

Reproductive behavior of *Pithecopus nordestinus* (Anura: Phyllomedusidae): a tree frog inhabiting the Brazilian semiarid

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Abstract. *Pithecopus nordestinus* is a small species of Phyllomedusidae that occurs in semi-arid zones, being the only one of the genus that occurs in Caatinga areas. In order to understand the ecological and behavioral traits responsible for the existence of the species in xeric environments we study a population of *P. nordestinus* of a Caatinga area in southwest Bahia, Brazil. Males of *P. nordestinus* used preferably the vegetation (mainly Euphorbiaceae and Poaceae) inside semi-permanent and temporary water bodies as calling sites. The dependence of aquatic environments, the arboreal habit, and reproductive mode may lead to these preferences, being that choice related to the species fitness in the semiarid region. The species has elaborated courtship, including the use of visual signals, with females that inspect the oviposition site and split their clutch (spatial partition), probably spawning in more than one occasion during the reproductive season (temporal partition). This set of behaviors points to a sophisticated control mechanism during egg laying and sperm release, as well as the existence of a repertoire of complex reproductive behavioral displays related to the occupation of environments with characteristics as peculiar as those of the Brazilian Northeastern semi-arid.

Key words. Amphibia, Caatinga, courtship, visual signal.

Introduction

Natural history brings a multifaceted description of nature and is a contemporary and critical science for the conservation of the biodiversity (SCHMIDLY 2005). In addition to inspiring theories, it provides crucial data for responses to comprehensive problems in ecology, ethology, evolution and conservation biology (GREENE 1986). Moreover, it foment hypotheses and assists in the projection of researches with awareness of the singularities of the organisms (FUTUYMA 1998), guaranteeing data more consistent with the complexity of nature (BARROWS et al. 2016).

In anurans, studies about natural history generally focus on aspects of the geographic distribution of the organisms, habitat use and intra and interspecific social interactions (e.g., DOMENICO et al. 2014, JORGE et al. 2015, NUNES et al. 2015). This data set is, in most cases, important for the establishment of taxonomic relationships and, often helps in understanding the mechanisms of segregation in the community (e.g. ZINA & HADDAD 2006a, b). However, within the list of basic biological data available in the literature about anuran, the description of behaviors is the most insipient, especially regarding the description of reproduc-

tive behavior. Data on this nature are particularly important in Anura, since the reproduction seems to be an important organizing force of populations and communities (e.g. BECKER et al. 2007, LOYOLA et al. 2008).

One of the striking aspects of the reproductive behavior of anurans, but still poorly understood for most of the species, is the selection of oviposition sites (PEARMAN & WILBUR 1990; REFSNIDER & JANZEN 2010), which may be associated both with behavioral issues, such as types of reproductive modes presented by the species and morphology and physiology of its representatives (e.g. HADDAD & PRADO 2005, SILVA & GIARETTA 2008, HADDAD et al. 2013). It is a choice made mostly by the females that, in most part of the species, will do it after inspecting the environment for suitable attributes and will enter in amplexus, not necessarily in this order (e.g. *Aplastodiscus* spp.: ZINA & HADDAD 2006a, *Pithecopus azureus* (COPE, 1862): DIAS et al. 2014). The selection of the oviposition site can be also indirect, through the selection of the male (see MARTINS 1988, ZINA & HADDAD 2007, LANTYER-SILVA et al. 2014). This is common in the case of species in which the male constructs nests and/or vocalizes in phytotelmata (e.g. ZINA & HADDAD 2007, NALI et al. 2012).

Besides the selection of the oviposition site, the very description of the egg deposition behavior is not very present in the literature. However, it brings interesting biological information, both from the sexual selection point of view and from the ecological view, which involves aspects regarding the adaptation of a given species to its environment and behavioral strategies aimed to maximize the eggs fertilization (e.g. DIAS et al. 2014, ZINA & HADDAD 2007). The movements and displays used during the courtship and eggs deposition, may also serve as an interesting matrix for observing groups synapomorphies and evolutionary convergences. Phyllomedusidae, for example, employ similar strategies when using leaves as oviposition sites (HADDAD & PRADO 2005). Most species of the family occupy humid forest environments, with few occurring in semi-arid zones, such as *Phyllomedusa bahiana* and *Pithecopus nordestinus* (GARDA et al. 2017, FROST 2019).

Pithecopus nordestinus is the only specie of the genus that occur in areas of Caatinga (CARAMASCHI 2006, FROST 2019). Similarly to other Phyllomedusidae its reproductive mode (number 24, sensu HADDAD et al. 2013) is characterized by the oviposition in suspended leaves in lentic water bodies and development of exotrophic tadpoles, which after hatching fall in the aquatic environment. In the present study we aimed to describe the reproductive behavior of the species, with emphasis on the use of the environment and reproductive seasonality. These data may help to understand the biological and behavioral mechanisms that enable the species to occupied xeric environments such as the Caatinga.

Material and methods

Study area

The present study was carried out in water bodies located in the Floresta Nacional Contendas do Sincorá (FLONA) (13°55'27.4"S; 41°06'57.6"O, 377 m), located in the municipality of Contendas do Sincorá, southwest region of the State of Bahia, Brazil. FLONA is located in a depression area with small undulations in the relief, between 300 and 400 m of altitude, which contributes to the low relative humidity of the air and high temperatures (IBAMA 2006), which is aggravated by being inserted in a "rain shadow" region, which results in low values of rainfall and absence of water surplus, aggravating the typical aridity of the region (IBAMA 2006).

The climate of the region is of the BSh type (KÖPPEN 1936), characterized by being hot, dry and seasonal. Between the years of 2012 to 2016 the municipality of Contendas do Sincorá presented 517.03 mm of annual precipitation, concentrated in few months of the year (November to March). It also had high temperatures (annual average of 24.9°C) and low relative air humidity (55.9%) (CPTEC/INPE 2017, present study). The rainy period in the region normally occurs from November to March, however there are annual variations of this period.

Collection and data analysis

Between July 2012 and July 2013 we performed bi-monthly surveys (July and September 2012 and May and July 2013) and monthly surveys (from November 2012 to March 2013) to obtain data on the seasonality, abundance of the individuals and reproductive characteristics of the males (vocalization season, weight and length of the testicles). Additionally, the characterization of the use of the environment by the individuals of *P. nordestinus* (perch type and height in relation to the soil), description of some of their reproductive behaviors (spawning and courtship behavior) and obtainment of data regarding the fecundity of the species (weight of the oocytes (mature and immature) and number of eggs in the spawning) were carried out from individuals collected between December 2014 and January 2016. Although we had collected different data at different moments, we believe that there were no difference in the use of environment or in the behavioral repertory of the species along the seasons since the structural of the ponds (size, plant composition and distribution) did not differ between the survey periods.

The campaigns had duration of three consecutive nights during the rainy season and two consecutive nights during the dry season, totaling 23 nocturnal field trips. During these visits, we inspected two water bodies: one semi-permanent pond, called Pond 1 (P1) (13°55'18.6"S; 41°06'57.0"O) (50.0 m maximum diameter and 1.5 m maximum depth, measured in the rainy season), and a temporary pond, called Pond 2 (P2) (13°55'20.3"S; 41°07'03"O) (30.0 m maximum diameter and 1.0 m maximum depth, measured in the rainy season), both located in an open area, with the presence of tree-shrub vegetation, closed shrub and herbaceous-shrub complex in the surroundings, in good conservation condition. The water body that suffered a reduction in its water volume until the complete dryness during the dry season was considered temporary and the water body that presented a drastic reduction in the water volume during the dry season (reaching approximately 5% of its maximum volume) was considered semi-permanent.

In the vicinity of P1 (up to 2 m distance from the water) herbaceous-shrub vegetation of the families Asteraceae, Acanthaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Hydroleaceae, Juncaceae, Malvaceae, Poaceae, Rubiaceae, Salviniaceae, Sapindaceae, Solanaceae, and Verbenaceae are equivalently distributed. While in the interior of P1, herbaceous of the Cyperaceae family predominated, and herbaceous of Asteraceae, Juncaceae, Poaceae, Fabaceae, and Salviniaceae were in less quantity.

In the surroundings of P2 shrubs of the Verbenaceae family predominated, followed by shrubs of the Cyperaceae and Poaceae family, and to a lesser extent, herbaceous-shrub vegetation of Alismataceae, Asteraceae, Commelinaceae, Euphorbiaceae, Hydroleaceae, Lamiaceae, Malvaceae, Verbenaceae, and Oxalidaceae. Within the water body herbaceous of Alismataceae predominated and, to a lesser extent Cyperaceae, Poaceae, Hydroleaceae, and Asteraceae. The vegetation configuration between the ponds differs, with P1

the environment with the highest vegetation density. These water bodies are about 50 m apart from each other.

In order to collect the data, we used the visual and acoustic active search in breeding sites (HEYER et al. 1994). For the behavioral observations we used focal animal method (LEHNER 1996), with the aid of headlights with weak batteries in order to reduce the amount of light emitted and, consequently, a possible interference in the behavior of the individuals observed. As soon as we finished each observation, we measured the SVL of each individual (snout-vent length), with a pachymeter of 0.1 mm precision and then returned the individuals to the place where they were captured. We totaled 66 h of observations of the behaviors in the field and in laboratory.

We collected four couples in amplexus throughout the study. These four couples were allocated in terrariums (dimension of 60 × 40 cm) to observe their displays during oviposition. In the terrarium we placed, in a very similar way to the registered in the field, the richness of plant species present in the place where the couple had been collected. After spawning, the males were returned to the collection site. From the observation of one of these couples we described the behavioral displays performed by the species during the oviposition. 60 eggs from three of the four spawning (20 eggs each one) obtained from these couples were measured (maximum diameter) under an optical microscope with an ocular micrometer with 0.1 μm precision. From the ovate females we obtained the mass of their ovaries and oocytes (matures and immatures). We weighed all the materials in an analytical balance with precision of 0.0001 g. We used the percentage of the mature ovarian and testicular mass in relation to the body mass as measure of the reproductive investment (RI). We used the proportion of males and females registered by campaign to determine the operational sex ratio (OSR) of the species.

In the laboratory we sacrificed five males and five females with lidocaine 5%, they were fixed in formaldehyde 10%, conserved in alcohol 70% and listed in the zoological collection of the Universidade Estadual do Sudoeste da Bahia (UESB), campus of Jequié (MHNJCH-0475, MHNJCH-0476, MHNJCH-0514, MHNJCH-0796, MHNJCH-1005, MHNJCH-1007, MHNJCH-1012, MHNJCH-1013, MHNJCH-1058, MHNJCH-1059).

In order to verify the possible correlation between the abiotic variables and the activity of *P. nordestinus*, we performed the Pearson correlation test between the air temperature and abundance of males in reproductive activity. The same test was applied between the abundance of males and monthly-accumulated rainfall. The rainfall data were obtained from the Proclima online platform (CPTEC/INPE 2017). To verify if the position of the calling male was altered given the density of competitors, we applied the Pearson correlation test with the male angulation data (estimated from soil or water). The same test was applied between the leaf area and the number of eggs deposited in the leaves, to verify the possible correlation between them. The leaf area was verified in the software ImageJ (RUEDEN et al. 2017).

To verify the relationship between the males SVL and the size of the testicles, as well as the SVL and testicular mass, we carried out linear regression tests (ZAR 2010). This same test was applied in a possible relationship between the body mass of the males with the mass and testicular size. In the cases of tests with distinct magnitudes, we logarithmized the values with log 10. We also used the Mann Whitney test to verify the presence of sexual dimorphism in relation to SVL. The same test was used to compare the perch height used by the males in P1 and P2.

For all the statistical analyses we tested the normality of the data and homogeneity of the variance in the software Sigma Stat 3.5 (2006). We considered significant values of $P < 0.05$ (ZAR 2010).

Results

Males of *Pithecopus nordestinus* presented mean SVL of 37.33 mm (SD = 2.98; range = 21.12–41.50 mm; N = 53) and mass of 2.06 g (SD = 0.36; range = 1.64–2.50 g; N = 05). The mean SVL of the females was 42.32 mm (SD = 2.98; range = 38.6–48.3 mm; N = 16). The mean mass of the ovated females was 3.91 g (SD = 0.58; range = 3.13–4.51; N = 4). All ovated females presented oocytes at different development stages. The mass of these oocytes corresponded, on average, to 22% of the females mean weight (SD = 0.30; range = 21.43–22.1; N = 05), while the mean RI of the males is of 0.34% (SD = 0.03; range = 0.31–0.38; N = 5). *Pithecopus nordestinus* presents sexual dimorphism in size ($t = 914.5$; $P < 0.001$).

The relationship between the SVL, testicular size, body mass and testicular mass are summarized in Table 1.

Reproductive period and habitat use

We observed males of *P. nordestinus* in vocalization activity both in months considered dry and in months considered rainy (Fig. 1), although the greatest number of calling males was recorded in the months of highest rainfall. We did not observe correlation between the maximum number of calling males and the monthly-accumulated rainfall ($r = 0.278$; $P = 0.342$) (Fig. 1). Nevertheless, this metric was positively correlated with the monthly mean air temperature ($r = 0.648$; $P = 0.029$) (Fig. 1). The peak of vocalization activity of the species occurred between 20:00 and 21:30 h, and at the end of the dawn, shortly before the daybreak, it was still possible to hear males of *P. nordestinus* in vocalization activity, especially during the rainy season. The maximum number of males in vocalization activity varied from 1 to 16. P1 was the environment in which we observed the highest number of males in vocalization activity (N = 16).

Both in P1 and in P2, the most used environment by the calling males was the vegetation present in the interior of the water body (54.3%; N = 19 and 92.9%; N = 26, respectively). Secondly, the calling males in P1 and P2 preferred the vegetation around the water body (45.7%, N = 16 e 7.1%; N = 2, respectively). The main vegetation used within P1 as

Table 1. Pearson's correlation of the body mass of the males in relation to the mass and testicular size and SVL (snout vent length) of the males in relation to the mass and testicular size *Pithecopus nordestinus*. We considered significant values of $P < 0.05$. Data from Contendas do Sincorá National Forest (FLONA), municipality of Contendas do Sincorá, State of Bahia, registered in the years of 2012 to 2013.

| | Testicles | | Males | |
|------|--------------------------------|--------------------------------|--------------------------------|------|
| | Mass | Mass | Mass | Size |
| Mass | --- | $r^2 = 0.980$; $P = 0.001$ | $r^2 = 0.231$; $P = 0.413$ | |
| Size | $r^2 = 0.346$; $P = 0.163$ | $r^2 = 0.452$; $P = 0.238$ | $r^2 = 0.459$; $P = 0.049$ | |

vocalization site, were the species of Euphorbiaceae (*Croton* sp.1 and *Croton* sp. 2) (42.1%), while in P2, the preferred were the herbaceous of Poaceae (76.9%). Secondly, the most used herbaceous within P1 was Hydroleaceae (*Hydrolea spinosa*) (31.6%), and within P2 herbaceous of Hydroleaceae (*Hydrolea spinosa*), Verbenaceae and Alismataceae (*Limnocharis flava*) (23.1%).

The mean height of the perches used by the calling males in P1 was 40.3 cm (SD = 47.8 cm; range = 0–140 cm; N = 35). In P2 the calling males perched at a mean height of 16.6 cm (SD = 14.0 cm; range = 5–50 cm; N = 28). We did not verify significant difference between the heights of the perches used by the calling males in both ponds ($t = 820.0$; $P = 0.29$). In the same way, there was no correlation between the number of calling males and their angulation on the perches in relation to the water or soil, both in P1 ($r = -0.995$; $P = 0.07$) and in P2 ($r = 0.0307$; $P = 0.97$). The operational sex ratio of *P. nordestinus* is aimed at the male, whose number is superior to the females throughout the study. The mean operational sex ratio for the species was 0.37 (SD = 0.17; range = 0.29–0.57; N = 3) in P1 and 0.24 (SD = 0.10; range = 0.14–0.36; N = 4) in P2.

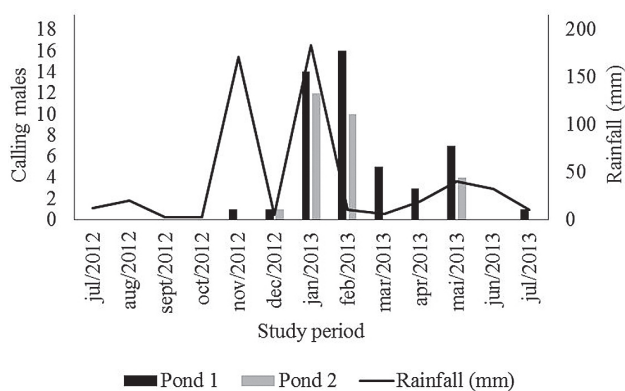


Figure 1. Number of calling males observed per campaign in the ponds and monthly accumulated rainfall (mm) registered in the Contendas do Sincorá National Forest (FLONA), during the months of July 2012 to July 2013, municipality of Contendas do Sincorá, State of Bahia.

Note about the courtship

We only saw one courtship, which was interrupted and, therefore, did not culminate in amplexus. At the moment we started our observations the female was already close to the male (approximately 8 cm apart from each other) on a separate leaf of the same plant. The female had its head facing the male, which in its turn had the posterior region laterally facing the female (Fig. 2). In this position the male performed movements of foot flagging (sensu HÖDL & AMÉZQUITA 2001) slightly modified (pedaling) (Fig. 2A).

Oviposition behavior and spawning characteristics

Throughout the study we collected four couples in amplexus and took them to the laboratory. Only one couple was observed in oviposition process, summarized in the steps described below.

1. During the amplexus, of the cephalic type, the male remained with the gular region on the cephalic region of the female. We observed that the male tapped slightly with the gular region on the female's head. While the male held the female, its legs remained parallel to the female's legs (Figure 2B);
2. During the amplexus, we observed the female touching some leaves of the terrarium (Fig. 2C), apparently inspecting them. Throughout this process, the male remained in amplexus at the same position described in 1. This event was also observed in two other couples in the field;
3. After the election of the oviposition site, the female started what we will call the "leaf preparation" process (Fig. 2D), event in which the female was responsible for the deposition of gelatinous capsules, without oocytes, from the apex of the leaf until approximately $\frac{3}{4}$ of the leaf length. For this, the female slightly projected its body forward, contracting it and opening the legs, always holding firmly the selected leaf with the feet. At the beginning of this process, the male moved partially, still maintaining the arms around the female's head, but with the legs in an adjacent leaf of the same plant, remaining in this position until the preparation was finished (Fig. 2D);
4. After the deposition of the gelatinous material, the male returned to the position on the back of the female to initiate the eggs deposition at $\frac{3}{4}$ height of the leaf (Fig. 2E);
5. During the oviposition, we observed the couple performing the same body contraction movements exhibited by the female in the deposition of the gelatinous capsules (described in 3) (Fig. 2E and F). During the whole process the male remained with the legs parallel to the female's legs and the feet rested on the female's feet;
6. The eggs were oviposited along the central vein of the leaf. Concomitantly, the couple, clinging with their feet on the leaf, performed the process of closing it (Fig. 2F), pressing the leaf with the fingers and toes. This process, herein denominated the spawning cycle, was repeated in another leaf, with 41 eggs deposited in the first leaf and 58 eggs in the second. The leaves presented similar measures (1st leaf: 53.7 mm long by 25.2 mm maximum width; 2nd leaf: 54.4 mm by 26.6 mm maximum width);

7. After completely finished the eggs deposition, the male undid the amplexus. No signs were emitted by the couple that indicated the end of the oviposition process, which lasted approximately 44 min. Only one of the four couples was observed splitting its clutch (ovipositing in more than one leaf in the same amplexus/oviposition process).

The spawning of *P. nordestinus* consists of a gelatinous mass with a mean of 123.75 (SD = 27.29, range = 99–157; N = 04) depigmented eggs of 3.66 mm of mean diameter (SD = 0.46 mm; range = 4.52–2.80 mm; N = 60). The leaves listed as spawning sites by the couples had 177.2 mm in area (SD = 108.5 mm; N = 05) and belonged to the species *Hydrolea spinosa* (Hydroleaceae) (split spawning) and *Stachytarpheta* sp. (Verbenaceae), *Croton* sp. 1 and *Croton* sp. 2 (Euphorbiaceae). The spawning of the species consists of a fertilized eggs, apparently unfertilized eggs and gelatinous capsules. About 70% of the eggs oviposited in these leaves were fertilized. The fertilized eggs are arranged from the region near the petiole to a little more than half of the

central region of the leaf. The unfertilized eggs were clustered in the apical region of the leaf. The eggs (fertilized eggs and unfertilized eggs) occupied about 75% of the leaf area. Here we found a positive correlation between the leaf area and the number of eggs deposited in the leaves ($r = 0.978$; $P = 0.021$). After they laid their eggs some of the eggs of different size remained in the ovaries of the females.

Discussion

Pithecopus nordestinus presented sexual dimorphism in size, females being larger than males, a very common attribute in all the Anura group. We also observed that the reproductive investment of the females of this species is very high, whereas the males are relatively low. The high RI observed herein, seems to be characteristic of females of Phyllomedusidae (e.g. *Pithecopus azureus* (RI = 29.1%) (RODRIGUES et al. 2007), *Phyllomedusa sauvagii* BOULEN-

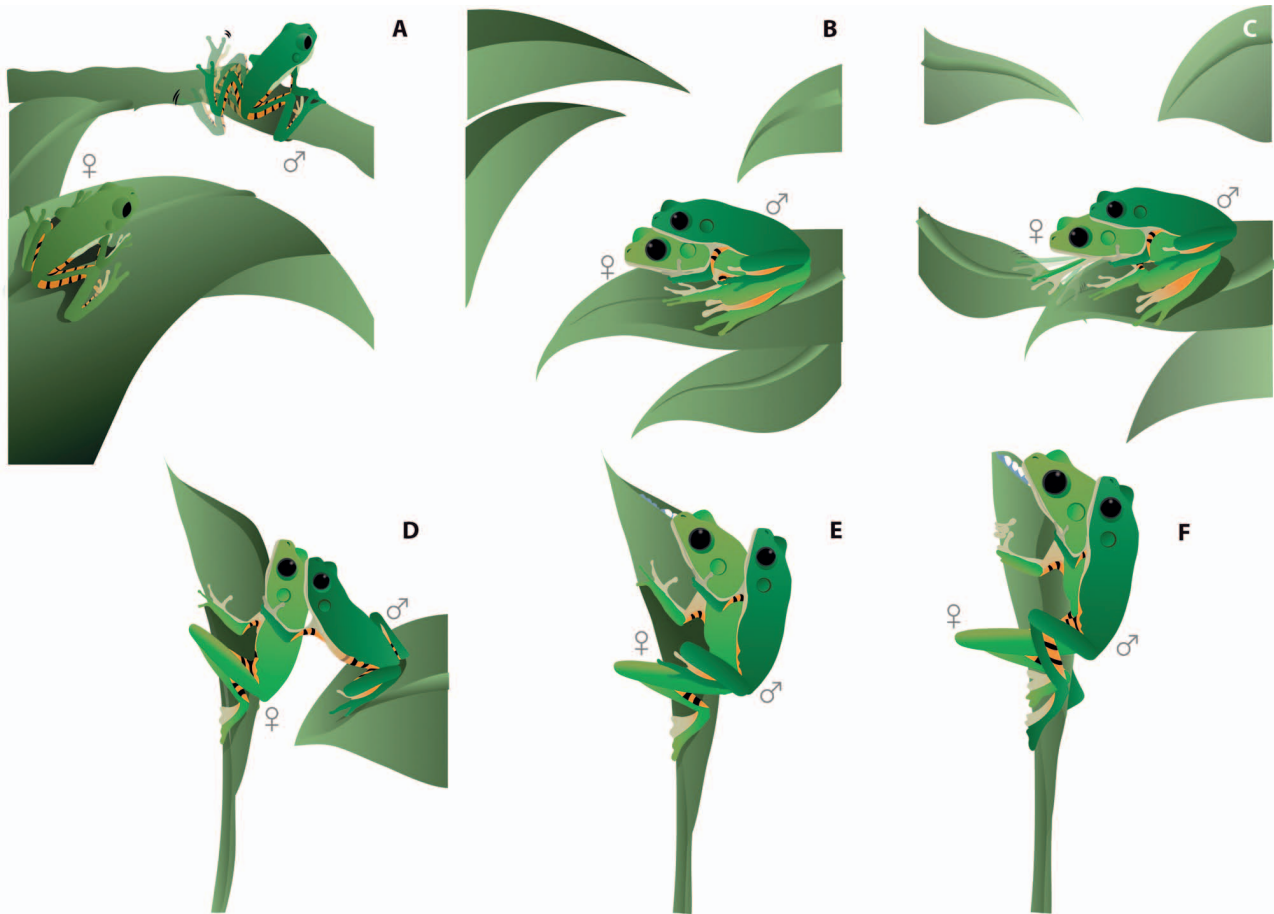


Figure 2. Reproductive behavior of *Pithecopus nordestinus* during the courtship (A) and oviposition (B–F) registered in the Contendas do Sincorá National Forest (FLONA), municipality of Contendas do Sincorá, State of Bahia between the years of 2014 and 2016. A) Courtship of *P. nordestinus* with visual signal (foot flagging); B) Male holding the female by the head, with its legs parallel to the female's legs; C) Female groping some leaves of the terrarium during the amplexus, apparently inspecting them; D) Female during the process of “leaf preparation”, depositing gelatinous capsules at the edge of the leaf, with the male in amplexus, partially displaced; E) Oviposition process along the central vein of the leaf, with the male positioned in the back of the female; F) Couple clinging with the feet on the leaf, performing the process of closing it.

GER, 1882 (RI = 21.1%) (RODRIGUES et al. 2007) and *P. hypochondrialis* (DAUDIN, 1800) (RI = 16.2%) (PRADO & HADDAD 2005)) and females of species that belongs to other families of anuran that live in seasonal environments (e.g. *Leptodactylus chaquensis* CEI, 1950 (RI = 16%) (PRADO & HADDAD 2005), *Physalaemus albonotatus* STEINDACHNER, 1864 (RI = 16.6%) (PRADO & HADDAD 2005), *Elachistocleis bicolor* (GUÉRIN-MÉNEVILLE, 1838) (RI = 18.0%) (PRADO & HADDAD 2005) and *Bokermannohyla ibitiguara* (CARDOSO, 1983) (RI = 20.26%) (NALI & PRADO 2012)). If we consider that the reproductive success is unpredictable in any environment, but is especially uncertain in xeric environments, the maximization of the RI becomes a useful strategy for species that inhabit semi-arid environments. In the case of *P. nordestinus*, the RI maximization associated to the reproductive mode should favor the survival of the offspring in the Caatinga, thus increasing the reproductive fitness of the species in this xeric environment. Unlike the females, the males of the species presented low reproductive investment. The low reproductive investment of the males of *P. nordestinus* in relation to some species of anurans (e.g. KUSANO et al. 1991, PRADO & HADDAD 2003) is probably due to the interaction of females with only one male during the complex courtship, eggs deposition in hidden places, as well as the occurrence of aggressive calls and physical combats, which reduces the opportunities for multi-males spawning and spermatoc competition (see NALI & PRADO 2012, BORGES et al. 2018). This pattern of low reproductive investment in gonads seems to be widespread among several species of anurans with similar reproductive characteristics (e.g. NALI & PRADO 2012, BORGES et al. 2018).

The lack of correlation between the body size and testicle weight of the males, may mean that small males with large testis are composing the reproductive chorus. The early recruitment of these males of smaller body size and supposedly younger, may be an advantage for the occupation of seasonal and xeric environments such as the Caatinga, since favorable periods for the reproduction are limited and their duration are uncertain (see SINSCH et al. 2010, SINSCH & DEHLING 2017, SZÉKELY et al. 2018). The positive relationship between the males' body size and testicular mass may be resultant from influence of gonadal hormones in the determination of secondary characteristics (e.g. muscle mass) which, therefore would increase the weight. Having more muscular mass, for example, can improve the male's fitness during a melee dispute, since the thickness of the forearm for the displacement of other males can be advantageous (e.g. HOWARD & KLUGE 1985).

Reproductive period and habitat use

Although the activity pattern of this species follows the seasonality of the Caatinga (reproduction in the rainy season), the lack of correlation with the rainfall is probably due to the absence of reproductive activity of *P. nordestinus* immediately after the first rains of the rainy season. This absence of activity is possibly due to, after a long drought

period, the first rains only percolate the soil without forming floods adequate to be used as vocalization sites and that serve for the development of aquatic larvae. The positive correlation of the species activity with air temperature, even with a small variation during the year (mean variation of 10°C) (personal data), may be the result of the relationship between the period of highest rainfall (rainy season) and highest temperatures of the year. That is, even that statistically the rainfall rate does not present a direct influence in the population dynamics of the species due to particular morphoclimatic issues, it is determinant in the configuration of the spaces used by the species such as, vocalization and oviposition sites (i.e. water availability (presence of water body), hydroperiod (influenced by the depth and configuration of the water body), presence, structures and diversity of the vegetation) (e.g. SILVA & GIARETTA 2008, VIEIRA et al. 2008, CALDAS et al. 2016). Moreover, the own structure of the population is influenced by biological factors (e.g. reproductive mode and reproductive pattern) typical of the species, which, in turn, also suffer the influence of the climatic factors described above. This is a cyclic process that, in the case of Caatinga, characteristically varies year by year due to the annual variation of the drought period and rainfall rate. In addition, each stochastic perturbation of these factors may have consequences for the others (see ARZABE 1999, PROTÁZIO et al. 2014, CALDAS et al. 2016).

CALDAS et al. (2016) studying a population of *P. nordestinus* in a Caatinga area verified a significant correlation between the number of calling males and the rainfall and air temperature. The area in which the author conducted their study is in the same ecoregion as the FLONA Contendas do Sincorá (see VELLOSO et al. 2002), and would have, in theory, similar ecological features. However, FLONA Contendas do Sincorá is located in an area of rain shadow (see IBAMA 2006), and therefore is subject of more intense and prolonged drought. Besides the rain period in Caatinga may be extremely variable throughout the year and over its geographical distribution. Therefore, each region and environment may present a climate particularity that influences the strategies adopted by the species.

P₁, environment with greater diameter and depth, longer hydroperiod and denser vegetation configuration, both inside and around the pond, was the environment most chosen by the males as vocalization site. The species used preferably low perch in the interior of the water body. Apparently, for *P. nordestinus*, the characteristics of the macro environment (type of water body and hydroperiod) and micro environments (structure and diversity of the vegetation, inside or around the water body) used by the species as sites of vocalization and oviposition, seem to be determinant factors in the choice of reproductive sites by the species. The set of traits related to the biology of the species (i.e. humidity dependency, arboreal habit, morphophysiological and reproductive mode) may be the guide of this choice, been that election crucial for the fitness of the species in the Caatinga, a strongly seasonal environment (see CRUZ 1982, FREITAS et al. 2008, CALDAS et al. 2016).

The oviposition in a longer lasting environment and in the portion less susceptible of water/humidity loss decreases the probability of egg desiccation and increases the probability of tadpoles' survival. The use of these characteristics to choose the reproductive sites was already observed for other species in Caatinga environments (see VIEIRA et al. 2008) and seems to be related the time for the development of the tadpoles until their metamorphosis.

For *P. nordestinus*, the females seem to be the determining agent in the choice of the oviposition site, as observed for other species of anurans (see *Engystomops pustulosus* (COPE, 1864) (RYAN 1983), *Dryophytes chrysoscelis* (COPE, 1880) (RESETARITS JR. & WILBUR 1989), *Phyllomedusa sauvagii* (GARCÍA et al. 2013). Nonetheless, although males do not choose the oviposition sites per se, they have notoriously vocalized in an environment where there is a hall of suitable plants to be listed by the females as oviposition site. The vegetation used as vocalization site present striking characteristics such as the presence of trichomes and rugosities, and *P. nordestinus*, has deposited its eggs mainly in those with trichomes. The behavior of laying eggs on leaves with trichomes was previously viewed for *P. nordestinus* (see CALDAS et al. 2016) in area of Caatinga and seems to be widely diffused in the genus *Pithecopus*, as observed in *P. rohdei* (MERTENS, 1926) (WOGEL et al. 2005), *P. azurea* (RODRIGUES et al. 2007, DIAS et al. 2014), and *P. ayeaye* LUTZ, 1966 (DE OLIVEIRA 2017, BORGES et al. 2018). Foliar cover seems to be essential for the survival of the Phyllomedusidae spawns during their embryonic development outside the water (ABRUNHOSA & WOGEL 2004). Trichomes and leaf spines can aid the fixation of the spawning, providing insulation by air retention, and protection and maintenance of the moisture for the eggs and embryos (DIAS et al. 2014). We suggest the same relationship to the rugosities present in the leaves. Thus, the use of plants with trichomes, as reported in this study, may be associated to the selection of an environment of high quality (e.g. vegetation with trichomes inside the water body) for the development of the spawning and maintenance of the ideal conditions for the reproduction in the Caatinga. Trichomes, together with caducifolia, microfilia and spines are known morphological adaptation of plants to xeric conditions (PRADO 2003), but only trichomes represent to be an advantage for *P. nordestinus* eggs development. That implies that in a hall of many plants with morphological characteristics unsuitable for reproduction propose males of *P. nordestinus* must select an ideal site for calling.

Pithecopus nordestinus presents a characteristic typical of species of prolonged breeding, such as: territorial males and OSR directed towards the males (e.g. WELLS 1977). The degree of competitions between males and the opportunity for partner selection by the females are strongly influenced by this pattern of reproduction (WELLS 2007). These patterns, in turn, will influence the force of the selection of important traits for the reproduction, such as territoriality of males, spawning size and investment in gonads (PRADO & HADDAD 2005). CALDAS et al. (2016) studying *P. nordestinus* in Caatinga area classified the species as ex-

plosive breeder (sensu WELLS 1977) and a population of the same species from an Atlantic forest area as prolonged breeder, indicating that the species may adjust its reproductive period according to the biome in which it is found. We believe that the species may performed behavioral and ecological adjustments according to local climatic conditions. That plasticity goes beyond the biome type.

Courtship

Males of *P. nordestinus* perform foot flagging during the courtship. According to HÖLD & AMÉZQUITA (2001), during this display the individuals "Raise one or both hind legs, extending it/them slowly out and back in an arc above the substrate level, and returning it/them to the body side", there are many peculiarities in its execution, related to the presence and coloration contrasting of the digits, degree of extension of the limbs and, if the movement is performed with one or more limbs. In the case of *P. nordestinus* we observed that the movements of the legs describe a 360° arc as a pedaling, not extending the whole leg or digits during the movement, as observed for other species of anurans (see HÖLD & AMÉZQUITA 2001, DE SÁ et al. 2016). Foot flagging has also been observed for other species of Phyllomedusidae, but in distinct contexts, as territorial sign between co-specifics (*Phyllomedusa boliviana* BOULENGER, 1902 (JANSEN & KÖHLER 2008), *Phyllomedusa sauvagii* (HALLOY & ESPINOZA 2000)) and attraction of preys (*Phyllomedusa burmeisteri* BOULENGER, 1882 (BERTOLUCI 2002)), this being the first time that it is described in a courtship context. Likewise, this is the first description of the visual signal for the genus *Pithecopus*. Foot flagging may be an "out of context" activity either in a courtship or in the territorial and foraging context. As cannot determined the phylogenetic origin of this behavior we cannot go further the merely description of the behavior and its context. However, we recommend the use of this trait as a phylogenetic signal to explain the taxonomic relationship among the phyllomedusids.

Foot flagging of *P. nordestinus* can facilitate the location of males by the females at short distances (attract the female when entering its territory), without calling the attention of co-specifics about the presence of the female. Moreover, the presence of contrasting coloration on the occult parts of the thigh may potentiate the signal emission, acting as sensory exploitation that, indirectly, may indicate the physical condition of the male. The foot flagging itself can add information about the male beyond those (e.g. size) already get by the female. That is, while dorsal coloration acts as camouflage for predators, the contrasting pattern of areas poorly exposed or exposed during the movement (e.g. belly, flanks and inner part of the thigh) may serve to transmit intra-specific cues, especially during visual exhibitions (e.g. postures and movements of the body and head), commonly related to subordination, territoriality and stimulation of the female during the intra-specific interactions (see HÖDL & AMÉZQUITA 2001, DE

SÁ et al. 2016). Foot flagging movements observed in the present study are probably a way for the male to evidence to the female the bright and contrasting colors of the orange with black stripes present on its flanks and inner part of its thighs. One of the examples of visual exhibitions involving movements in association with colors, visible due to the movements [for more examples and information see *Brachycephalus ephippium* (SPIX, 1824) (POMBAL JR. et al. 1994), *Atelopus zeteki* DUNN, 1933 (LINDQUIST & HETHERINGTON 1998), *Hylodes japi* DE SÁ et al., 2015 (DE SÁ et al. 2016)].

The hypothesis already proposed to explain the evolution of the visual displays in anurans concerns overcoming environmental noises (HÖDL & AMÉZQUITA 2001), being more common in diurnal species. Nonetheless, the selective pressures that lead to the evolution of the visual communication in nocturnal anurans are more difficult to understand. Albeit we cannot foreclose the environmental noise hypothesis, considering that there are vocalizations of other species of anurans (biological noise) that also take advantage of the short period favorable to the reproduction in the Caatinga, other hypothesis can be raised regarding the evolution of visual signals. Visual signal may have evolved both in the territorial context (in order to avoid physical combat between males, ensuring the maintenance of spacing between them) and in the context of partner selection (to ensure well positioned leaves or facilitate the localization of males by the females) (see HARTMANN et al. 2005, JANSEN & KÖHLER 2008, DE SÁ et al. 2016, PRESENT STUDY). Therefore, in nocturnal anurans, visual signals may potentiate, by adding a signal of a non-sound nature, the accuracy of the receptor's evaluation on aspects of the signaling's condition (e.g., potential fighting condition, motivational or health state of the individual) (see JOHNSTONE 1996).

In addition to the visual stimuli during the courtship, we also noticed throughout the amplexus a series of tactile stimuli of the gular region of the male on the head of the females. The type and extension of the information that is passed on and collected through the physical contact is still unknown in Anura. One possible explanation for the tactile stimulus in *P. nordestinus* is a possible chemical communication. However, our data do not support greater inferences. We suggest that futures studies verify the existence of a secondary sex characteristic in the males: the sexually dimorphic skin glands (SDSG), described for some species of anurans (see BRUNETTI et al. 2015).

Oviposition behavior and spawning characteristics

The behavior of ovipositing in more than one leaf in the same amplexus/oviposition process, characterizes *P. nordestinus* as a species that splits its clutch. The report of split spawning in the same reproductive event is relatively uncommon in anurans (e.g. *Oloolygon* spp. ALVES-SILVA & SILVA 2009), and this is the first report in Phyllomedusidae. This reproductive characteristic may be related to the

capacity of physiological control of the amount of eggs deposited subsequently by the females in the spawning sites and ability of the males in controlling the sperm release, so that they are able to perform fertilizations of each one of the shares (ALVES-SILVA & SILVA 2009). Clutch splitting also may be related to the reproductive mode of the species and/or the characteristics of the oviposition sites available in the environment (e.g. size of the leaves suitable for oviposition that are available in the water body). Since the most adequate leaves listed as oviposition sites are frequently small, and the spawning of *P. nordestinus* occupies a significant portion of the foliar area, the capacity of dividing the spawning is especially advantageous, particularly if we consider xeric environments, since it can contribute to minimize the loss of eggs by desiccation. Additionally, gelatinous capsules around the fertilized eggs, characteristic common to all the species of Phyllomedusidae, can provide moisture for the spawning during the embryos development (PYBURN 1980, DUELLMAN & TRUEB 1994) while it helps in the closure of the leaves. Besides the dissection advantage, splitting clutch may also be related to the dilution effect of predation (e.g., NECKEL OLIVEIRA & WACHLEWSKI 2004).

CALDAS et al. (2016) did not observed splitting clutch behavior for *P. nordestinus*, however they observed a significant difference between the maximum number of eggs laid by *P. nordestinus* from Caatinga (130 eggs) when compared to populations from an Atlantic forest area (100 eggs). Those values are in accordance to what we observed in the present study (157 eggs). According to CALDAS et al (2016), the population of Caatinga adopted the strategies of "putting all its eggs in one basket" by laying a bigger number of eggs when compared to the Atlantic forest population. As we observed here, the strategies may goes beyond the number of eggs, and the species may choose between lay the eggs in one leaf or splitting it in two. CALDAS et al. (2016) also observed a great range in the values of eggs laid in the Caatinga population; from (22 to 130), we believe that, although not mentioned by the authors, they may had collected splitting clutches as well. This plasticity associated to the oviposition behavior, as observed for the temporal pattern of reproductive season of *P. nordestinus*, may be success key for the occupation of xeric environments by the species.

In Anura, the oviposition frequency of each female per reproductive season is dependent on a combination of the ovulation pattern of the female and the local climate (TSUJI & LUE 2000). In tropical regions it is possible to find females with multiple spawns (ovules that mature asynchronously) given the favorable conditions to the reproduction for a long period of the year (see TSUJI & LUE 2000). The presence of a large amount of oocytes in several development stages in the ovary of the females of *P. nordestinus* points to the hypothesis of multiple spawns along the same reproductive season, as proposed by VAIRA (2001) for *Phyllomedusa boliviana*. Multiple spawns occurred typically in species of prolonged breeding season (see WELLS 1976, KLUGE 1981, PERRILL & DANIEL 1983, TSUJI & LUE

2000), and it was reported a maximum frequency of nine spawns in the same reproductive season (in a period of 81 days), with at least three days of interval between the amplexus with the same female (*Limnectes kuhlii*: TSUJI & LUE 2000). However, reports of such high values of multiple spawns are unusual, being this the only case in field observations. Usually the females spawn two or three times per reproductive season, with mean interval of 15 days (see KLUGE 1981, PERRILL & DANIEL 1983, TSUJI & LUE 2000). The opportunity to spawn more than one time in a single breeding season may be one of the main adaptive advantages of a prolonged reproductive season (WELLS 1976) in xeric environments, where the hydroperiod is normally very short. In this case, the advantages for the females of *P. nordestinus* presenting multiple spawning per reproductive season are: 1. Increase its reproductive success increasing the genetic variability of offspring and their chance of survival; 2. Reduce the impact of predation, unpredictable environmental conditions and competition for food resources between the offspring (see TSUJI & LUE 2000).

The set of information available here about the reproductive biology of *P. nordestinus* may serve as basis for more detailed analyses of the reproductive strategies of anurans, as well as to study the allocation of energy resources for the reproduction and different environmental scenarios, in order to evidence patterns closer to the nature complexity. Further, the data provided in this study exemplifies how little we know about the biology of relatively common and well-distributed species of anurans, with higher deficit in those that inhabit seasonal and semi-arid environments. Prestonian deficit (see CARDOSO et al. 2011), which is the lack of knowledge on the abundance of species and dynamics in space and time, unfortunately is very common among the species of anuran and result in a loss of fundamental information to elucidate ecological issues (HORTAL et al. 2014) (e.g. how a certain community was formed, organized and how it is currently structured) or propose conservation actions.

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References

- ABRUNHOSA, P. A. & H. WOGEL (2004): Breeding behavior of the leaf-frog *Phyllomedusa burmeisteri* (Anura: Hylidae). – *Amphibia-Reptilia*, **25**: 125–135.
- ALVES-SILVA, R. & H. R. SILVA (2009): Life in bromeliads: reproductive behaviour and the monophyly of the *Scinax perpusillus* species group (Anura: Hylidae). – *Journal of Natural History*, **43**: 205–217.
- ARZABE, C. (1999): Reproductive activity patterns of anurans in two different altitudinal sites within the Brazilian Caatinga. – *Revista Brasileira de Zoologia*, **16**: 851–864.
- BARROWS, C. W., M. L. MURPHY-MARISCAL & R. R. HERNANDEZ (2016): At a crossroads: the nature of natural history in the twenty-first century. – *BioScience*, **66**: 592–599.
- BECKER, C. G., C. R. FONSECA, C. F. B. HADDAD, R. F. BATISTA & P. I. PRADO (2007): Habitat split and the global decline of amphibians. – *Science*, **318**: 1775–1777.
- BERTOLUCI, J. (2002): Pedal luring in the leaf-frog *Phyllomedusa burmeisteri* (Anura, Hylidae, Phyllomedusinae). – *Phyllomedusa*, **1**: 93–95.
- BORGES, M. M., R. C. NALI, B. F. FIORILLO & C. P. A. PRADO (2018): Site fidelity, reproductive behavior and investment in the Brazilian Reticulate leaf Frog, *Pithecopus ayeaye* Lutz, 1966 (Anura: Phyllomedusidae). – *Herpetozoa*, **31**: 61–68.
- BRUNETTI, A. E., G. N. HERMIDA, M. C. LUNA, A. M. G. BARSOTTI, C. JARED, M. M. ANTONIAZZI, M. RIVERA-CORREA, B. V. M. BERNECK & J. FAIVOVICH (2015): Diversity and evolution of sexually dimorphic mental and lateral glands in Cophomantini treefrogs (Anura: Hylidae: Hylinae). – *Biological Journal of the Linnean Society*, **114**: 12–34.
- CALDAS, F. L. S., B. D. SILVA, R. A. SANTOS, C. B. DE-CARVALHO, D. O. SANTANA, F. F. A. GOMES & R. G. FARIA (2016): Autoecology of *Phyllomedusa nordestina* (Anura: Hylidae) in areas of the Caatinga and Atlantic Forest in the State of Sergipe, Brazil. – *North-Western Journal of Zoology*, **12**: 271–285.
- CARAMASCHI, U. (2006): Redefinição do grupo de *Phyllomedusa hypochondrialis*, com redescoberta de *P. megacephala* (Miranda-Ribeiro, 1926), revalidação de *P. azurea* Cope, 1862 e descrição de uma nova espécie (Amphibia, Anura, Hylidae). – *Arquivos do Museu Nacional*, **64**: 159–179.
- CARDOSO, P., T. L. ERWIN, P. A. V. BORGES & T. R. NEW (2011): The seven impediments in invertebrate conservation and how to overcome them. – *Biological Conservation*, **144**: 2647–2655.
- CPTEC/INPE (2017): Centro de Previsão de Tempo e Estudos Climáticos/ Instituto Nacional de Pesquisas Espaciais. – Available at <http://proclima.cptec.inpe.br/>
- CRUZ, C. A. G. (1982): Conceituação de grupos de espécies de Phyllomedusinae brasileiras com base em caracteres larvários (Amphibia, Anura, Hylidae). – *Arquivos da Universidade Federal Rural do Rio de Janeiro*, **5**: 147–171.
- DE OLIVEIRA, F. F. R. (2017): Mating behaviour, territoriality and natural history notes of *Phyllomedusa ayeaye* Lutz, 1966 (Hylidae: Phyllomedusinae) in south-eastern Brazil. – *Journal of Natural History*, **51**: 657–675.
- DE SÁ F. P., J. ZINA & C. F. B. HADDAD (2016): Sophisticated communication in the Brazilian torrent frog *Hylodes japi*. – *PLoS ONE*, **11**: 1–24.
- DIAS, T. M., F. P. MARAGNO, C. P. A. PRADO & S. Z. CECHIN (2014): Reproductive site selection in the leaf frog *Phyllomedusa azurea* COPE, 1862 (Anura: Hylidae) in altered areas of the Brazilian Cerrado. – *Journal of Natural History*, **48**: 2689–2699.
- DOMENICO, E. A., C. F. B. HADDAD & H. ZAHER (2014): Natural History of *Paratelmatobius gaigeae* (Amphibia, Anura, Leptodactylidae): Description of the Tadpole and Advertisement Call. – *Journal of Herpetology*, **48**: 430–433.

- DUELLMAN, W. E. & L. TRUEB (1994): Biology of Amphibians. – The Johns Hopkins University Press, Baltimore and London, 670 pp.
- FREITAS, E. B., C. B. DE-CARVALHO, R. G. FARIA, R. C. BATISTA, C. C. BATISTA, W. A. COELHO & A. BOCCHIGLIERI (2008): Nicho ecológico e aspectos da história natural de *Phyllomedusa azurea* (Anura: Hylidae, Phyllomedusinae) no Cerrado do Brasil Central. – *Biota Neotropica*, **8**: 1–10.
- FROST, D. (2019): Amphibian Species of the World: an Online Reference: Version 6.0. American Museum of Natural History, New York. – Available at <http://research.amnh.org/vz/herpetology/amphibia/Amphibia/Anura/Phyllomedusidae/Pithecopus/Pithecopus-nordestinus>
- FUTUYMA, D. J. (1998): Wherefore and whither the naturalist? – *The American Naturalist*, **151**: 1–6.
- GARCÍA, C. G., J. N. LESCANO & G. C. LEYNAUD (2013): Oviposition-site selection by *Phyllomedusa sauvagii* (Anura: Hylidae): An arboreal nester inhabiting arid environments. – *Acta Oecologica*, **51**: 62–65.
- GARDA, A. A., M. G. STEIN, R. B. MACHADO, M. B. LION, F. A. JUNCÁ & M. F. NAPOLI (2017): Ecology, biogeography and conservation of amphibians of the Caatinga. – pp. 133–149 in: SILVA, J. M. C., I. R. LEAL & M. TABARELLI (eds): Caatinga: The largest tropical dry forest region in South America. – Springer, Miami.
- GREENE, H. W. (1986): Natural history and evolutionary biology. – pp. 99–108 in: FEDER, M. E. & G. V. LANDER (eds): Predator-prey relationships: perspectives and approaches from the study of lower vertebrates. – University of Chicago Press, Chicago-Illinois.
- HADDAD, C. B. F., C. P. A. PRADO (2005): Reproductive modes in frogs and their unexpected diversity in the Atlantic forest of Brazil. – *Bioscience*, **55**: 207–217.
- HADDAD, C. B. F., L. P. TOLEDO, C. P. A. PRADO, D. LOEBMANN, J. L. GASPARINI & I. SAZIMA (2013): Guia dos anfíbios da Mata Atlântica: diversidade e biologia. – Anolis Books Editora, 544 pp.
- HALLOY, M. & R. ESPINOZA (2000): Territorial encounters and threat displays in the Neotropical frog *Phyllomedusa sauvagii* (Anura: Hylidae). – *Herpetological Natural History*, **7**: 175–180.
- HARTMANN, M. T., L. O. M. GIASSON, P. A. HARTMANN & C. F. B. HADDAD (2005): Visual communication in Brazilian species of anurans from the Atlantic Forest. – *Journal of Natural History*, **39**: 1675–1685.
- HEYER, W. R., M. A. DONNELLY, R. W. MCDIARMID, L. C. HAYEK & M. S. FOSTER (1994): Measuring and monitoring biological diversity: standard methods for amphibians. – Smithsonian Institution Press, Washington, 384 pp.
- HÖDL, W. & A. AMÉZQUITA (2001): Visual signaling in anuran amphibians. – pp. 121–141 in: RYAN, M. J. (ed.): Anuran communication. – Smithsonian Institution Scholarly Press, Washington.
- HORTAL, J. F. BELLO, J. A. F. DINIZ-FILHO, T. M. LEWINSOHN, J. M. LOBO & R. J. LADLE (2014): Seven shortfalls that beset large-scale knowledge of biodiversity. – *Annual Review of Ecology, Evolution, and Systematics*, **46**: 523–49.
- HOWARD, R. D. & A. G. KLUGE (1985): Proximate Mechanisms of Sexual Selection in Wood Frogs. – *Evolution*, **39**: 260–277.
- IBAMA (2006): Plano de manejo Floresta Nacional de Contendas do Sincorá: informações gerais sobre a Floresta Nacional. – Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, 186 pp.
- JANSEN, M. & J. KÖHLER (2008): Intraspecific combat behavior of *Phyllomedusa boliviana* (Anura: Hylidae) and the possible origin of visual signaling in nocturnal treefrogs. – *Review*, **39**: 290–293.
- JOHNSTONE, R. A. (1996): Multiple displays in animal communication: ‘backup signals’ and ‘multiple messages’. – *Philosophical Transactions of the Royal Society B: Biological Sciences*, **351**: 329–338.
- JORGE, J., R. SALES, M. KOKUBUM & E. M. FREIRE (2015): On the natural history of the Caatinga Horned Frog, *Ceratophrys joazeirensis* (Anura: Ceratophryidae), a poorly known species of northeastern Brazil. – *Phyllomedusa: Journal of Herpetology*, **14**: 147–156.
- KLUGE, A. G. (1981): The life history, social organization, and parental behavior of *Hyla rosenbergi* Boulenger, a nest-building gladiator frog. – University of Michigan Museum of Zoology: Miscellaneous publications, 170 pp.
- KÖPPEN, W. (1936): Das geographische system der klimatologie. – pp. 1–44 in: KÖPPEN, W. & R. GEIGER (eds): Handbuch der Klimatologie. – Gebrüder Bornträger, Berlin.
- KUSANO, T., M. TODA & K. FUKUYAMA (1991): Testes size and breeding systems in Japanese anurans with special reference to large testes in the tree-frog, *Rhacophorus arboreus* (Amphibia: Rhacophoridae). – *Behavioral Ecology and Sociobiology*, **29**: 27–31.
- LANTYER-SILVA, A. S. F., M. SOLÉ, J. ZINA (2014): Reproductive biology of a bromeligenous frog endemic to the Atlantic Forest: *Aparasphenodon arapapa* Pimenta, Napoli and Haddad, 2009 (Anura: Hylidae). – *Anais da Academia Brasileira de Ciências*, **86**: 867–880.
- LEHNER, P. N. (1996): Handbook of ethological methods. Cambridge University Press, Cambridge, 672 pp.
- LINDQUIST, E. D. & T. E. HETHERINGTON (1998): Semaphoring in an earless frog: the origin of a novel visual signal. – *Animal Cognition*, **1**: 83–87.
- LOYOLA, R. D., C. G. BECKER, U. KUBOTA, C. F. B. HADDAD, C. R. FONSECA & T. M. LEWINSOHN (2008): Hung out to dry: choice of priority ecoregions for conserving threatened neotropical anurans depends on life-history traits. – *PLoS ONE* **3**: 1–8.
- MARTINS, M. B. (1988): Biologia reprodutiva de *Leptodactylus fuscus* em Boa Vista, Roraima (Amphibia: Anura). – *Revista Brasileira de Biologia* **48**: 969–977.
- NALI, R. C. & C. P. A. PRADO (2012): Habitat use, reproductive traits and social interactions in a stream-dweller treefrog endemic to the Brazilian Cerrado. – *Amphibia-Reptilia*, **33**: 337–347.
- NECKEL-OLIVEIRA, S. & M. WACHLEVSKI (2004): Predation on the arboreal eggs of three species of *Phyllomedusa* in Central Amazônia. – *Journal of Herpetology*, **38**: 244–248.
- NUNES, I., D. LOEBMANN, C. A. G. CRUZ & C. F. B. HADDAD (2015): Advertisement call, colour variation, natural history, and geographic distribution of *Proceratophrys caramaschii* (Anura: Odontophrynidae). – *Salamandra*, **51**: 103–110.
- PEARMAN, P. B. & H. M. WILBUR (1990): Changes in population dynamics resulting from oviposition in a subdivided habitat. – *The American Naturalist*, **135**: 708–723.

- PERRILL, S. A. & R. E. DANIEL (1983): Multiple egg clutches in *Hyla regilla*, *H. cinerea* and *H. gratiosa*. – *Copeia*, **1983**: 513–516.
- POMBAL JR., J. P., I. SAZIMA & C. F. B. HADDAD (1994): Breeding behavior of the Pumpkin Toadlet, *Brachycephalus ephippium* (Brachycephalidae). – *Journal of Herpetology*, **28**: 516–519.
- PROTÁZIO, A. S., R. L. ALBUQUERQUE, L. M. FALKENBERG & D. O. MESQUITA (2014): Acoustic ecology of an anuran assemblage in the arid Caatinga of northeastern Brazil. – *Journal of Natural History*, **49**: 1–20.
- PRADO, C. P. A. & C. F. B. HADDAD (2003): Testes size in leptodactylid frogs and occurrence of multiple spawning in the genus *Leptodactylus* in Brazil. – *Journal of Herpetology*, **37**: 354–362.
- PRADO, C. P. A. & C. F. B. HADDAD (2005): Size-fecundity relationships and reproductive investment in female frogs in the Pantanal, South-Western Brazil. – *Herpetological Journal*, **15**: 181–189.
- PRADO, D. E. (2003): As Caatingas da América do Sul. – pp. 3–74 in: LEAL, I. L., M. TABARELLI, & J. N. C. SILVA (eds): *Ecologia e Conservação da Caatinga*. – Editora Universitária UFPE, Recife.
- PYBURN, W. F. (1980): The function of eggless capsules and leaf in nests of the frog *Phyllomedusa hypochondrialis* (Anura: Hylidae). – *Proceedings of the Biological Society of Washington*, **93**: 153–167.
- REFSNIDER, J. M. & F. J. JANZEN (2010): Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. – *Annual Review of Ecology Evolution and Systematics*, **41**: 39–57.
- RESETARITS JR., W. J. & H. M. WILBUR (1989): Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors. – *Ecology*, **70**: 220–228.
- RODRIGUES, D. J., M. UETANABARO & F. S. LOPES (2007): Breeding biology of *Phyllomedusa azurea* COPE, 1862 and *P. sauvagii* Boulenger, 1882 (Anura) from the Cerrado, Central Brazil. – *Journal of Natural History*, **41**: 1841–1851.
- RUEDEN, C. T., J. SCHINDELIN, M. C. HINER, B. E. DEZONIA, A. E. WALTER, E. T. ARENA & K. W. ELICEIRI (2017): ImageJ2: ImageJ for the next generation of scientific image data. – *BMC Bioinformatics*, **18**: 529.
- RYAN, M. J. (1983): Sexual selection and communication in a neotropical frog, *Physalaemus pustulosus*. – *Evolution*, **37**: 261–272.
- SCHMIDL, D. J. (2005): What it means to be a naturalist and the future of natural history at american universities. – *Journal of Mammalogy*, **86**: 449–456.
- SigmaStat 3.5 for Windows Copyright© (2006) Systat Software. Inc. United States of America.
- SILVA, W. R. & A. A. GIARETTA (2008): Seleção de sítios de oviposição em anuros (Lissamphibia). – *Biota Neotropica*, **8**: 243–248.
- SINSCH, U., J. M. DEHLING (2017): Tropical anurans mature early and die young: evidence from eight Afrotropical *Hyperolius* species and a meta-analysis. – *PLoS ONE*, **12**: e0171666.
- SINSCH, U., F. MARANGONI, N. OROMI, C. LESKOVAR, D. SANUY & M. TEJEDO (2010): Proximate mechanisms determining size variability in natterjack toads. – *Journal of Zoology*, **281**: 272–281.
- SZÉKELY, D., P. SZÉKELY, F. STĂNESCU, D. COGĂLNICEANU & U. SINSCH (2018): Breed fast, die young: demography of a poorly known fossorial frog from the xeric Neotropics. – *Salamandra*, **54**: 37–44.
- TSUJI, H. & K. Y. LUE (2000): The reproductive ecology of female *Rana (Limnonectes) kuhlii*, a fanged frog of Taiwan, with particular emphasis on multiple clutches. – *Herpetologica*, **56**: 153–165.
- VAIRA, M. (2001): Breeding biology of the leaf-frog *Phyllomedusa boliviana* (Anura, Hylidae). – *Amphibia-Reptilia*, **22**: 421–429.
- VIEIRA, W. L. S., G. G. SANTANA, C. ARZABE (2008): Diversity of reproductive modes in anurans communities in the Caatinga (dryland) of northeastern Brazil. – *Biodiversity and Conservation*, **18**: 55–66.
- VELLOSO, A. L., E. V. S. B. SAMPAIO, A. M. GIULIETTI, M. R. V. BARBOSA, A. A. J. F. CASTRO, L. P. QUEIROZ, A. FERNANDES, D. C. OREN, L. A. CESTARO, A. J. E. CARVALHO, F. G. C. PAREYN, F. B. R. SILVA, E. E. MIRANDA, S. KEEL & R. S. GONDIM (2002): Ecorregiões propostas para o bioma Caatinga. – *The Nature Conservancy of Brasil, Recife*, 76 pp.
- WELLS, K. D. (1976): Multiple egg clutches in the green frog (*Rana clamitans*). – *Herpetologica* **32**: 85–87.
- WELLS, K. D. (1977): The social behavior of anuran amphibians. – *Animal Behaviour*, **25**: 666–693.
- WELLS, K. D. (2007): *The ecology and behavior of amphibians*. – The University of Chicago Press, Chicago, 1400 pp.
- WOGEL, H., P. A. ABRUNHOSA & J. P. POMBAL JR. (2005): Breeding behaviour and mating succes of *Phyllomedusa rohdei* (Anura, Hylidae) in south-eastern Brazil. – *Journal of Natural History*, **39**: 2035–2045.
- ZAR, J. (2010): *Biostatistical analysis*. – Prentice Hall, New Jersey, 960 pp.
- ZINA, J. & C. F. B. HADDAD (2006a): Ecology and reproductive biology of two species of *Aplastodiscus* (Anura: Hylidae) in the Atlantic Forest, Brazil. – *Journal of Natural History*, **40**: 1831–1840.
- ZINA, J. & C. F. B. HADDAD (2006b): Acoustic repertoire of *Aplastodiscus arildae* and *A. leucopygius* (Anura: Hylidae) in Serra do Japi, Brazil. – *South American Journal of Herpetology*, **1**: 227–236.
- ZINA, J. & C. F. B. HADDAD (2007): Courtship behavior of two treefrog species, *Aplastodiscus arildae* and *A. leucopygius* (Anura, Hylidae), from the Atlantic rainforest, southeastern Brazil. – *Herpetological Review*, **38**: 282–285.