Dissimilarities in auditory tuning in midwife toads of the genus *Alytes* (Amphibia: Anura)

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The auditory sensitivity in three species of the anuran genus *Alytes* (Alytidae) was examined to determine patterns of intra- and interspecific variation, relating these measurements to behavioural preferences measured in previous studies and to the adaptive and evolutionary significance of this sensory function. The audiograms obtained with multi-unit recordings in the torus semicircularis of 13 *Alytes cisternasii*, 10 *Alytes obstetricans*, and eight *Alytes dickhilleni* show two regions of enhanced sensitivity, between approximately 100–500 and 1200–2400 Hz, with minimum thresholds at approximately 40 and 45 dB SPL, respectively. The mean and range of the high-frequency region differed among species, although the sensitivity, measured as minimum thresholds, was similar. The region of high-frequency sensitivity was centred at approximately the frequency of the advertisement call in *A. cisternasii* but, in *A. obstetricans* and *A. dickhilleni*, was centred at frequencies higher than the conspecific calls. These results contrast with preferences for lower frequencies exhibited by *Alytes* in female phonotactic and in male evoked vocal responses. Such loose relationships between signals and receivers suggest that the divergence of the sound communication system in *Alytes* has implied environmental and phylogenetic factors in addition to sexual selection processes. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, 116, 41–51.

**ADDITIONAL KEYWORDS:** acoustic communication – auditory sensitivity – torus semicircularis.

**INTRODUCTION**

Animal communication involves the exchange of information between emitters and receivers, a process favoured by a broad correspondence between signal structure and receptor’s sensitivity.

The notion of a strict matching between signals and receptors oriented pioneering research in sound communication of anurans (Capranica, 1965; Capranica & Moffat, 1983). Males of the original experimental subject of this research, *Rana catesbeiana*, now *Lythobates catesbeianus*, give evoked vocal responses only to stimuli containing spectral energy both in low and high-frequency regions, matching the spectral design of the conspecific advertisement call. These two components in turn match the sensitivities of the auditory receptors, the amphibian, and the basilar papilla of this species (Frishkopf, Capranica & Goldstein, 1968).

In general, such a trend has been confirmed in more recent studies (Gerhardt & Schwartz, 2001); however, various instances with a lack of correspondence between vocalizations and auditory tuning have been reported. Gross mismatches occur in anurans that communicate with calls having their main spectral components restricted to the high-frequency region, with their low frequency sensitivity having no apparent communicative role (Ryan & Rand, 1993; Bosch & Boyero, 2003a). At a finer scale, in a number of anuran species communicating with calls in the high-frequency region, females prefer frequencies lower than the mean of the dominant frequency of the conspecific call, originating processes of directional selection by which conspecific calls of relative low frequencies within the high-frequency range are favoured (Ryan, Perrill & Wilczynski, 1992; Wollerman,
The advertisement calls of toads of the genus *Alytes* of the family Alytidae structure (Ryan & Rand, 1999; Meenderink, 1992; Anderson, 1994; Endler & Basolo, 1998). These variants from a strict correspondence between emitters and receivers have been explained in terms of constraints imposed by environmental factors (Endler, 1992) or by sensory systems (Ryan, 1990; Arak & Enquist, 1995). In particular, the sensory bias hypothesis proposes that natural selection models female preferences and males take advantage on these sensory designs for mate attraction (Fuller, Houle & Travis, 2005).

Explorations of correspondences between signal structure and receptor sensitivity have been conducted mostly in derived anurans, which, in some cases, communicate by means of signals having complex structure (Ryan & Rand, 1999; Meenderink, Kits & Narins, 2010). Anurans of the family Alytidae, having a sister status relative to Neobatrachia, have proved to be advantageous subjects for research on sound communication (Marquez & Bosch, 2001). The advertisement calls of toads of the genus *Alytes* are low-amplitude, short-duration pure tones lacking frequency and amplitude modulation. The signals of four species found in the Iberian Peninsula differ in their dominant frequency and duration (Heinzmann, 1970; Marquez & Bosch, 1995; Bush, 1996). Phono-taxis studies on *Alytes cisternasii* and *Alytes obstetricans* have reported that females orient preferentially to low-frequency calls within the conspecific range, a preference pattern congruent with mating advantages of larger males found in nature (Marquez, 1995a, b; Marquez & Bosch, 1997; Bosch & Marquez, 2005; Marquez, Bosch & Eekhout, 2008). Males also call more actively in response to synthetic call dummies having lower dominant frequencies (Bosch & Marquez, 1996), indicating the relevance of this trait for intrasexual competition.

Measurements of auditory sensitivity have been conducted for two species of *Alytes*. Audiograms of *A. obstetricans* reported by Bosch & Wilczynski (2003) show a region of high-frequency sensitivity tuned at approximately 1400 Hz, which closely matches the dominant frequency of the call of the population analyzed. However, it is not clear how comparable these measurements are because each species was studied in different settings, different laboratories and with an interval of decades in between. In addition to these explorations, two studies on auditory midbrain single and few-unit recordings have been carried out in *A. obstetricans* (Mohneke, 1982, 1983).

In the present study, we conducted measurements of the auditory sensitivity of *Alytes cisternasii*, *A. obstetricans* and *Alytes dickhillenii*, aiming to examine patterns of variation within and among species, relating these sensitivities to the spectral structure of their calls and to behavioral preferences for these signals reported in previous studies on this genus. We aimed to determine whether the tuning of the auditory sensitivity of these anurans has a correspondence with the preferences of males and females for calls of low dominant frequencies within the conspecific ranges reported in the literature. In addition, the current knowledge of the phylogenetic relationships among these taxa (Martinez-Solano et al., 2004; Maia-Carvalho et al., 2014) allows interpretations on evolutionary trends of correspondences between auditory sensitivity and vocal traits. These explorations were carried out using a common set-up between the three taxa, aiming to provide interpretations on potential interspecific differences in auditory sensitivity devoid of difficulties of comparisons based on two previous independent studies (Mohneke & Schneider, 1979; Bosch & Wilczynski, 2003).

**MATERIAL AND METHODS**

**EXPERIMENTAL SUBJECTS AND SURGICAL PROCEDURES**

The adult individuals used for the present study were captured in different localities in Spain between 2003 and 2006: 13 *A. cisternasii* (nine females and four males from Merida, 38.917°N, 6.333°W), 10 *A. obstetricans* (three females and seven males from Toro and Picos de Europa National Park, 41.525°N, 5.392°W and 43.187°N, 4.871°W, respectively), and eight *A. dickhillenii* (three females and five males from Cazorla Natural Park, 38.158°N, 2.738°W). The frogs were carried to the Laboratory of Neuroethology at the University of Chile in Santiago, Chile where they were housed in terraria under an inverted 12 : 12 h light/dark cycle at 15 ± 1 °C and fed with tenebrionid larvae once per week. The
animals were used for neurophysiological recordings within 1 month of their arrival in Chile. The subjects were anaesthetized by immersion in a 0.4% aqueous solution of MS-222 (Sigma). Under anaesthesia, the skin on the dorsal surface of the head was incised and a hole was drilled into the underlying skull. Dura and pia membranes covering the brain were dissected away to expose the surface of the optic tectum. After surgery, animals were allowed to recover from anaesthesia for 12–24 h. Next, the subjects were immobilized with an injection of d-tubocurarine chloride (A. cisternasi: 45 μg g⁻¹ body weight; A. obstetricans 55 μg g⁻¹ body weight; A. dickhilleli: 40 μg g⁻¹ body weight), placed on a Peltier plate (Cambion) and covered with light moist gauze to facilitate cutaneous respiration. The recordings were conducted in a sound-attenuated booth having walls and ceiling covered with 10-cm height foam wedges. Immobilization was maintained throughout the recording session with periodic injections of d-tubocurarine chloride, and lidocaine (2%) was applied topically to the wound margins. A DC current of approximately 1 A was supplied to the Peltier plate to maintain the frog’s body at 15 °C, a temperature at which these frogs normally breed and call in their natural settings. Frog temperature was monitored with a miniature thermometer probe (Digi-sense 8528-20; Cole-Parmer) inserted into the cloaca. These procedures comply with the bioethical regulations of the University of Chile (Protocol CBA# 061 FMUCH) and with the authorization of different local governments of Spain (Junta de Extremadura, CN04/0118; Junta de Castilla y León, EP35/04 MG; Junta de Andalucía, N/Ref. SCFFS/AFR/CMM, RS:28/04).

After the experiments were completed, the snout–vent length (SVL) of the toads was measured to the nearest millimeter by pressing the animals flat ventrally against a ruler, followed by weighing (Acculab 333) to the nearest 0.1 g. The toads were euthanized with an anaesthetic overdose to comply with the regulations of the Chilean legislation for the use of imported animals for experimentation (Permit 1022006). The sex of the individuals was determined by surgical inspection of the gonads.

**Stimuli generation**

Synthetic calls and pure tones were generated with SOUND MAKER, version 1.0.3 (Ovobal) on a PowerBook 520c (Apple Inc.). Pure, 175-ms tones having an envelope following the design of a typical advertisement call of *Alytes*, with a 10-ms sinusoidal rise time, a gradual amplitude decline thereafter, and a steeper decrease in the last 20 ms, were used to obtain audiograms. Representative calls of the three species recorded are shown in Fig. 1. The pure tones were set at frequencies between 100 and 5000 Hz, with 100-Hz steps between 100 and 3000 Hz, 200-Hz steps between 3000 and 4000 Hz, and 500-Hz steps between 4000 and 5000 Hz.

The stimuli amplitude was controlled with a programmable attenuator (PA4; Tucker-Davis Technologies), amplified (NAD C 320 BEE or Nakamichi PA-202) and broadcast with a two-way loudspeaker (Dynaudio BM 6, frequency response 38–20 000 Hz), positioned at 1 m in front of the experimental subject. At the beginning of each experimental session, the root mean square sound pressure level (RMS SPL fast) of the pure tones of 100–5000 Hz was measured to the nearest 0.1 dB SPL with the microphone of a sound level meter (Brüel & Kjaer 2238) positioned 2–3 mm above the subject’s head. For these initial measurements, the electrical signal delivered from the amplifier to the loudspeaker had a constant amplitude and the attenuator was set at a fixed value of 10 dB across the frequencies tested. The absolute SPLs of auditory thresholds were determined during the subsequent experimental session by varying the attenuation in 3-dB steps. The threshold values in dB SPL were calculated by subtracting the attenuations in dB at which they occurred (plus 10 dB) from the initial SPLs measured at a constant attenuation of 10 dB. The thresholds obtained were thus expressed with the one-decimal accuracy of the initial measurements. The frequency response of the system was within ± 8 dB in the 100–5000 Hz range and ± 6 dB in the 300–5000 Hz range.

**Recording procedures**

Multi-unit responses from the torus semicircularis were recorded with custom-made gross glass-insulated tungsten electrodes (diameter 75 μm, approximately 50 μm of exposed tip, < 1 MΩ). The electrode was attached to a hydraulic microdrive (Narishige MO-8), positioned on the dorsal surface of the optic tectum and advanced into the brain. The large size of the electrode relative to the optic lobe facilitated a consistent positioning of the electrode on the brain surface at approximately the intersection of the limit between the mid and caudal third and the limit between medial and lateral half of the hemisphere. This placement consistently yielded the largest auditory responses; recordings for each subject were made at this single approximate location. The magnitude of the auditory responses also depended on the electrode depth; the largest responses were obtained at depths of 800–1300 μm, depending on the subject. For most individuals, recordings were obtained from both midbrain hemispheres.
The neural responses were passed through a preamplifier (Dagan 2400) and filtered between 10 and 3000 Hz for multiunit recordings. Neural responses were monitored by means of an oscilloscope and a loudspeaker, and thresholds were determined by audiovisual criteria. Auditory responses were considered to cease when no audible neuronal discharge occurred for two out of three consecutive presentations of a pure tone at a given level. During the recording sessions, measurements conducted independently by different experimenters were always highly coincident, never differing by more than a 3-dB step, which is the minimum amplitude interval used throughout the present study. This methodology has been used in previous studies and yields reliable measurements of auditory thresholds in different anurans (Penna et al., 1990; Penna, Velásquez & Solis, 2008; Penna, Plaza & Moreno-Gómez, 2013; Penna & Moreno-Gómez, 2014).

A search stimulus consisting of a white noise burst with the same envelope structure as the pure tones was presented at an intensity of approximately 80 dB RMS SPL when the electrode was lowered into the brain to determine the depth at which the strongest auditory response was evoked. Stimuli were presented at a rate of 0.5 stimulus s⁻¹.

In all specimens, the lowest threshold observed in the low frequency region of high sensitivity (i.e. low thresholds) corresponded to the 100-Hz tone and this was termed the best threshold of the low frequency region (BTL). The lowest threshold in the high-frequency region of enhanced sensitivity corresponded to different tone frequencies observed in individual audiograms and was termed the best threshold of the high-frequency region (BTH). A centre frequency for the high-frequency region (CFH) was calculated from a weighted mean of thresholds for frequencies between 1000 and 5000 Hz that were within 6 dB above the BTH. To calculate the CFH of an individual audiogram, the differences between an SPL 6 dB above the BTH and the SPLs at different frequencies were computed and added in linear units (N m⁻²), and this total was divided by the sum of the differences between the SPL 6 dB above the BTH and thresholds at different frequencies. This weighted mean obtained from the frequencies at which low thresholds occurred is intended to provide a better estimate of the spectral sensitivity in an audiogram compared to using a single frequency at which the BTH occurs (Penna et al., 2013; Penna & Moreno-Gómez, 2014). An example of the procedure is provided in Fig. 2B.

**CALL DATA**

The calls analyzed correspond to 60 males of *A. cisternasii* recorded in Mérida at temperatures of 11.8–19.4 °C (Márquez & Bosch, 1995) and to the calls of 38 males of *A. dickhilleni* recorded in Cazorla Natural Park at temperatures of 9.8–17.8 °C (Márquez & Bosch, 1996). Because the *A. obstetricans* specimens for which midbrain recordings were obtained were from two populations for which their calls have not been reported, we used the dominant frequencies of the calls reported in four populations of this species encompassing a wide distribution in Spain (Márquez...
Figure 2. A, audiograms obtained from multiunit recordings in the two midbrain hemispheres of exemplars of *Alytes cisternasii* [female, snout–vent length (SVL): 37 mm], *Alytes obstetricans* (female, SVL: 42 mm), and *Alytes dickhilleni* (female, SVL: 45 mm). Filled circles: left hemisphere, open circles: right hemisphere. B, midbrain dorsal surface showing the approximate electrode positions used for recordings of multiunit auditory responses in both hemispheres (crosses) and borders of the hole on the cranium (dotted line). C, method used to obtain the best threshold and centre frequency in the high-frequency range (BTH and CFH, respectively) in the audiogram of the left hemisphere of *A. obstetricans*. Filled circles represent thresholds at different tone frequencies and the open circle represents BTH, corresponding to a threshold of 34.1 dB SPL (horizontal arrow) at 1800 Hz. Vertical bars above circles represent differences between 40.1 dB SPL (i.e. 6 dB above BTH) and thresholds at different frequencies. A CFH of 1627 Hz (vertical arrow) was obtained multiplying each of these values by the corresponding frequency, adding these products and dividing them by the sum of the differences between 40.1 dB SPL and thresholds at different frequencies. Only thresholds within 6 dB above BTH were considered for these calculations, which, in this case, correspond to tones of 1100–2200 Hz.
& Bosch, 1995). From these samples, we considered the calls of 37 A. obstetricans having sizes within the range of SVLs of the male individuals recorded in the present study (41–49 mm) and recorded at temperatures of 6.7–18.4 °C.

**STATISTICAL ANALYSIS**

To obtain mean thresholds of multiunit responses at each of the frequencies tested, threshold values of individuals in dB SPL were converted to N m⁻², and the resulting means were reconverted to dB SPL.

To explore the variation in CFHs and BTs among the three species, general linear models were applied, using species and sex as categorical predictors and SVL as a continuous predictor ($\alpha = 0.05$). SVL instead of weight was used as a covariate because a high correlation occurred between both attributes, although body size had a lower intraspecific variation than body weight (coefficient of variation = 20.2 and 60.7, respectively). Post-hoc comparisons for CFHs and BTs among the three species were performed with Tukey’s tests. Student’s $t$-tests ($\alpha = 0.05$) were used to compare BTLs, BTHs, and CFHs of audiograms obtained in both brain hemispheres within each of the three species.

**RESULTS**

Audiograms of the three species of *Alytes* showed regions of high sensitivity at a low-frequency range, between approximately 100–500 Hz and, at a high-frequency range, between approximately 1200–2400 Hz. The BTLs reached values of approximately 40 dB SPL, occurring at the lowest frequency tested (100 Hz), whereas minima in the high-frequency range (i.e. BTHs) were between 40 and 50 dB SPL. These minimum thresholds in the high-frequency range occurred in the different individuals at frequencies ranging from 1200 to 2000, 1200 to 2400, and 1300 to 2300 Hz in *A. cisternasi*, *A. obstetricans* and *A. dickhilleni*, respectively.

Audiograms measured in both hemispheres of an individual were very similar, although differences for some frequencies reaching approximately 10 dB were occasionally measured (Fig. 2A). Comparisons for BTH and CFH between audiograms of both hemispheres performed for six *A. cisternasi*, eight *A. obstetricans*, and eight *A. dickhilleni* showed no significant differences (Student’s $t$-test for related samples: $t = -0.23$, $P = 0.817$ for BTH; $t = -0.48$, $P = 0.637$ for CFH). However, BTL differed between recordings obtained from both hemispheres (Student’s $t$-test for related samples: $t = -2.94$, $P < 0.010$). For further comparisons, and to homogenize our sampling method, only recordings from the left hemisphere were considered.

Audiograms for the three species are shown in Fig. 3, and mean values of BTLs, BTHs, and CFHs are listed in Table 1. Mean thresholds at CFH and at
Table 1. Body size, tuning and sensitivity of multi-unit audiograms in three species of *Alytes*

<table>
<thead>
<tr>
<th>Species, locality</th>
<th>SVL ranges (females, males)</th>
<th>N (females, males)</th>
<th>T at DF (dB SPL)</th>
<th>BTH (dB SPL)</th>
<th>T at CFH (dB SPL)</th>
<th>BTH (dB SPL)</th>
<th>DF (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alytes cisternasii</em>, Merida</td>
<td>(36–49, 32–45)</td>
<td>13 (3,4)</td>
<td>51.0 (38.1–65.0)</td>
<td>49.8 (38.1–68.3)</td>
<td>51.0 (38.1–65.0)</td>
<td>51.0 (38.1–65.0)</td>
<td>1507 (1305–1724)</td>
</tr>
<tr>
<td><em>Alytes obstetricans</em>, Toro, Picos de Europa</td>
<td>(38–42, 41–49)</td>
<td>10 (3,7)</td>
<td>42.4 (24.3–53.0)</td>
<td>49.0 (38.4–55.8)</td>
<td>49.0 (33.2–46.2)</td>
<td>49.8 (38.1–68.3)</td>
<td>1571 (1245–2033)</td>
</tr>
<tr>
<td><em>Alytes dickhilleni</em>, Cazorla</td>
<td>(36–46, 37–46)</td>
<td>8 (3,5)</td>
<td>41.6 (32.0–45.9)</td>
<td>46.1 (42.4–50.5)</td>
<td>41.6 (32.0–45.9)</td>
<td>46.1 (42.4–50.5)</td>
<td>1571 (1245–2033)</td>
</tr>
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</table>

*SVL* = snout–vent length; *BTL* = best threshold in the low frequency range; *BTH* = best threshold in the high frequency range; *CFH* = centre frequency in the high-frequency range; *DF* = dominant frequency.

Numbers in parenthesis for SVL BTL, BTH, CFH, and DF correspond to individual ranges. Values for DFs correspond to calls of 60 males of *A. cisternasii*. The DFs of the calls recorded in Merida (Marquez & Bosch, 1995), and to the calls of 38 *A. dickhilleni* recorded in Cazorla (Marquez & Bosch, 1996). Because the audiograms yielded significant differences for species (F = 7.22, P = 0.004). No significant differences in CFH dependent on SVL and sex occurred (F = 3.43, P = 0.076) and F = 3.6, P = 0.557, respectively. Post-hoc Tukey’s tests showed that the CFH of *A. dickhilleni* was significantly higher than in *A. cisternasii* (P = 0.003) and *A. obstetricans* (P = 0.026).

The CFHs of the audiograms of the three species were compared with the values of call dominant frequencies obtained in populations of *Alytes* in previous studies. The dominant frequency means and ranges for calls of the three species are listed in Table 1. Comparisons between the call dominant frequencies and CFH showed similar values for *A. cisternasii* but not for *A. obstetricans* and *A. dickhilleni* in which the spectral component of the calls was well below the corresponding CFH. In *A. cisternasii*, CFH was 0.4 SDs above the mean dominant frequency of conspecific males. By contrast, CFH of the audiograms of *A. obstetricans* is approximately 350 Hz (4.2 SDs) above the mean dominant frequency of 1243 Hz calculated for the calls of males of this species recorded in four localities in Spain. Similar to *A. obstetricans*, the CFH obtained for the audiograms of *A. dickhilleni* was approximately 400 Hz (6.9 SDs) above the mean dominant frequency of 1404 Hz reported for the call of males of *A. dickhilleni* from the same population.
quite similar: for *A. cisternasii*, these thresholds were the same because both frequencies coincided and, for *A. obstetricans* and *A. dickhilleni*, differences between both thresholds were within 1 dB (Table 1).

**DISCUSSION**

**AUDITORY SENSITIVITY AND ACOUSTIC SIGNALS**

Overall, the audiograms of the species of *Alytes* analyzed have regions of low- and high-frequency sensitivity with minimum thresholds between 39 and 47 dB SPL, which do not differ among species. However, the tuning in the high-frequency range, as evaluated by the CFH, shows significant differences among species.

Two previous studies of the auditory sensitivity of *Alytes* have reported results concordant with those in the present study: a correspondence between the tuning of the high-frequency region of auditory sensitivity and call dominant frequency for *A. cisternasii* (Bosch & Wilczynski, 2003) and an auditory sensitivity in the high-frequency range in *A. obstetricans* tuned to higher values than the conspecific call dominant frequency (Mohneke & Schneider, 1979). The present study confirms these divergent patterns, showing that they are not a result of different experimental procedures in the distant laboratories and times at which measurements were conducted. The comparisons of the audiogram CFHs with the call DFs in each of these two species indicate a mismatch between auditory sensitivity and spectral contents of the calls. However, as observed in Table 1 and Fig. 3, thresholds at the mean DF of the conspecific vocalizations are similar to thresholds at the CFH in the audiograms of the three species.

Matching between auditory tuning and dominant frequency of the calls is a common occurrence in anurans, a correspondence providing support for the matched filter hypothesis (Capranica, 1965). However, considerable deviations from this trend have been reported in some species. Gerhardt & Schwartz (2001) note that deviations larger than 15% between these two measures have been reported for 19 out of 36 species, of which approximately two-thirds (*N* = 12) correspond to mismatches with auditory sensitivity tuned to low values relative to conspecific call dominant frequency. Deviations of auditory sensitivity to lower frequencies relative to the dominant frequencies of the calls support female phonotactic preferences for low frequencies and for large sized males reported in various anurans (Keddy-Hector, Wilczynski & Ryan, 1992; Ryan et al., 1992; Wilczynski, Keddy-Hector & Ryan, 1992). Also, better sensitivities centred at low frequencies within the range of conspecific signals could be adaptive for improving detection of propagating signals in which the high-frequency components suffer greater attenuation (Penna & Solis, 1998).

In the review by Gerhardt & Schwartz (2001), seven species are reported to have auditory sensitivities centred at high frequencies within the range of conspecific signals, although no adaptive relationships are mentioned to account for this mismatch. A case of deviation of auditory tuning towards the high end of spectral components of the calls has been reported in the South American anuran *Eupsophus roseus* (Moreno-Gómez et al., 2013). The sensory displacement in this frog is related to the concurrent vocal activity of the congeneric *Eupsophus vertebralis*, having a call with low-frequency components, which overlap partially with the low end of the spectrum of the call of the focal species. Mismatches between signal spectra and tuning of receptors to higher frequencies reported for insects have been related to detection of predators in the ultra-sound range (Bailey & Romer, 1991; Schul & Patterson, 2003). However, this frequency domain is well above the auditory capabilities of *Alytes* toads and therefore is not relevant to the mismatches reported in the present study. A preliminary characterization of the acoustic environment during the breeding activity of *A. cisternasii* has shown concurrent biotic components at frequencies above and below the spectrum of this toad (Penna et al., 2012). Further characterizations of the soundscape of biotic and abiotic nature during the breeding activity of *A. obstetricans* and *A. dickhilleni* are needed to establish possible environmental correlates of the detuning between the call spectra and auditory sensitivity in these species.

A possible adaptive role of an auditory sensitivity extended towards the high-frequency end is related to the detection of distress calls, which have been reported for *A. obstetricans* (Heinzmann, 1970). These vocalizations contain noisy and tonal components in the range of 2000 Hz and beyond, and no reports on such vocalizations are available for the other species of *Alytes*.

Sound communication in *Alytes* is peculiar because females also produce vocalizations of low intensity during the final phonotaxis stages. However, female vocalizations analyzed in *A. cisternasii* (Márquez & Verrel, 1991; Bosch & Márquez, 2015) *A. obstetricans* (Heinzmann, 1970) and *Alytes muletensis* (Bush, 1993) show dominant frequencies that are somewhat lower on average relative to those of male advertisement calls. In addition, these calls are considerably smaller in amplitude than those of males. It is therefore apparent that the auditory sensitivity to high frequencies in *Alytes* is not related to the detection and processing of these signals.
AUDITORY SENSITIVITY AND BEHAVIOURAL RESPONSES

Studies of phonotaxis with females of *A. cisternasii* and *A. obstetricans* have shown preferences for low frequencies within the conspecific range (Márquez, 1995a; Márquez & Bosch, 1995, 1997; Márquez et al., 2008) and field studies have shown mating advantages for larger males (Márquez, 1993; Lodé & Le Jacques, 2003). Also, males and females of *A. cisternasii* vocalize more actively in response to calls of low frequency, relative to calls of high frequency within the conspecific range (Bosch & Márquez, 1996; Bosch, 2001). The lack of dependence of auditory tuning in the high-frequency range (CFH) on size in the three species examined does not support these behavioural preferences. Furthermore, results on mean auditory tuning in the high-frequency range, showing a matching with the conspecific call dominant frequency of *A. cisternasii* and a tuning to higher frequencies in *A. obstetricans*, are not congruent with the behavioural data on preferences for low call dominant frequencies within the natural conspecific range.

Nevertheless, as Gerhardt & Schwartz (2001) have noted, mismatches between auditory sensitivity and call spectra may not necessarily have straightforward functional consequences as a result of inherent nonlinear properties of the auditory system. Auditory thresholds do not strictly predict the responsiveness of anurans to signals presented at higher intensities to which animals are more often exposed in breeding aggregations: for example, frequency preferences and auditory tuning in female treefrogs depend on stimulus intensity (Schwartz & Gerhardt, 1998; Gerhardt & Schwartz, 2001).

In general, loose relationships of correspondence between signals and receptors of the kind reported here for *A. obstetricans* and *A. dickhilleni* may result from constraints acting on emitter and/or sensory systems. In addition to potential environmental determinants, phylogenetic factors have been shown to be influential in other anuran groups (Zimmerman, 1983; Gerhardt, 1994; Kime et al., 2000; Funk, Cannatella & Ryan, 2009; Amézquita et al., 2009). In *Alytes*, phylogenetic analyses (Martínez-Solano et al., 2004; Maia-Carvalho et al., 2014) have shown that *A. cisternasii* is a sister group to *A. obstetricans*, *A. dickhilleni*, *A. muletensis*, and *A. maurus*. *Alytes obstetricans* is a highly diversified taxon, comprising four subspecies, being the sister taxa to *A. muletensis*, *A. maurus*, and *A. dickhilleni*, with the last two species being more closely related (Fig. 3A) (Maia-Carvalho et al., 2014). This reconstruction suggests that the absence of a strict matching between auditory sensitivity for high frequencies and call dominant frequency is a secondary event in the history of the genus. In consonance with this tenet, a study on auditory sensitivity incorporating the phylogenetic analysis in the Neotropical frog genus *Physalaemus* reveals that tuning of the basilar papilla sensitivity to higher frequencies in one species is a secondary occurrence (Wilczynski, Rand & Ryan, 2001).

Our data do not support the application of an effective phylogenetic analysis because of the limitations inherent to a low number of taxonomical entities (Garland & Adolph, 1994).

Electrophysiological measurements in the unexplored *A. muletensis* and *A. maurus* are needed for a phylogenetic analysis of auditory sensitivity. A phylogenetic analysis considering male acoustic signals and female phonotactic preferences applied to *Alytes* and the related genera *Discoglossus* and *Bombina* (formerly *Discoglossidae*, now *Alytidae* and *Bombinatoridae*; Frost, 2014) has revealed a phylogenetic load for these characters (Bosch & Boyero, 2003b). Evidence on auditory sensitivity would contribute to a more comprehensive assessment of the evolution of acoustic communication in these anurans.

The phylogenetic factors outlined, together with environmental determinants discussed earlier, may restrict the effect of sexual selection processes on the extent correspondence between communicating emitters and receivers in *Alytes*.

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