Herpetology Notes, volume 10: 197-203 (2017) (published online on 25 April 2017)

# Description of vocalisations and analysis of intra- and inter-individual variation in *Pristimantis ramagii* (Boulenger, 1888) in an upland swamp, Northeast Brazil

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**Abstract.** Vocalisation is used by frogs to attract females and to establish spaces. Evaluation of variation of the call is necessary for investigating the social and ecological role of the species. In addition, call patterns are influenced by body mass and snout-vent length. In this study we analysed the call pattern of *Pristimantis ramagii*, to verify types of call variation of inter- and intra-individual patterns, and its relationship with body mass and SVL. The study was carried out in a protected area in Belo Jardim, 240 calls were registered (advertisement calls and territorial calls). Advertisement calls had 2:55 ± 0.69 notes / call, lasting 0.151 ± 0.052 seconds, while the territorial call had 0.516 ± 0.111 seconds. Note duration and number of pulses is presented as dynamic properties, while duration and dominant frequency are intermediate properties. Dominant frequency was significantly correlated with body mass and SVL, while call duration was only related to the SVL. We observed that the variety in advertisement calls is higher between individuals than between calls of the same individual. Furthermore, calls may be influenced by environmental factors rather than by their morphology. In complex calls, dynamic variation is expected in the amount and intensity of notes in relation to the time period.

**Keywords:** Advertisement Call, Territorial Calls, Altitude, Anuran, Temperature, Humidity

**Resumo.** A vocalização é usada por anuros para a atração das fêmeas e estabelecimento do espaço inter e intraespecífica. Avaliação da variação intraespecífica do canto, faz-se necessária para a investigação do papel social e ecológico. Além disso, padrões podem mudar de acordo com massa e tamanho rostro-cloacal, constituindo um fator para seleção do acasalamento, determinar o sítio de vocalização e afugentar invasores. No presente estudo foi realizado análises nos padrões do canto da espécie *Pristimantis ramagii*, objetivando-se verificar tipos de canto, variação dos padrões inter e intraindividual, e relação com a massa e CRC. O estudo foi desenvolvido no refúgio de vida silvestre Belo Jardim. Foram registrados 240 cantos, podendo ser observados cantos de anúncio e territorial. O canto de anúncio de *P.ramagiii* apresentou 2.55±0.69 notas/canto, com duração de 0.151±0.052 segundos, enquanto o canto territorial apresentou duração de 0.516 ±0.111 segundos. Dos testes de covariância, a duração das notas, e a quantidade dos pulsos apresentaram-se como propriedades dinâmicas, enquanto duração do canto e frequência dominante, foram classificados como propriedades intermediárias. A frequência dominante apresentou relação siginificativa para massa e CRC, enquanto a duração do canto somente para CRC. No presente estudo foi possível observar que a vocalização de anúncio tem maior variedade entre os indivíduos do que internamente.

**Palavras-Chave:** Chamado, Anúncio, Território, Anuros

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## Introduction

Acoustic communication is an important aspect of the social behaviour of different animal species (Wells, 2007). This also applies to many species of anurans, especially during the reproductive period. In this perspective, acoustic communication can be used in various contexts, such as in attracting mates, marking territories, in male-male spacing and also avoiding intraspecific conflicts (Bastos et al., 2011; Bilate and Lack, 2011; Morais et al., 2015; Morais et al., 2016).

Acoustic signals are also of great importance, as they may be linked to both biotic aspects such as the morphology of the individual (Morais et al., 2012), environmental factors (Lignau and Bastos, 2007), or the abiotic and anthropogenic sounds (Jennifer and Narins, 2005; Mazerolle, 2005). Some studies have shown a link between the body size of an animal and the dominant frequency of its vocalisation (De-Orense and Tejedo, 1990; Howard and Young, 1998; Bastos et al., 2011; Morais et al., 2016). For anurans, the dominant frequency is determined by the mass and tension of vocal cords, because vocal cords with larger mass tend to vibrate at lower frequencies (Morais et al., 2016).

In this context, dominant frequency is a major indicator of a species' behavioural ecology, such as mate selection (De-Orense and Tejedo, 1990; Howard and Young, 1998), and intraspecific agonistic interactions (Bastos et al., 2011; Morais et al., 2016). Call type and frequency is species-specific, but also influenced by the environment and the caller's morphology (Morais et al., 2012).

The *Pristimantis* genus is commonly found in northeastern Brazil, in remaining areas of the Atlantic Forest, and it is composed of three species in this region: *P. paulodutrai* (Bokermann, 1975), with records from the state of Alagoas to the south of Bahia; *P. vinhai* (Bokermann, 1975), with records only to the southern part of Bahia and east of the state of Sergipe and Pernambuco (Santos et al., 2016) and *P. ramagii* from Mamanguape, in the state of Paraiba to the south of Prado, in Bahia (Silvano and Pepper, 2003; Juncá, 2006; Morato et al, 2011).

The species *P. ramagii* is a small frog which can be found in areas at up to 800m of altitude, living on the leaf litter in primary and/or secondary forests, around forest edges and in rocky areas (Juncá, 2006; Napoli et al., 2009). Despite being a species with wide distribution and high abundance, little information is available about its ecological characteristics. The vocalisation of this species is claimed to have been heard by populations from the state of Sergipe (Heyer and Carvalho, 2000), but it is not clear whether such vocalisation tends to vary within species depending on the morphology of each individual, and the occupied microhabitats.

From the aforementioned, the aim of this study is to describe the types of vocalisations produced by *P. ramagii* and to analyse inter- and intra-individual call variation with respect to morphology and in comparison to the original call description.

## Materials and Methods

### *Study site*

This study was conducted at Vale do Tabocas farm, in the middle region of the state of Pernambuco, in the city of Belo Jardim (8°13'50" S e 36°22'52' W). The place is the remaining area of an upland swamp, at 907 meters above sea level. The area is covered with Atlantic Forest vegetation characteristic of upland swamps with an area of 205 ha distributed in the Borborema plateau. The climate is dry with the rainy season from May to August, an average annual rainfall of about 1,000 mm and an average temperature of 23 °C.

### *Methodology*

Specimens of *P. ramagii* were captured by the active search method. The air temperature and humidity on the collection days were noted down. .We recorded at least one minute of the vocalisations of male *P. ramagii* with a recorder SONY PCM 10 (96 kHz sampling rates and 24 bits), and a unidirectional microphone at a distance of about 0.5 meters. We measured the snout-vent length (SVL) of each captured individual using an electronic caliper (accuracy 0.01 mm), and the body mass using a digital balance (precision 0.1 g). Each specimen was euthanised with lidocaine, fixed in 10% formalin, preserved in alcohol (70%) and subsequently deposited in the Herpetological and Paleoherpetological Collection of UFRPE.

The temporal and spectral call parameters were analysed with the software Avisoft and Cool Edit Pro 2.1 respectively. For each specimen, six calls were analysed. We analysed the following call parameters: call duration (s), note duration (s), number of notes (notes / minute), pulse duration (s), dominant and fundamental frequency (Hz), repetition rate (vocalisation / minute). To determine the relation between call parameters, body mass and SVL we used a correlation test. Call samples were edited in the Sound Ruler program.

To determine the call variability between all of the specimens, we calculated the intra-subject mean and

the standard deviation (SD) for each parameter. To determine the variability between males, we set a general overall mean and standard deviation. Thus, we calculated two coefficients of variation: Intra-male and inter-male. We considered parameters static whenever they had a variation coefficient of less than 5%, while those with a variation above 12% were considered dynamic (Gehardt, 1991).

We also calculated the ratios between the coefficients (CV / CVW) to determine the inter-male variability (Bee et al., 2001; Marquez and Eekhout, 2006). An acoustic parameter has a greater inter-male than intra-male variability when the ratio is greater than one (Bee et al., 2001) and can therefore function as a sign of recognition (Jouventin et al., 1999). To finalise the analysis, the nonparametric Kruskal-Wallis test was used to assess whether the acoustic properties vary more among males than among multiple calls of the same individual (Zar, 1996 apud Morais et al., 2012). All these variability patterns were considered only for advertisement calls, since this was the most common vocalisation.

To determine the influence of an individual's body mass and SVL on its vocalisations, we used the non-normal distributions from the Spearman correlation coefficient (rs). This test was performed using Statistica version 8.0 software to verify the level of relationship between morphology compared to some standards vocalisations as in common types of calls. Among the compared patterns, we chose the types of vocalisation used and the standards found in the vocalisations considered static. Correlations with a *p* value below 0.05 were considered significant. This type of assay is preferred in this situation due to lower distortion for the species data, which in turn reduces the impact of uncertainty about small differences (Sokal and Rohlf, 1995; Kidwell, 2008).

## Results

The specimens of *P. ramagii* were found calling in the soil near the roots of trees and shrubs, or on the leaf litter along the edges of marked trails, these specimens were also found vocalising in the dense forest on fringes of the streams of water. The average SVL and body mass were 24.55 mm ± 1:39 and 1:02 ± 0:12 g respectively. Variations in temperature and humidity during the collection days were 26.6 ± 0.89 ° C and 92 ± 4.24%, respectively.

We recorded 240 vocalisations emitted by 9 males. The recorded vocalisations consisted of two different call types (advertisement and territorial calls). Advertisement calls were more common (ca. 80% of vocalisations), while territorial calls were rarely produced by a few individuals.

Once identified, an isolated analysis of each call was performed. The advertisement call consisted of short pulses with very short inter-pulse interval, ranging from one to four notes (2.55 ± 0.69 notes / call; n = 9 males) (Figs. 1 and 2). The average advertisement call duration was 0.151 ± 0.052 seconds (0073-0245 seconds; n = 138 notes). The average number of pulses per note was 24.58 ± 15:29 (7.8-53.5 pulses / note, n = 138 notes), with a duration ranging from 0.001 to 0.0033 sec (0.0017 ± 0.0006 seconds n = 414 pulses). Recurrence rate was 8.33 ± 3.5 vocalisations per minute (6-17 vocalisations / min, n = 9), and the mean dominant frequency was 3941.7 ± 490.78 Hz (2217- 4898 Hz, n = 138 notes).

The territorial call had no notes, but was a unique sound with six calls per minute (n = 1 male). The average call duration was 0.516 ± 0.111 seconds (0.37-0.64 s, n = 5). The number of pulses per call ranged from five to nine pulses (09.07 ± 1.13; n = 11) with a duration of 0.027 ± 0.01 seconds (0.018-0.06 s; n = 13). The dominant frequency of this call was at 3611.8 ± 213.9 Hz (Fig. 3).

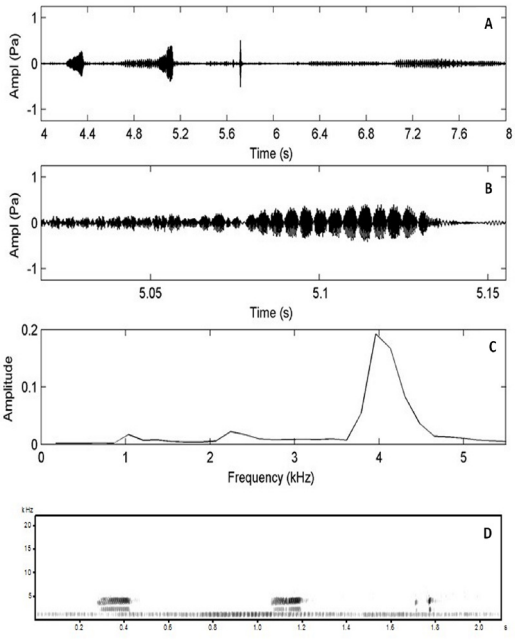


**Fig. 1.** Advertisement call of *P. ramagii*. A) Call amplitude, B) Oscillogram, C) Dominant frequency, D) spectrogram, with three and four notes respectively.

**Table 1.** Mean, standard deviation and intra- and inter-male variation of call parameters and the coefficient of variation of the advertisement call in *Pristimantis ramagii* in Belo Jardim, Pernambuco.

| Acoustic parameter | mean±sd | Variation | CV Intraindividual (%) | CV Interindividual (%) | Mean CVa/CVw | Kruskal Wallis | |
|---|---|---|---|---|---|---|---|
| | | | | | | H | p |
| Call duration (s) | 0.151±0.052 | 0.073-0.245 | 5.7 | 34.9 | 6.12 | 46.69 | <0.001 |
| Number of Pulses/call | 25.45±16.22 | 7.8-53.5 | 18.3 | 63.8 | 3.48 | 88.55 | <0.001 |
| Pulse duration(s) | 0.0017±0.0006 | 0.001-0.0033 | 12.97 | 35.3 | 2.72 | 347.73 | <0.001 |
| Dominant frequency (Hz) | 3941.7±490.78 | 2217- 4898 | 5.54 | 12.5 | 2.25 | 37.32 | <0.001 |
| Note duration (s) | 0.036±0.0082 | 0.019-0.069 | 15.67 | 22.2 | 1.41 | 41.91 | <0.001 |

According to the coefficient of variation (12%) of intra-male averages calculated in the covariance tests, note and call duration as well as the number of pulses are dynamic properties (Table 1). Call length and dominant frequency were classified as intermediate properties in intra-male evaluation, since their coefficient value is between 5 and 12% (Tab. 1). In the analysis of inter-male coefficients, all the parameters analysed are now dynamic, with values greater than 30%, except the dominant frequency and note duration. All coefficients had results above one. It was possible to observe a wide inter-individual variation separately for each individual (Table 1; $p < 0.05$).

The morphology of the observed individuals enabled us to static parameters inversely and directly proportional to the body mass and the SVL. Spearman's test revealed a significant correlation between dominant frequency and body mass ($sr = -0.57$, $n = 36$, $p < 0.05$) and between dominant frequency and SVL ($sr = -0.51$, $n = 36$, $p < 0.05$), while the call length was only significantly correlated to SVL ($mr = 0:35$, $n = 36$, $p < 0.05$).

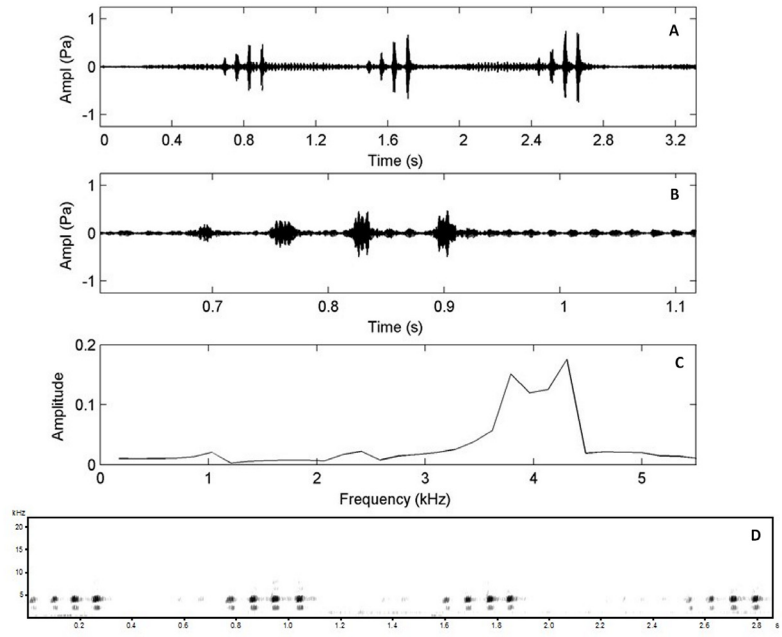


**Fig. 2.** Territorial call of *P.ramagii*. A) Call amplitude, B) Oscillogram, C) Dominant frequency, D) spectrogram.

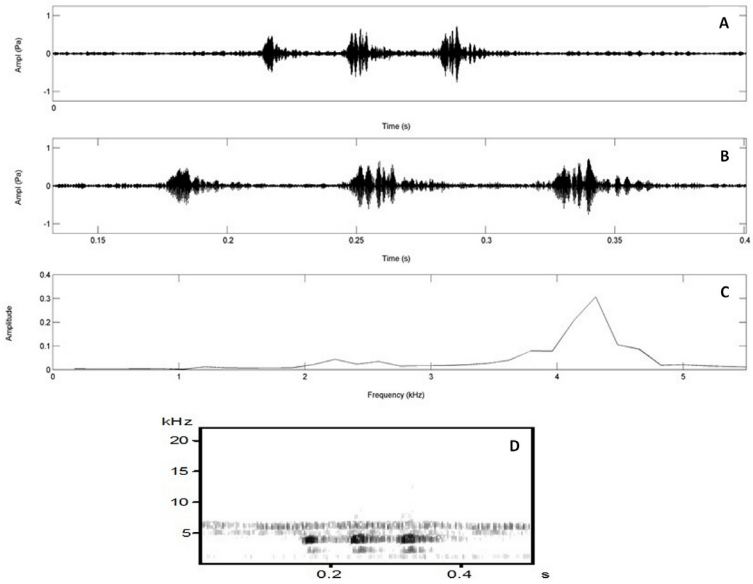


**Fig. 3.** Territorial call of *P. ramagii*. A) Call amplitude, B) Oscillogram, C) Dominant frequency, D) spectrogram.

## Discussion

As described above, the advertisement call of *P. ramagii* consists of a fast rise of the frequency, containing varying peak frequency (Heyer and Carvalho, 2000). However, this study showed a greater variation in dominant frequency pulses, as well as the number of notes found in the vocalisation of the same species elsewhere (Heyer and Carvalho, 2000). Regarding territory calls, there is no published record for the locations where *P. ramagii* occurs. The territory call is a long continuous call without notes and well-differentiated from this species advertisement call.

Our results indicate that environmental conditions, such as temperature and altitude, can influence the vocalisation of the species (Gerhardt, 1991). Especially high altitude may select for call parameters which increase the reach of the advertisement call and therefore improve the detectability by females. Changes in call parameters whether advertisement or territorial, have been observed also in studies on other species, due to their variations between geographically distinct populations (Trepanier et al., 1999; Castellano et al., 2002; Heyer and Reid, 2003; Bernal et al., 2005).

In the present study, we were able to describe the conditions of calling sites as well as vocalisation in a chorus. Since our data was collected during the rainy season and under similar conditions to studies in distinct and lower areas (Heyer and Carvalho, 2000), it is suitable for comparison. As Heyer and Carvalho's description in 2000 is the only work about calls for the present species, information about their calls is still rather scarce.

Previous call descriptions included an individual difference in the number of notes ranging from one to four (Mitchell et al., 2012), while others found the use of one-note calls only (Martins and Haddad, 1988; Bilate and Lack, 2011). Call differences, among populations, are often found in species that exhibit territorial behaviour, and they may change not only the number of notes, but also call frequency and duration (Martins and Haddad, 1988). Some males tend to use these vocalisations also to avoid physical combat (Brigs, 2010; Bastos et al., 2011; Morais et al., 2012), using alternately territorial calls and advertisement calls (Martins and Haddad, 1988), as was observed for *P. ramagii*.

We found that the inter-individual call variation was greater than intra-individual, which is in accordance with findings for several species (Bee et al., 2001; Castellano et al., 2002; Brigs, 2010; Morais et al., 2012), and suggests that advertisement call properties might enable conspecifics to identify an invidual caller (Bee et al., 2001). Of the analysed parameters, the dominant

frequency has proved to be the most static property, which is compatible with other frog species (Morais et al., 2012; Morais et al., 2015). The call duration also remained static. Dominant frequency, call duration and the dynamic number of pulses are important parameters for female choice in this species (Gerhardt, 1991). In this specie, unlike previously thought, temporal patterns can also be used for specific recognition (Gambale and Bastos, 2014).

The intra- and inter-individual variability is presented as a direct tendency in this study. As found in previous studies (Gerhadt, 1991; Bee et al., 2001; Mitchell et al., 2012), the more static parameters had the smaller coefficients among males, while the dynamic parameters tended to vary more in both. In contrast to previous findings, this study revealed a high variability of the ratio of call duration, but not for the dominant frequency (Table 1). Therefore, some static parameters are more likely to play a role in the identification of individual male than others (Gerhadt, 1991, Bee et al., 2001). Moreover, some dynamic parameters might also be used to identify individuals if the extent of variability among males is sufficiently high (Sullivan and Hinshaw, 1990; Gerhadt, 1991; Bee et al., 2001).

Note duration, number of pulses and pulse duration are dynamic factors within the species *P. ramagii*. Such factors may change constantly at short time intervals. This can occur as a result of social interactions, whether intra- or interspecific (Gehardt, 1991; Castellano et al., 2002). Since call production is highly energy consuming, it is a major indicator for a male's condition (Zimitti, 1999). Thus, *P. ramagii* were observed interacting with at least another specie, *Hypsiboas faber* (Wied-Neuwied, 1821).

Regarding the morphology, the results support the hypotheses that static patterns are, generally, connected with morphology, hence they are static. Call duration depends mainly on the size and strength of the muscles which fill the lungs with air. The larger and more robust these muscles are, the greater amount of air is needed for call production, resulting in an increased call duration, which makes the dominant frequency lower (Castellano et al., 2002). As *P. ramagii* is a species of small size, this vocal tract morphology tends to vary little among individuals compared to other anurans (Napoli et al., 2009). This may indicate a greater influence of the environment on the call parameters.

The present study indicates that call variation in *P. ramagii* may be influenced by various environmental factors such as altitude, temperature and humidity. The advertisement call of *P. ramagii* is a call with a potentially dynamic variation of note duration and number of notes. However, additional studies are needed to better understand call function and ecological aspects related to switching between the advertisement calls and the territorial calls.

**Acknowledgements.** We thank the PhD student Marco Freitas, and the Doctor Márcio Frazão, The professors of Universidade Federal Rural de Pernambuco Wallace Telino, Ana Carla Pinho and Thiago Gonçalves for the support and criticism of the manuscript. The master student Paulo Mateus Martins-Sobrinho for his contributions when he was an undergraduate student, and all undergraduate students enrolled at the amphibians and reptiles field course.

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*Accepted by Iris Starnberger*