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The diet of *Chiasmocleis hudsoni* and *C. shudikarensis* (Anura, Microhylidae) of *terra firme* forests in the Brazilian Amazonia

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Myrmecophagy has been described in anurans belonging to the families Bufonidae, Dendrobatidae and Microhylidae (Toft, 1981; Isacch and Barg, 2002; Darst et al., 2004; Mebs et al., 2018). This specialization is found in searching foragers that are more active and with high dietary proportion of ants and mites (Toft, 1981). Certain species that specialize in ants and mites are able to sequestrate dietary alkaloids to be used as chemical defences (Mebs et al., 2010). The diet of different genera of Gastrophryninae microhylid frogs in the Neotropical region is composed mainly of ants, termites, mites and collembolans in addition to lower proportions of other arthropods species (Duellman, 1978; Schlüter and Salas, 1991; Parmelee, 1999; Van Sluys et al., 2006; Lopes et al., 2017). *Chiasmocleis* Méhely, 1904 is the most speciose genus of Gastrophryninae with 36 described species (Frost, 2019). However, detailed information regarding the diet is restricted to seven species (Duellman, 1978; Parmelee, 1999; Van Sluys et al., 2006; Araújo et al., 2009; Lopes et al., 2017). Data on the diet of *C. jimi* Caramaschi and Cruz, 2001 are available to only one specimen (Caramaschi and Cruz, 2001).

Chiasmocleis hudsoni Parker, 1940 and *Chiasmocleis shudikarensis* Dunn, 1949 are widely distributed in the Amazonia region (Frost, 2019), occurring in *terra firme* forests of central Amazonia (Lima et al., 2012). Both species are fossorial and reproduce during the rainy season (Lescure and Marty, 2000; Lima et al., 2012). No information is available on the diet of both species. Herein we describe the dietary composition of *Chiasmocleis hudsoni* and *C. shudikarensis* from several individuals captured in different sites of the Brazilian Amazonia. We also provide detailed taxonomic identification for the subfamilies and genera of Formicidae found in the stomach contents.

The individuals analysed in this study were obtained from the Amphibia Section of the Paulo Bührnheim Zoological Collection, Universidade Federal do Amazonas, Manaus, state of Amazonas, Brazil (vouchers CZPB-AA 27–39, 80, 137–154, 439, 769, 770). Specimens were collected in three areas at Amazonas State, Brazil: (1) municipality of São Sebastião do Uatumã, Jatapú River (2.0253° S, 58.1900° W), (2) municipality of Santa Isabel do Rio Negro, Daraá River (0.3992º S, 64.7867 W), and (3) municipality of Tapauá, Purus River (4.9804° S, 62.9601° W and 5.7116° S, 63.2178° W; datum = WGS84), totalizing 21 specimens of *C. hudsoni* (1 juvenile, 15 females and 5 males), and 13 juvenile specimens of *C. shudikarensis*. All sites were mainly *terra firme* forests, characterized by a well-drained forest without seasonal flooding, with closed canopy, emergent trees and abundant sessile palms (Oliveira et al., 2014; Menin et al., 2017). Sites

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Jatapú and Purus Rivers are under the tropical monsoon climate domain (climate symbol "Am"), while the site Daraá River has a tropical rainforest climate (climate symbol "Af"), with precipitation ≥ 60 mm during the driest month, according to Köppen-Geiger system (Peel et al., 2007).

We removed and identified stomach contents of each specimen by Order, Suborder or Family following the identification keys of Triplehorn and Johnson (2011) and Rafael et al. (2012). Subfamilies and genera of Formicidae were identified following Baccaro et al. (2015). The length and width of each prey item were measured using an ocular micrometre connected to a Zeiss Stemi SV 11 stereomicroscope. We estimated the volume of each item using the formula $V = (\pi \times \text{length} \times \text{width}^2)/6$ (Colli et al., 1992). The index of relative importance (IRI; Pinkas et al., 1971) was determined using the formula $IRI = (N + V) \times F$, in which N = numerical percentage, V = volumetric percentage, F = frequency of occurrence percentage of each prey category. We transformed quantitative results of the IRI into percentage following López et al. (2007). We also measured the snout-vent length (SVL) and the mouth width of each specimen using a digital calliper, and used Pearson's correlation to evaluate the relationship between these two variables and the volume of the largest prey consumed.

We found 152 prey items belonging to 20 taxonomic categories in *Chiasmocleis hudsoni,* and 197 prey items from 19 categories in *C. shudikarensis* (Table 1). Six specimens (28.6%) of *C. hudsoni* had empty stomachs. The number of prey items per stomach varied from 1 to 45 items (mean = 8.44 ± 11.07) in *Chiasmocleis hudsoni* and from 1 to 40 items (mean = 15.15 ± 13.37) in *C. shudikarensis*. Overall, in descending order, Hymenoptera was the most abundant item in the stomachs of both species followed by Isoptera, Acari, Coleoptera, unidentified Hymenoptera, Ricinulei and Collembola (Table 1). Formicidae represented 75% of the overall prey content in the stomachs of *Chiasmocleis hudsoni* and 51.2% in *C. shudikarensis*. Formicidae was represented by five subfamilies and 18 genera (Table 1). Myrmicinae was the most abundant subfamily followed by Dorylinae (Table 1). Plant material was found in the stomach of 38.9% and 38.5% of *Chiasmocleis hudsoni* and *C. shudikarensis* specimens, respectively. The most important prey items to *Chiasmocleis hudsoni*, in descending order, were Isoptera Termitidae, Myrmicinae unidentified genus, and Myrmicinae *Crematogaster/Pheidole*, while to *C. shudikarensis* were Isoptera Termitidae, Myrmicinae ants of the genus *Crematogaster*, *Solenopsis* and *Sericomyrmex* (Table 1). We did not find a significant correlation between SVL (*Chiasmocleis hudsoni*: $r = 0.284$, $p = 0.286$; *C. shudikarensis*: $r = 0.241$, $p = 0.428$) or mouth width (*C. hudsoni*: $r = 0.037$, $p = 0.891$; *C. shudikarensis*: $r = -0.014$, $p = 0.963$) and size of their largest prey item consumed. The mean SVL for *Chiasmocleis hudsoni* and *C. shudikarensis* was 24.72 ± 3.87 mm (range = 12.43 to 25.33 mm) and 16.15 ± 1.42 mm (range = 14.08 to 18.41 mm), respectively. The volume of the largest prey item consumed by the individuals of *Chiasmocleis hudsoni* varied from 0.02 to 116.97 mm^3 (mean = 12.41 ± 28.59) and for *C. shudikarensis* from 0.26 to 19.40 mm^3 (mean = 4.98 ± 5.39).

Although feeding mainly on ants and termites, *Chiasmocleis hudsoni* and *C. shudikarensis* also consumed other prey categories including beetles, collembolans, mites and ricinuleid. Some *Chiasmocleis* species feed exclusively on ants (*C. anatipes* Walker and Duellman, 1974) or on ants and termites (*C. jimi* Caramaschi and Cruz, 2001 and *C. ventrimaculata* (Andersson, 1945); Duellman, 1978; Schlüter and Salas, 1991; Parmelee 1999; Caramaschi and Cruz, 2001). Additionally, a high proportion of ants compared to other prey categories was also reported in diets of *Chiasmocleis albopunctata* (Boettger, 1885), *C. antenori* (Walker, 1973), *C. bassleri* Dun, 1949, *C. capixaba* Cruz, Caramaschi and Izecksohn, 1997, *C. leucosticta* (Boulenger, 1888) and in other genera in the subfamily Gastrophryninae [e.g. *Ctenoprhyne geayi* Mocquard, 1904, *Hamptophryne alios* (Wild, 1995), *H. boliviana* (Parker, 1927), *Elachistocleis ovalis* (Schneider, 1799)] (Duellman, 1978; Schlüter and Salas, 1991; Parmelee, 1999; Solé et al., 2002; Van Sluys et al., 2006; Araújo et al., 2009; Lopes et al., 2017), corroborating a dietary specialization in the consumption of ants in Microhylidae (e.g. López et al., 2007; Lopes et al., 2017). Thus, we speculate that there is a phylogenetic conservatism effect on the diet of *Chiasmocleis* species, as suggested to the genus *Elachistocleis* Parker, 1927 (Marques-Pinto et al., 2018).

Diet composition of Neotropical microhylid frogs that includes the identification of subfamilies and genera of Formicidae are available only to the species *Chiasmocleis leuscosticta*, *E. bicolor* (Guérin-Méneville, 1838) and *E. ovalis* (Solé et al., 2002; López et al., 2007; Lopes et al., 2017). Similarly, we observed a predominance of Myrmicinae ants in the diet of both *Chiasmocleis hudsoni* and *C. shudikarensis*. Myrmicinae is the most common subfamily of ants in soils of *terra firme* forest, comprising high abundance

Table 1. Prey items of *Chiasmocleis hudsoni* and *Chiasmocleis shudikarensis* of *terra firme* forests in the Brazilian Amazonia. N: number of prey items; %N: relative abundance; F: frequency of occurrence; %F: relative frequency; V: volume (in mm^3); %V: relative volume; IRI: index of relative importance; %IRI: percent IRI.

Prey Category	***Chiasmocleis hudsoni* (N = 18)**								***Chiasmocleis shudikarensis* (N = 13)**							
	N	**%N**	**F**	**%F**	**V**	**%V**	**IRI**	**%IRI**	**N**	**%N**	**F**	**%F**	**V**	**%V**	**IRI**	**%IRI**
Arachnida																
Acari																
Oribatida	7	4.61	3	16.67	1.40	0.58	86.36	2.42	8	4.06	2	15.38	0.70	0.29	66.98	0.86
Ricinulei	1	0.66	1	5.56	4.06	1.68	12.98	0.36	-	-	-	-	-	-	-	-
Entognatha																
Collembola																
Neanuridae	1	0.66	1	5.56	0.01	0.002	3.67	0.10	-	-	-	-	-	-	-	-
Insecta																
Coleoptera	1	0.66	1	5.56	0.89	0.37	5.70	0.16	12	6.09	3	23.08	6.95	2.90	207.59	2.67
Hymenoptera																
Formicidae																
Myrmicinae																
Blepharidatta	3	1.97	1	5.56	0.19	0.08	11.41	0.32	1	0.51	1	7.69	0.39	0.16	5.15	0.07
Crematogaster	32	21.05	4	22.22	9.54	3.94	555.38	15.58	22	11.16	5	38.46	8.55	3.57	567.00	7.28
Cyphomyrmex	3	1.97	2	11.11	0.55	0.23	24.45	0.69	7	3.55	5	38.46	5.65	2.36	227.54	2.92
Myrmicocrypta	3	1.97	2	11.11	0.47	0.19	24.09	0.68	1	0.51	1	7.69	0.92	0.38	6.87	0.09
Octostruma	1	0.66	1	5.56	0.08	0.04	3.85	0.11	-	-	-	-	-	-	-	-
Pheidole	20	13.15	7	38.89	1.92	0.79	542.51	15.22	5	2.54	3	23.08	0.53	0.22	63.69	0.82
Sericomyrmex	-	-	-	-	-	-	-	-	6	3.05	6	46.15	13.67	5.72	404.16	5.19
Solenopsis	-	-	-	-	-	-	-	-	40	20.30	3	23.08	1.13	0.47	479.47	6.16
Strumigenys	3	1.97	2	11.11	0.15	0.06	22.60	0.64	1	0.51	1	7.69	0.04	0.01	4.02	0.05
Trachymyrmex	5	3.29	3	16.67	6.82	2.82	101.74	2.85	1	0.51	1	7.69	3.04	1.27	13.68	0.17
Wasmannia	-	-	-	-	-	-	-	-	10	5.08	4	30.77	0.75	0.31	165.77	2.13
Unidentified	14	9.21	6	33.33	33.11	13.67	762.60	21.39	2	1.06	1	7.69	0.12	0.05	8.19	0.10
Dolichoderinae																
Azteca	-	-	-	-		-	-	-	1	0.51	1	7.69	0.47	0.20	5.42	0.07
Dolichoderus	-	-	-	-		-	-	-	1	0.51	1	7.69	0.06	0.03	4.11	0.05
Ponerinae																
Ectatomma	1	0.66	1	5.56	24.50	10.11	59.85	1.68	-	-	-	-	-	-	-	-
Neoponera	1	0.66	1	5.56	0.44	0.18	4.67	0.13	-	-	-	-	-	-	-	-
Odontomachus	-	-	-	-	-	-	-	-	1	0.51	1	7.69	0.97	0.40	7.02	0.09
Unidentified	-	-	-	-	-	-	-	-	1	0.51	1	7.69	0.20	0.08	4.56	0.06
Dorylinae																
Acanthostichus	17	11.18	1	5.56	116.97	48.28	330.38	9.27	-	-	-	-	-	-	-	-
Unidentified	3	1.97	2	11.11	0.71	0.29	25.17	0.71	1	0.51	1	7.69	0.21	0.09	4.56	0.06
Ectatomminae																
Gnamptogenys	8	5.26	3	16.67	11.07	4.57	163.88	4.60	-	-	-	-	-	-	-	-
Unidentified	8	5.26	2	11.11	8.42	3.47	97.08	2.72	-	-	-	-	-	-	-	-
Isoptera																
Termitidae	20	13.16	6	33.33	20.95	8.65	726.90	20.39	76	38.58	6	46.15	194.97	81.47	5,540.64	71.16

and social complexity (Bolton, 1995). Interestingly, the most common ant genera in the diet of the studied species were *Solenopsis*, *Pheidole* and *Crematogaster*, in accordance with other species that occur in different biomes (*Chiasmocleis leucosticta* in Atlantic Forest Biome, *E. bicolor* in Wetlands and Hydrophilous Forests at Argentina and *E. ovalis* in Araucaria Forest; e.g. Solé et al., 2002; López et al., 2007; Lopes et al., 2017). Moreover, other ant genera reported in the diet of *Chiasmocleis leucosticta* and *E. ovalis*, such as *Acanthostichus*, *Cyphomyrmex*, *Strumigenys*, *Wasmannia*, *Gnamptogenys* and *Odontomachus* (Solé et al., 2002; Lopes et al., 2017) were also found in the diet of *C. hudsoni* and *C. shudikarensis*. *Solenopsis*, *Pheidole* and *Crematogaster* are relatively small-sized ants and the most locally diverse genera due to their abundance and distribution in different habitats when compared to other genera of Myrmicinae (Wilson, 1976). *Solenopsis* and *Pheidole* comprise epigean ants, while *Crematogaster*, comprises arboreal ants that forage in leaf litter (Wilson, 1976). Despite of the absence of data on food resource availability in our study, we suggest that *Chiasmocleis hudsoni* are actively selecting ants while *C. shudikarensis* feed on these ants probably due to their availability in the environment. Additionally, other Myrmicinae ants such as *Cyphomyrmex*, *Strumigenys* and *Wasmannia* inhabit mainly the forest leaf litter (Baccaro et al., 2015). *Acantosthicus* (Dorylinae), only present in the stomach content of *Chiasmocleis hudsoni*, has underground habits (Baccaro et al., 2015). Our study indicates that these ants are important to the diet of *C. hudsoni*, which also have a fossorial habitat (Lima et al., 2012), or perhaps its foraging behaviour is similar to that of *E. bicolor*: frogs dig with their heads and introduce it under leaf litter in which the ant trails are present (López et al., 2017). *Gnamptogenys* ants (Ectatomminae) are found in the soil or herbaceous vegetation and forage individually, making them an easy prey (Lattke et al., 2007). On the other hand, *Odontomachus* ants (Ponerinae) are more difficult to be preyed upon because they use jaws with touch-sensitive bristles that help to scare away predators (Brown, 1976; Baccaro et al., 2015).

Termites were the second most consumed item in the diet of *Chiasmocleis hudsoni* and *C. shudikarensis*. Termitidae is the most representative family of Isoptera, corresponding to 85% of the Brazilian termite fauna (Rafael et al., 2012). These insects build great colonies with complex nests on the leaf litter and decayed material on the forest floor, which explains their large consumption by anurans (Constantino, 1992, 2012). In *Chiasmocleis hudsoni*, termites were preyed upon only by females and one juvenile; no termite was found in the stomach content of the males of this species (N = 5 individuals). The consumption of termites by females could be related to high energetic demands for reproduction, as reported to *Ameerega braccata* (Steindachner, 1864) of the Cerrado biome in Brazil (Forti et al., 2011). According to Biavatti et al. (2004), termites contain less sclerotised material than ants, making them more energetically rewarding preys.

Differently from *Chiasmocleis antenori*, *C. leucosticta* and *C. capixaba* (Duellman, 1978; Van Sluys et al., 2006; Lopes et al., 2017), we found a small number, frequency and volume of mites (Acari) in the diet of *C. hudsoni* and *C. shudikarensis*. According to Simon and Toft (1991), there are high densities of mites in the soil and leaf-litter of many terrestrial habitats, and the degree of mite consumption is inversely related to body size of anurans. Since in central Amazonia juveniles and adults of some small-sized and leaf-litter anuran species showed positive electivity to mites (Lima, 1998), we may have underestimated the importance of mites in the diet of *Chiasmocleis hudsoni* and *C. shudikarensis* due to our small sample size. Despite the association between the consumption of ants and mites and alkaloid sequestration in some anuran species (e.g., Dendrobatidae), species of *Elachistocleis* are alkaloid-free (Mebs et al., 2010), a characteristic that is still to be determined for *Chiasmocleis* species.

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