Ontogeny of long-range vocalizations in a Neotropical fossorial rodent: the Anillaco Tuco-Tuco (*Ctenomys* sp.)

Juan Pablo Amaya [Corresp., 1, 2], **Juan Ignacio Areta** [3]

[1] Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR-CONICET), Anillaco, La Rioja, Argentina

[2] Universidad Nacional de La Rioja (UNLAR), La Rioja, Argentina

[3] Instituto de Bio y Geociencias del Noroeste Argentino (IBIGEO-CONICET), Rosario de Lerma, Salta, Argentina

Corresponding Author: Juan Pablo Amaya
Email address: juanentuculandia@gmail.com

Tuco-tucos (*Ctenomys* spp.) are subterranean rodents that produce territorial, high intensity long-range vocalizations (LRVs) of broadband and low frequency that are essential for long-distance communication between individuals in different tunnel systems. Despite their importance, the development of LRVs remains poorly understood. In adult Anillaco Tuco-Tucos (*Ctenomys* sp.) the LRV is composed by two types of syllables (series and individual notes) that are repeated a variable number of times. We studied the development of the LRVs in 8 juveniles of the Anillaco Tuco-Tuco ranging from 14 to 118 days after birth. We 1) tested whether the syllables followed any of three alternative developmental modes (retention of juvenile vocalizations, modification of juvenile precursors or *de novo* appearance in adults), 2) evaluated the development of structural and acoustic features of syllables, and 3) tested the prediction that juveniles should produce a greater proportion of atypical series in precursors of the LRV than adults, due of lack maturation and/or precise coupling of neuromuscular and anatomical structures. The LRV of the Anillaco Tuco-Tuco exhibited a mixed developmental mode: while series developed from juvenile precursors whose acoustic features gradually approached those of adults, individual notes appeared later in the ontogeny and *de novo* with acoustic features indistinguishable from those of adults. The number of series per vocalization increased through development and varied from 1 to 25 in juvenile males and from 1 to 6 in juvenile females. The structure of the most common series type (triad) did not exhibit ontogenetic changes and was present as such at the onset of the emission of vocalizations. On the contrary, acoustic features of juveniles triad notes changed with age in both sexes (duration 90% increased through development, while bandwidth 90% and peak frequency decreased). Furthermore, juveniles emitted a higher proportion of atypical series than adults (7.4% vs. 0.3%), as expected in the development of any complex behavior that requires practice to be mastered. The maturation of the LRV occurred well before the

sexual maturation, presumably due to the protracted time needed to acquire or build a burrow system long before mating is possible. We propose that protracted vocal development is another component in the slow developmental strategy of *Ctenomys* and subterranean rodents in general.

2 **Ontogeny of long-range vocalizations in a Neotropical fossorial**

3 **rodent: the Anillaco Tuco-Tuco (*Ctenomys* sp.)**

4 Juan Pablo Amaya[1,2], Juan Ignacio Areta[3]

5 [1] Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR-CONICET), Anillaco,
6 La Rioja, Argentina
7 [2] Universidad Nacional de La Rioja (UNLAR), La Rioja, Argentina.
8 [3] Instituto de Bio y Geociencias del Noroeste Argentino (IBIGEO-CONICET), Rosario de Lerma, Salta, Argentina

9 Corresponding Author: Juan Pablo Amaya
10 Email address: jamaya@crilar-conicet.gob.ar

Abstract

Tuco-tucos (*Ctenomys* spp.) are subterranean rodents that produce territorial, high intensity long-range vocalizations (LRVs) of broadband and low frequency that are essential for long-distance communication between individuals in different tunnel systems. Despite their importance, the development of LRVs remains poorly understood. In adult Anillaco Tuco-Tucos (*Ctenomys* sp.) the LRV is composed by two types of syllables (series and individual notes) that are repeated a variable number of times. We studied the development of the LRVs in 8 juveniles of the Anillaco Tuco-Tuco ranging from 14 to 118 days after birth. We 1) tested whether the syllables followed any of three alternative developmental modes (retention of juvenile vocalizations, modification of juvenile precursors or *de novo* appearance in adults), 2) evaluated the development of structural and acoustic features of syllables, and 3) tested the prediction that juveniles should produce a greater proportion of atypical series in precursors of the LRV than adults, due of lack maturation and/or precise coupling of neuromuscular and anatomical structures. The LRV of the Anillaco Tuco-Tuco exhibited a mixed developmental mode: while series developed from juvenile precursors whose acoustic features gradually approached those of adults, individual notes appeared later in the ontogeny and *de novo* with acoustic features indistinguishable from those of adults. The number of series per vocalization increased through development and varied from 1 to 25 in juvenile males and from 1 to 6 in juvenile females. The structure of the most common series type (triad) did not exhibit ontogenetic changes and was present as such at the onset of the emission of vocalizations. On the contrary, acoustic features of juveniles triad notes changed with age in both sexes (duration 90% increased through development, while bandwidth 90% and peak frequency decreased). Furthermore, juveniles emitted a higher proportion of atypical series than adults (7.4% vs. 0.3%), as expected in the development of any complex behavior that requires practice to be mastered. The maturation of the LRV occurred well before the sexual maturation, presumably due to the protracted time needed to acquire or build a burrow system long before mating is possible. We propose that protracted vocal development is another component in the slow developmental strategy of *Ctenomys* and subterranean rodents in general.

Introduction

40 Like many other aspects of biological systems, vocalizations follow a developmental trajectory

41 during ontogeny. In principle, adult vocalizations could develop following three alternative

42 modes. Adults may just retain the vocalizations of juveniles (Winter et al. 1973; Campbell et al.

43 2014), vocalizations may develop through the modification of juvenile precursors that eventually

44 reach adult features (Clemmons & Howitz 1990; Grimsley et al. 2011), or they may appear *de*

45 *novo* in adults without the need of homologous juvenile precursors (Campbell et al. 2014).

46 Vocalizations that develop through precursors may follow two non-mutually exclusive

47 developmental pathways: vocal learning, that involve the modification of vocalizations based on

48 the copy of sounds from an external auditory input (Tyack 2016); and vocal tract maturation,

49 that involves the capacity of juveniles to generate adult-like vocalizations by the tuning of an

50 innate motor program (Winter et al. 1973; Tyack 2016).

51   The acquisition of adult vocal patterns requires practice, since it is necessary to

52 synchronize the neuromuscular system and anatomical structures, either to match the vocal

53 output to those signals acquired through external input or to achieve the successful deployment

54 of instructions contained in the internal innate motor program (Boughman & Moss 2003). The

55 lack of maturation and/or precise coupling between the morphology and the neuromuscular

56 system of the vocal apparatus leads to the production of atypical vocalizations. Consequently,

57 regardless of the developmental pathway followed, juveniles are expected to produce a greater

58 proportion of atypical vocalizations during their practice period than adults during the emission

59 of their mature vocalization (Konishi 1965).

60   Tuco-tucos (*Ctenomys* spp.) are subterranean rodents that produce long-range

61 vocalizations (LRVs) of high intensity, broadband and low frequency. These vocalizations are

62 essential for long-distance communication between individuals in different tunnel systems

63 (Schleich & Busch 2002, Amaya et al. 2016). Multiple lines of evidence suggest that LRVs are

64 territorial signals that facilitate the maintenance of individual territories and minimize aggressive

65 encounters, especially between males (Francescoli 1999; Schleich & Busch 2002; Amaya et al.

66 2016). Despite their behavioral importance, the LRVs of adults of only three of the more than 60

67 recognized species of *Ctenomys* (Bidau 2015) have been characterized in some detail

68 (Francescoli 1999, Schleich & Busch 2002, Amaya et al. 2016). Juvenile LRVs were mentioned in

69 *C. talarum* (Zenuto et al. 2002) and *C. mendocinus* (Camin 2010), but there is no detailed

70 information on the developmental modes, pathways or developmental times of LRVs. Learning

71   how and when the LRVs reach maturity can improve our understanding of how life-history traits

72   interact and influence each other in fossorial rodents. For example, since burrows are

73   energetically expensive resources (Vleck 1979, 1981) it is conceivable that solitary species of

74   *Ctenomys* secure or build their own burrow system before mating. This would imply the need of

75   having mature LRVs to help defend the burrows during the process of acquiring a mate, even

76   before reaching sexual maturity.

77   The LRV of adult Anillaco Tuco-Tucos (*Ctenomys* sp.) is composed of two types of

78   syllables: series and individual notes, that are repeated a variable number of times in each

79   vocalization (Figure 1). All LRVs contain series, but not always include individual notes (Amaya et

80   al. 2016). Series are composed by two sound types: notes, loud sounds that initiate and finish

81   the series; and soft-notes, softer sounds that are sandwiched by notes. Depending on the

82   number of notes and soft notes, series can belong to any of three series-patterns: dyads (two

83   notes, one soft note), triads (three notes, two soft notes), or tetrads (four notes, three soft

84   notes). Triads constitute the most common series pattern. However, adults occasionally produce

85   atypical series by adding a soft-note before the initial note. The individual notes are single notes

86   emitted at a faster rate in succession and are always given after the series (Figure 1) (Amaya et

87   al. 2016).

88   Structural and quantitative acoustic traits of vocalizations may experience different

89   developmental trajectories (Elowson 1992; Brittan-Powell et al. 1997; Hammerschmidt et al.

90   2000, 2001; Grimsley et al. 2011; Campbell et al. 2014). Structurally, triads are the most

91   common series pattern in adults of the Anillaco Tuco-Tuco. These triads could conceivably

92   develop through the initial emission of separate notes and soft-notes, passing to their joint

93   emission in the shape of monads, subsequently leading through the repetition of this basic

94   structure to the creation of the other types of series composed by more notes and soft-notes.

95   Alternatively, the structure of series could be a hard-wired feature that would experience no

96   ontogenetic changes, with triads and other series types existing since early ontogenetic stages.

97   Likewise, quantitative acoustic features of LRVs such as frequency and duration could develop

98   gradually or may simply appear with adult parameters. Since in most mammals body size is

99   inversely correlated with peak frequency and juveniles tend to produce less precise vocalizations

100   (Morton 1977, Owings and Morton 1998), we expect peak frequency and bandwidth to

101   decrease and length of syllable types to approach adult values.

102   This paper aims to describe the developmental trajectory of the LRVs in the Anillaco

103   Tuco-Tuco based on recordings of captive-reared pups, in order to 1) discuss which

104   developmental mode (juvenile retention, juvenile precursor, or *de novo* appearance) may

105   explains the ontogeny of the two types of syllables of the LRV; 2) evaluate the development of

106   structural and quantitative acoustic features of syllables in LRVs and compare them with those in

107   adults; 3) test the prediction that juveniles should produce a greater proportion of atypical

108   series in precursors of the LRV than adults; and 4) discuss the relationship between the

109   development of LRVs and other life-history traits.

110   **Materials and methods**

111   **Experimental animals**

112   We studied the development of the LRV in 8 juveniles (5 males and 3 females) of the

113   Anillaco Tuco-Tuco. Taxonomy of this species is still cursory and multiple lines of evidence

114   indicate that our study area is occupied by a single and yet unidentified *Ctenomys* species that is

115   closely allied to species described from similar dry habitats in neighboring provinces (Amaya et

116   al. 2016).

117   Juveniles of the Anillaco Tuco-Tuco were trapped at Anillaco, La Rioja, Argentina

118   (28°48'50"S, 66°55'54"O; 1365 m asl) in November and December yearly between 2013 and

119   2016. Immediately after trapping, juveniles were carried to the Chronology and Ethology lab in

120   CRILAR to a room (2.5m x 4m) with a fluorescent light source (36 W) controlled by a mechanical

121   timer (TBCin-China) providing a light-dark cycle of 12:12. Each juvenile was housed in a

122   transparent glass enclosure (90 x 40 x 40 cm, see Amaya et al. 2016) with abundant wood chips

123   on the floor and PVC tubes used as burrows. Food was offered *ad lib* and consisted of sweet

124   potatoes, carrots, sunflower seeds and natural food items collected weekly from the field

125   (*Larrea* sp. and *Opuntia* sp.).

126   Juveniles were acoustically isolated from adults during their development in captivity.

127   Male 1 and female 1 were kept alone in 2013 and 2014 respectively; while males 2 and 3, and

128   female 2 were kept together in 2015; and males 4 and 5, and female 3 were kept together in

129   2016. Juveniles were weighed four times during our study (when trapped and three times at

130   intervals of 30-35 days in captivity; Table 1) and sexed *a posteriori* because the genitalia were

131   not mature at the time of capture.

132   Since we captured juveniles of unknown biological age, we estimated their absolute ages

133   at trapping by comparing their weights to those of juvenile *C. mendocinus* of known age (Camin

134   2010). Adults of the Anillaco Tuco-Tuco are 10% heavier than adults of *C. mendocinus* (Camin

135   2010, Amaya et al. 2016). Thus, we assumed a linear age-weight relationship such that 10%

136   heavier juveniles of the Anillaco Tuco-Tuco where aged as equivalent to 10% lighter juveniles of

137   *C. mendocinus* (e.g., at day 14 juveniles of *C. mendocinus* weigh on average 33 g, while at this

138   age the weight of juvenile Anillaco Tuco-Tucos was estimated to be around 36.3 g). The

139   minimum estimated age at trapping was 14 days (female 1; Table 1) and the maximum 28 days

140   (male 1; Table 1). To conduced statistical comparisons we merged data of juvenile males and

141   females in four age classes: 14-28, 44-58, 74-88, and 104-118 days after birth.

142   All procedures followed the guidelines of the American Society of Mammologists for the

143   use of wild mammals in research (Sikes & Gannon, 2011). All experiments were performed at

144   the CRILAR and were authorized by the Environmental Department of La Rioja (exp. P40015215).

145   All individuals were returned to their natural environment after the study was complete.

146   **Recording of vocalizations**

147   We studied the development of LRVs of 8 juveniles of Anillaco Tuco-Tuco. Each juvenile was

148   recorded during four 6-8hr sessions in alternate days within a week every four weeks (i.e., a

149   week of recording with four sessions was separated by three weeks without recording). Sessions

150   began during the first week that individuals were trapped and finished after twelve weeks. Thus,

151   each individual was recorded in 16 sessions spread along 12 weeks, except for male 3 that died

152   in the ninth week and was recorded in only 12 sessions.

153   Recordings were made from outside the transparent glass enclosure using a Zoom H4n

154   digital hand recorder system with built-in microphones (sample rate of 44.1 kHz and 24 bit

155   depth) mounted on a tripod with the microphones facing the enclosure. The gain setting of the

156   recorder was the same for all recordings. Our recording protocol allowed us to detect and record

157   the emission of the LRVs, which occur at a low rate and unpredictably. All the recording sessions

158   were made during the night (between 22:00 and 6:00hs Argentina time, GMT -5), due the low

159   level of ambient noise and to the high nocturnal activity of the species in laboratory conditions

160   (Tachinardi et al. 2014). In total, we recorded 5 males for 448 hours in 76 sessions and 3 females

161   for 276 hours in 48 sessions.

162   We searched for LRVs in recordings by manually examining on-screen spectrograms built

163   with Raven pro 1.4 (http://www.birds.cornell.edu/Raven). We used the following spectrogram

164   parameters to prioritize resolution in time to be able to easily detect vocalizations: Window:

165   Hann; size: 256 samples (= 5,8 ms); 3 dB bandwith-filter: 248 Hz; Time grid-overlap: 50%; hop

166   size: 128 samples (= 2,9 ms); Frequency grid–DFT size: 256 samples; grid spacing: 172 Hz.

167   **Acoustic characterization of the long-range vocalization**

168   We analyzed all juvenile vocalizations to quantify the number of series and individual notes

169   emitted per vocalization and to acoustically characterize them. Of all the types of series in

170   juveniles, we measured only the triads because they are the most common and characteristic

171   series type of the LRV in adults of these species (Amaya et al. 2016). We characterized one triad

172   and three individual notes per vocalization. In total, we analyzed 47 triads in males and 19 in

173   females; and 9 individual notes in males (see Table S1 for sampling in each age class) To compare

174   triads and individual notes of juveniles with those of adults we characterized them in five adult

175   males and three adult females (one triad and three individual notes per adult individual), using

176   adult recordings from Amaya et al. (2016). We measured duration 90% (ms), bandwidth 90%

177   (Hz) and peak frequency (Hz) of each triad note and individual notes in juveniles and adults. We

178   calculated single duration 90%, bandwidth 90% and peak frequency values for each individual in

179   each vocalization by averaging the values of the three triad notes and the three individual notes.

180   These acoustic parameters were defined as follows: 1-duration 90%, is the difference between

181   time 5% (time that divides the selection border in two time intervals containing 5% and 95% of

182   the energy) and time 95% (time that divides the selection border into two time intervals

183   containing 95% and 5% of the energy); 2-bandwidth 90%, is the difference between frequency

184   5% (frequency that divides the selection into two frequencies intervals containing 5% and 95%

185   of the energy) and frequency 95% (frequency that divides the selection border into two

186   frequencies intervals containing 95% and 5% of the energy); and 3-peak frequency, is the

187   frequency at which the peak energy is produced within the selection border.

188   We delimited the triad notes and individual notes for measurements of duration 90%

189   (ms), bandwidth 90% (Hz) and peak frequency (Hz) as follows. For triads, we delimited an initial

190 selection border A (400 ms and 0-22.05 kHz) (Figure 2 A) that was subdivided in 20 equal

191 selection borders B (20 ms and 0-22.05 kHz) (Figure 2 B), and we defined a selection border C

192 (40 ms and 0-22.05 kHz) delimiting each triad note by choosing the two adjacent selection

193 borders B with the highest energy values (Figure 2 B). We performed the same procedure for

194 individual notes, where an initial selection border D (80 ms and 0-22.05 kHz) (Figure 2 C) was

195 subdivided into 4 equal selection borders E (20 ms and 0-22.05 kHz) (Figure 2 C), and we defined

196 a selection border F (40 ms and 0-22.05 kHz) delimiting each individual note by choosing the

197 two adjacent selection borders E with the highest energy values (Figure 2 D).

198   All acoustic measurements were made with Raven Pro 1.4. We used different

199 spectrograms parameters that allowed us to obtain accurate time and frequency domain

200 measurements. For duration 90% we used the spectrogram parameters used to search for LRVs

201 in recordings (see above). For bandwidth 90% and peak frequency the spectrogram parameters

202 were: Window: Hann, size: 1024 samples (= 52.4 ms), 3 dB bandwith-filter: 27.4 Hz; Time grid–

203 overlap: 50%, hop size: 512 samples (= 26.2 ms); Frequency grid–DFT size: 4,096 samples, grid

204 spacing: 4.7 Hz. All recordings were band-pass filtered between 80–5,000 Hz in Raven Pro 1.4 to

205 eliminate sources of disturbance and distortion in acoustic measurements as in Amaya et al.

206 (2016).

207 **Series-patterns and atypical series**

208 We analyzed all juvenile vocalizations to quantify the occurrence of each of the three normal

209 series-patterns (dyads, triads and tetrads). We first identified the series-patterns in the

210 corresponding waveform and spectrogram by counting the number of notes and soft-notes per

211 series, and then counted the number of series-patterns per vocalization. Following the same

212 procedure, we quantified the occurrence of atypical series in juvenile vocalizations. We

213 considered a series to be atypical if it had a soft-note before the note 1 in dyads, triads and

214 tetrads or if it was composed by a single note followed by a soft-note (i.e., it was a monad)

215 (Figure 3).

216   In order to test the prediction that juveniles produce proportionally more atypical series

217 than adults, we compared the proportion of atypical series in adults using data from Amaya et

218 al. (2016) to the proportion of atypical series in juveniles. To calculate these proportions

219   (expresed as %) we counted the number of atypical series and divided these counts by the total

220   number of series in juveniles and in adults. In total, 325 juvenile series were recorded for the

221   present study and compared against 703 adult series from Amaya et al. (2016).

222   **Statistical analyses**

223   To evaluate if the acoustic features of juvenile male triad notes and if the number of series per

224   vocalization changed during their development, we performed a non-parametric repeated

225   measures analysis of variance (Friedman test) of duration 90%, bandwidth 90%, peak frequency

226   and number of series per vocalization across four age classes (14–28, 44–58, 74–88 and 104–118

227   days after birth). Post-hoc analyses were conducted using Bonferroni multiple comparison tests.

228   To compare at which point acoustic features of juvenile male triad notes reached adult

229   parameters during their vocal development, we performed a Wilcoxon Signed-Rank test

230   comparing duration 90%, bandwidth 90%, and peak frequency of triad notes of each juvenile

231   age class against values in adult males. Few vocalizations of juvenile males contained individual

232   notes and all appeared at the 104-118 dab age class. Thus, we visually compared values of

233   acoustic parameters of juvenile individual notes against those in adults.

234   Due to the low number of juvenile females, comparisons among age classes and between

235   age classes and adults were restricted to visual comparisons of acoustic parameters along their

236   ontogeny.

237   For each variable analyzed, we used the average value of measurements taken from each

238   individual within each age class. All analyses were carried out in R (R Core Team 2017) with

239   alpha set at 0.05.

240   **Results**

241 Seven juveniles of the Anillaco Tuco-Tuco (five males and two females) produced 71 LRVs in the

242 12 study weeks; while an eighth juvenile (female 3) did not produce any vocalization (Table 1).

243 The two syllable types of LRVs, series and individual notes, appeared sequentially in the vocal

244 development (Figure 4). Series appeared first and were produced by juveniles as young as 14

245 days old (39 g). Individual notes were produced considerably later in the development, when

246 juveniles were as young as 108 days old (156 g) (Figure 4, Table 1). Accordingly, most

247 vocalizations were composed only by series (95.77%, N=68), and fewer vocalizations were

248 composed by series and individual notes (4.22%, N=3) (Table 1).

249 **Development of series**

250 Developmental changes caused juvenile triads to progressively approach the acoustic

251 parameters of adult triads (Figure 5). The seven juveniles that vocalized produced series (Table

252 1). The number of series per vocalization increased through development and varied from 1 to

253 25 in juvenile males (Table 1) and from 1 to 6 in juvenile females (Table 1). The first vocalizations

254 of all juveniles contained a single series, and the highest numbers of series were recorded in

255 juvenile males between 108 to 118 days old, and in juvenile females between 104 to 113 days

256 old (Table 1).

257 Juveniles produced the three series-patterns present in the adults. Triads were by far the

258 most common series-pattern (82.76%, N=269), followed by dyads (16.61%, N=54), and tetrads

259 (0.61%, N=2) (Table 1). Triads were produced by all vocalizing juveniles, dyads were produced by

260 all juveniles (except male 3 which died prematurely), and tetrads were produced by only two

261 juveniles (female 1 and female 2) (Table 1).

262 All three acoustic features of triad notes and the number of series per vocalization

263 changed along the ontogenetic trajectories of juvenile males and females (Figures 4, 5, Table 2

264 and Table S1). In juvenile males, duration 90% of triad notes exhibited a significant directional

265 increase through development (Friedman test; n=5, p<0.04) (Figure 5, Table 2 and Table S1);

266 bandwidth 90% and peak frequency of triad notes exhibited a significant directional decrease

267 during the development (Friedman tests; bandwidth 90%: n=5, p<0.01; and peak frequency:

268 n=5, p<0.01) (Figure 5, Table 2 and Table S1). Finally, the number of series per vocalization

269 increased through development (Friedman test; n=5, p<0.007) (Figure 5, Table 2 and Table S1).

270 Although we could not perform statistical comparisons in females, our data show that through

271   development duration 90% of triad notes increased (Figure 5, Table 2 and Table S1), bandwidth

272   90% and peak frequency of triad notes decreased (Figure 5, Table 2 and Table S1), and the

273   number of series per vocalization increased (Table 1). Thus, juvenile males and females showed

274   similar ontogenetic changes in their series.

275   Acoustic features of triad notes of juvenile males progressively approached features of

276   adult males and achieved mature features at different developmental stages. Pairwise

277   comparisons (Wilcoxon Signed-Rank test) of duration 90%, bandwidth 90% and peak frequency

278   of juvenile and adult males showed significant differences at different ages (Figure 5, Table 2 and

279   Table S1). Thereby, acoustic features of triad notes passed through a juvenile developmental

280   stage in which they were different from adults before reaching adult values (Figure 5, Table 2

281   and Table S1). We were unable to compare the acoustic features of triad notes between juvenile

282   and adult females statistically. However, visual inspection of average acoustic values show that

283   triad notes of juvenile females had shorter duration 90% and higher bandwidth 90% and peak

284   frequency than those in adult females. Thus, just like juvenile males, juvenile females seem to

285   gradually acquire acoustic features of triad notes of adult females during their vocal

286   development (Figure 5, Table 2 and Table S1).

287   **Atypical series in juveniles and adults**

288   Juveniles emitted two types of atypical series during the development of the long-range

289   vocalization. In the first type of atypical series a soft note preceded the first series note, and

290   included atypical triads (Figure 3A), dyads (Figure 3B) and tetrads (Figure 3C). In the second

291   type, the series was conformed by a single monad that consists of a single note followed by a

292   soft-note (Figure 3D). Monads are unknown in adults (see Amaya et al. 2016).

293   The percentage of atypical to normal series was more than an order of magnitude larger

294   in juveniles than in adults. Juveniles produced 7.38% of atypical series (8 atypical triads, 11

295   atypical dyads, 3 atypical tetrads, and 4 monads vs. 299 normal series) while in adults only

296   0.28% of the series were atypical (one atypical triad and one atypical dyad vs. 701 normal

297   series).

298   **Development of individual notes**

299   Only 3 of the 5 males and none of the two females that vocalized gave individual notes. These

300   notes were given by males 1, 2 and 4 when they had between 156-162 g and were 108-118 days

301   old (Table 1); while male 3 died at an early age, male 5 approached this weight and was within

302   this age range but never gave individual notes (Table 1). Individual notes were recorded in only a

303   single vocalization in each male, and ranged in number from 22-31 notes always emitted after a

304   sequence of series and never in isolation (Table 1). Visual comparisons of average values of

305   duration 90%, bandwidth 90% and peak frequency of individual notes (Figure 5, Table S1) show

306   that acoustic parameters of individual notes of juveniles were undistinguishable from those of

307   adults (Figure 5 and Table S1).

308   **Discussion**

309   In this paper we have shown that the long-range vocalization (LRV) of the Anillaco Tuco-Tuco

310   (*Ctenomys* sp.) exhibits a mixed developmental mode in which the two syllables types of the

311   vocalization develop differently: while series developed from juvenile precursors whose acoustic

312   features gradually approach those of adults, individual notes appeared later in the ontogeny and

313   *de novo*, with acoustic features undistinguishable from those of adults. The most common series

314   type did not exhibit ontogenetic changes in its structure: triads appeared structurally as such at

315   the onset of the emission of vocalizations at early developmental stages. Finally, we found that

316   juveniles emit a higher proportion of atypical series than adults, as expected in the development

317   of any complex behavior that requires practice to be mastered.

318   **Development of the long-range vocalization**

319   The LRV in the Anillaco Tuco-Tuco exhibits a mixed developmental mode, since it includes a

320   syllable type (series) that develops gradually from structurally conserved ontogenetic precursors

321   in the juveniles and another syllable type (individual notes) that appears *de novo* with adult

322   features. This sort of mixed development is novel and has not been reported in the vocalizations

323   of other rodents. For example, while the vocalization of *Mus musculus* develops from precursors

324   of the syllables that increase in duration and decrease in fundamental frequency (Grimsley et al.

325   2011), the acoustic characteristics of the notes in *Scotinomys* spp. do not experience

326   ontogenetic changes (Campbell et al. 2014) and are therefore the product of retention of

327   juvenile vocalizations.

328   Early in the ontogeny, juveniles of the Anillaco Tuco-Tuco produce the three series-
329   patterns present in adults (dyads, triads and tetrads). The subsequent lack of structural changes
330   in the structure of series is interesting since *a priori*, other developmental mechanisms could
331   have occurred. For example, series could have developed by the sequential addition of simple
332   notes and soft-notes, subsequently coupled to create the series-patterns. Triads were the most
333   common series-pattern in adults (Amaya et al. 2016) and in juveniles (this work). We interpret
334   the early appearance of triads and their ubiquitous presence as evidence of a substantial genetic
335   makeup in the structural composition of series in *Ctenomys*. In fact, other series-patterns
336   predominate in other species of tuco-tucos. For example, monads are characteristic of *Ctenomys*
337   *talarum* (Schleich & Busch 2002) and dyads are characteristic of *Ctenomys mendocinus* (Amaya
338   et al. 2016, Amaya & Areta, unpublished data). Further studies in these species could help clarify
339   the genetic contribution to the determination of the structure of series-patterns in *Ctenomys*.

340   Although triads appear early in the development of juveniles, their acoustic features
341   differ from those of adults. Juvenile precursor triad notes increase duration; and decrease
342   bandwidth and peak frequency gradually until reaching the values of adult triads. Thereby,
343   juvenile triad precursors develop into adult triads by gradual changes in their acoustic
344   parameters but without changing their basic structure.

345   The lower degree of morphological and neuromuscular maturation and tuning in the
346   vocal apparatus of juvenile in comparison to adult tuco-tucos may account for the higher
347   proportion of atypical series in juvenile individuals. This explanation may be more general and
348   could also apply to juveniles of two species of singing mice (*Scotinomys teguina and S.*
349   *xerampelinus*). Five types of notes were described in juvenile *Scotinomys* spp. (Campbell et al.
350   2014); one very frequent note (note A) exists at all ages and is almost the only note sang by
351   adults, while four less frequent notes are more frequent in the first 12 days of life and become
352   almost non-existent after 30 days (Campbell et al. 2014). We speculate that these four types of
353   less frequent notes are not different notes *per se*, but that they could simply represent atypical
354   renditions of the note A. Hence, their decrease in frequency could be explained by the improved
355   performance of the vocal tract and associated neuromuscular apparatus through development.

356   Individual notes of LRVs appear late, *de novo* and with acoustic parameters
357   indistinguishable from those of adults, showing a developmental mode that is strikingly different
358   to that of series. The first individual notes are given by juveniles when the series have already

acquired adult acoustic characteristics through maturation from precursors. This suggests that the appearance of individual notes with their definitive acoustic characteristics without the existence of ontogenetic precursors may be possible because the neuromuscular system and the vocal apparatus have acquired the necessary "fine tuning" through practice of the series during their development. The passive maturation of the individual notes might be, at least in part, a co-product of the active maturation of the series.

The LRVs in the Anillaco Tuco-Tuco exhibit a large variety of syntactic patterns (Amaya et al. 2016). These syntactic patterns undergo some general and predictable ontogenetic changes that include, first an increase in both the number of series and series-patterns per vocalization, and then an increase in syllable types given by the late appearance of individual notes. The increment in vocalization length and the late appearance of individual notes might be widespread in *Ctenomys*. These patterns appear to occur also in the vocal development of *C. mendocinus*, where "short calls" presumably conformed only by series occur at 33 days and "long calls" presumably conformed by series and individual notes occur at 70 days (Camín 2010). In contrast, the highly stereotyped pattern of *Scotinomys* adult song appears *de novo* in the first vocalization of juveniles after a period of vocal inactivity (Campbell et al. 2014).

Vocalizations of rodents appear to develop by maturation of the vocal tract and not by vocal learning since adult vocalizations develop normally without the need of an external auditory model from which to copy (Scherrer & Wilkinson 1993, Grimsley et al. 2011, but see Arriaga et al. 2012). In this paper we demonstrated that juveniles of the Anillaco Tuco-Tuco develop normal LRVs without an adult external auditory input between 14 and 108 days. This would indicate that the development of this vocalization does not depend on vocal learning processes (Efremova et al. 2011). However, juveniles were exposed to adult LRVs from birth until they were captured, and *Ctenomys* are born with an open auditory meatus (Zenuto et al. 2002, Camín 2010). Thus, it remains possible that adult vocalizations heard at an early age have served as an auditory model for the subsequent vocal development. More thorough future tests on the importance of vocal learning in the development of LRVs in *Ctenomys* should focus on evaluating if deafened juveniles develop normal LRVs when devoid from acoustic self-stimulation (Kroodsma & Konishi 1991; Campbell et al. 2014).

Life-history traits and territorial vocalizations in subterranean rodents

389 Subterranean rodents exhibit slow life-histories, which are characterized by slow speed of tissue
390 growth due to a low basal metabolic rate selected for by the high energetic demands of
391 burrowing (Henneman, 1983, 1984; Hofman, 1983; Glazier 1985) leading to delayed sexual
392 maturity (Busch et al. 2000). In *Ctenomys talarum* and *C. mendocinus* gestation lasts around 95
393 days (Zenuto et al. 2002; Camin 2010); sexual maturity takes 180-240 days in *C. talarum* (Busch
394 et al. 2000) and presumptively adult vocalizations have been reported long before sexual
395 maturity at 65-70 days (Zenuto et al. 2002) and the same pattern is evident in *C. mendocinus*
396 which reaches sexual maturity around 180-270 days (Camín 2010) and supposed vocal maturity
397 at 70 days (Camín 2010). We lack precise information on the age of sexual maturity in the
398 Anillaco Tuco-Tuco, but the patterns described for *C. talarum* and *C. mendocinus* suggest that
399 vocalizations mature well in advance of sexual maturity in *Ctenomys*. The solitary bathyergids,
400 *Bathyergus janetta*, *B. suillus* and *Georychus capensis* exhibit a moderate gestation period of
401 around 50 days and presumably delayed sexual maturation (Jarvis 1969; Bennett & Jarvis 1988;
402 Bennett et al. 1991, 2000). In *B. janetta* and *B. suillus*, juveniles begin to produce seismic signals
403 (foot-drumming) at 80 days, after dispersion at 60-65 days from the maternal burrow system;
404 while in *Georychus capensis* juveniles produce foot-drumming at 50 days of age before
405 dispersion at 55-60 days of age (Bennett & Jarvis 1988). Dispersion in these bathyergids, occurs
406 when they have the capacity to dig and protect their own burrow systems (Bennett & Jarvis
407 1988). Thus, territorial acoustic signals of solitary ctenomyids and bathyergids seem to mature
408 approximately at the time of dispersion but long before sexual maturity.

409 The protracted development of the LRV of the Anillaco Tuco-Tuco (ca. 110-120 days)
410 herein reported contrasts markedly with the short developmental times of vocalizations of
411 rodents that live in the surface such as *Scotinomys* (35 days; Campbell et al. 2014) and *Mus*
412 *musculus* (at 13 days individuals had some adult vocal characteristics, Grimsley et al. 2011). The
413 altricial *Ctenomys* contrast with the precocial *Scotinomys* in which gestation takes 30 days
414 (Hopper & Carleton 1976) and which reach sexual maturity after 30-40 days concomitantly with
415 the maturation of adult vocalizations (Hooper & Carleton 1976, Campbell et al. 2014).

416 We propose that protracted vocal development is another component in the slow
417 developmental strategy of *Ctenomys* and subterranean rodents in general. The underground
418 burrow systems have a central role in the ecology of subterranean rodents (Busch et al. 2000)
419 and their defense is of key importance. Burrows are costly resources that serve for breeding and

420   that are defended by the use of long-range vocalizations in *Ctenomys* (Amaya et al. 2016). The

421   maturation of vocalizations in advance of sexual maturation might be explained by the

422   protracted time needed to acquire or build a burrow system long before mating is possible.

423   **Acknowledgements**

424   We are grateful to Pablo Lopez, Tatiana Sanchez, Johana Barros and Veronica Valentinuzzi for

425   help with care and feeding of the tuco-tucos in the lab. Hynek Burda, Elke Zimmermann and two

426   anonymous reviewers provided critical reviews and suggested numerous venues for

427   improvement.

428   **Bibliography**

429   **Amaya JP, Areta JI, Valentinuzzi VS, Zufiaurre E. 2016.** Form and function of long-range

430   vocalizations in a Neotropical fossorial rodent: the Anillaco Tuco-Tuco (*Ctenomys* sp.). *PeerJ*,

431   4, e2559

432   **Arriaga G, Zhou EP, Jarvis ED. 2012**. Of mice, birds, and men: the mouse ultrasonic song system

433   has some features similar to humans and song-learning birds. *PloS One* **7(10)**: e46610.

434   **Bennett NC, Jarvis JUM. 1988.** The reproductive biology of the Cape mole-rat, *Georychus*

435   *capensis* (Rodentia, Bathyergidae). *Journal of Zoology* **214(1):**95-106.

436   **Bennett NC, Jarvis JUM, Aguilar GH, McDaid EJ. 1991.** Growth and development in six species of

437   African mole-rats (Rodentia: Bathyergidae). *Journal of Zoology* **225(1):** 13-26

438   **Bennett NC, Faulkes CG, Molteno AJ. 2000.** Reproduction in subterranean rodents In Lacey EA,

439   Cameron G, Patton JL, eds. *Life underground: the biology of subterranean*

440   *rodents*:University of Chicago Press, Chicago, Illinois, 145-177.

441   **Bidau CJ. 2015**. Family Ctenomyidae Lesson, 1842. In: Patton J L, Pardiñas U F J, D'Elia G, eds.

442   Mammals of South America, Volume 2-Rodents. University of Chicago Press, Chicago, 818-

443   877.

444   **Boughman W, Moss C. 2003.** Social sounds: Vocal learning and development of mammal and

445   bird calls. In Simmons A, Popper AN, Fay RR eds. *Acoustic Communication*. Springer Press :

446   138–224.

447   **Brittan-Powell EF, Dooling RJ, Farabaugh SM. 1997.** Vocal development in budgerigars

448   (*Melopsittacus undulatus*): contact calls. *Journal of Comparative Psychology* **111(3):** 226-

449   241.

450   **Busch C, Antinuchi CD, del Valle JC, Kittlein M, Malizia AI, Vassallo AI, Zenuto RR. 2000.**

451   Population ecology of subterranean rodents. In Lacey EA, Cameron G, Patton JL, eds. *Life*

452   *underground: the biology of subterranean rodents*: University of Chicago Press, Chicago,

453   Illinois, 183-226.

454   **Camín S. 2010.** Gestation, maternal behaviour, growth and development in the subterranean

455   caviomorph rodent *Ctenomys mendocinus* (Rodentia, Hystricognathi, Ctenomyidae). *Animal*

456   *Biology* **60(1):** 79-95.

457   **Campbell P, Pasch B, Warren AL, Phelps SM. 2014**. Vocal ontogeny in Neotropical singing mice

458   (*Scotinomys*). *PloS one* **9(12):** e113628

459   **Clemmons J, Howitz JL. 1990.** Development of Early Vocalizations and the Chick-a-dee Call in

460   the Black-capped Chickadee, Parus atricapillus. *Ethology* **86(3)**: 203-223.

461   **Efremova KO, Volodin IA, Volodina EV, Frey R, Soldatova NV, Lapshina EN, Gorbunov KS. 2011.**

462   Sex and age effects on the structural features of nasal calls and body size in the goitred

463   gazelle (*Gazella subgutturosa*, Artiodactyla, Bovidae) calves. *Zoologicheskii Zhurnal* **90:** 603-

464   615.

465   **Elowson AM, Snowdon CT, Sweet CJ. 1992.** Ontogeny of trill and J-call vocalizations in the

466   pygmy marmoset, Cebuella pygmaea. *Animal Behaviour* **43(5):** 703-715.

467   **Francescoli G. 1999.** A preliminary report on the acoustic communication in Uruguayan

468   *Ctenomys* (Rodentia, Octodontidae): basic sounds types. *Bioacoustics* **10(2–3):**203–218 DOI

469   10.1080/09524622.1999.9753431.

470   **Glazier DS. 1985.** Energetics of litter size in five species of *Peromyscus* with generalizations for

471   other mammals. *Journal of Mammalogy* **66(4):**629-642.

472   **Grimsley JMS, Monaghan JJM, Wenstrup JJ. 2011.** Development of social vocalizations in mice.

473   *PloS one* **6(3):** 1–15.

474   **Hammerschmidt K, Newman JD, Champoux M, Suomi SJ. 2000.** Changes in rhesus macaque

475   'coo'vocalizations during early development. *Ethology* **106(10):** 873-886.

476   **Hammerschmidt K, Jürgens U, Freudenstein T. 2001.** Vocal Development in Squirrel Monkeys.

477   *Behaviour* **138(9):** 1179–1204.

478   **Henneman WW. 1983.** Relationship between body mass, metabolic rate and the intrinsic rate of

479   natural increase. *Oecologia* **56:**104 - 108 .

480   **Henneman WW. 1984.** Intrinsic rate of natural increase of altricial and precocial eutherian

481   mammals: the potential price of precociality . *Oikos* **43:**363 - 367

482   **Hofman MA. 1983.** Evolution of brain size in neonatal and adult placental mammals: a

483   theoretical approach . *Journal of theoretical biology* **105(2):**317-332.

484   **Hooper ET, Carleton MD. 1976.** Reproduction, growth and development in two contiguously

485   allopatric rodent species, genus *Scotinomys*. Miscellaneous publications

486   Museum of Zoology, University of Michigan: 151

487   **Jarvis JU. 1969.** The breeding season and litter size of African mole-rats. *Journal of Reproduction*

488   *and Fertility* **6:**237-248.

489   **Konishi M. 1965.** The role of auditory feedback in the control of vocalization in the white-

490   crowned sparrow. *Ethology* **22(7):** 770-783.

491   **Kroodsma DE, Konishi M. 1991.** A suboscine bird (eastern phoebe, *Sayornis phoebe*) develops

492   normal song without auditory feedback. *Animal Behaviour* **42(3):** 477-487.

493   **Morton ES. 1977.** On the occurrence and significance of motivation-structural rules in some bird

494   and mammal sounds. *The American Naturalist* **111(981):** 855-869.

495   **Owings DH, Morton ES. 1998.** Animal vocal communication: a new approach. *Cambridge*

496   *University Press*.

497   **R Core Team (2017)**. R: A language and environment for statistical computing. R Foundation for

498   Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

499   **Scherrer JA, Wilkinson GS. 1993.** Evening bat isolation calls provide evidence for heritable

500   signatures. *Animal Behaviour* **46(5):**847-860.

501   **Sikes RS, Gannon WL. 2011.** The animal care and use committee of the American Society of

502   Mammalogists. Guidelines of the American Society of Mammalogists for the use of wild

503   mammals in research. *Journal of Mammalogy* **92(1):**235–253

504   **Schleich C, Busch C. 2002.** Acoustic signals of a solitary subterranean rodent *Ctenomys talarum*

505   (Rodentia: Ctenomyidae): physical characteristics and behavioral correlates. *Journal of*

506   *Ethology* **20(2):**123–131 DOI 10.1007/s10164-002-0064-9.

507 **Tachinardi P, Bicudo JEW, Oda GA, Valentinuzzi VS. 2014.** Rhythmic 24 h variation of core body

508   temperature and locomotor activity in a subterranean rodent (*Ctenomys* aff. *knighti*) the

509   tuco-tuco. *PLoS ONE* **9(1):**e85674 DOI 10.1371/journal.pone.0085674.

510 **Tyack PL. 2016.** Vocal Learning and Auditory-Vocal Feedback. In Suthers RA, Fitch WT, Fay RR,

511   Popper AN, eds. *Vertebrate Sound Production and Acoustic Communication*. Springer, 261–

512   295.

513 **Vleck D. 1979.** The energy cost of burrowing by the pocket gopher *Thomomys bottae.*

514   *Physiological Zoology* **52(2):**122–136 DOI 10.1086/physzool.52.2.30152558.

515 **Vleck D. 1981.** Burrow structure and foraging costs in the fossorial rodent, *Thomomys bottae.*

516   *Oecologia* **49(3):**391–396 DOI 10.1007/BF00347605

517 **Winter P, Handley P, Ploog D, Schott D. 1973.** Ontogeny of squirrel monkey calls under normal

518   conditions and under acoustic isolation. *Behaviour* **47:** 230–239.

519 **Zenuto RR, Antinuchi CD, Busch C. 2002.** Bioenergetics of reproduction and pup development in

520   a subterranean rodent (*Ctenomys talarum*). *Physiological and Biochemical Zoology*

521   **75(5):**469-478.

Figure 1

General structure of the long-range vocalization (LRV).

A. General structure of the long-range vocalization (LRV) of the Anillaco Tuco-Tuco (Ctenomys sp.). B. Syllable types (series and individual notes notes). Triad series are composed by three notes (N1, N2 and N3) and two soft-notes (Sa and Sb), and individual notes are simple sounds. Waveforms are shown above and corresponding spectrogram below in both figures.

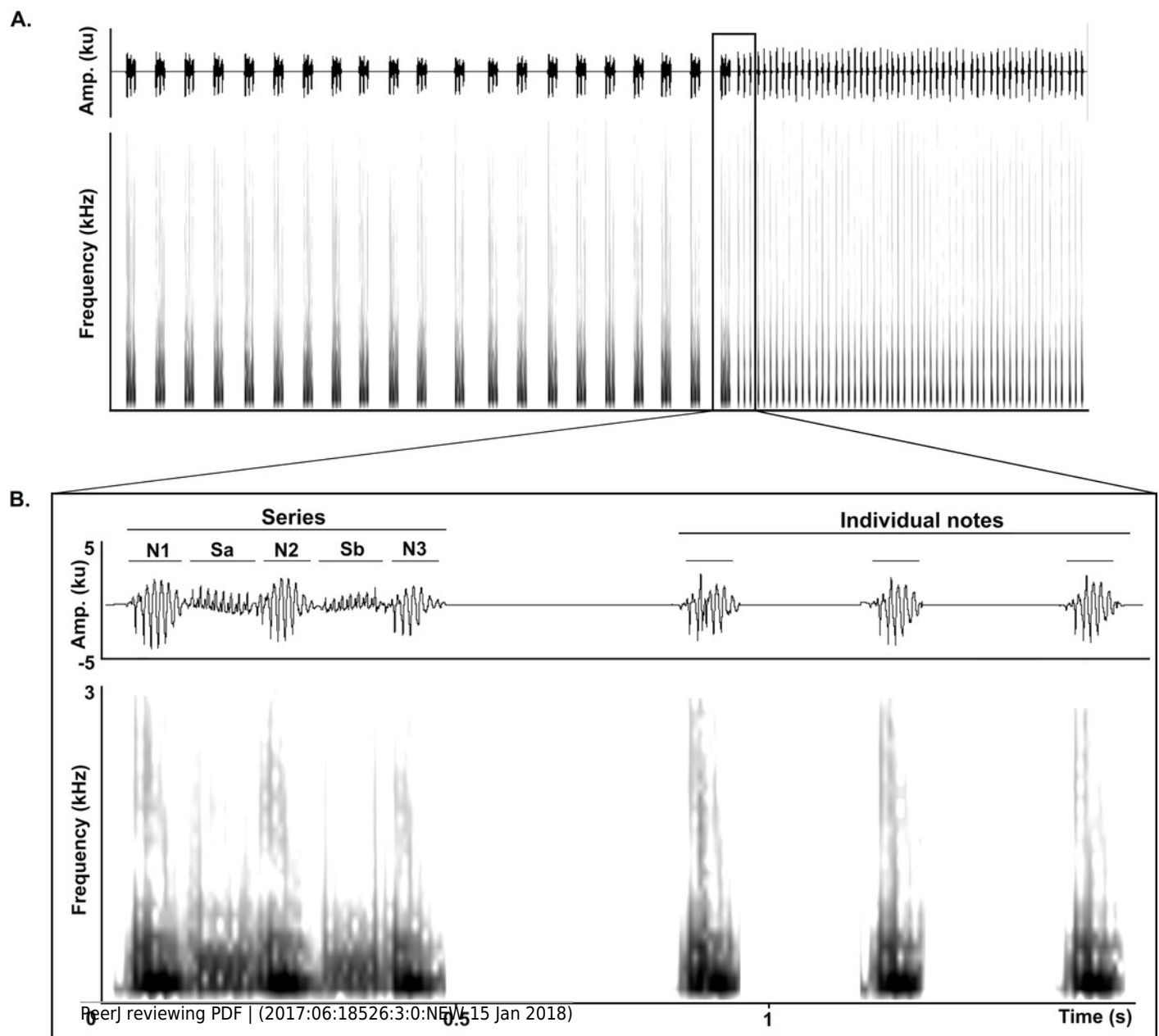



Figure 2

Acoustic characterization of the long-range vocalization (LRV).

Delimitation of selection borders used for the quantitative acoustic characterization of triads (A and B) and individual notes (C and D) of the long-range vocalization (LRV) during the vocal development of juveniles and in adults of the Anillaco Tuco-Tuco (*Ctenomys* sp.). Waveforms are shown above and corresponding spectrogram below in all figures. A. Selection border A delimiting a triad. B. Selection borders B and selection border C delimited by the two adjacent selection borders B with the highest energy values. C. Selection border D delimiting an individual note. D. Selection borders E and selection border F delimited by the two adjacent selection borders B with the highest energy values. We measured duration 90%, bandwidth 90% and peak frequency in selection borders C (in triads) and F (in individual notes).

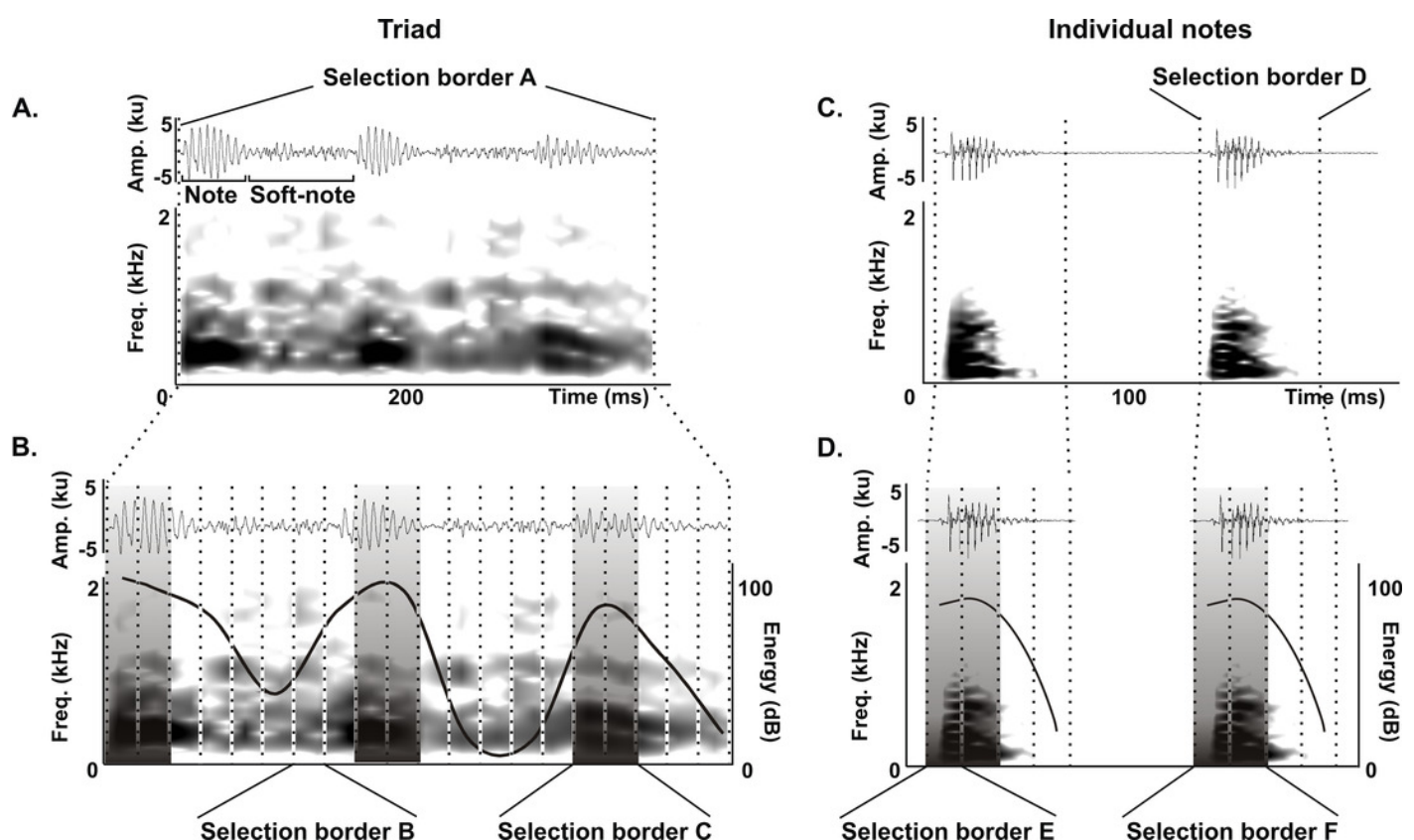



Figure 3

Abnormal series.

Abnormal series produced by juveniles of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during the development of the long-range vocalization (LRV). Waveforms are shown above and corresponding spectrogram below in all figures. A. Abnormal triads. B. Abnormal dyads. C. Abnormal tetrad. D. Monad. Previous soft-note (P S); note (N); soft-note (S). Numbers indicate the corresponding note or soft-note in the series.

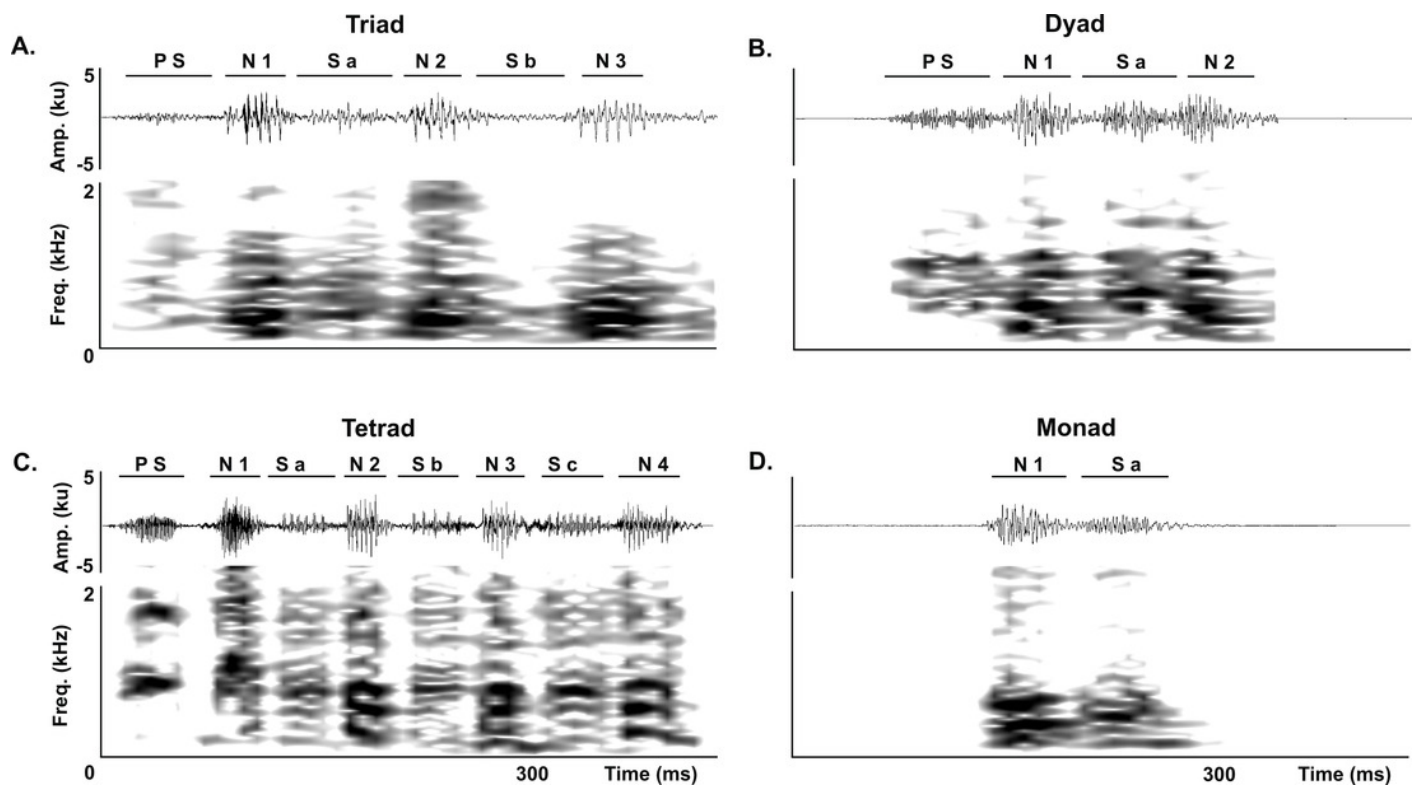



Figure 4

Development of the long-range vocalization (LRV).

Example of the development of the long-range vocalization (LRV) in a male of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during 4 sampling weeks over 12 study weeks. During the sampling period this individual (male 2) gave 11 vocalizations (numbered boxes). Note the increase in the number of series per vocalization and the late appearance of individual notes. These two features are representative of the development of the LRV in this species. For each sampling week we indicate weight (g) and estimated age (days after birth). Vx�d

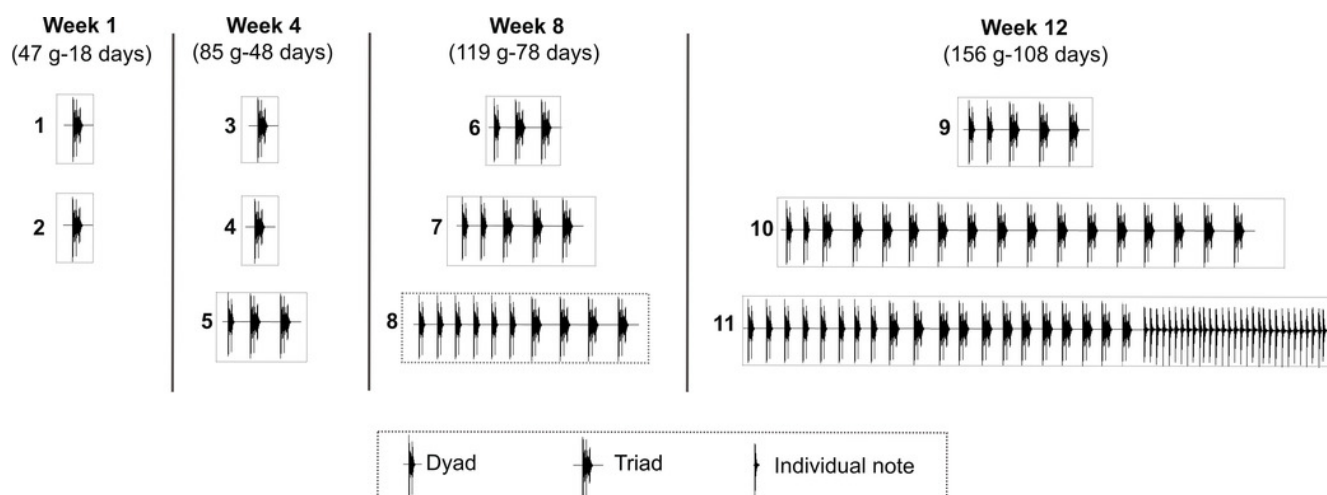



Figure 5

Developmental changes in acoustic features in the long-range vocalization.

Developmental changes in acoustic features of triad notes and individual notes in the long range vocalization (LRV) of the Anillaco Tuco-Tucos (*Ctenomys* sp.) and comparison to adult values. All data from juveniles and adults in Table S1. A. Duration 90%. B. Bandwidth 90%. C. Peak frequency.

A. Duration 90%

Juveniles · Adults

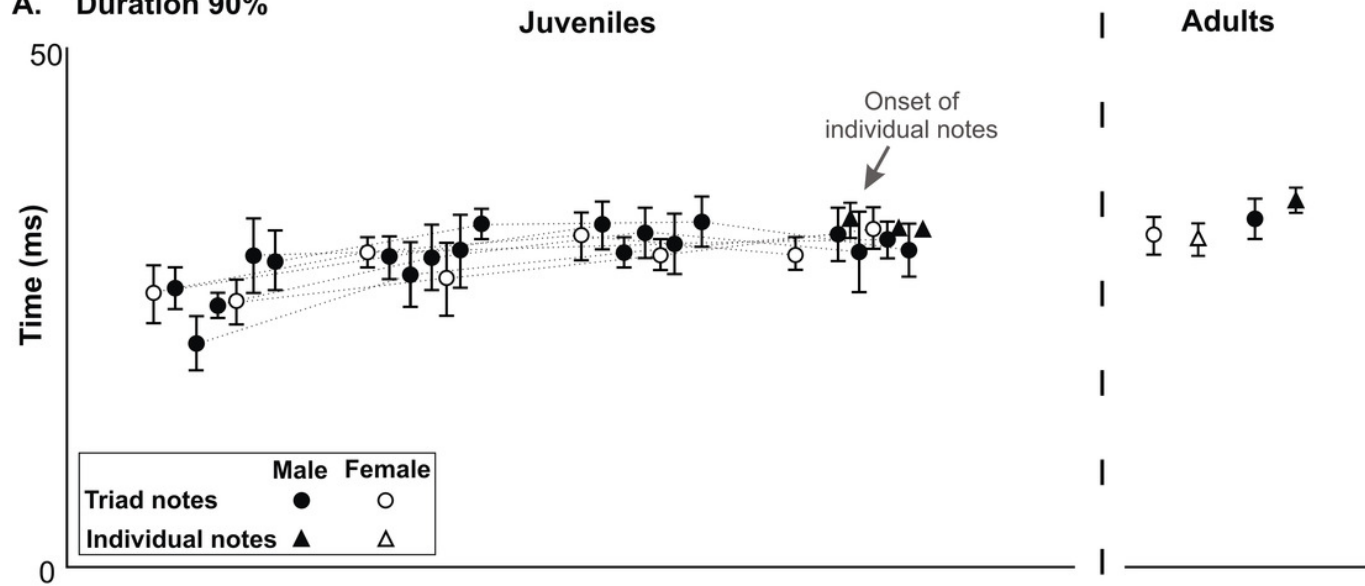
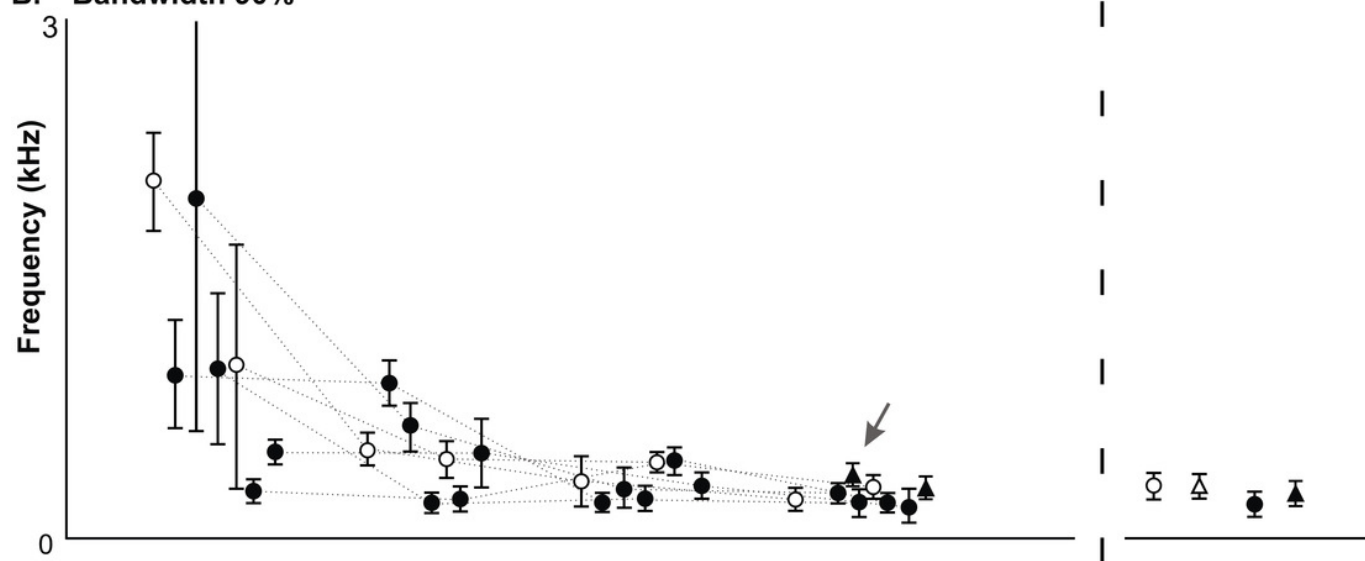
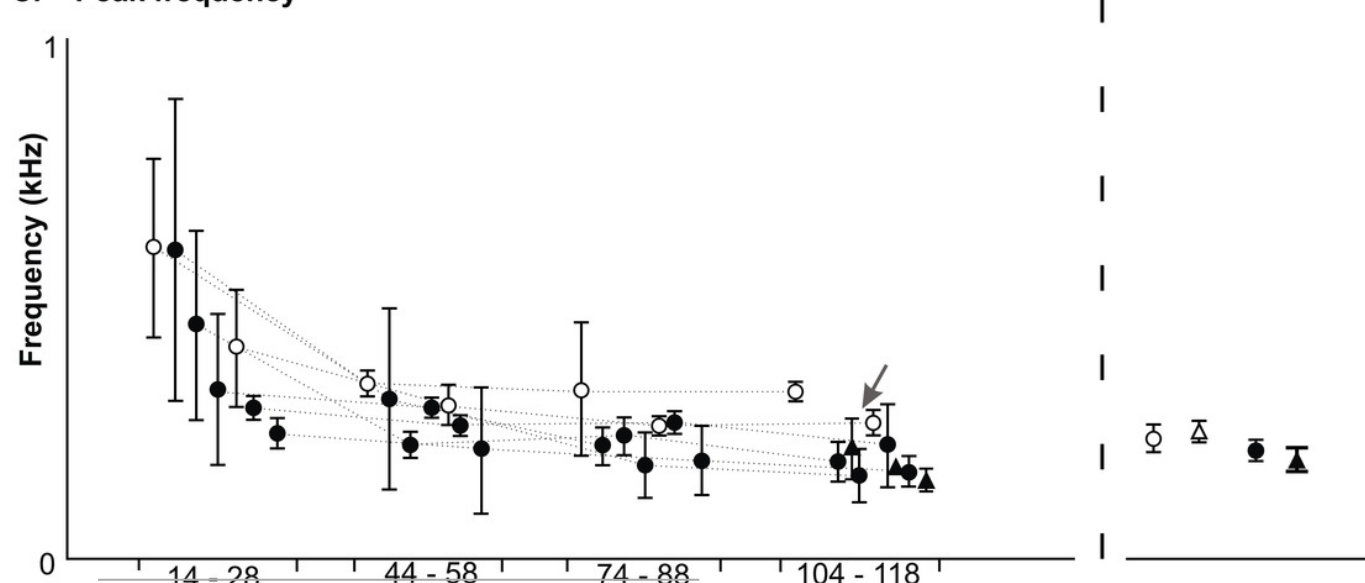
Onset of individual notes

Male	Female
Triad notes ●	○
Individual notes ▲	△

B. Bandwidth 90%

C. Peak frequency

14 - 28 · 44 - 58 · 74 - 88 · 104 - 118

Age classes (days after birth)

Long-range vocalizations emitted by juveniles during vocal development.

Structural features of the long-range vocalizations (LRVs) emitted by eight juveniles (five males and three females) of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during 4 sampling weeks over 12 study weeks. For each juvenile individual we indicate weight (W) (g), estimated age in days (D), and recording time in hours (R). For each vocalization recorded (Roman numerals) we indicate number of each normal series-pattern (triads in bold font; dyads in normal font; and tetrads with underline), and number of individual notes per vocalization (underlined). Deceased (†), no vocalization (-).

Juveniles individual	Week 1			Vocalizations				Week 4			Vocalizations				Week 8			Vocalizations				Week 12			Vocalizations			
	W	D	R	I	II	III	IV	W	D	R	I	II	III	IV	W	D	R	I	II	III	IV	W	D	R	I	II	III	IV
Male 1	73	28	22	**1**	1	**1**	-	101	58	24	1	**1**	11	-	125	88	18	23	23	16	-	162	118	24	24	**10**	21826	-
Male 2	47	18	22	**1**	**1**	-	-	85	48	20	**1**	**1**	12	-	119	78	24	12	23	64		156	108	24	23	216	6816231	-
Male 3	45	17	20	**1**	**1**	-	-	80	47	22	**1**	**2**			117	77	20	**4**	**7**	-	-	†	†	†	†	†	†	†
Male 4	71	27	24	**1**	**1**	**3**	-	97	57	24	11	**3**	5	-	123	87	28	25	**13**	29	-	158	117	28	**13**	42122	-	-
Male 5	49	19	24	**1**	1	-	-	81	49	24	**2**	**4**	-	-	111	79	28	11	25	43	-	150	109	28	5	16	**15**	-
Female 1	39	14	22	**1**	**1**	1	-	68	44	24	**1**	**1**	11	-	95	74	22	**1**	**2**	**3**	-	125	104	20	5	**6**	-	-
Female 2	59	23	24	**1**	**2**	1	-	85	53	24	**1**	**1**	11	-	110	83	28	**1**	**3**	-	-	142	113	28	**2**	**3**	**6**	-

Female 3	55	21	20	-	-	-	-	70	5 1	1 8	-	-	-	-	92	81	22	-	-	-	-	11 5	10 1	24	-	-	-	-

Statistical analyses.

Quantitative comparisons of acoustic parameters (duration 90%, bandwidth 90% and peak frequency) of triad notes and number of series in the long-range vocalizations (LRVs) between four age classes of juvenile males of the Anillaco Tuco-Tuco (*Ctenomys* sp.) and acoustic parameters of triad notes within juvenile males and females age class against adults of the corresponding sex. Friedman test and Bonferroni post hoc-test results comparing acoustic parameters of triad notes and number of notes per vocalization between different juvenile males age classes; shared letters indicate no statistical differences and different letters indicate significant statistical differences between age classes. Wilcoxon Signed-Rank test results compare acoustic parameters of all triad notes of different age classes of juvenile males against adults; P-values in bold indicate statistically significant differences between an age class in comparison to adult values. Data presented is mean±sd [range]. See table S1 for each juvenile and adult values.

	Statistical test	Series notes parameters	Juvenile age classes (days after birth)				Adults
			14–28	44–58	74–88	104–118	
Males	Friedman test (Bonferroni post-hoc test)	Duration 90% (ms)	A	B	B	B	-
		Bandwidth 90% (Hz)	A	AB	B	B	-
		Peak frequency (Hz)	A	AB	B	B	-
		Series per vocalization	A	AB	B	C	-
		N	5	5	5	4	-
	Wilcoxon Signed-Rank test	Duration 90% (ms)	**p<0.001** 28.2±2.1 [25.1-31.9]	p<0.3 30.5±2.2 [27.9-33.8]	p<0.6 31.9±1.5 [29.9-34.8]	p<0.8 30.8±2.0 [27.0-32.9]	- 31.4±1.7 [29.3-34.6]
		Bandwidth 90% (Hz)	**p<0.001** 832.6±771.5 [234.4-2637.8]	**p<0.01** 479.1±186.8 [201.0-778.8]	p<0.06 268.8±55.5 [186.6-351.7]	p<1 207.6±82.5 [96.9-305.1]	- 192.7±67.8 [105.5-257.8]
		Peak frequency (Hz)	**p<0.001** 376.1±193.4 [222.5-832.6]	**p<0.001** 254.4±47.1 [208.2-367.2]	**p<0.03** 217.4±36.3 [157.9-269.1]	p<0.1 207.6±82.5 [96.9-305.1]	- 187.5±15.6 [164.1-210.9]
		N	5	5	5	4	5
Females	-	Duration 90% (ms)	26.1±1.3 [25.2-28.1]	29.7±1.9 [27.1-31.9]	31.9±0.7 [31.0-32.9]	31.7±0.1 [30.0-32.9]	31.3±0.1 [29.3-32.0]
		Bandwidth 90% (Hz)	1545.9±769.8 [452.2-2128.2]	497.7±63.3 [426.1-556.3]	337.9±124.5 [215.3-463.0]	260.2±65.5 [168.7-315.8]	293.0±57.4 [246.1-363.3]
		Peak frequency (Hz)	508.7±139.4 [337.4-674.7]	322.2±35.6 [269.5-348.1]	315.8±99.6 [244.1-463.0]	274.7±59.3 [210.9-333.8]	249.0±17.5 [234.4-269.5]
		N	2	2	2	2	3