

## Diet and morphometry of two poison frog species (*Anura*, *Dendrobatidae*) from the plateaus surrounding the Pantanal of Mato Grosso do Sul state, Brazil

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### ABSTRACT

The plateaus surrounding the Pantanal in Mato Grosso do Sul are highly threatened by land-use conversion and are home to *Ameerega berohoka* and *Ameerega picta*, two species of poison frogs renowned for their aposematic coloration and toxic skin. The species have diurnal and terrestrial habitats and are often observed among dead tree branches, leaf litter or under rocks. Herein, we investigated the diet and sexual dimorphism of body size in *A. berohoka* and *A. picta*, aiming to increase our understanding of the natural history of both species. We collected *A. berohoka* specimens from the Bonito municipality and *A. picta* from the Rio Negro Municipality and obtained 1,600 prey items organized into 12 categories. We found that formicid insects had the highest index of relative importance and were the most frequent prey category for both species. Despite prey items such as Acari and Isoptera being present in the diet of these species, the niche breadth of these species was low. We found no evidence of sexual dimorphism in body size or body shape for *A. berohoka* and *A. picta*. Based on our findings, we conclude that both species are ant specialists, as proposed for other *Ameerega* species.

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## Introduction

Studies about natural history of species are fundamental for understanding basic ecology (Ricklefs 1990) and provide essential information about organisms' interactions (Greene 1994). In the current scenario of loss of natural habitats, natural history plays an important role as the basis for studies about species preservation and conservation (Bastos 2007), especially those sensitive to environmental changes, such as amphibians (Duellman & Trueb 1994; Rothermel & Semlitsch 2002).

Anurans are considered opportunistic and generalist predators (Teixeira & Coutinho 2002; Santos et al. 2004) and have an important role in maintaining ecosystems and the food chain, providing a significant part of animal biomass for several animal groups (Toledo et al. 2007) and acting in the control of invertebrate populations (Attademo et al. 2005; Toledo et al. 2007; Wells 2007). Their foraging strategies (both active and sit-and-wait) are influenced by morphological traits (Biavati et al. 2004; Pacheco et al. 2017), energy demand (Grayson et al. 2005) and prey availability (Hirai 2004; López et al. 2009). Moreover, it is expected a positive relationship between the size of the anuran and the size of its prey (Sanabria et al. 2005; López et al. 2007).

Another important aspect of anuran's natural history is body size as a fundamental morphological characteristic, which is important in a physiological, ecological, and social context (Baraquet et al. 2012). Anurans usually present sexual size dimorphism (SSD), where females are usually bigger than males (Shine 1979; Liao et al. 2013). This pattern in anurans is associated with size-dependent fecundity advantage, which can drive the evolution of female body size and consequently leads to the evolution of bigger females (Han & Fu 2013). Nevertheless, several studies have also shown sexual dimorphism for body shape and have demonstrated, for instance, greater forelimbs in males, which improves their reproductive efficiency (Yu et al. 2010) and females with larger heads than males, which reduces competition for prey between sexes (Guimarães et al. 2011).

Dendrobatidae Cope, 1865 has 199 species and the genus *Ameerega* Bauer, 1986 has the most species (30 spp.) in the Colostethinae subfamily (Frost 2019) with a broad range in South America (Grant et al. 2006; Neves et al. 2017; Frost 2019). Species of this genus are diurnal and recognized for their aposematic coloration and presence of toxins in the skin (Lötters et al. 2000). This aposematic color is an important driver of sexual

selection for *Ameerega* species, where females choose to mate brighter males (Maan & Cummings 2009; Dreher & Pröhl 2014). In addition, *Ameerega* species also present parental care, which is rare in anurans (McDiarmid 1978; Lehtinen & Nussbaum 2003), that prevent the predation of their offspring and enhance dispersal by carrying out tadpoles on their backs, even for a number of days before finding a temporary pool (Wells 1980; Acioli & Neckel-Oliveira 2014; Summers & Tumulty 2014). Nevertheless, there is scarce information about the aspects of natural history for *Ameerega* species (e.g. Forti et al. 2011; Lima & Eterovick 2013; Summers & Tumulty 2014).

The Pantanal of Mato Grosso do Sul, Brazil is surrounded by plateaus covered by Cerrado *sensu strictu*, Cerradão, and semi-deciduous forests, which are threatened by land-use conversion, such as agriculture and cattle ranching (Scariot et al. 2005; Alho 2008). In these plateaus, two species of *Ameerega* occur in different uplands. *Ameerega berohoka* (Vaz-Silva and Maciel 2011) (Figure 1a) is restricted to Brazil and distributed in Mato Grosso, Mato Grosso do Sul and Goiás States (Frost 2019) and was recently registered in Serra de Maracaju (Sant'Anna et al. 2017). *Ameerega picta* (Tschudi, 1838) (Figure 1b) has a wider distribution in South America, occurring in Bolivia, Colombia, Peru, and Venezuela (Frost 2019). In Brazil, the species is distributed in Bodoquena and Urucum plateaus in Mato Grosso do Sul (Souza et al. 2017), eastern Amazon, and adjacent areas of Acre, Rondônia, and Mato Grosso states (Lötters et al. 2000).

Both species occur in various terrestrial microhabitats, such as dead tree branches, litter, or under rocks, and despite the close occurrence of these species in Mato Grosso do Sul state, *A. berohoka* is easily distinguished from *A. picta* by its smaller hand size,

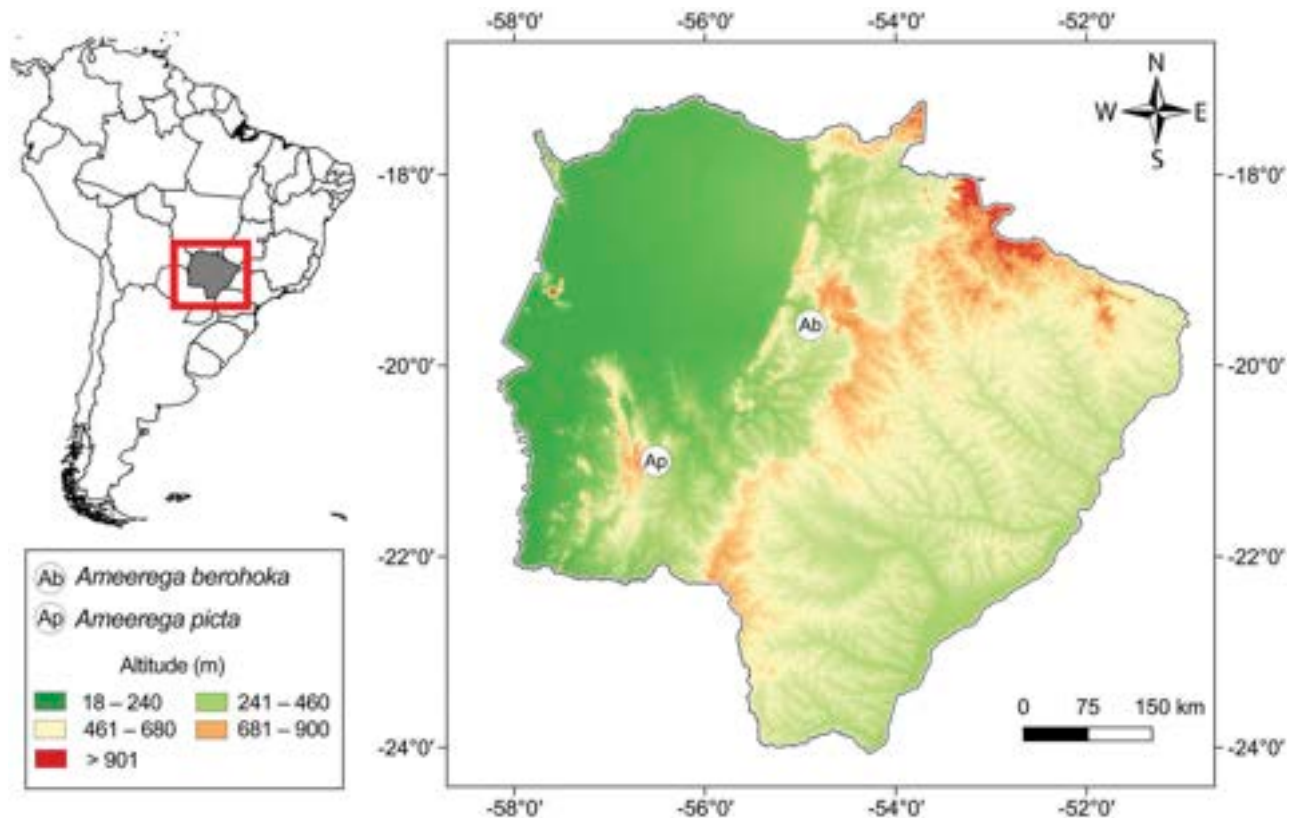
nostril shape, and irregular spots on the dorsal region (Vaz-Silva & Maciel 2011). Additionally, there is a paucity of studies that address diet or sexual dimorphism for these species, with one study reporting myrmecophagy in a population of *A. picta* from Bolivia (Mebs et al. 2010) and another describing sexual size dimorphism in a population of *A. berohoka* from the Brazilian Cerrado (Vaz-Silva & Maciel 2011). In this study, we (i) describe the diet composition of two populations of *A. berohoka* and *A. picta* and highlight their most relevant prey categories; and (ii) analyze if sexual size dimorphism is present in both species.

## Materials and methods

We captured 24 individuals (14 males and 10 females) of *Ameerega berohoka* from the Rio Peixe Waterfall (19°34'31"S, 54°53'37"W), a hillside area of the Serra de Maracaju in the Rio Negro municipality, Mato Grosso do Sul state, Brazil. For *A. picta*, we captured 22 individuals (15 males and 07 females) from the Estância Mimosa (20°58'49"S, 56°30'32"W), located in the Serra da Bodoquena, Bonito municipality, Mato Grosso do Sul state, Brazil (Figure 2). The specimens were collected during two field trips, one in April and the other in May 2016. The individuals were found and collected through visual encounter surveys (Crump & Scott 1994). The specimens were killed using topical anesthetic (lidocaine 5%) and then fixed in 10% formaldehyde before analyzing their stomach content. All individuals were considered adults, as we confirmed the development of gonads during the dissection process. The captured specimens were collected with permission from the Brazilian wildlife regulatory service (SISBIO #49080-1) and are housed at Coleção



**Figure 1.** Poison frogs from Pantanal surrounding uplands of Mato Grosso do Sul state (a) *Ameerega berohoka*, from Serra de Maracaju, in Rio Negro municipality and (b) *Ameerega picta*, from Serra da Bodoquena, in Bonito municipality.



**Figure 2.** Localization of sampling areas of (Ab) *Ameerega berohoka*, in Serra de Maracaju, Rio Negro municipality, and (Ap) *Ameerega picta*, in Serra da Bodoquena, Bonito municipality. The dark green indicates the Pantanal area in Mato Grosso do Sul state.

Zoológica of the Universidade Federal de Mato Grosso do Sul ([Appendix I](#)).

We utilized a stereomicroscope to identify the prey and classify them to the lowest possible taxonomic level. We measured prey length ( $l$ ) and width ( $w$ ) using millimeter paper placed underneath a petri dish and estimated the prey's volume ( $V$ ) from the ellipsoid formula (where  $V$  = volume,  $W$  = width, and  $L$  = length) (Griffiths & Mylotte 1987):  $V = \frac{4}{3}\pi\left(\frac{W}{2}\right)^2\frac{L}{2}$ .

For each anuran specimen, we calculated the numerical and volumetric percentages of each prey category and the same percentages for all examined stomachs. We then calculated the Index of Relative Importance (IRI) (Pinkas et al. 1971) to determine the relative importance of each prey item in the diet using the following formula:  $IRI = (\%N + \%V)\%FO$ . This formula effectively shows the main and rare food items, where  $FO\%$  is the mean percentage of prey occurrence,  $N\%$  is the numerical percentage of prey and  $V\%$  is the volumetric percentage of prey (Krebs 1989). Higher values of IRI regarding other prey items indicate a greater importance of the prey category in the diet. To facilitate the comparison among prey categories, we calculated the IRI percentage ( $\%IRI$ ).

We also analyzed the niche breadth using Levin's Measure of Niche Breadth (Krebs 1989). This measurement permits the calculation of the diet's amplitude, particularly considering the quantitative distribution of each prey item. To facilitate comparisons with other studies, we calculated Levin's standardized measure of niche breadth (BA) according to Hurlbert (1978), which limits the value on a scale from 0 to 1 according to the following equation:  $BA = (B-1)/(n-1)$ , where  $n$  represents the number of resources (prey species) registered and  $B$  represents the Levin's measure of niche breadth. Values closer to 0 are attributed to a more specialist diet, while values closer to 1 represent a more generalist diet (Krebs 1989).

To investigate sexual dimorphism in size and shape, we only measured sexually mature individuals, which yielded 29 males and 17 females for analysis (14 males and 10 females for *A. berohoka*, and 15 males and 7 females for *A. picta*). We measured morphometric variables following Vaz-Silva and Maciel (2011) using digital calipers (nearest 0.01 mm): snout to vent length (SVL); head length (HL); head width (HW); internarial distance (IND); eye to nostril distance (END); eye diameter (ED); tympanum diameter (TD); hand length (HAL);

thigh length (THL); tibia length (TBL); foot length (FL) (from the tip of the longest toe to the articulation of tibia–fibula and tarsus). To analyze morphometric variation in size, we defined body size as an isometric size variable (Rohlf & Bookstein 1987) following the procedure described by Somers (1986). We calculated an isometric eigenvector, defined *a priori* with values equal to  $p = 0.5$ , where  $p$  is the number of variables (Jolicoeur 1963), and obtained scores from this eigenvector, hereafter called Body Size, by post-multiplying the  $n \times p$  matrix of log<sub>10</sub>-transformed data, where  $n$  is the number of observations, by the  $p \times 1$  isometric eigenvector. To analyze morphometric variation in shape, we removed the size effect from the log<sub>10</sub>-transformed variables using Burnaby's method (Burnaby 1966). We post multiplied the  $n \times p$  matrix of the log<sub>10</sub>-transformed data by a  $p \times p$  symmetric matrix,  $L$ , defined as:  $L = I_p - V(VTV)^{-1}VT$ , where  $I_p$  is a  $p \times p$  identity matrix,  $V$  is the isometric size eigenvector defined above, and  $VT$  is the transpose of matrix  $V$  (Rohlf & Bookstein 1987). We tested for difference in body size between sexes using an analysis of variance (ANOVA) and tested for difference between sexes in Shape Variables using a Bayesian logistic regression in the *Arm* package for R (Gelman & Hill 2014). We performed all analyses in R v3.5 (R Core Team 2018). We used an  $\alpha = 0.05$ .

We state that this work and animal handling procedures were conducted with the approval of the Animal Ethics Committee from Universidade Federal

de Mato Grosso do Sul (CEUA/UFMS), protocol number 838/2017.

## Results

All the 46 analyzed stomachs from both species contained some items. We found 1,600 items, 754 in *A. berohoka* and 846 in *A. picta*, comprising 12 prey categories (11 orders and one family) and vegetal material (Table 1). For *A. berohoka*, we recorded nine prey categories and the most frequent preys were Formicidae insects (37.50%), which were present in all analyzed stomachs, representing 83.55% of ingested preys, 92.08% of total volume of stomach content and the highest index of relative importance (65.86%). Araneae, Isopoda, and Thysanoptera were the least frequent items, with a single occurrence each. Formicidae was also the most frequent item for *A. picta* (27.16%) and had the highest index of relative importance for the species (41.24%), representing 59.10% of the stomach content and 92.77% of the stomach volume. Ten prey categories were recorded and the least frequent items were Hemiptera, with a single occurrence, and Pulmonata, Ixodida, Araneae, and Isoptera, with two occurrences each. The niche breadth was 0.04 and 0.05 for *A. berohoka* and *A. picta*, respectively.

There was no sexual dimorphism in body size ( $p = 0.17$ ;  $p = 0.70$ ) or in body shape ( $p = 0.44$ ;  $p = 0.44$ ) for *A. berohoka* and *A. picta*, respectively. Average body size score for *A. berohoka* was

**Table 1.** Prey categories found in the stomachs of *Ameerega berohoka* and *Ameerega picta* in Pantanal surrounding uplands, Brazil. N = number of individuals registered (absolute number and %); F = frequency of occurrence of prey category (absolute number and %); V = volume occupied by prey item in the entire sample (in mm<sup>3</sup> and %); IRI = Index of Relative Importance. \*Except Formicidae.

Prey category	N	N%	F	F%	V (mm <sup>3</sup> )	V%	%IRI
<b><i>Ameerega berohoka</i></b>							
Acari	50	6.63	15	23.44	976.82	0.006029	1.55
Araneae	1	0.13	1	1.56	3.53	0.000022	<0.01
Coleoptera	23	3.05	12	18.75	542.30	0.003347	0.57
Formicidae	630	83.55	24	37.50	14,919,893.57	92.080104	65.86
Hemiptera	3	0.40	2	3.13	9.16	0.000057	0.01
Hymenoptera*	2	0.27	2	3.13	2.96	0.000018	<0.01
Isopoda	1	0.13	1	1.56	19.63	0.000121	<0.01
Isoptera	40	5.31	4	6.25	1,281,700.75	7.910186	0.82
Thysanoptera	1	0.13	1	1.56	0.16	0.000001	<0.01
<b><i>Ameerega picta</i></b>							
Acari	193	22.81	18	22.22	701,565.28	0.3695782	5.15
Araneae	2	0.24	2	2.47	2.58	0.0000014	<0.01
Coleoptera	32	3.78	13	16.05	7690.73	0.0040514	0.60
Diptera	5	0.59	4	4.94	138.85	0.0000731	0.02
Formicidae	500	59.10	22	27.16	176,109,222.42	92.772732	41.24
Hemiptera	1	0.12	1	1.23	2.06	0.0000011	<0.01
Hymenoptera*	12	1.42	10	12.35	70.70	0.0000372	0.17
Isoptera	91	10.76	2	2.47	13,009,880.95	6.8534866	0.43
Ixodida	2	0.24	2	2.47	0.59	0.0000003	0.01
Pulmonata	2	0.24	2	2.47	1.27	0.0000007	<0.01

**Table 2.** Morphometric measures of *A. berohoka* and *A. picta* in mm. Means  $\pm$  Standard deviation and ranges.

Measurements	<i>A. berohoka</i>		<i>A. picta</i>	
	Males (n = 14)	Females (n = 10)	Males (n = 15)	Females (n = 7)
Snout-vent length	19.10 $\pm$ 1.57 (16.07–21.89)	19.15 $\pm$ 2.06 (16.48–22.93)	23.48 $\pm$ 3.34 (18.09–32.84)	23.70 $\pm$ 3.37 (17.61–28.75)
Head length	5.36 $\pm$ 0.57 (4.41–6.26)	5.60 $\pm$ 0.79 (4.04–6.79)	5.68 $\pm$ 0.80 (4.37–7.84)	5.88 $\pm$ 1.33 (3.31–7.84)
Head width	6.31 $\pm$ 0.44 (5.76–7.19)	6.65 $\pm$ 0.56 (5.52–7.57)	6.49 $\pm$ 0.84 (5.04–8.96)	6.61 $\pm$ 0.95 (4.95–7.91)
Internarial distance	2.14 $\pm$ 0.17 (1.86–2.51)	2.25 $\pm$ 0.19 (1.86–2.50)	2.69 $\pm$ 0.40 (2.15–3.72)	2.59 $\pm$ 0.24 (2.20–2.91)
Eye-nostril distance	2.16 $\pm$ 0.07 (2.05–2.30)	2.39 $\pm$ 0.25 (1.90–2.85)	2.60 $\pm$ 0.31 (1.75–2.94)	2.24 $\pm$ 0.33 (1.82–2.82)
Eye diameter	2.43 $\pm$ 0.22 (2.11–2.88)	2.45 $\pm$ 0.29 (2.08–3.08)	2.26 $\pm$ 0.28 (1.92–2.73)	2.26 $\pm$ 0.26 (1.71–2.40)
Tympanum diameter	1.01 $\pm$ 0.16 (0.74–1.27)	1.14 $\pm$ 0.20 (0.82–1.46)	1.64 $\pm$ 0.22 (1.11–2.08)	1.43 $\pm$ 0.23 (1.33–1.96)
Hand length	5.19 $\pm$ 0.36 (4.49–5.69)	5.45 $\pm$ 0.37 (4.59–5.65)	6.46 $\pm$ 1.30 (5.30–10.78)	6.63 $\pm$ 0.75 (5.35–7.79)
Thigh length	8.49 $\pm$ 0.84 (7.74–10.36)	8.91 $\pm$ 0.83 (8.17–10.67)	11.07 $\pm$ 1.00 (9.06–13.30)	11.38 $\pm$ 1.35 (8.22–12.35)
Tibia length	9.51 $\pm$ 0.54 (8.50–10.34)	9.99 $\pm$ 0.62 (8.76–10.35)	11.57 $\pm$ 1.25 (9.21–14.97)	11.91 $\pm$ 1.43 (8.82–13.57)
Foot length	8.71 $\pm$ 0.80 (7.28–9.97)	8.32 $\pm$ 0.85 (8.03–10.12)	10.28 $\pm$ 1.23 (8.36–13.80)	10.53 $\pm$ 1.43 (7.47–11.77)

2.25  $\pm$  0.07 (SD; range = 2.08–2.48) and for *A. picta* was 2.48  $\pm$  0.17 (SD; range = 2.07–2.86) (Table 2). Furthermore, we did not observe external morphological differences between males and females in both species, except for the presence of vocal sacs in males.

## Discussion

Our results showed ants as the most frequent prey category, with the greatest total volume of stomach content and the highest index of relative importance, ingested by *A. berohoka* and *A. picta* in the plateaus surrounding the Pantanal of Mato Grosso do Sul. Similar results have been documented for *Ameerega picta* (Ramon et al. 2010; Landgraf-Filho et al. 2019) and other populations of *Ameerega* from different biomes (Caldwell 1996; Forti et al. 2011; Lima & Eterovick 2013; Luiz et al. 2015). The niche breadth of both species was low, which is attributed to species with specialist diet and relatively limited niches (Levins 1968). Although there are no arthropod availability data in environments occupied by these populations, if we consider data on diet and niche breadth for *A. berohoka* and *A. picta* from this study, both species seem to be ant-specialists, as putatively proposed for other *Ameerega* species (Toft 1980; Caldwell 1996; Luiz et al. 2015).

Dendrobatidae is a family widely recognized for having skin toxins and aposematic coloration as its main defense mechanisms (Caldwell 1996; Darst et al. 2005). Some Dendrobatidae, including those from *Ameerega*, are diurnal active foragers, which exposes

them for longer amounts of time outside of shelter and increases their risk of predation mainly due to their diurnal habits, which could be related the bright color and skin toxins of these organisms (Toft 1980). Toxic substances in the skin of these animals are synthesized from alkaloid bases obtained exogenously (diet) from ants, mites, and other arthropods (Daly et al. 2000; Mebs 2002).

Other representative prey categories in *Ameerega* diet were Coleoptera and Isoptera. They are considered more palatable and easier to catch since they are less aggressive than ants (Juncá & Eterovick 2007). In addition, coleopterans are important protein sources for anurans, since they have more protein content than other invertebrate taxa (Anderson & Smith 1998). Coleoptera is the largest order of insects in the world and is present in most environments in Brazil (Rafael et al. 2012), presenting a high availability for consumption, and Isoptera, such as termites, are energetically valuable because they contain less sclerotized material and a higher carbohydrate content than ants (Biavati et al. 2004). Coleoptera, Isoptera, and other registered orders such as Diptera, Acari, and Araneae were also representative prey categories for other populations of *Ameerega* from different biomes (Caldwell 1996; Forti et al. 2011; Lima & Eterovick 2013; Luiz et al. 2015). Such orders and Formicidae are abundant in the litter (Sakchoowong et al. 2008), where both of the *Ameerega* species studied here usually forage, which could explain the high contribution of these prey categories to the diet of *A. berohoka* and *A. picta*. We also recorded vegetal material in the stomach contents of both

species. Anderson et al. (1999) listed some benefits for anurans to actively select plant material as dietary items, such as elimination of intestinal parasites, an additional resource of water and even nutrition resource, if digestible. However, due to the low frequency and index of relative importance, here we assume as accidentally the consumption of vegetal material by *A. berohoka* and *A. picta* (Whitaker et al. 1977).

Regarding the sexual size dimorphism of the *Ameerega* populations studied, we did not observe differences in morphometric variables, indicating that these populations do not present differences in size and shape between males and females, except for the vocal sac in males. This result opposes previous studies that reported larger body size through SVL analysis (e. g. *A. berohoka*, Vaz-Silva & Maciel 2011; *A. braccata*, Forti et al. 2013; *A. trivittata*, Acioli & Neckel-Oliveira 2014) and body shape (*A. flavopicta*, Lima & Eterovick 2013) in females. Sexual dimorphism in size for anurans, in general with males bigger than females, has been justified by sexual selection, since female fecundity is positively correlated to size (Woolbright 1983), thus allowing for bigger ovules and/or a greater number of oocytes (Crump & Kaplan 1979; Prado et al. 2000; Acioli & Neckel-Oliveira 2014) or the presence of parental care (Vaz-Ferreira & Gehrau 1975; Wells & Bard 1988). However, any pressure that affects one of the sexes may trigger a delay or acceleration of anuran growth, for example, female-male breeding age differences (Monnet & Cherry 2002), and hence the absence of sexual dimorphism in size or even males bigger than females. When sexual dimorphism in size is absent, the sexual selection in dendrobatid generally is related to the bright coloration of the males (Maan & Cummings 2009) and by the advertisement calls performed by males (Dreher & Pröhl 2014).

We also did not find evidences for external sexual dimorphism of shape in our studied *Ameerega* populations. Sexual dimorphism of shape is often observed in populations where male anurans are larger than females, which is strongly correlated to male combatting behavior and the occurrence of weaponry in males, such as spines and tusks (Shine 1979). This important trait increases the probability of defeating other males in a fight, since combatant anurans tend to be larger than noncombatant ones (Shine 1979), thus improving their reproductive success. Despite the fact that males of *A. picta* and *A. berohoka* in our study did not possess defensive or other external structures, it is common that both male and female dendrobatids provide parental care to offspring by periodically visiting nests and transporting them to

the water to complete their developmental cycle (Uetanabaro 2008; Summers & Tumulty 2014). Such shared parental care may lead females to invest less in energy intake for growth, and hence contributing to the absence of sexual dimorphism. Thus, we assume that sexual selection is also driving the absence of sexual dimorphism in both species.

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## Appendix I

The reference specimens can be found at ZUFMS under numbers: *Ameerega berohoka*: Brazil: Mato Grosso do Sul state, Rio Negro municipality: ZUFMS 3763–3786. *Ameerega picta*: Brasil: Mato Grosso do Sul state, Bonito municipality: ZUFMS 3787–3808.