up the remaining 16.4% of calls. A single call was emitted when a mother was carrying
223 a juvenile, although the caller could not be distinguished. We could measure the distance
224 between callers 83 times, with distances ranging from 0-110 m ($\bar{x}=11.8$ m \pm SD 16.3). Of
225 these distances, 67% were associated with the behavioural category affiliative social
226 behaviour (e.g. were 10 m or less).

227

228 Information Encoded in Doublet Click Call

229

230 We could enter 89 calls ($n_{\text{adult}} = 58$; $n_{\text{subadult}} = 14$; $n_{\text{juvenile}} = 17$; $n_{\text{male}} = 53$; $n_{\text{female}} = 36$) of 14
231 individuals into the QDFA. A QDFA model including both sex and age classes as grouping
232 factors generated three components. A follow up MANOVA test indicated a significant
233 difference in call types between males and females (Wilks' lambda = 0.816, $F_{2,82} = 8.570$, $p =$
234 0.015) with the second component (which expresses an association with doublet click
235 variation) and the third component (which expresses an association with dominant
236 frequency) explaining the variation (second component: $F_{1,88} = 17.239$, $p < 0.001$; third
237 component: $F_{1,88} = 5.019$, $p < 0.028$) (Table 2). The MANOVA model indicated a significant
238 difference in call types between age classes (Wilks' lambda = 0.876, $F_{4,164} = 2.609$, $p = 0.038$)
239 with the first component (which expresses an association with inter-doublet click duration,
240 dominant frequency, and call duration) explaining the variation ($F_{1,88} = 4.097$, $p = 0.020$).

241

242 Discussion

243

244 We have confirmed that *N. javanicus* produces one type of vocalisation purely in the
245 ultrasonic range: the doublet click call. The doublet click call has a similar acoustic and
246 temporal structure to the ultrasonic "doubles" described for *T. tarsier* by Gursky [2015]. The
247 dominant frequency [$x = 46.0$ kHz] of the doublet click call suggests *N. javanicus* has a higher
248 frequency sensitivity than Ramsier and Dominy's [2010] finding of 45.3 kHz for captive slow
249 lorises (probably *N. bengalensis*, previously lumped into one species *N. coucang*). This
250 finding marks the third time that a pure ultrasonic call has been documented in primates,
251 and the first time that an ultrasonic call has been documented in strepsirrhine primates
252 [Ramsier et al., 2012a; Gursky, 2015].

253

254 Ours is the first study to record the ultrasonic vocalisations of a primate in a completely wild
255 setting using active recording techniques whereby animals were followed and observed
256 throughout their entire activity period to understand the context of the call. We found that
257 significantly more vocalisations were produced during affiliative social behaviour and that
258 doublet click calls contained information on sex and age, most prominently in doublet click
259 duration and dominant frequency, suggesting an affiliative function for this call. We made
260 these recordings during behavioural observations that formed part of a long-term ecological

261 study on *N. javanicus*, allowing us to test several hypotheses regarding the function of
262 ultrasonic calls in primates.

263

264 Echolocation

265 Researchers have documented different forms of echolocation in several species including
266 shrews, birds, tenrecs, humans, and most notably bats and dolphins [Gould et al., 1964;
267 Gould, 1965; Sales and Pye, 1974; Forsman and Malmquist, 1988]. The doublet click call
268 described here is very similar in acoustic and temporal structure to that of the echolocation
269 calls of several bat and dolphin species [Au, 1997; Thomas et al., 2004]. If the calls serve to
270 echolocate prey, conspecifics, or are used for general navigation, we would not expect to
271 see such highly developed visual and olfactory anatomy present in *N. javanicus* and the call
272 would be emitted far more frequently during foraging behaviour [Hill, 1953; Loo and
273 Kanagasuntheram, 1973; Kavanau and Peters, 1979]. The potential for use of the call for
274 navigation could be further explored, but unlike mouse lemurs and tarsiers, slow lorises
275 move stealthily and use the same travel routes with high fidelity [Nekaris et al., 2017]. Thus,
276 its use for navigation is also not likely. Therefore, an echolocation function is unlikely but
277 requires further investigation, potentially using sensory deprivation experiments [c.f.
278 Pariente, 1974].

279

280 Resource Advertisement

281 Many taxa use vocalisations to advertise their reproductive status to the opposite sex and to
282 attract mates, or to advertise food availability [Montgomerie and Thornhill, 1989; Cocroft
283 and Ryan, 1995; Semple and McComb, 2000]. We never heard *N. javanicus* produce the
284 doublet click in a mating context, and rarely heard it during feeding. Regarding reproductive
285 advertisement, the doublet click call is produced by all mobile age-classes and both sexes,
286 and only within a social group. We did not collect data on female reproductive status
287 frequently enough to test for variation in call occurrence relative to reproductive status.
288 Regarding advertisement of food availability, by informing conspecifics of where food is
289 found, individuals may lose out on a monopoly on the food source. Alternatively food
290 advertisement may benefit individuals by increasing reproductive chances or reducing
291 aggression from dominant individuals [Judd and Sherman, 1996; Gros-Louis, 2004].
292 *Nycticebus javanicus* does not appear to live in hierarchical social groups and foraging is a

293 largely solitary behaviour. Furthermore, their two main food sources, nectar and gum,
294 occur in discrete defendable patches that animals use on a regular basis [Cabana et al.,
295 2018]. The rarity of the doublet click call during feeding behaviour, and the types of foods
296 exploited by *N. javanicus*, suggest that the call does not play a role in advertising food
297 resource availability.

298

299 *Social Group Cohesion*

300 Contact calls are common among birds and primates to maintain cohesion with conspecifics
301 that are beyond visual contact [Oda, 1996; Kondo and Watanabe, 2009]. Improved hearing
302 sensitivity to detect complex calls has also been suggested to have co-evolved with social
303 complexity [Ramsier et al., 2012b]. The significant use of the doublet click call by *N.*
304 *javanicus* during affiliative social behaviour suggests that its main function is for social group
305 cohesion. The typical social group of *N. javanicus* comprises an adult male and female with
306 two to three offspring. Infant slow lorises are carried for two to four weeks following birth.
307 After that time, the young loris is parked near the sleeping site while other group members
308 forage [Nekaris, 2003; Wiens and Zitzmann, 2003]. At this time, group members frequently
309 visit the infant during the night, and we observed animals using the doublet click to join up
310 with parked juveniles [Nekaris, unpublished data]. For this infant care strategy to be
311 effective, the social group may need to remain in contact even when visual contact is lost,
312 whilst at the same time avoiding predator detection.

313

314 An effective contact call should be encoded with information about the caller's identity and
315 distance [Kondo and Watanabe, 2009]. We showed that the doublet click calls were
316 encoded with information about age-class and sex. We also found that the calls could be
317 detected by our recording equipment until at least 131 m, a distance suitable for carrying
318 across the average length of agricultural fields bordered by rows of tree in our study area.
319 The limited transmission distance of these ultrasonic calls may force social groups to remain
320 in close proximity, thereby reducing predation risk, reducing extra-pair copulation,
321 strengthening pair-bonds, and reducing aggressive encounters with aggressive extra-group
322 conspecifics [Arch and Narins, 2008] . Further studies should investigate if information on
323 the callers' identity is also encoded in the call.

324

325 *Predator Avoidance and Conspecific Defense*

326 The doublet click call has an added advantage to facilitate predator avoidance, and may
327 form an additional strategy within a cryptic behavioural repertoire of slow lorises (silent
328 movement, camouflage, etc.) [Wiens and Zitzmann, 2003; Nekaris et al., 2006]. In
329 comparison to sonic calls [c.f. Zimmermann, 1985], ultrasonic vocalisations are relatively
330 common throughout their active period. Despite the presence of a range of potential
331 predators at our study site, we have not yet observed predation. These observations confer
332 limited predation on *Nycticebus* across their range [Wiens et al., 2006; Starr et al., 2012;
333 Nekaris et al., 2013]. Another obvious animal to avoid are neighbouring slow lorises. The
334 venom of slow lorises is used to defend territory and can be deadly to other slow lorises
335 [Nekaris et al., 2013; Fuller et al., 2018] Ultimately, the doublet click call may have evolved
336 to communicate with group members cryptically while avoiding advertisement to predators,
337 and either warning off conspecifics, or monitoring the location of vulnerable young.

338

339 Despite their frequent designation as solitary, strepsirrhine primates have long been known
340 to maintain complex social relations. We provide compelling evidence that the doublet click
341 call may facilitate maintaining and seeking contact. For the highly-cryptic slow lorises, an
342 inconspicuous call has advantages for seeking conspecifics in parenting, especially in
343 relation to infant parking and independent foraging from older offspring. Contact calls that
344 allow constant communication with offspring whilst evading the ability for potential
345 predators to hear the call, represent a clever trade-off for increased crypsis while
346 maintaining social cohesion.

347

348

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474

475 Figure Headings

476 Fig. 1 - Spectrograms of the pure ultrasonic call emitted by *N. javanicus* the 'doublet click',
477 showing some acoustic and temporal variables

478 Fig. 2: (a) Barplot showing expected occurrence of vocalisations versus observed occurrence
479 of vocalisations for each behavioural category; (b) Barplot showing the distribution of
480 residuals following Pearson's X^2 test for each behavioural category (FE – feeding, FO –
481 foraging, CA – capture, SY – solitary resting, TR – travel, AL – alert, AF – affiliative, AG –
482 aggressive). Asterisks (*) indicates residuals greater than 1 standard deviation (1.96), at 95%
483 confidence intervals.

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