HERPETOZOA 31 (1/2): 61 - 68
Wien, 30. August 2018

Site fidelity, reproductive behavior and investment in the Brazilian Reticulate Leaf Frog, *Pithecopus ayeaye* Lutz, 1966

(Anura: Phyllomedusidae)

Ortstreue, Fortpflanzungsverhalten und -aufwand bei *Pithecopus ayeaye* Lutz, 1966
(Anura: Phyllomedusidae)

Marília M. Borges & Renato C. Nali
& Bruno F. Fiorillo & Cynthia P. A. Prado

KURZFASSUNG

Die Untersuchung zur Fortpflanzungsbiologie einer Population von *Pithecopus ayeaye* Lutz, 1966, einem für den südostbrasilianischen Cerrado typischen Laubfrosch, erfolgte in Exkursionen, die in den Monaten der Regenzeit der Jahre 2013/2014 durchgeführt wurden. Von 41 gefangenen Männchen wurden 14 im Abstand von 13 Monaten ein- bis viermal wiedergefangen. Einige Wiederfänge erfolgten nahe (< 2 m) dem ursprünglichen Rufstandort. Die Körpergrößen der nur einmal gefangenen Männchen und jene von Wiederfängen waren ähnlich, was nahelegt, daß die Teilnahme am Rufgeschehen und die Rufplatztreue nicht von deren Körpergröße abhingen. Jedenfalls riefen die Männchen meist von oder nahe bei *Leandra* - Büschen, und alle erfaßten Gelege befanden sich in gefalteten Blättern dieser Pflanze, welche Trichome tragen, die zur Befestigung der Eier und dem Schutz der Embryonen bis zum Schlupf dienen könnten. So kann die Anwesenheit eines Männchens an und seine Ortstreue zu einer bestimmten Stelle einen Eiablageplatz von hoher Güte bezeichnen, was die Wahrscheinlichkeit der Partnerwahl durch ein Weibchen erhöht. Der Fortpflanzungsaufwand ausgedrückt als Gonadosomatischer Index von Männchen (0.35 ± 0.05 %; $N = 14$) und Weibchen (4.25 ± 0.35 %; $N = 3$) war allgemein geringer als bei anderen Phyllomedusenarten. Das kann erklärt werden mit (1) einer höheren Lebensdauer von *P. ayeaye*, aus der sich mehr Laichgelegenheiten für Weibchen ergeben, und (2) dem Vorkommen von aggressiven Rufen und physischen Kämpfen, welche die Gelegenheit für die Besamung durch mehrere Männchen und Spermienwettbewerb verringert.

ABSTRACT

In this study, the authors describe the reproductive biology of a population of *Pithecopus ayeaye* Lutz, 1966, a leaf-frog typical of the Cerrado of southeastern Brazil. Field expeditions were conducted monthly during the rainy season of 2013/2014. From 41 males captured, fourteen were recaptured from one to four times, up to 13 months apart. Some recaptures occurred close to the original calling site (< 2 m). The body size of males captured just once, and that of recaptured males were similar, suggesting that male chorus attendance and calling site fidelity are not influenced by male body size. However, males called mainly from or close to shrubs of *Leandra* sp., and all clutches were found inside folded leaves of this plant, which contain trichomes that could assist in fixing the eggs and protecting embryos until hatching. Thus, male attendance and fidelity to such locations may represent a high-quality oviposition site, increasing the likelihood of female choice. The reproductive investment of males (0.35 ± 0.05 %; $N = 14$) and females (4.25 ± 0.35 %; $N = 3$) was in general lower than that of other phyllomedusid species. This could be explained by (1) a comparatively long lifespan of *P. ayeaye*, which could represent more breeding opportunities for females; and (2) the occurrence of aggressive calls and physical combats, which could decrease the opportunities for multi-male spawning and sperm competition.

KEY WORDS

Amphibia: Anura: Phyllomedusidae; *Pithecopus ayeaye*, reproduction, behavior, reproductive investment, site fidelity, natural history, Cerrado, Brazil

INTRODUCTION

The greatest anuran richness in the world is found in Brazil, with 1,039 described species (Segalla et al. 2016). Nevertheless, the natural history of most species remains unknown (e.g., Rodrigues et al. 2007; Oliveira et al. 2011). With regard to anuran male traits, such as body size, territorial behavior, fidelity to calling sites, persistence in calling activity, as well as gonadal investment, all are related to the variation in reproductive success and survival due to corresponding variation in the degree of

exposure to predators (e.g., Wells 1977a, 1979; Roithmair 1992; Prado & Haddad 2003). Specifically considering chorus attendance and site fidelity, studies that analyze data from Brazilian anuran mark and recapture studies in their breeding territories are rare (e.g., Oliveira et al. 2011), thus leaving the relationship between reproductive strategies and reproductive investment and success largely unknown.

The genus *Pithecopus* Cope, 1866 (family Phyllomedusidae) is composed by eleven species, formerly classified as the *Phyllomedusa hypochondrialis* species group (Caramaschi 2006; Faivovich et al. 2010; Duellman et al. 2016; Frost 2018). The Reticulate Leaf Frog, *Pithecopus ayeaye* Lutz, 1966, was previously considered to be restricted to its type-locality in Morro do Ferro (21°48' S, 46°35' W, 1400-1540 m a. s. l.), municipality of Poços de Caldas, Minas Gerais, southeastern Brazil (Araújo et al. 2007); however, further studies showed a broader distribution with new records of this species within Conservation Units (Araújo et al. 2007; Giovanelli et al. 2008; Baêta et al. 2009; Nali et al. 2015). Although the authors have information about the reproductive biology of many species from this family (e.g., Wogel et al. 2005; Costa et al. 2010; Oliveira et al. 2011), this is not the case for *P. ayeaye* (Nali et al. 2015). This species shows a complex genetic structure, comprising at least three separate Evolutionarily Significant Units (ESU) (Magalhães et al. 2017); aspects of its natural history were uncovered for only one of them (Oliveira 2017). Research on the different ESUs, including their reproductive investment and behavior, are mandatory to assess the conservation status of *P. ayeaye* (Magalhães et al. 2017), which is still classified as Critically Endangered by the IUCN, likely due to lack of data (Caramaschi et al. 2016).

In the present study the authors investigated male chorus attendance and calling site fidelity, as well as reproductive traits and habitat use in a population of *P. ayeaye* belonging to the "Canastra" ESU (Magalhães et al. 2017). Thus, the present results are both complementary at the species level, and novel at the ESU level.

MATERIALS AND METHODS

The population studied inhabited a temporary stream in the municipality of Sacramento, Minas Gerais State, southeastern Brazil (20°13'10" S, 47°06'21" W, 880 m a. s. l.), located within the Brazilian Cerrado, a savanna formation with a rainy season from October to March (Queirolo & Motta-Junior 2007). The authors sampled about 75 meters along the stream, totaling ca. 270 person-hours of direct observations that occurred approximately from 18:00 to 01:00 h, two to four nights per month, during the reproductive season of *P. ayeaye* (October 2013 to February 2014), and additionally in December 2014. Eight individuals collected from the same locality during the reproductive season of 2010/2011 were used to quantify the morphological and reproductive traits as described in detail below.

At each visit to the study site, the authors characterized the microhabitats where females were found, the microhabitats used as calling sites by males and oviposition sites according to the type of substrate. Females and calling males were manually captured and body mass and snout-vent length (SVL) were measured in the field with a spring balance (accuracy: 0.1 g) and an analogical caliper (accuracy: 0.05 mm). The frogs were then photographed with a digital camera for subsequent individual recognition by natural color pattern marks present on their legs and flanks (Fig. 1). To verify male fidelity to the calling sites, the calling sites were marked using colored tape, and the males' photographs were compared to identify the recaptures. Recaptures up to a radius of 2 m from the place of previous capture, as measured with a measuring tape, were considered as evidence of calling site fidelity. Instead of considering the exact calling site, the area of 2 m radius was used to define the calling site because amplectant pairs may move around the selected shrub before choosing an ovipo-

Fig. 1. An individual of *Pithecopus ayeaye* Lutz, 1966, exposing the colorful regions used as natural marks for individual recognition in the present mark-recapture study. The specimen was collected in the municipality of Sacramento, southeastern Brazil, and photographed by M. M. Borges.

Abb. 1. Das abgebildete Exemplar von *Pithecopus ayeaye* Lutz, 1966 zeigt jene farbenfrohen Körperstellen, die in der vorliegenden Fang-Wiederfangstudie zur individuellen Erkennung dienten. Es wurde im südostbrasilianischen Gemeindegebiet von Sacramento gesammelt und von M. M. Borges photographiert.

sition site (R. C. Nali, pers. obs.; Oliveira 2017).

Clutches were collected and fixed in formalin 5 %; all the eggs from each clutch were counted and the diameter of each egg was measured under a Nikon SMZ 1500 stereomicroscope. For the morphological analyses, adult males and females were collected, euthanised with lidocaine spray 10 %, initially fixed in formalin 10 % and then transferred to ethanol 70 % (McDiarmid 1994). In the laboratory, their body and gonad mass was measured using a digital balance (accuracy: 0.001 g). Gonad mass was measured from preserved specimens; although the results could be biased due to tissue shrinkage resulting from preservation, there is evidence that gonad size does not differ significantly between preserved and freshly collected material (Byrne et al. 2002).

The reproductive investment (gonadal investment, gonadosomatic index, RI, GSI) of the males and females was calculated as the percentage of gonad mass relative to body mass [(gonad mass/body mass)*100] (Prado & Haddad 2005). Collected individuals were deposited at the Célio F. B. Haddad Amphibian Collection, Universidade Estadual Paulista, Rio Claro, São Paulo state, Brazil (vouchers CFBH 32772–79, CFBH 36024–27, CFBH 36030–32, CFBH 36050–52). Measures below are reported as arithmetic mean ± standard deviation.

Table 1: Captures and recaptures for males of *Pithecopus ayeaye* Lutz, 1966, in the sampling campaigns of the reproductive periods October 2013 to February 2014, and December 2014. Each capture or recapture event is represented by the letter X. Out of the 41 male frogs captured, 14 (males # 1 to 14 in the table) were repeatedly recaptured in overall 27 recapture events. Sampling effort was two to four nights (approximately from 18:00 to 01:00 h) per month totaling about 270 person-hours. ∗ - The male was recaptured at the same calling site where it was captured before. Σ - Number of captures per male.

Tab. 1: Fänge und Wiederfänge der Männchen von *Pithecopus ayeaye* Lutz, 1966 in den Sammelaktionen der Fortpflanzungsperioden Oktober 2013 bis Februar 2014 und Dezember 2014. Jedes Fang- und Wiederfangereignis ist durch den Buchstaben X dargestellt. Von 41 gefangenen Männchen wurden 14 (Männchen Nummer 1 bis 14 in der Tabelle) bei insgesamt 27 Wiederfang-Vorgängen mehrmals wiedergefangen. Der Sammelaufwand betrug zwei bis vier Nächte (etwa von 18:00 bis 01:00 Uhr) pro Monat, insgesamt etwa 270 Personenstunden. ∗ - Dieses Männchen wurde an demselben Rufplatz wiedergefangen, an dem es davor bereits einmal gefangen worden war. Σ - Anzahl der Fänge je Männchen.

	Males / Männchen Nr.													
Date / Datum	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14
19 Oct 2013	X													
21 Oct 2013	X	X												
05 Nov 2013	X	X*	X	X	X	X	X	X	X					
06 Nov 2013	X				X	X	X	X	X*					X
16 Dec 2013	X									X	X			
17 Dec 2013											X			
18 Dec 2013							X		X*		X	X		
21 Jan 2014		X	X	X										
22 Jan 2014												X	X	
23 Jan 2014									X*				X	
18 Feb 2014									X	X	X*		X	
01 Dec 2014														X
03 Dec 2014														X
04 Dec 2014														X
Σ	5	3	2	2	2	2	3	2	5	2	4	2	3	4

RESULTS

Breeding site characteristics.- The authors found 47 adult males and four adult females, three of which were gravid. Males of *P. ayeaye* called mostly perched on vegetation along the stream's margin, using branches, leaves, grass and rarely the ground and bromeliads. Different males called from the same shrub, but never on the same branch. Males called on branches and leaves of *Leandra* sp. (family Melastomataceae; 54.4 %, $N = 37$ observations) or at most about two meters away from them (45.6 %, $N = 31$ observations). Two females were found on the grass, close to the water, and the other two were found perched on the trees surrounding the water body.

Breeding site fidelity (recaptures).- Fourteen males were recaptured between one and four times during the reproductive season of 2013/2014 ($N = 27$ recaptures; Table 1). Males number 1, 5, 6, 7, 8, 9, 11, 13, and 14 were recaptured in the following days during the same sampling campaign (month). Each time male # 9 was recaptured in subsequent sampling campaigns (months) it was found on the same shrub of *Leandra* sp.; at the end of the reproductive season (18 February 2014) this individual was detected about 2.20 m away from this shrub. The maximum interval between the first and the last recapture was found for male # 14, captured in November 2013 and recaptured in December 2014, hence, in two subsequent breeding seasons. Two males (# 1 and # 14) moved as far as 40 meters from where they were captured first.

Body and gonad measures, sex specific differences, reproductive investment.- Measures of SVL, body mass, and reproductive investment of males and females are stated on Table 2. Males captured just once (SVL = 33.48 ± 1.92 mm, body mass = 2.31 ± 0.50

Table 2: Body size and reproductive characteristics of *Pithecopus ayeaye* Lutz, 1966, located in the municipality of Sacramento, Minas Gerais State, Brazil. Measures are reported as arithmetic mean ± standard deviation (minimum – maximum; sample size).

Tab. 2: Körpergröße und Fortpflanzungsparameter von *Pithecopus ayeaye* Lutz, 1966 aus dem Gemeindegebiet von Sacramento, Bundesstaat Minas Gerais, Brasilien. Die Meßwerte sind in der Reihenfolge arithmetischer Mittelwert ± Standardabweichung (Minimum – Maximum; Stichprobenumfang) angegeben.

	Females / Weibchen	Males / Männchen
Snout-vent length (mm) Kopf-Rumpf-Länge (mm)	38.96 ± 1.34 (36.75 – 42.70; *N* = 4)	33.53 ± 1.95 (28.70 – 37.90; *N* = 47)
Body mass (g) Körpermasse (g)	3.44 ± 0.61 (1.89 – 4.90; *N* = 4)	2.30 ± 0.50 (1.10 – 3.70; *N* = 47)
Reproductive investment (% gonad mass/body mass) Fortpflanzungsaufwand (% Gonadenmasse / Körpermasse)	4.25 ± 0.35 (3.65 – 4.88; *N* = 3)	0.35 ± 0.05 (0.16 – 0.80; *N* = 14)
Egg diameter (mm) Eidurchmesser (mm)	2.84 ± 0.02 (2.19 – 3.70; *N* = 109)	
Number of eggs per clutch Anzahl der Eier je Gelege	29 ± 2.38 (20 – 35; *N* = 7)	

g, $N = 33$) and recaptured males (SVL = 33.65 ± 2.08 mm, body mass = 2.29 ± 0.53 g, $N = 14$) had similar SVL (Mann-Whitney $U = 230$, $P = 0.98$) and body mass ($U = 208$, $P = 0.59$). A significant difference between the sexes was found for SVL, with females being larger (Mann-Whitney $U = 4$; $P < 0.05$). This difference was, however marginally nonsignificant for body mass ($U = 44.5$; $P = 0.06$).

The authors collected six clutches laid on folded leaves of *Leandra* sp. and one that was laid in the plastic bag used to collect the pair. Clutches were composed by yellowish eggs and many empty gelatinous capsules. In some cases, several embryos at a pre-hatching stage and newly hatched tadpoles were simultaneously present on the same leaf. Further characteristics of clutches and eggs are stated on Table 2.

DISCUSSION

Breeding site characteristics.- The calling sites of *P. ayeaye* varied in substrate, but most males called from branches and leaves pendant above the stream, as reported for another ESU of this species, and for other *Pithecopus* species (Freitas et al. 2008; Brandão et al. 2009; Oliveira 2017). Also, grass was observed as calling site of *P. ayeaye* in the present study, as reported for *Pithecopus azureus* (Cope, 1862) by Costa et al. (2010) and Dias et al. (2014). However, clutches of *P. ayeaye* were exclusively found on the hairy leaves of *Leandra* sp.. The oviposition on leaves that have trichomes, prickles and spines, like those of the plant families Melastomataceae and Solanaceae, was observed also in other Phyllomedusidae species and another ESU of *P. ayeaye* (Pombal Jr. & Haddad 1992; Rodrigues et al. 2007; Costa et al. 2010; Dias et al. 2014; Oliveira 2017). These structures could assist in fixing the clutches, providing thermal insulation by air retention, and maintaining moisture for eggs and embryos (Dias et al. 2014). Thus, the use of leaves with trichomes seems to be important to ensure the development and survival of the eggs and embryos of species from this family (Dias et al. 2014), increasing their reproductive success (Freitas et al. 2008). Also, the fact that males of *P. ayeaye* called most frequently from such shrubs suggests the benefit of defending this type of territory for oviposition, using physical combat and aggressive vocalisations (Nali et al. 2015; Oliveira 2017).

Breeding site fidelity (recaptures).- Males of *P. ayeaye* were recaptured from one to four times, and some of them were recaptured in as many as four consecutive months (Table 1), unlike anoth-

er ESU of the species, in which individuals were less frequently recaptured (Oliveira 2017). In the present study, some recaptured males called from the same sites where they had been captured earlier (Table 1). Anuran males that defend high quality breeding sites or remain within the same territory for longer periods can gain increased reproductive success, which can also be affected by body size (e. g., Wells, 1977a; Howard 1978; Roithmair 1992). In the present study however, males captured just once and males recaptured were of similar body size, possibly indicating that male chorus attendance and calling site fidelity are not influenced by body size in *P. ayeaye*. In prolonged-breeding species, females usually search for and select males based on their call traits and calling site characteristics (Wells 1977b; Sullivan et al. 2005; Nali & Prado 2012). The fact that some males stay and defend a selected calling site for a prolonged period could indicate high quality of the territory and effect an increased probability of female choice (Bertram et al. 1996; Wogel et al. 2005; Wogel & Pombal Jr. 2007).

Body and gonad measures, sex specific differences, reproductive investment.- Females of *P. ayeaye* were larger than males, as commonly observed within the family Phyllomedusidae (Rodrigues et al. 2007; Costa et al. 2010; Oliveira et al. 2011; Oliveira 2017). Recent studies on sexual size dimorphism in frogs indicate that the pressure to increase body size is higher in females, especially in small species, even for those with territorial males such as *P. ayeaye* (Han & Fu 2013; Nali et al. 2014, 2015). This pressure was suggested to be related with the "fecundity advantage" hypothesis, according to which larger females can produce larger clutches or eggs (Howard 1978; Shine 1989; Nali et al. 2014). In contrast and regardless of the small sample size in the present study, females of *P. ayeaye* showed a lower reproductive investment than other phyllomedusids (e.g., *Pithecopus azureus* and *Phyllomedusa sauvagii* Boulenger, 1882 - Rodrigues et al. 2007). The amount of energy invested in reproduction is inversely proportional to adult survival rate (Pough et al. 1998), i.e., small species tend to have shorter life spans and less breeding opportunities, so higher fecundity would be advantageous to ensure maximum production of offspring in a single bout (Rodrigues et al. 2007; Nali et al. 2014). However, even the small-bodied *P. ayeaye* individuals have at least two breeding seasons to reproduce, given that the authors' capture and recapture of one paticular individual at the breeding site was 13 months apart. Similarly, a study with mark-recaptures of *Pithecopus megacephalus* (Miranda-Ribeiro, 1926), showed that individuals survived for at least three breeding seasons (Oliveira et al. 2011). The present results highlight the importance of data on mortality and lifespan to understand anuran reproduction, including reproductive investment (e.g., Oliveira et al. 2011).

Regarding the reproductive investment of males, different species may suffer selective pressures to increase testes size, depending on aspects of the mating system, such as the occurrence of multi-male spawning and consequently sperm competition (Parker 1970; Rodrigues et al. 2007; Zamudio et al. 2016). Multi-male spawning has been reported for some Phyllomedusidae species (e.g., *Phyllomedusa distincta* Lutz, 1950 - Prado et al. 2006; *Pithecopus megacephalus* - Oliveira et al. 2011; *Phyllomedusa bahiana* Lutz, 1925 - Santos-Silva et al. 2012), and at least in *Phyllomedusa distincta*, the reproductive investment was larger than that of *P. ayeaye* (Zamudio et al. 2016). The territorial behavior and physical combats observed in *P. ayeaye* could decrease the opportunities for multi-male spawning, which in turn could lead to the observed low reproductive investment of its males, compared to other phyllomedusid treefrogs (Nali et al. 2015; Zamudio et al. 2016; Oliveira 2017).

ACKNOWLEDGMENTS

The authors thank I. A. Martins and C. A. Brasileiro for the comments and criticisms on this manuscript, and all the colleagues that helped with fieldwork. The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided fellowships to M. M. Borges (proc. # 130278/2013-0) and R. C.

Nali (# 130737/2010-0). The São Paulo Research Foundation (FAPESP) provided fellowships to R. C. Nali (proc. # 2010/03656-6, # 2012/06228-0 and # 2017/20299-1) and B. F. Fiorillo (# 2011/00507-2); C. P. A. Prado is grateful to both CNPq (proc. # 471106/2010-0) and FAPESP (proc. # 2009/12013-4) for research grants. Capture of individuals was authorized by the Chico Mendes Institute for Biodiversity Conservation (ICMBio, license # 19269-1).

REFERENCES

Araújo, C. O. & Condez, T. H. & Haddad, C. F. B. (2007): Amphibia, Anura, *Phyllomedusa ayeaye* (B. Lutz, 1966): Distribution extension, new state record, and geographic distribution map.- Check List, São Paulo; 3: 156-158.

Baêta, D. & Caramaschi, U. & Cruz, C. A. G. & Pombal Jr., J. P. (2009): *Phyllomedusa itacolomi* Caramaschi, Cruz & Feio, 2006, a junior synonym of *Phyllomedusa ayeaye* (B. Lutz, 1966) (Hylidae, Phyllomedusinae).- Zootaxa, Auckland; 2226: 58-65.

Bertram, S. & Berrill, M. & Nol, E. (1996): Male mating success and variation in chorus attendance within and among breeding seasons in the gray treefrog (*Hyla versicolor*).- Copeia, Washington, DC; 3: 729-734.

Brandão, R. A. & Álvares, G. F. R. & Crema, A. & Zerbini, G. J. (2009): Natural history of *Phyllomedusa centralis* Bokermann 1965 (Anura: Hylidae: Phyllomedusinae): Tadpole and calls.- South American Journal of Herpetology, São Paulo, Washington, DC; 4: 61-68.

Byrne, P. G. & Roberts, J. D. & Simmons, L. W. (2002): Sperm competition selects for increased testes mass in Australian frogs.- Journal of Evolutionary Biology, Oxford; 15: 347-355.

Caramaschi, U. (2006): Redefinição do grupo de *Phyllomedusa hypochondrialis*, com redescrição de *P. megacephala* (Miranda-Ribeiro, 1926), revalidação de *P. azurea* Cope, 1962 e descrição de uma nova espécie (Amphibia, Anura, Hylidae).- Arquivos do Museu Nacional do Rio de Janeiro, Rio de Janeiro; 64: 159-179.

Caramaschi, U. & Cruz, C. A. G. & Lima, R. & Brandão, R. (2016): *Pithecopus ayeaye*. The IUCN Red List of Threatened Species. doi:10.2305/IUCN.UK.2016-3.RLTS.T55839A107295713.en. [last downloaded on 19 July 2017].

Costa, T. B. & Guimarães, L. D. & Bastos, R. P. (2010): Territorial and mating behavior in *Phyllomedusa azurea* (Anura: Hylidae) at a temporary pond in west-central Brazil.- Phyllomedusa, Piracicaba; 9: 99-108.

Dias, T. M. & Maragno, F. P. & Prado, C. P. A. & Cechin, S. Z. (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, London; 48: 43-44.

Duellman, W. E. & Marion, A. B. & Hedges, S. B. (2016): Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae).- Zootaxa, Auckland; 4104: 1-109.

Faivovich, J. & Haddad, C. F. B. & Baêta, D. & Jungfer, K. & Álvares, G. F. R. & Brandão, R. A. & Sheil, C. & Barrientos, L. S. & Barrio-Amóros, C. L. & Cruz, C. A. G. & Wheeler, W. C. (2010): The phylogenetic relationships of the charismatic poster frogs, Phyllomedusinae (Anura, Hylidae).- Cladistics, London; 26: 227-261.

Freitas, E. B. & De-Carvalho, C. B. & Faria R. G. & Batista, R. C. & Batista, C. C. & Coelho, W. A. & Bocciglieri, A. (2008): Nicho ecológico e aspectos da história natural de *Phyllomedusa azurea* (Anura: Hylidae, Phyllomedusinae) no Cerrado do Brasil Central. [Ecological niche and aspects of the natural history of *Phyllomedusa azurea* (Anura: Hylidae, Phyllomedusinae) in the Cerrado of Central Brazil].- Biota Neotropica, Campinas; 8: 101-110. doi:10.1590/S1676-06032008000400009.

Frost, D. (2017): Amphibian Species of the World: an Online Reference. Version 6.0 (Date of access 28-12-2017). Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA. Accessed on 27.12.2017.

Giovanelli, J. G. R. & Araújo, C. O. & Haddad, C. F. B. & Alexandrino, J. (2008): Modelagem do nicho ecológico de *Phyllomedusa ayeaye* (Anura: Hylidae): Previsão de novas áreas de ocorrência para uma espécie rara.- Neotropical Biology and Conservation, São Leopoldo; 3: 59-65.

Han, X. & Fu, J. (2013): Does life history shape sexual size dimorphism in anurans? A comparative analysis.- BMC Evolutionary Biology, London; 13: 27. doi: 10.1186/1471-2148-13-27

Howard, R. D. (1978): The evolution of mating strategies in bullfrogs, *Rana catesbeiana*.- Evolution, Hoboken; 32: 850-871.

Magalhães, R. F. & Lemes, P. & Camargo, A. & Oliveira, U. & Brandão, R. A. & Thomassen, H. & Garcia, P. C. A. & Leite, F. S. F. & Santos, F. R. (2017): Evolutionarily significant units of the critically endangered leaf frog *Pithecopus ayeaye* (Anura, Phyllomedusidae) are not effectively preserved by the Brazilian protected areas network.- Ecology and Evolution, electronic resource. John Wiley & Sons Inc.; 7: 8812-8828.

McDiarmid, R. W. (1994): Preparing amphibians as scientific specimens; pp. 289-297. In: Heyer, W. R. & Donnelly, M. A. & McDiarmid, R. W. & Hayek, L. C. & Foster, M. S. (Eds.): Measuring and monitoring biological diversity. Standard methods for amphibians; Washington, (Smithsonian Institution).

Nali, R. C. & Prado, C. P. A. (2012): Habitat use, reproductive traits and social interactions in a stream-dweller treefrog endemic to the Brazilian Cerrado.- Amphibia-Reptilia, Leiden; 33: 337-347.

Nali, R. C. & Zamudio, K. R. & Haddad, C. F. B. & Prado, C. P. A. (2014): Size-dependent selective mechanisms on males and females and the evolution of sexual size dimorphism in frogs.- The American Naturalist, Chicago; 184: 727-740.

Nali, R. C. & Borges, M. M. & Prado, C. P. A. (2015): Advertisement and release calls of *Phyllomedusa ayeaye* (Anura: Hylidae) with comments on the social context of emission.- Zoologia, Curitiba; 32: 263-269.

Oliveira, F. F. R. & Nogueira, P. A. G. & Eterovick, P. C. (2011): Natural history of *Phyllomedusa megacephala* (Miranda-Ribeiro, 1926) (Anura: Hylidae) in southeastern Brazil, with descriptions of its breeding biology and male territorial behaviour.- Journal of Natural History, London; 46: 117-129.

Oliveira, F. F. R. (2017): Mating behaviour, territoriality and natural history notes of *Phyllomedusa ayeaye* Lutz, 1966 (Hylidae: Phyllomedusinae) in southeastern Brazil.- Journal of Natural History, London; 51: 657-675.

Parker, G. A. (1970): Sperm competition and its evolutionary consequences in the insects.- Biological Reviews, Oxford; 45: 525-567.

Pombal Jr., J. P. & Haddad, C. F. B. (1992): Espécies de *Phyllomedusa* do grupo *burmeisteri* do Brasil oriental, com descrição de uma espécie nova (Amphibia, Hylidae).- Revista Brasileira de Biologia, Rio de Janeiro; 52: 217-229.

Pough, F. H. & Andrews, R. M. & Cadle, J. E. & Crump, M. L. & Savitsky, A. H. & Wells, K. D. (1998): Herpetology; Upper Saddle River (Prentice-Hall).

Prado, C. P. A. & Haddad, C. F. B. (2003): Testes size in leptodactylid frogs and occurrence of multiple spawning in the genus *Leptodactylus* in Brazil.- Journal of Herpetology, Houston; 37: 354-362.

Prado, C. P. A. & Haddad, C. F. B. (2005): Size-fecundity relationships and reproductive investment in female frogs in the Pantanal, South-Western Brazil.- Herpetological Journal, London;15: 181-189.

Prado, C. P. A. & Toledo, L. F. & Woehl Jr., G. & Castanho, L. M. (2006): *Phyllomedusa distincta* (Leaf Frog). Multimale spawning.- Herpetological Review, New York; 37: 206-207.

Queirolo, D. & Motta-Junior, J. C. (2007): Prey availability and diet of maned wolf in Serra da Canastra National Park, southeastern Brazil.- Acta Theriologica, Heidelberg; 52: 391-402.

Rodrigues, D. J. & Uetanabaro, M. & Lopes, F. S. (2007): Breeding biology of *Phyllomedusa azurea* Cope, 1962 and *P. sauvagii* Boulenger, 1882 (Anura) from the Cerrado, Central Brazil.- Journal of Natural History, London; 41: 1841-1851.

Roithmair, M. E. (1992): Territoriality and male mating success in the dart-poison frog, *Epipedobates femoralis* (Dendrobatidae, Anura).- Ethology, Oxford; 92: 331-343.

Santos-Silva, C. R. & Santos, É. D. S. & Ferrari, S. F. (2012): Multiple amplexus on *Phyllomedusa bahiana* (Hylidae: Phyllomedusinae) in the Caatinga Northeast Brazil.- Herpetology Notes, Braunschweig; 5: 171-172.

Segalla, M. V. & Caramaschi, U. & Cruz, C. A. G. & Grant, T. & Haddad, C. F. B. & Garcia, P. C. A. & Berneck, B. V. M. & Langone, J. A. (2016): Brazilian amphibians: List of species.- Herpetologia Brasileira, São Paulo; 5: 34-46.

Shine, R. (1989): Ecological causes for the evolution of sexual dimorphism: A review of the evidence.- The Quarterly Review of Biology, Chicago; 64: 419-461.

Sullivan, B. K. & Ryan, M. J. & Verrel, P. A. (1995): Female choice and mating system structure; pp. 469-517. In: Heatwole, H. & Sullivan, B. K. (Eds.): Amphibian biology. Chipping Norton (Surrey Beatty).

Wells, K. D. (1977a): Territoriality and male mating success in the green frog (*Rana clamitans*).- Ecology, Washington; 58: 750-762.

Wells, K. D. (1977b): The social behavior of anuran amphibians.- Animal Behaviour, Amsterdam; 25:666-693.

Wells, K. D. (1979): Reproductive behavior and male mating success in a Neotropical toad, *Bufo typhonius*.- Biotropica, Oxford; 11: 301-307.

Wogel, H. & Pombal Jr., J. P. (2007): Comportamento reprodutivo e seleção sexual em *Dendropsophus bipunctatus* (Spix, 1924) (Anura, Hylidae).- Papéis Avulsos de Zoologia, São Paulo; 47: 165-174.

Wogel, H. & Abrunhosa, P. A. & Pombal Jr., J. P. (2005): Breeding behaviour and mating success of *Phyllomedusa rohdei* (Anura, Hylidae) in south-eastern Brazil.- Journal of Natural History, London; 39: 2035-2045.

Zamudio, K. R. & Bell, R. C. & Nali, R. C. & Haddad, C. F. B. & Prado, C. P. A. (2016): Polyandry, predation, and the evolution of frog reproductive modes.-The American Naturalist, Chicago; 188: S41-S61.

DATE OF SUBMISSION: July 27, 2017 Corresponding editor: Heinz Grillitsch

AUTHORS: Marília M. Borges [1)] & Renato C. Nali (Corresponding author < r_nali@yahoo.com.br >) [1, 4)] & Bruno F. Fiorillo [3)] & Cynthia P. A. Prado [1, 2)]

[1)] Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências, Rio Claro, São Paulo, Brazil.

[2)] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias, Departamento de Morfologia e Fisiologia Animal, Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, São Paulo, Brazil.

[3)] Programa de Pós-Graduação em Ecologia Aplicada, Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, São Paulo, Brazil.

[4)] Universidade de São Paulo, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Departamento de Biologia, Av. Bandeirantes 3900, 14040-901, Ribeirão Preto, São Paulo, Brazil.