Breeding biology, territoriality, and reproductive site use by *Phyllomedusa iheringii* (Anura: Phyllomedusidae) from the South American Pampa in Brazil

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Abstract. The breeding behaviour of anurans can be associated with environmental variables, availability of suitable reproductive sites, and the number of individuals in a breeding area. We describe the social and breeding behaviour of *Phyllomedusa iheringii*, characterize the reproductive period and reproductive sites, and investigate the presence of assortative mating and calling site fidelity in ponds in southern Brazil. The breeding season was found to be prolonged and associated with the warmest months. Mating was not assortative in terms of body size of amplectant pairs. Patrolling behaviour and multiple amplexi were occasionally observed, which appears to be a density-dependent shift related to high male abundance in the choruses. Calling sites were mainly on grasses above the ground, in contrast to the oviposition sites that were mainly shrubs above the water, suggesting a lek mating system. The positions of reproductive sites varied between months, but only locations of calling sites varied between ponds. Male body size correlated significantly with perch height, and males exhibited territorial behaviour despite having low fidelity to calling sites. Males did not defend oviposition sites, dismissing a resource defence mating system, even though they did exhibit aggressive behaviour, meaning that territoriality plays some role in this species' reproductive strategy.

Key words. Amphibia, habitat use, behaviour, assortative mating, site fidelity.

Introduction

The reproduction of anurans can be influenced by environmental conditions, availability of suitable calling and oviposition sites, and the number of individuals in the chorus (Silva & Giaretta 2008, Canavero et al. 2009, Nali & Prado 2012). In anurans, mating is often driven by female preference (Morris 1989). Assortative mating can be advantageous when it results in higher fertilization rates due to the closer juxtaposition of the cloacae of the partners (Licht 1976). Anurans, in a social context, may exhibit territoriality associated with the defence of resources needed for survival, calling sites or oviposition sites (Wells 1977). Males may be influenced by the vocalizations of their neighbours, eventually changing their acoustic behaviour and/or engaging in physical combat (Wells 1977, Bastos et al. 2011, Lemes et al. 2012). Thus, size may determine an individual male's ability to persist in a certain microhabitat. Defending such a specific site can be advantageous if it provides the male with exclusive or increased access to limited resources (Wells 1977, Howard 1978).

Environmental conditions such as climatic factors and availability of reproductive sites may be important determinants of calling activity and reproductive behaviour in anurans, influencing the number of calling males and their selection of reproductive sites (Bertoluci & Rodrigues 2002, Prado & Pombal Jr 2005, Canavero et al. 2009). These environmental conditions can be related to reproductive success, for example, by affecting sound dissemination, and determine the visual field of individuals (Wells & Schwartz 1982, Abrunhosa & Wogel 2004). Furthermore, the morphological and physiological characteristics

of males can influence their use of calling sites (e.g., Crump 1971, Cardoso et al. 1989). For females, the sites chosen for oviposition are those with the most favourable characteristics for the survival and development of their offspring (see Silva & Giaretta 2008).

The genus *Phyllomedusa* Wagler, 1830 includes 15 species that use vegetation for both vocalization and spawning (Haddad & Prado 2005, Faivovich et al. 2010, Duellman et al. 2016, Frost 2016). *Phyllomedusa iheringii* Boulenger, 1885 belongs to the *Phyllomedusa burmeisteri* group (sensu Lutz 1950), and is a leaf frog endemic to the forest and grassland ecosystems of the Uruguayan and Brazilian Pampa (Maneyro & Carreira 2012, Frost 2016). Accounts of the breeding biology of *P. iheringii* are scarce and restricted to brief observations (de Sá & Gerhau 1983, Langone et al. 1985, Langone 1993). In the present study, we describe the social behaviour and characterize the breeding period and sites of *P. iheringii*. We answer the questions: i) What is the extent of the breeding season of *P. iheringii*? ii) Which environmental variables explain male calling activity? iii) Does the use of reproductive sites vary between ponds and months? iv) Do males exhibit calling site fidelity? v) Is mating assortative regarding body size? Additionally, we characterize calling and oviposition sites by vegetation type and position in the vegetation, and clutches by egg number, egg size, and predation rate.

Materials and methods
Study area

We conducted this study in the grassland Pampa biome (Ibge 2004) of the Rio Grande do Sul state, Brazil. Here, the vegetation is characterized by a natural mosaic of grassland, shrubs and forests and also includes cultivated lands (e.g., wheat, soybean, and ryegrass). The climate is temperate, with hot summers and no clearly defined dry season (Cfa of Köppen-Geiger' classification) (Peel et al. 2007). We collected data at two sites. The first site (A1) comprised six ponds on a private property in the municipality of São Sepé (30°15'03.9" S, 53°35'05.1" W, 198 m a.s.l.), which we sampled from August 2012 through July 2013. The second site (A2) comprised three ponds in the grounds of the campus of the Universidade Federal de Santa Maria in the municipality of Santa Maria (29°43'15.68" S, 53°43'35.58" W, 92 m a.s.l.), which we sampled in December 2011, January, September and October 2012, and January 2013. This second area was sampled only during the hot season, which is the period of high anuran activity for this type of climate (Santos et al. 2008).

Data collection

The breeding season of *Phyllomedusa iheringii* was identified based on monthly monitoring of the ponds at A1. At each pond, we assessed the abundance of calling males, number of clutches, and occurrence of tadpoles. Calling males were counted around the banks of the pond from 19:00 h, when males started calling (sensu Scott Jr & Woodward 1994). For clutch sampling, we inspected vegetation on the banks during the day, and we marked each leaf nest with permanent ink to prevent recounts. Tadpoles were sampled using a wire mesh dip net (3 mm² mesh size) and search for in all available microhabitats (e.g., water column and margins of ponds with and without vegetation) from the bottom to the surface (Santos et al. 2009, Vasconcelos et al. 2011). Tadpoles were anaesthetized, fixed in 10% formalin and identified in the laboratory. At this site, we also measured the snout–vent lengths (SVL) of males and females of 12 amplectant pairs to test for the presence of an assortative mating system.

To test for the relationships between the number of males and environmental variables, we counted the males calling from the banks of the ponds at A1 and measured the following variables: temperature and relative humidity, photoperiod, moon phase, and rainfall. Air temperature and relative humidity were measured during sampling nights using a thermo-hygrometer (Equitherm model TH439). Photoperiod data for the sampling days were obtained from the Observatório Nacional Brasileiro (available online in Portuguese at http://euler.on.br/ephemeris/index.php), and accumulated precipitation information was obtained from the Secretaria de Agricultura of the municipality of São Sepé.

Throughout the sampling period at A1 and A2, we characterized 235 calling sites and 371 oviposition sites. To characterize calling sites, we actively searched for males at the ponds from sunset to the end of calling activity (approximately 24:00 h). For each male found, we recorded perch height (cm), distance (cm) from the water (using positive values for males inside the pond, and negative values for males outside the pond, respectively), distance (cm) to the nearest neighbouring calling male, and vegetation type at the calling site. We characterized the oviposition sites during a daytime inspection of the vegetation around the ponds. For each leaf nest, we recorded vegetation height (cm), distance (cm) from the water (using positive values for nests inside the pond, and negative values for nests outside the pond, respectively), distance (cm) to the nearest neighbouring clutch, number and size of leaves used in the nest, and vegetation type. To quantify clutch size, we collected 26 nests and fixed them in 10% formaldehyde. We categorized vegetation types based on height and complexity according to Vidal & Vidal (2003): herbs (little or no lignification), lianas (climbing vines), shrubs (with branches from the base and less than 5 m in total height), small trees (tree architecture, with a maximum of 5 m in height), and trees (clear trunk, taller than 5 m). We also classified plants by family if they were Bromeliaceae, Cyperaceae, Juncaceae or Poaceae (Gramineae).

To test for calling site fidelity, we marked 30 males at A2 using subcutaneous implants (alphanumeric fluorescent tags that can be read under ultraviolet light). Calling sites were marked with green ribbons with knots attached to the trees, with each ribbon with a specific number of

Table 1. Structural characterization of the study ponds (P1–P6) in area A1 serving as reproduction sites for the southern walking leaf-frog (*Phyllomedusa iheringii*) in southern Brazil.

Environmental factors	P1	P2	P3	P4	P5	P6
Altitude (m)	184	192	214	201	200	183
Area (m²)	340	125	512	276	2430	185
max. Depth (cm)	50	81	70	140	126	70
Hydroperiod (months)	12	12	12	12	10	12
Emerse vegetation (%)	50	75	45	0	10	80
Canopy cover (%)	5	3	10	0	0	0
Number of vegetation strata on the banks	3	3	4	3	3	2
Mean height (min–max) of the embankment vegetation (cm)	190.33 (22–500)	146.33 (80–390)	216.00 (4–450)	115.67 (21–280)	100.67 (21–230)	184.50 (130–350)

knots corresponding to one particular marked individual. Each time a marked individual was found at a site, a corresponding ribbon was placed there. Each night, ponds were inspected for individuals and marked sites.
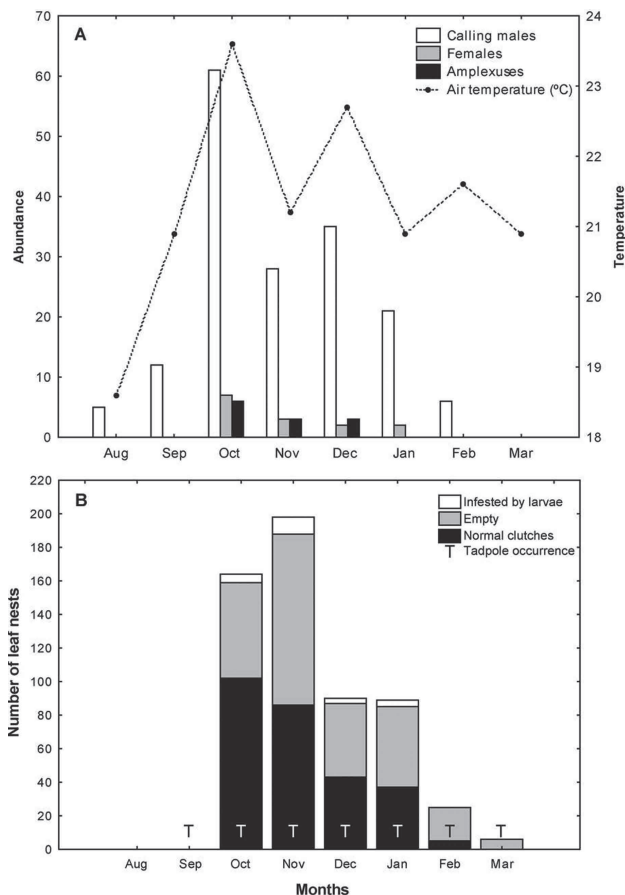

Figure 1. (A) Breeding season of the southern walking leaf-frog (*Phyllomedusa iheringii*) and air temperature. (B) Tadpole occurrence and the number of leaf nests of the southern walking leaf-frog (*Phyllomedusa iheringii*) recorded with clutches, empty or infested by insect larvae in southern Brazil, from August 2012 through March 2013 at six ponds in research area A1.

In addition, we occasionally recorded field observations and described the breeding behaviour of *Phyllomedusa iheringii* by analysing photos and short video clips taken with a digital camera Sony® Handycam DCR-DVD 508. Expressions of breeding behaviour were recorded from 22:00 to 04:30 h with torchlights or infrared light at pond P3 of A1, where the highest abundance of *P. iheringii* was recorded.

Data analysis

To test whether environmental variables influence the number of calling males, we performed a multiple linear regression using a forward stepwise selection procedure. Before performing the regression analyses, all of the variables were log-transformed [ln(x+1)] and tested for normal distribution (ZAR 1999).

To compare reproductive sites between ponds and months, we used a one-way ANOVA. We performed the test with the variables "perch height" and "distance from the water" and separated the data for calling and oviposition sites. We used a Bonferroni post-hoc test. For calling and oviposition sites, we included only ponds with heterogeneous vegetation (summarized in Table 1), totalling six ponds.

To examine whether male SVL was correlated with calling site position, we performed three simple linear regressions: between male SVL and perch height, and between male SVL and the inside or outside distance from the water edge. Prior to analysis, all the variables were log-transformed [ln(x+1)] and tested for normal distributions with the software Statistica 8 (ZAR 1999).

Finally, to analyse whether mating was assortative by body size, we performed a Spearman's Rank Correlation test between the SVLs of amplectant males and females.

Results
Breeding season and breeding behaviour

We recorded the calling activity of *Phyllomedusa iheringii* from August through February, finding calling activity to

peak from October to January (86% of records). Amplectant pairs were found from October through December (n=12), and leaf nests were present from October through March (with ~95% of leaf nests found between October and January). We failed to find leaf nests in August and September, only recorded empty leaf nests in March. Tadpoles were recorded from September through March (Fig. 1).

The monthly number of calling males was related only to the monthly air temperature ($F_{[2,4]}$=7.54, p=0.044, R^2_{adj}=0.68, beta temp.= 0.96, p=0.039). Most males started calling approximately 1 hour after sunset. Males would usually call from perches on vegetation and emit territorial calls. When the density of calling males was high (from 17 to 35 individuals in the same night), we observed males in frenetic activity, leaving their calling perches and constantly moving through the vegetation [patrolling behaviour sensu Wogel et al. (2005)], which is indicative of a scramble-competition mating system. During these phases, it was common to observe males grasping the backs of other males (and even interactions among three males), resulting in vocal interactions and eventually in physical combat between males, which included mutual kicking with their hind legs (Fig. 2). In at least five male–male combats, we observed the opponents falling to the ground.

The body sizes of amplectant couples were not correlated (r_s=0.2, p=0.54). Males had an average SVL of 53.9±4.1 mm (range = 46–69 mm; n=162), and females had an average SVL of 63±5.1 mm (range = 52–75 mm, n=21). Amplectant pairs usually walked through the vegetation before spawning. However, the movements of a pair seemed to attract

Figure 2. Males of the southern walking leaf-frog (*Phyllomedusa iheringii*) fighting on leaves of the shrub *Miconia hiemalis* (Melastomataceae) in southern Brazil. Photo: T.G. Santos.

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intruder males that frequently chased, intercepted and tried to clasp the attached male. At least twice, we recorded the amplectant males performing leg-stretching behaviour (by stretching and then retracting their hind legs transversally to the body in alternation) while chasing an intruder. In one event, we observed an amplectant male avoiding the approach of an intruder by extending his hind legs straight back and using his feet to immobilise the forelimbs of the competitor male. Males in multi-male amplexi interacted by means of aggressive calls. Intruder males tried to dislodge mating males from the female, and both males fought on the female's back (trying to dislodge each other by kicking with the hind legs) until the intruder male gave up. All the while the female kept moving, with both males on her back.

The female of an amplectant pairs would eventually position her body on a tipped leaf (we did not notice any selectivity by leaf surface), grasping the leaf base and leaf apex with her arms and legs, respectively. She would then deposit her first eggs close to the leaf apex, and add the others of a clutch an ascending movement. Egg-less capsules were deposited intermittingly. For is part, the male would curve his body to align his cloaca with that of the female and assist her during spawning by folding the leaf with his hind legs. However, all males observed left the oviposition site before the female, leaving closing the leaf nest to her.

Calling sites

The plants most commonly used as calling perches were Poaceae (32%, n=75), followed by dicotiledoneous herbaceous plants (24%, n=55), shrubs (22%, n=51), small trees (11%, n=26), trees (6%, n=13), Bromeliaceae (3%, n=8), and Cyperaceae + Juncaceae + lianes (2%, n=4; Fig. 3A). The position on the vegetation varied: perch height ranged from 0 cm (pond 4) to 288 cm (average = 63±40 cm, n=235) (pond 9), and the distance of calling males from the bank ranged from -933 cm (pond 3) to +150 cm (pond 8) (Table 2). On average, males vocalized from positions distant from the banks (i.e., above the ground), rather than above the water (average = -29±115 cm, n=234).

Male SVL was significantly correlated with perch height ($F_{[1,221]}$=16.85, R^2_{adj}=0.07, beta=0.27, p<0.0001) but not with positive/negative distance from the edge of the pond ($F_{[1,81]}$=1.13, R^2_{adj}=0.001, beta=0.12, p=0.29; $F_{[1,137]}$=0.83, R^2_{adj}=-0.001, beta=0.08, p=0.36, respectively). The mean distance to the nearest calling male indicated that males were spaced widely in choruses (average = 222±302 cm, n=130; ranging from 0 to 1861 cm).

Calling site positions (distance from the water edge and perch height) varied significantly between months ($F_{[5, 197]}$=14.04, *p*<0.0001; $F_{[5, 197]}$=3.67, p=0.003, respectively), but not between ponds ($F_{[5, 134]}$=1.59, p=0.16; $F_{[5, 134]}$=1.70, p=0.13, respectively). In February, males called from farther from the water (p<0.0001). In October, males occupied lower perches as compared to January (p=0.011).

Table 2. Mean perch heights and mean distances from water (DW) registered for calling males and leaf nests of the southern walking leaf-frog (*Phyllomedusa iheringii*) at each pond in southern Brazil. N = sample size. Positive values indicate distances from the pond margin and above the water, and negative values indicate distances from the pond margin and above the ground on land. Ranges are given in parentheses.

Pond	Leaf nests (N)	Leaf nest height (cm)	Leaf nest DW (cm)	Males (N)	Male height (cm)	Male DW (cm)
1	50	32 (7–90)	17 (-32–179)	28	47.5 (7–164)	-37 (-500–63)
2	6	16.2 (0–31)	4.25 (-14–15)	13	53.7 (27–134)	-93.7 (-415.4–41)
3	207	89.5 (0–302)	5.4 (-50–95)	67	62.4 (2–202.2)	-23.4 (-933–71)
4	35	15 (2–29)	12.6 (-35–105)	17	38.3 (0–90)	-37 (-426.7–87)
5	44	43 (9–77)	-36 (-140–76)	11	43 (10.5–98)	-126 (-667.1–63)
6	–	–	–	3	52 (23–83)	-40.3 (-75–19)
7	7	50.8 (30–60)	27 (0–50)	50	75.7 (20–160)	-18 (-300–140)
8	22	78 (35–135)	12 (-55–40)	40	72.2 (20–140)	7 (-220–150)
9	–	–	–	6	108 (40–288)	-51 (-150–20)
Total	371			235		

Of the 30 males investigated for calling site fidelity, one was found at the same site during two consecutive nights, and one was found at the same site during five non-consecutive nights. Two males were found in different calling spots in the same night, and six males were found in different ponds during the breeding season.

Oviposition sites

Clutches were most often deposited on the leaves of shrubs (66%, n=246), followed by herbaceous plants (25%, n=92), small trees (8%, n=30), and Poaceae (1%, n=3) (Fig. 3B). The shrub most commonly used as an oviposition site was *Miconia hiemalis* (Melastomataceae). The average lengths and widths of the leaves used for oviposition were 10.8±4.18 cm (range = 2.3–26 cm, n=497) and 4.20±1.55 cm (range = 1–9.5 cm; n=497), respectively. *Phyllomedusa iheringii* used from one to eight leaves to make a nest (average = 1.55±0.96, n=371). Approximately 4% of all clutches that we sampled were parasitised, always by Diptera larvae (Phoridae and Calliphoridae). In addition, we recorded ant predation on live tadpoles that had hatched and fallen to the ground from nests with no water below. Hatching was likely triggered by rainfall during the previous day and humid weather conditions. Tadpoles fell on the ground and performed irregular spasmodic movements in an attempt to reach a nearby pond. Some succeeded in this quest while others fell victim to ants that were already on the ground.
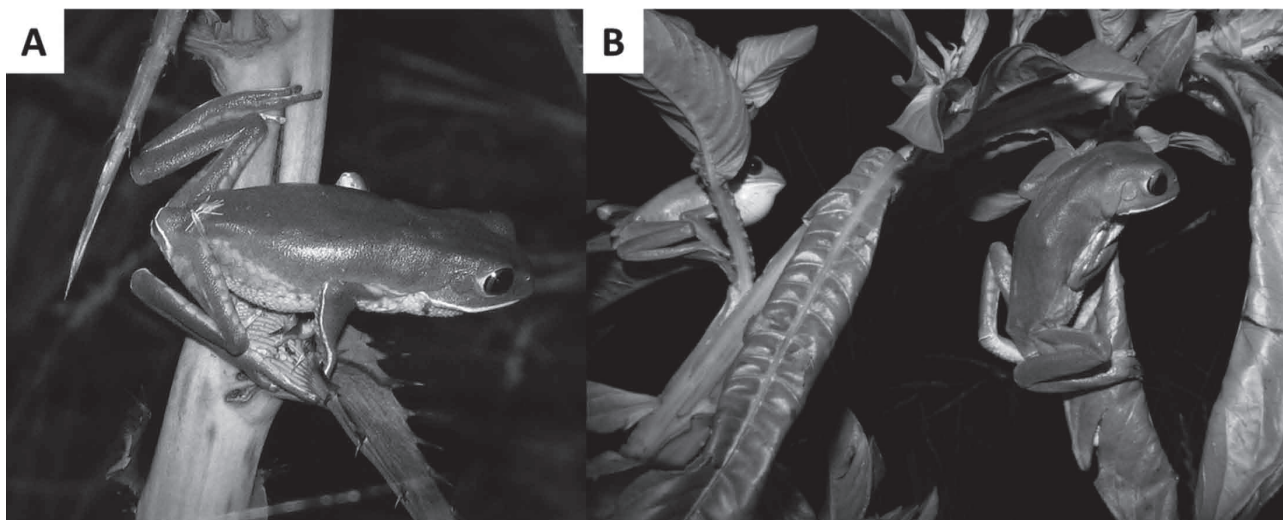

Figure 3. Reproductive sites of the southern walking leaf-frog (*Phyllomedusa iheringii*) in southern Brazil. (A) Male using a bromeliad as a calling site. (B) A female (right) has just finished wrapping her clutch into a leaf, with the male (left) that fertilized the ovules resuming vocalization. Photos: T.G. Santos.

The average number of eggs per clutch was 106±28.78 (range = 57–216, n=26). The average egg size, including the gelatinous envelope, was 3.22±0.72 mm (range = 2.02–4.75 mm, n=90), and the average egg size without the gelatinous envelope was 2.57±0.25 mm (range = 2.14–3.20 mm, n=90).

Leaf nest heights ranged from 0 cm (ponds 2 and 3) to 302 cm (pond 3) (average = 67±55, n=371), and their distances from the water varied from -140 cm (pond 5) to +179 cm (pond 1) (Table 2). On average, leaf nests were found above water, rather than above dry ground (average = 3.5±34, n=371). The mean distance to the nearest neighbouring leaf nest indicated clustered oviposition sites (average = 28±101 cm, n=325) and ranged from 0 to 725 cm.

Leaf nest height varied significantly between ponds, with the highest clutches found at pond 3 (pond 3 versus pond 2: p=0.003; pond 3 versus all others p<0.0001), but it did not vary between months ($F_{[4, 337]}$=32.08, p<0.0001; $F_{[5, 350]}$=0.82, p=0.53, respectively). The distances of leaf nests from the water varied between ponds and months ($F_{[4, 337]}$=20.04, p<0.0001; $F_{[5, 350]}$=2.45, p=0.033, respectively). Leaf nests were farther from the water at pond 5 as compared to the other ponds (pond 5 versus pond 2: p=0.038; pond 5 versus all others p<0.0001), and farther inside the pond in January compared to November and December (p=0.04; p=0.03, respectively).

Discussion
Breeding season and breeding behaviour

Phyllomedusa iheringii has a prolonged breeding season sensu WELLS (1977) since calling activity, oviposition and the presence of tadpoles continued for several months. The breeding period we observed was longer than that observed for this species at other locations (KLAPPENBACH 1961, ACHAVAL & OLMOS 2007, MANEYRO & CARREIRA 2012). The duration of anuran calling seasons can be locally determined by the landscape as well as the scale of sampling, because some anuran species may vocalize for longer periods of time in ponds with more favourable terrestrial habitats (SILVA & ROSSA-FERES 2011), and the calling season was reported to be longer when a greater number of water bodies were available (SANTOS et al. 2007). Here, the breeding season was identified by sampling nine ponds, unlike the studies in Uruguay, where the breeding season was identified based on the observation of only a few ponds. The shorter duration observed in Uruguay can be explained by latitude, since amphibian activity is restricted to a shorter period of suitable conditions at higher latitudes (DONNELLY & GUYER 1994, CANAVERO et al. 2009).Temperature appears to be one of the most important factors influencing the breeding activity of anurans, especially in subtropical climates where rains are usually well distributed throughout the year whereas temperature is seasonal (BERTOLUCI & RODRIGUES 2002, BOTH et al. 2008, CANAVERO et al. 2009), and *Phyllomedusa iheringii* appears to be no exception. Females might be able to use the calls to evaluate male size and thus fitness (LICHT 1976, MORRIS 1989). Females might also select males of appropriate sizes to allow a close juxtaposition of cloacae (LICHT 1976), but the correlation between the body sizes of amplectant *P. iheringii* was not significant. Random mating regarding size is common in anurans (see review in WOGEL et al. 2005), including at least three other phyllomedusid species (ROBERTS 1994, VAIRA 2001, WOGEL et al. 2005). The absence of size-assortative mating can be explained by the ability of pairs to closely align cloacae by assuming a conducive posture during oviposition (i.e., by the male adjusting his body curvature while the females releases her eggs), ensuring a high rate of fertilization regardless of the pairs' size ratio (WOGEL et al. 2005).

The breeding behaviour of *Phyllomedusa iheringii* is similar to that of related species. Pyllomedusids have intermediate to prolonged breeding seasons, with males being territorial, interacting antagonistically (acoustically, visually and/or physically), and sometimes presenting patrolling behaviour (ABRUNHOSA & WOGEL 2004, OLIVEIRA et al. 2012, WOGEL et al. 2005). Patrolling behaviour (or scramble-competition) is more common in explosive breeders, but can also occur in anuran species with prolonged breeding seasons as a density–dependent shift between searching and calling that is associated with high densities in the choruses (e.g., PYBURN 1970, WOGEL et al. 2005). The specialized nature of the oviposition sites may result in localised congregations of males around the best sites, favouring multi-male mating in which males attempt to displace others from amplexus, as reported for racophorid frogs (see references in WELLS 2007). Thus, we hypothesize that both the high abundance of male *P. iheringii* recorded at P3 in October and November and the species' dependence on suitable leaves for spawning here acted together and led to a switch from calling to patrolling behaviour, the antagonistic interactions between males, and the occurrence of multi-male amplexi that has recently been reported for this species (OLIVEIRA et al. 2014).

Amphibians that deposit their eggs outside the water most likely reduce the rate of predation by aquatic invertebrates, but instead expose their eggs to attack from a variety of other predators (e.g., crabs, spiders, beetles, wasps, crickets, and dipteran larvae; see the review in WELLS 2007), and also to the risks of desiccation and fungal infections (e.g., SIMON 1983, POO & BICKFORD 2013). In fact, various species have been observed predating upon the eggs of phyllomedusids (NECKEL-OLIVEIRA & WACHLEVSKI 2004, FREITAS et al. 2008, FIGUEIREDO-DE-ANDRADE & KINDLOVITS 2012). Predation on eggs by ants and snakes has been recently reported for *Phyllomedusa iheringii* (DIAS et al. 2012), and in the present study, we recorded that ant predation can also occur on the larval phase, which was previously reported only for beetles and birds (IOP et al. 2015). Ants are considered to be opportunistic predators (VITTAR 2008), but Phoridae fly larvae (which infested 4% of *P. iheringii* clutches) are particularly destructive and have been associated with drastic tadpole mortality in some localities around the world (e.g., DAVIS & DISNEY 2003, MENIN & GIARETTA 2003, VONESH 2000).

Calling sites

The Pampa biome is characterized by a predominance of low vegetation with the Poaceae family being one of the main constituents (Overbeck et al. 2006), and male *Phyllomedusa iheringii* vocalized mainly from grasses in this study. Males of this species were also observed calling from reeds, which are abundant at the ponds (Langone 1985), and two other species of phyllomedusids, which also occur in open areas, use Poaceae as vocalization sites (Vaira 2001, Dias et al. 2014). Male *Phyllomedusa* in open areas seem to use the best available vegetation at the ponds as calling sites, and this may be the case in *P. iheringii* as well.

Most males called from sites that were spatially separated from oviposition sites (inferred by differences in plant type, perch height, and distance from the water edge), suggesting that the spacing recorded between calling sites is more indicative of a lek mating system than of a resource defence mating system. In fact, resource defence in frogs depends on the male's ability to monopolize resources that are essential to the reproductive success of females (i.e., oviposition sites), which may not always be possible (Wells 2007). We observed a positive relationship between the SVL of males and the height of their calling sites. In territorial species, larger males may defend the best calling sites (Howard 1978, Bastos & Haddad 1996, Wogel et al. 2002). Higher perches may facilitate the sound of their calls to be disseminated more widely, because vegetation will be denser near the ground and so act as a barrier that attenuates the acoustic signals (Wells & Schwartz 1982). Therefore, males vocalizing from the highest sites can potentially attract more females (Bastos & Haddad 1999). On the other hand, no relationship between the distance from the water and SVL of the males could be detected in our study. This can be explained simply by the males of *Phyllomedusa iheringii* forming their choruses on the banks of ponds at some distance from the water.

Males of *Phyllomedusa iheringii* occupied lower perches in October, which was when we registered the highest temperature. Some hylids vary in the use of vertical strata of vegetation according to environmental variables, and this position can be positively or negatively correlated with temperature (Prado & Pombal Jr. 2005). This would explain why males of *P. iheringii* occupied microhabitats nearer to the ground in October, as these would then provide improved protection and more adequate levels of warmth and humidity during very hot nights (Bartelt & Peterson 2005). In February, males were found farther from the water than in other months. This was the last month during which we registered calling activity, and males may have been leaving the breeding arena, possibly moving to areas where they may alternate between the search for resources, such as shelter and food, and periods of dormancy until the next breeding season (Bull & Hayes 2001, Lemckert & Slatyer 2002, Wells 2007). Calling sites did not vary between ponds. Males may select calling sites that are optimal in terms of distance from other males, exposure, sound dissemination, and can be maintained even when environments are heterogeneous (Whitney & Krebs 1975, Wells & Schwartz 1982, Abrunhosa & Wogel 2004). Males of *P. iheringii* could therefore vary their use of vegetation for calling according to the vegetation available at each pond. This was also observed for the phyllomedusid *Phytecopus azureus* (Dias et al. 2014).

The defence of calling sites is often the most conspicuous trait of territorial anurans, but site fidelity is also a critical component of territoriality (Mathis et al. 1995). Fidelity to calling sites has been reported for at least four other phyllomedusid species (Abrunhosa & Wogel 2004, Wogel et al. 2006, Costa et al. 2010, Oliveira et al. 2012). The lack of fidelity recorded here could be explained by any one of four hypotheses about mobility and should be further evaluated. Namely, males may change from a strategy of calling to active searching; they may switch to another microhabitat depending on the density of males in the chorus; they could respond to the availability of suitable calling sites; or they may adjust to the presence of females (Whitney & Krebs 1975, Tárano 2009, Nali & Prado 2012, Oliveira et al. 2012). Individuals then defend their calling sites, but may adopt a patrolling behaviour to actively search for females (as suggested by our observations of males during nights with high male density), thus shifting to a scramble-competition mating system under certain conditions.

Oviposition sites

The vegetation types most often used as oviposition sites for *Phyllomedusa iheringii* were shrubs, unlike the preferred calling sites. A similar pattern has been observed for *Phyllomedusa sauvagii* Boulenger, 1882 in the Cerrado (Rodrigues et al. 2007). In this study, the height at which leaf nests were found did not vary between months, but varied between ponds. At pond 3, for example, clutches were deposited higher above the ground, most likely because the best leaves for spawning [e.g., those with suitable sizes and shapes, with the presence of trichomes (Dias et al. 2014)] belonged to tall plants (authors' observation). As the vegetation suitable for spawning varied between ponds, so did the height at which clutches were deposited.

On the other hand, the distance from the water edge varied between months and ponds. This seems be related to the variation in the hydroperiods of ponds. In December, for example, clutches were found beyond the water edge more often than in other months, most likely because the accumulated rainfall was low in November and the pond surface area retracted. Conversely, in January, ponds expanded in size as a result of the accumulated rainfall in December and clutches were found higher above the water. Regarding the clutches found above the ground, Langone (1993) also found supraterrestrial clutches of *Phyllomedusa iheringii* and suggested that larvae were able to make their way to the water, as we observed in the present study. The capacity of the vigorous lateral contractions of tadpole tail

muscles to free themselves from the jelly of their egg capsules during hatching was reported for *Agalychnis dacnicolor* (Pyburn 1970). This type of displacement seems to be efficient enough for *P. iheringii* larvae that fall on the ground to move to the edge of their pond by their own efforts.

The *Phyllomedusa iheringii* in our study used one to eight individual leaves to make their nests. Leaf use seems to be variable between the populations and localities already studied (Langone et al. 1985, Langone 1993) and could be related to the vegetal composition at each water body of the studied areas. Furthermore, the numbers and sizes of the eggs found in the present study are similar to data previously reported for this species, and this seems to be a consistent characteristic throughout the populations of *P. iheringii* (Klappenbach 1961, Langone et al. 1985, Langone 1994).

The breeding activity of *Phyllomedusa iheringii* responds strongly to the influence of air temperature and is associated with the hottest period of the year. This species is considered to be a prolonged breeder. The lack of association between calling and oviposition sites suggests a lek mating system rather than one driven by resource defence. However, males do defend sites through territorial interactions, such as vocal interactions, visual displays, and physical fighting, and may exhibit patrolling behaviour as well as multiple amplexi, suggesting a scramble-competition mating system.

Our results raise interesting questions about the mating system of *Phyllomedusa iheringii*. Males did not defend oviposition sites, which counter-indicates a resource defence mating system, but exhibited marked territorial behaviour, which means that male aggression and territoriality are related to this species' reproductive strategy. Further studies should be performed to: i) better understand the extent of resource defence in this species under different conditions (e.g., male density, resource scarcity) and its relation to the species' mating system; ii) verify the occurrence of sperm competition and multiple paternity; and iii) measure the mating success of males (e.g., identify whether the result of physical contests between males is related to body size).

Acknowledgements

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