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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, adolfomartino@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±2ºC) 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-maximun)) 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).

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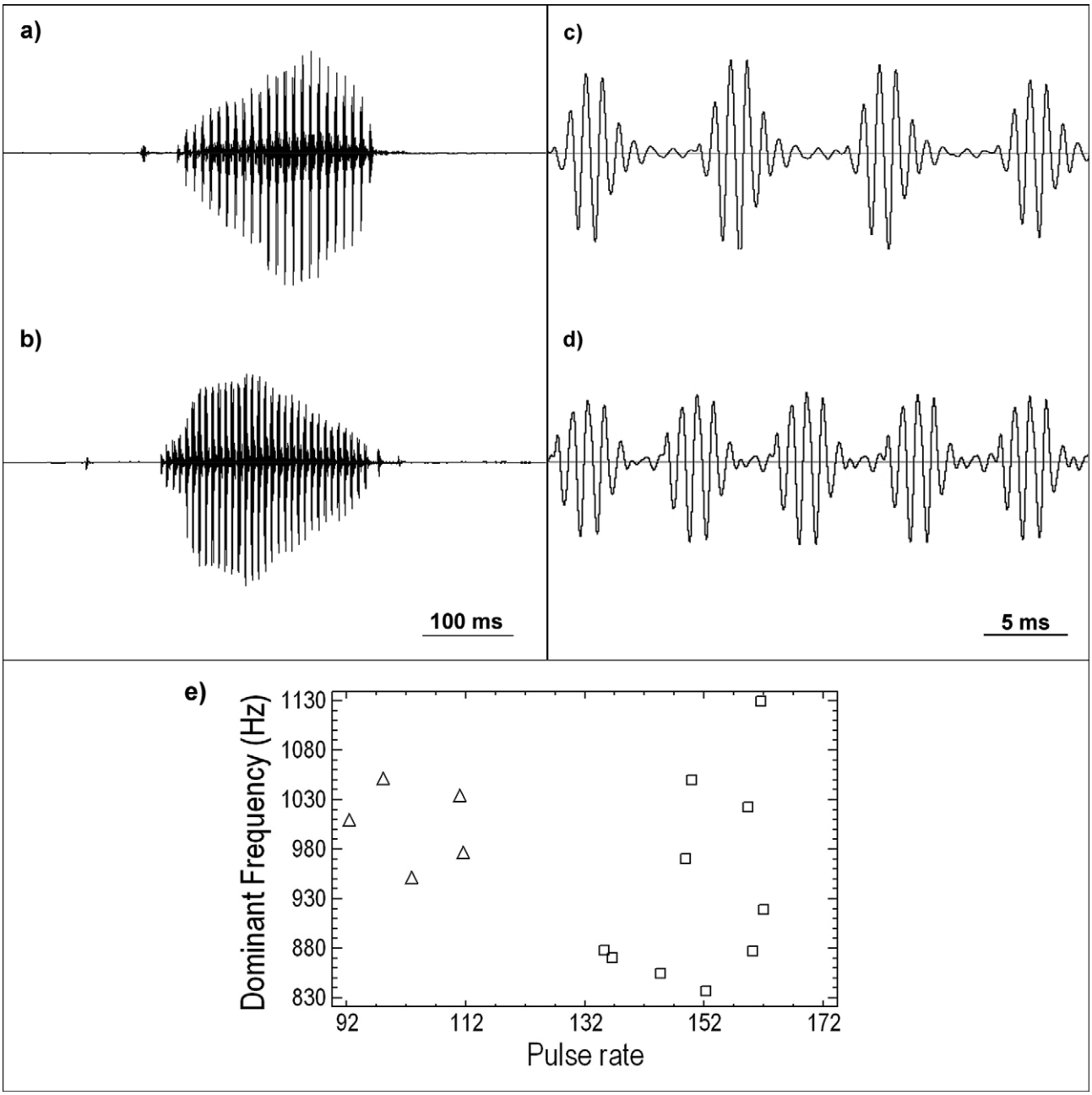

FIGURE 1. Comparisons of release calls of *Odontophrynus americanus* (a) and *O. cordobae* (b); and pulse and interpulse duration of their respective calls (c and d). The scatterplot (e) illustrate the acoustic distinction among release calls of *Odontophrynus cordobae* (squares) and *O. americanus* (triangles) in terms of pulse rate.

Descriptive statistics of acoustic parameters for *O. cordobae* release calls were: CD = 204.6 ± 48.0 ms (range: 128–34); P/C = 29.4 ± 6.9 pulses/call (16–47); DF = 939.5 ± 112.8 Hz (704.7–1149.5); PD = 3.3 ± 0.7 ms (2.1–5.2); IPI = 3.3 ± 0.6 ms (2.1–4.6); PR = 151.7 ± 11.0 (120.8–172.1); PQ = 1.1 ± 0.4 (0.4–2.2).

Overall, the release calls of *O. cordobae* and *O. americanus* showed a similar structure but differed statistically in all acoustic parameters when univariated analyses of variance were performed ($p<0.01$). DFA based on three uncorrelated acoustic parameters (CD, DF and PR) yielded a single highly significant function (Eigenvalue=9.23; Canonical correlation=0.95; Wilks' $\lambda=0.10$; $p=0.000$). The function obtained was most strongly correlated with PR (Fig. 1). We obtained a reclassification rate of individuals within its own species of 100%.

Advertisement calls of *O. cordobae* and *O. americanus* have the same general structure: a single pulse group, whose oscillogram shows a spindle shape in which the pulses located at the beginning of the call have a low intensity, followed by increasing intensity pulses, and a decrease in the intensity of the pulses at the end of the call (Martino and Sinsch

2002). Thus, we would expect that their release calls were also similar. Barrio (1964) noted a correspondence in the structure of advertisement calls and release calls of *O. americanus*, as we observed in this study, and as was also reported for *O. cordobae* (Grenat *et al.* 2012). We compared our results with the acoustic parameter values of *O. americanus* advertisement calls reported by Martino & Sinsch (2002). The duration of release call was approximately half that of their respective advertisement calls. Pulse duration and interpulse interval were lower in the release call although the differentiation between these two parameters was larger than that found in the advertisement call. This resulted in a higher value for pulse rate than reported by Martino & Sinsch (2002).

In despite of their same general structure, Martino & Sinsch (2002) reported notable differences in the acoustic parameters of advertisement calls of these species, which could act as a reproductive barrier between them. In contrast, Leary (2001) suggested that selection favouring prompt release during heterospecific amplexus should result in convergent character displacement in release vocalizations. Di Tada *et al.* (2001) suggested that the structural similarity of release calls in the *Bufo* (*Rhinella*) *spinolusus* group may result in the avoidance of heterospecific amplexus between sympatric toads. Considering its function, it would be expected that in areas of syntopy, advertisement call characters of related species were influenced by divergent selection, while the release call characters should be under the influence of convergent selection (Rand 1988). Our results show a remarkable difference in the release calls between *Odontophrynus americanus* and *O. cordobae*, even when syntopic individuals of each species were included in the analysis. It is known that polyploidization can lead to changes in some properties of the advertisement call, such as pulse rate (Keller and Gerhardt 2001), as a direct result of the increase in cell size (Bogart and Wasserman 1972). Under the same basis, a polyploidization event may affect the properties of the release call. This could explain the observed differences between species in both types of vocalization. While the number of individuals examined in our study is low, we consider that the number of calls analyzed and the high distinction observed between species reinforces our results. These results, combined with the simplicity to induce this type of vocalizations, shows that release call could represent a powerful diagnostic character to distinguish between this pair of cryptic species.

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