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Dietary selectivity and sexual size dimorphism of *Chiasmocleis mehelyi* (Anura: Microhylidae) in a Cerrado area of southwest Brazil

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Abstract. We studied the feeding behavior and sexual size dimorphism of *Chiasmocleis mehelyi* from a Cerrado area in the state of Mato Grosso do Sul, Brazil. We collected 40 specimens during the rainy season and evaluated the availability of potential prey (terrestrial arthropods) using pitfall traps. The number, frequency, and volume of different prey found in stomachs were measured to determine the relative importance of each prey type in the species' diet. We assessed the degree of selectivity by comparing the relative abundances of prey types in the stomachs with the availability of potential prey in the environment. We recorded nine potential prey types in the habitat of *C. mehelyi*, Diptera and Formicidae being the most abundant with 36% and 18% of abundance, respectively. *Chiasmocleis mehelyi* fed on arthropods from four prey categories: Formicidae (91.18% frequency), Isoptera (11.76%), Coleoptera (5.88%), and Acari (2.95%). The index of food selection indicates that *C. mehelyi* feeds selectively on Formicidae and consumes other prey types randomly. There was sexual dimorphism in body size, with females being bigger than males. We suggest that dietary habits of *C. mehelyi* may be related to their active foraging behavior, while the sexual size dimorphism is attributable to sexual selection related to fecundity.

Key words: Microhylidae, dietary specialization, food selection, myrmecophagy, prey availability, natural history.

Introduction

The diet of an organism is related to its morphological, physiological, and behavioral characteristics, and those of the prey it consumes (Berazategui et al. 2007). In addition, spatial and temporal changes in prey availability can influence dietary characteristics significantly (Rosa et al. 2002). Feeding behavior ranges in a continuum from highly specialized to generalized, thus, the terms specialist and generalist describe individuals, populations, or species characterized by a restricted and large niche breadth, respectively, which reflects the interaction between particular traits of organisms and specific environmental factors (Toft 1985, Simon & Toft 1991, Devictor et al. 2010, Irschick et al. 2010). Anurans can be selective, exhibiting prey consumption in different proportions to those found in the environment, preferring certain types of food resources and avoiding others (López et al. 2007, Attademo et al. 2010). Alternatively, anurans can be opportunistic and presumably eat any prey they find in the proportions found in the environment (Chesson 1978, Menin et al. 2005, López et al. 2009, Caldart et al. 2012).

Neotropical anurans in the family Microhylidae selectively feed mainly on ants and termites (Solé et al. 2002, Van Sluys et al. 2006, Teixeira et al. 2006, Berazategui et al. 2007, López et al. 2007, 2017, Araújo et al. 2009, Atencia et al. 2017, Lopes et al. 2017). However, only in exceptional cases (e.g., López et al. 2007) the availability of potential prey in the environment is included in the analysis.

Another key aspect of anurans' natural history is body size, a morphological characteristic in the physiological, ecological, and social contexts (Baraquet et al. 2012). In anurans, although with some exceptions (López et al. 2017a), females are usually bigger than males (Shine 1979, Liao et al. 2013). This pattern is associated with a size-dependent fecundity advantage, which can drive the evolution of female body size and consequently lead to the evolution of bigger females (Han & Fu 2013, Arantes et al. 2015, Tolosa et al. 2015; De Magalhães et al. 2016).

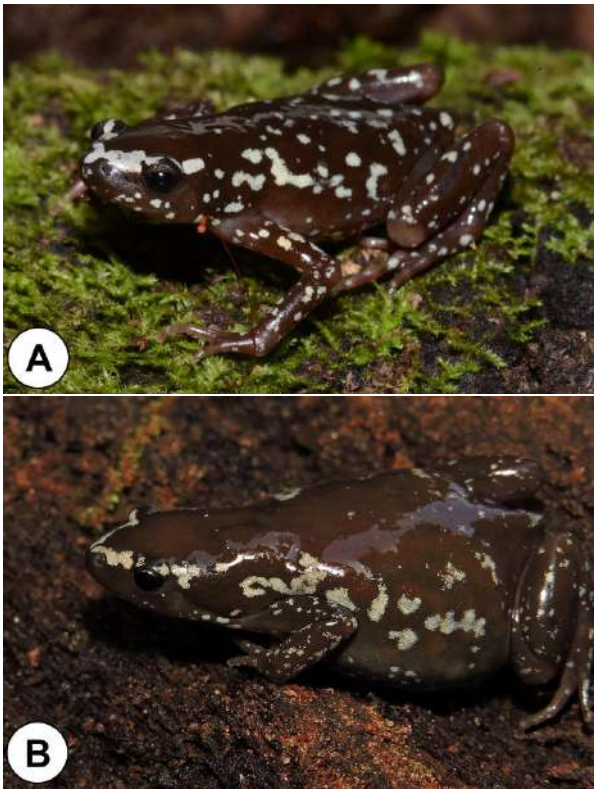

Figure 1. (A) Male and (B) ovigerous female adult specimens of *Chiasmocleis mehelyi* from the Parque Estadual das Nascentes do Rio Taquari, Brazil.

Chiasmocleis mehelyi Caramaschi & Cruz, 1997 (Fig. 1) is a small frog inhabiting areas of the Brazilian Cerrado in the

states of Mato Grosso and Mato Grosso do Sul (Caramaschi & Cruz 1997, De Sá et al. 2019). Only a defensive behavior has been described about the natural history of this species (Hartmann et al. 2002). Here, we investigate sexual size dimorphism and describe the diet of *C. mehelyi,* taking into account the availability of potential prey in a Cerrado area from southwest Brazil.

Material and Methods

We carried out this study at the Parque Estadual das Nascentes do Rio Taquari (PENRT) located in the state of Mato Grosso do Sul, Brazil (Fig. 2A). This conservation unit has an area of 30618 ha and constitutes an important ecological corridor between the Cerrado and Pantanal. The average annual rainfall is 2000 mm, with a dry season from June to September and a rainy season from October to May (Sette 2005, Carrijo 2005).

We sampled for *C. mehelyi* during two not consecutive nights in January 2018, in the rainy season. Frogs were captured manually at the margin of a temporary pond (53°24'40.19"W, 18°9'33.62"S, 421 m.a.s.l, Fig. 2B), located just on the side of a path inside of the PENRT; this temporary pond was shallow (~90 cm deep), ~6 m in diameter, with abundant herbaceous vegetation in and around the pond, with moderately flooded soil around the pond. Frogs were killed using lidocaine, fixed in 10% formaldehyde, and preserved in 70% ethanol (Pisani 1973). Anurans were killed because this work was part of two large research projects on anuran trophic networks and anuran-parasite networks whose methodology requires animal sacrifice. All specimens are housed at the "Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul" (ZUFMS-AMP13740 – ZUFMS-AMP13779). The research was under license number 007/2016 provided by the Instituto de Meio Ambiente de Mato Grosso do Sul (IMASUL). To access the availability of potential prey, we installed 20 pitfalls traps around the pond, separated 2 m from each other, from the edge up to 5 m distance to the pond, in the same microhabitats where frogs were seen active. Pitfalls consisted of 1000 ml plastic receptacles (diameter: 15 cm) filled with 70% ethanol (solution depth: 5 cm), opened for 12 hours, between 18:00 and 06:00 h, during the same two nights we collected the anurans.

Dietary analysis

We analyzed the stomach content of each frog under a stereomicroscope. We identified the stomach food items and arthropods from pitfalls traps to the level of order (Guillot 2005); Formicidae were identified to the genus level based on a specialized key (Palacio & Fernandez 2003). We measured the length and width of each prey using a digital caliper (0.01 mm precision) and estimated the volume of each stomach item using the ellipsoid formula: $V = 4/3 \times \pi \times (L/2) \times (A/2)^2$, where L is maximum length and A is maximum width of each prey (Dunham 1983). We determined the number, frequency, and volume of each stomach item and calculated its importance value using the index of relative importance (IRI; Pinkas et al. 1971). We calculated the IRI for the prey categories identified to the order level and the IRI for genera of Formicidae separately, and transformed the values of IRI to percentages in order to facilitate the interpretation.

Prey selectivity

To evaluate whether frog populations are selectively choosing a specific prey category, we calculated the Linear Food Selection Index (LI), according to the following equation: LI = ri - pi, where ri and pi are the relative abundances (expressed in this case as proportions) of prey item i in the stomachs and habitat, respectively (Strauss 1979). This index ranges from -1 to +1, with positive values indicating preference, values near 0 indicating random feeding, and negative values – avoidance or inaccessibility (Strauss 1979).

Figure 2. (A) Panoramic view of the Parque Estadual das Nascentes do Rio Taquari and (B) the surveyed temporary pond; egg masses of *Chiasmocleis mehelyi* visible floating on the surface.

Sexual dimorphism

To investigate sexual dimorphism, we measured only sexually mature individuals (characterized by the presence of vocal sacs in males and eggs in females), which yielded 34 males and 6 females. We measured morphometric variables to the nearest 0.01 mm using digital calipers, following Duellman & Trueb (1986): snout vent-length (SVL), head length (HL), interorbital distance (IOD), eye–nostril distance (END), internarial distance (ND), thigh length (THL), eye diameter (ED), and foot length (FL). 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). We obtained scores from this eigenvector, called body size, by post-multiplying the n × p matrix of $\log_{10}$-transformed data, where n is the number of observations, by the p × 1 isometric eigenvector. To analyze morphometric variation in shape, we removed the effect of size from the $\log_{10}$-transformed variables using Burnaby's method (Burnaby 1966). We post multiplied the n × p matrix of the $\log_{10}$-transformed data by a p × p symmetric matrix, L, defined as: $L = I_p \times V \times (V^T \times V)^{-1} \times V^T$, where I_p is a p × p identity matrix, V is the isometric size eigenvector defined above, and V^T is the transpose of matrix V (Rohlf & Bookstein 1987). Below, we refer to the resulting size-adjusted variables as shape variables. We tested for differences in body size between sexes using an analysis of variance (ANOVA) and tested for differences between sexes in shape variables using a Bayesian logistic regression in the 'Arm' package for R (Gelman & Hill 2007). Later, we compared the full model against a constant-only (null) model using a chi-square test of the scaled deviance to evaluate the statistical significance of the full model based on shape variables (Chambers & Hastie 1992, Faraway 2016). We also calculated the significance of the logistic regression model with a chi-square test on the value of the difference between the residual deviations from the full model and the null model (Tabachnick & Fidell 2007). We then assessed the importance of each variable in discriminating the two sexes by model selection via single term addition (Chambers & Has-

tie 1992). After the model selection analysis, we assessed the misclassification error of the reduced model using 1,000 bootstrap replications of a linear discriminant analysis with R package 'ipred' (Peters & Hothorn 2011). We further assessed variable importance using model averaging, retaining only models with ΔAICc < 4 (Crawley 2012). We conducted model averaging in the 'MuMIn' package in R (Burnham & Anderson 2002). We performed all analyses in R v3.5 (R Development Core Team 2018) and used an α = 0.05.

Results

We analyzed 40 specimens of *C. mehelyi* (34 males and 6 females), with a mean SVL of 26.3 mm (min = 23.59, max = 31.82, SD = 2.09). Twenty-nine males and five females had stomach contents with identifiable prey; the remaining six individuals (one female and five males) had stomachs with tiny remains of unidentifiable arthropods, which were excluded from the dietary analysis.

We recorded 584 prey items divided into four main taxonomic categories of Arthropoda. Hymenoptera (Formicidae) was the most important prey type in the diet, representing 93% of total prey number and occurring in 91% of individuals (31 frogs out of 34). Isoptera was the second most important prey, and Coleoptera and Acari were rare (Table 1).

Number of prey items eaten by males (mean = 17, SD = 23.2, min = 1, max = 109, n = 29) were similar to those of females (mean = 18.2, SD = 19.3, min = 1, max = 51, n = 5). However, the volume of total prey in females (mean = 22.9 mm^3, SD = 29.9, min = 0.088, max = 75.5, n = 5) was higher than that observed in males (mean = 8.19 mm^3, SD = 12.42, min = 0.076, max = 59.55, n = 29).

We identified 12 genera of Formicidae in the diet of *C. mehelyi*. The genera *Pheidole* and *Wasmannia* had the highest IRI values (Table 1). The mean number of ants consumed by *C. mehelyi* was 17.61 (min = 1, max = 109, SD = 23.49, n = 34).

For assessing the availability of trophic resources, we collected 112 arthropods of which 66 were classified as potential prey due to having an appropriate or ingestible size; we considered arthropods with a length less than 5 mm as potential prey because the maximum length of an ingested prey item was 4.19 mm. We identified nine orders of potential prey, with Diptera and Formicidae being the most abundant, representing 36% and 18%, respectively, of total available prey (Fig. 3). *Chiasmocleis mehelyi* foraged selectively on Formicidae and consumed other prey categories randomly (Linear Food Selection Index: Formicidae = 0.75; Acari = 0.0034, Coleoptera = -0.027; Isoptera = 0.060).

Body size scores of males averaged 2.38 ± 0.04 (SD; range

Table 1. Dietary composition of a population of sexually mature *Chiasmocleis mehelyi*. N = number of prey items, F = frequency, V = volume (mm^3), IRI = index of relative importance.

Prey categories	N	F	V	%N	%F	%V	IRI	%IRI
Isoptera	35	4	169.60	5.99	11.76	48.07	636	4.59
Acari	2	1	0.30	0.34	2.94	0.09	1	0.01
Coleoptera	2	2	0.56	0.34	5.88	0.16	3	0.02
Hymenoptera (Formicidae)	545	31	182.32	93.32	91.18	51.68	13221	95.38
Pheidole Westwood, 1839	237	14	70.90	40.58	41.18	20.10	2499	62.43
Wasmannia Forel, 1893	153	7	13.03	26.20	20.59	3.69	615	15.38
Crematogaster Lund, 1831	23	4	27.04	3.94	11.76	7.66	137	3.41
Neivamyrmex Borgmeier, 1936	17	1	10.92	2.91	2.94	3.09	18	0.44
Carebara Westwood, 1840	14	3	0.72	2.40	8.82	0.20	23	0.57
Nylanderia Emery, 1906	5	5	1.33	0.86	14.71	0.38	18	0.45
Dolichoderus Lund, 1831	6	1	9.07	1.03	2.94	2.57	11	0.26
Sericomyrmex Mayr, 1865	2	2	2.87	0.34	5.88	0.81	7	0.17
Trachymyrmex Forel, 1893	3	1	4.44	0.51	2.94	1.26	5	0.13
Gnamptogenys Roger, 1863	1	1	1.36	0.17	2.94	0.38	2	0.04
Cyphomyrmex Mayr, 1862	1	1	0.27	0.17	2.94	0.08	1	0.02
Strumigenys Smith, 1860	1	1	0.06	0.17	2.94	0.02	1	0.01
Not determined	1	1	0.48	0.17	2.94	0.14	1	0.02
Ants remains	81	9	39.86	13.87	26.47	11.30	666	16.65

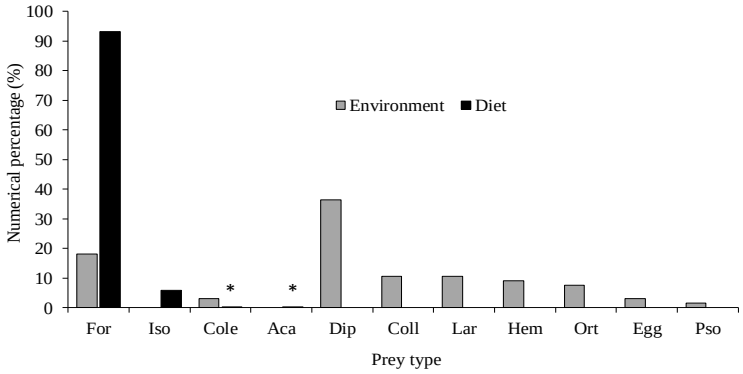

Figure 3. Proportional abundance of prey in the diet and potential prey of *Chiasmocleis mehelyi* in the study area environment (asterisks indicate the low amount of some consumed prey that are difficult to observe in the graph). For: Formicidae; Iso: Isoptera; Cole: Coleoptera; Aca: Acari; Dip: Diptera; Coll: Collembola; Lar: Larvae; Hem: Hemiptera; Ort: Orthoptera; Egg: Eggs of arthropods; Pso: Psocoptera.

Table 3. Results of model selection of shape variables differing between female and male *Chiasmocleis mehelyi*. The best model includes only the shape variables manually selected using the Akaike's Information Criterion (AIC). Model results are for Full Model = Full, Best Model = Best, Model-averaged Coefficient = MAC, and Relative Variable Importance = RVI. Abbreviations are I = Intercept, SVL = snout vent-length, HL = head length, IOD = interorbital distance, END = eye–nostril distance, ND = internarial distance, THL = thigh length, ED = eye diameter, FL = foot length. χ^2 = Chi-squared values, df = degrees of freedom, significant P value models indicated by asterisks.

Model	I	SVL	HL	IOD	END	ND	THL	ED	FL	AIC	χ^2	df	P
Full	140.79	-224.92*	18.71	26.68	-11.2	10.1	-23.07	-9.68	-9.51	24.11	4.11	6	0.93
Best	157.79	-214.56*	-	-	-	25.91	-	-	-	12.51	33.81	2	0.001
MAC	143.76	-216.88*	25.98	34.3	4.1	23.28	-26.84	-2.3	-13.25	-	-	-	-
RVI	-	1	0.14	0.12	0.11	0.3	0.09	0.37	0.11	-	-	-	-

Table 2. Mean and standard deviation (in mm) of morphometric variables for sexually mature *Chiasmocleis mehelyi*. Mean and standard deviation of size-adjusted shape variables are in parentheses.

Variable	Males (n = 34)	Females (n = 6)
Body size	25.61 SD=1.2	30.41 SD=1
	(0.65 SD=0.01)	(0.69 SD=0.01)
Head length	8.02 SD=0.59	8.31 SD=0.35
	(0.14 SD=0.02)	(0.13 SD=0.01)
Interorbital distance	5.1 SD=0.26	5.68 SD=0.28
	(0.14 SD=0.01)	(0.14 SD=0.01)
Eye–nostril distance	2.2 SD=0.12	2.3 SD=0.12
	(-0.04 SD=0.01)	(-0.03 SD=0.01)
Internarial distance	2.19 SD=0.15	2.32 SD=0.1
	(-0.41 SD=0.02)	(-0.42 SD=0.02)
Thigh length	8.97 SD=0.56	9.6 SD=0.51
	(-0.41 SD=0.02)	(-0.42 SD=0.01)
Eye diameter	2.24 SD=0.24	2.23 SD=0.26
	(-0.4 SD=0.04)	(-0.44 SD=0.04)
Foot length	13.27 SD=0.84	15 SD=0.74
	(0.36 SD=0.02)	(0.38 SD=0.02)

= 2.3–2.45) and females averaged 2.49 ± 0.03 (range = 2.43–2.52). There was sexual dimorphism in body size (F = 27.38, df = 1, $P < 0.001$), and in body shape ($\chi^2 = 33.81$, df = 39, $P < 0.001$). Females had longer body size than males (Table 2) and SVL explained shape differences between sexes (Table 3). Misclassification error based on the two-selected variables in the best model was 0.08.

Discussion

The diet of *C. mehelyi* analyzed in this study corresponds to a selective predator on ants (Formicidae). Its myrmecophagous diet is similar to that reported for other species in the genus such as *C. albopunctata*, *C. leucosticta, C. capixaba, C. alagoanus, C. hudsoni* and *C. shudikarensis* (Van Sluys et al. 2006, Araújo et al. 2009, Lopes et al. 2017, Leite-filho et al. 2017, Da Silva et al. 2019), and other neotropical microhylids such as *Elachistocleis bicolor, E. pearsei,* and *Stereocyclops incrassatus* (Solé et al. 2002, Teixeira et al. 2006, Berazategui et al. 2007, López et al. 2007, Atencia et al. 2017). This indicates that myrmecophagous feeding behavior of Neotropical microhylids prevails across different geographical areas and environmental characteristics.

Ants of the genus *Pheidole* were the most numerous and frequent prey in the diet of *C. mehelyi*, similar to the diets of *C. leucosticta* and *E. bicolor* (Solé et al. 2002, López et al. 2007, Berazategui et al. 2007, Lopes et al. 2017). *Pheidole* is the most species-rich genus in the New World and is abundant and dominant in terms of numbers of workers, colonies, and biomass in leaf litter and soil in many tropical habitats (Fowler 1993). This also occurs in the Cerrado biome where they are very abundant both in open and forest areas (Brandão et al. 2011, Camacho & Vasconcelos 2016). These ants nest mainly in the soil and their colonies contain a large number of individuals (Fowler 1993, Wilson 2003), which can be attractive for frogs. Once a nest or ant trail is located in the soil or leaf litter, the frog eats as many ants as possible to satiate its energetic requirements, a behavior registered in another microhylid frog (López et al. 2017b). This is supported in the present study by the fact that we observed many ants of the same morphospecies in the stomachs of several individual frogs. Microhylids usually dig with their heads, introducing it under the leaf litter right where the ant trails run and can remain in this position hunting ants for several minutes (López et al. 2017b).

On the other hand, nothing its known about the foraging activity of *C. mehelyi* (e.g., movement frequency, time in movement and total distance moved during foraging), but the fact that our studied population of this species consumes mainly ants suggests that individuals search actively for their prey, behavior observed for other microhilyd species (López et al. 2017b). Frogs in the genus *Chiasmocleis* exhibit an explosive reproduction, forming mating aggregations that sometimes involve hundreds of individuals in temporary water bodies during a very short period of time (Van Sluys et al. 2006). Active foraging may be the best way to capture small, slow moving and locally abundant or gregarious prey and may be a more efficient way to obtain and allocate energy for reproduction when reproductive period is short (Toft 1985, Nomura 2003).

We observed significant differences in body shape and body size between males and females of *C. mehelyi* (Fig. 1A-B). This is similar to other studies on *Chiasmocleis* (e.g., Caramaschi & Cruz 1997, 2001, Caramaschi & Pimenta 2003, Van Sluys et al. 2006). Sexual size dimorphism in anurans has been explained by sexual selection, due to female fecundity being positively related to size (Woolbright 1983), thus allowing bigger ovules or greater numbers of oocytes (Crump & Kaplan 1979, Prado et al. 2000). Related to this, we observed that females consume a greater volume of prey than males, which would perhaps be associated with such sexual differences in body size and higher energetic requirements by females for reproduction (Shine 1989, Kupfer 2007).

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