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The release call as a diagnostic character between cryptic related species *Odontophrynus cordobae* and *O. americanus* (Anura: Cycloramphidae)

P.R. GRENAT^{1,2} & A.L. MARTINO^{1,3}

¹*Ecología, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto. Ruta Nacional N° 36 - km 601, (X5804BYA) Río Cuarto, Argentina. E-mails: pgrenat@exa.unrc.edu.ar, amartino@exa.unrc.edu.ar*

²*CONICET Fellowships*

³*Corresponding author. E-mail: amartino@exa.unrc.edu.ar, adolfofmartino@gmail.com*

Diploid *Odontophrynus cordobae* and tetraploid *O. americanus* are morphologically cryptic species (Martino and Sinsch 2002). These species occurs in sympatry and syntopy in the SW of the Córdoba province, Argentina (Grenat *et al.* 2009). At present, individuals of *O. cordobae* and *O. americanus* can be only differentiated by mean cytogenetics (Salas 2006; Rosset *et al.* 2006), which involves the sacrifice of the animal, and erythrometry (Grenat *et al.* 2009). We propose the release call as a novel character to differentiate these species. Release vocalizations are produced by male anurans as a negative response to male mating attempts. Some studies demonstrated that the release calls of several anuran species differed specifically and that temporal structure of calls could be phylogenetically informative (Brown and Littlejohn 1972; Sullivan and Lamb 1988; Sullivan and Malmos 1994; di Tada *et al.* 2001). In the genus *Odontophrynus*, only the release call of *O. cordobae* has been described (Grenat *et al.* 2012). The sonograms of *O. americanus* and *O. occidentalis* release calls were showed by Barrio (1964), but call measurements were not reported. The aims of present study are: 1) to describe the release call of *Odontophrynus americanus*; 2) to compare release calls of cryptic species *O. cordobae* and *O. americanus*; 3) to evaluate release calls as diagnostic character to distinguish between these species.

We analyzed the calls of 10 individuals of *O. cordobae* and 5 individuals of *O. americanus* from Córdoba province, Argentina. Seven diploid individuals were sampled near of the locality of Alpa Corral while two tetraploid individuals were sampled in the locality of Río Cuarto. Three syntopic individuals of each species, from the locality of La Escondida, were included within this sample. The ploidy of syntopic individuals was previously confirmed by cytogenetic and erythrometric analysis (Grenat *et al.* 2009). Release calls were induced in the laboratory (air temperature=20±2°C) by a slight pressure behind the forelimbs simulating an axillary amplexus (Leary 2001; Grenat *et al.* 2012). Microphone was positioned to 20–30 cm of each individual and a series of release calls (between 30 s to five minutes) were recorded using a Walkman digital audiotape (DAT) Sony TCD-100™ and a Stereo Microphone Sony ECM-MS907™. The procedures of induction and registration of release calls were performed similarly for each of the individuals tested.

We analyzed 8–10 calls per individual. The acoustic signals were digitized using Adobe® Audition™ 1.0 (sampling rate: 44.1 KHz; bit depth: 16 bit) and analyzed using five parameters: (1) Call duration (CD), (2) Pulse / call (P / C), (3) Pulse duration (PD), (4) interpulse interval (IPI), (5) Dominant frequency (DF, FFT: 1024 points), all these measurement following di Tada *et al.* (2001). Moreover, we calculated the pulse rate (PR = 1 / (PD + IPI)) and duty cycle (DC = PD / IPI). We calculated the averages of each variable per individual and used these mean values in subsequent data analysis. We made comparisons between species using ANOVA. Discriminant function analyses (DFA) were performed to study the variation among groups previously defined, and obtain a reclassification rate of calls analyzed. Given that correlations between variables affect the results of DFA, we tested the association between acoustic parameters and included in the analysis only the uncorrelated variables ($p < 0.05$).

The release call of *O. americanus* consisted of a single pulse group, structurally similar but shorter than their advertisement call (*see* Martino and Sinsch 2002) (Fig. 1). Descriptive statistics (reported as mean ± standard deviation (minimum-maximum)) of acoustic variables of *O. americanus* release calls were: CD = 268.1 ± 56.9 ms (170–381); P/C = 26.1 ± 5.7 pulses/call (17–37); DF = 1006.4 ± 46.6 Hz (925.5–1094.5); PD = 3.6 ± 1.1 ms (2.4–6.3); IPI = 6.3 ± 1.6 ms (3.6–8.8); PR = 102.2 ± 7.9 (85.3–116.7); PQ = 0.7 ± 0.4 (0.3–1.7).