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Diversity of ants and mites in the diet of the Brazilian frog *Chiasmocleis leucosticta* (Anura: Microhylidae)

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Abstract: We describe here the diet of the microhylid frog *Chiasmocleis leucosticta* based on the stomach contents of 72 individuals (47 males and 25 females) collected in pitfall traps at the Reserva Florestal de Morro Grande, state of São Paulo, southeastern Brazil. We identified 1,981 food items distributed in 13 prey categories of arthropods, mainly ants, mites and collembolans. Formicidae was the most abundant and frequent prey category, including 16 genera from seven subfamilies, and data on ant availability in the habitat suggest that *C. leucosticta* selects ants actively. The second main prey category was Acari, predominantly represented by mites of the suborder Oribatida. This is the first work identifying mites to the family level in the diet of a Microhylidae. There was no statistical difference between males and females regarding diet composition.

Keywords: Acari, Anuran, Atlantic Rainforest, Food habits, Formicidae, Myrmecophagy.

Diversidade de formigas e ácaros na dieta do anuro brasileiro *Chiasmocleis leucosticta* (Anura: Microhylidae)

Resumo: Descrevemos neste trabalho a dieta do anuro *Chiasmocleis leucosticta*, pertencente à família Microhylidae, com base no conteúdo estomacal de 72 indivíduos (47 machos e 25 fêmeas) coletados em armadilhas-de-queda na Reserva Florestal de Morro Grande, SP. Foram identificados 1981 itens alimentares, distribuídos em 13 categorias de artrópodes, especialmente formigas, ácaros e colêmbolos. Formicidae foi a categoria de presas mais abundante e frequente, incluindo 16 gêneros pertencentes a sete subfamílias. Os dados de disponibilidade de formigas no ambiente sugerem que *C. leucosticta* seleciona formigas ativamente. A segunda categoria de presas mais relevante foi Acari, predominantemente representada por ácaros da subordem Oribatida. Este é o primeiro trabalho que identifica os ácaros ao nível de família na dieta de um Microhylidae. Não houve diferença estatística entre machos e fêmeas quanto à composição da dieta.

Palavras-chave: Acari, Anuros, Mata Atlântica, Hábitos alimentares, Formicidae, Mirmecofagia.

Introduction

The anuran family Microhylidae is composed of 13 subfamilies and 612 species (Frost 2017), comprising about 8% of the global anuran diversity (Sá et al. 2012), and distributed worldwide, except in Europe (Vitt & Caldwell 2013, Pough et al. 2016). Microhylids are extremely diversified in habits, and fossorial and semifossorial species tend to be myrmecophagous, feeding on ants and termites (Vitt & Caldwell 2013). The gastrophrynine genus *Chiasmocleis* (Méhely, 1904) is widely distributed and the most speciose genus among tropical microhylids (Van Sluys et al. 2006, Forlani, 2010). Currently, the genus comprises 29 species, which are spread from Panama to Southern Brazil (Frost 2017), including 11 species found in the Atlantic rainforest of southeastern Brazil.

Information about the food habits of *Chiasmocleis* species is scarce, ants being the most common item of the diet (Duellman 1978, Schlüter & Salas 1991; Caramaschi & Cruz 2001, Van Sluys et al. 2006).

Chiasmocleis leucosticta (Boulenger, 1888) is found in Brazilian Atlantic rainforest areas from São Paulo to Santa Catarina states (Cruz et al. 1997). Similarly to the other species of the genus, *C. leucosticta* is fossorial and an explosive breeder, characterized by large aggregations in temporary water bodies during a very short period of the year (Van Sluys et al. 2006). Thereby, due to their fossorial habits and annual breeding pattern, individuals are rarely found, and natural history data about the species are rare.

We describe here the diet of *Chiasmocleis leucosticta* based on stomach contents and test if there are dietary differences between the sexes.

Material and Methods

This study was conducted with individuals of *C. leucosticta* collected accidentally in pitfalls traps used to sample arthropods in a study on rodent ecology (Bovendorp 2013) in the Reserva Florestal de Morro Grande, Cotia municipality, state of São Paulo, southeastern

Brazil (23°39'–23°48' S, 46°55'– 47°01' W). Climate in this area is Cfb of Koeppen, warm temperate and humid, and mean annual rainfall is 1339 mm (Metzger et al. 2006). The reserve locates in a continuum of montane rainforest, between the Atlantic and the Semideciduous forests (Aragaki & Mantovani 1998, Catharino et al. 2006), occupying an area of 9,400 ha; vegetation is composed by a mosaic of secondary forests in different stages of conservation and mature forests (Metzger et al. 2006).

Sampling occurred monthly (five days) between January 2008 and January 2010. Two additional samples were performed in July 2010 (16 days) and January 2011 (13 days). Traps (400 mL plastic cups) were buried at soil level and filled with 200 mL 92% alcohol in three areas with different successional stages in a grid consisting of six lines with 100 m separated by 20 m (six equidistant traps in each line), totalling 36 traps per area.

All frogs had their snout-vent length (SVL) measured with digital callipers to the nearest 0.5 mm. Animals were dissected, and food items were determined to the lowest possible taxonomic level under stereo microscopes (Motic K-700L and Zeiss Discovery V20) with the aid of dichotomy keys (Rafael et al. 2012, for insect orders, Fernández 2003, for subfamilies and genera of Formicidae). Mites were mounted in Hoyer's medium on microscope slides and identified to family using keys provided in Krantz & Walter (2009). Total number of each prey category was counted, and frequency of occurrence was calculated by the ratio between the number of stomachs with the prey category and the total number of stomachs (empty stomachs were excluded).

All results were compared between males and females with t-student test (Zar 1999). The sexes were determinate by the presence or the absence of ovaries and oviducts. Voucher material (frogs and stomach contents) was deposited in the herpetological collection of the Laboratório de Zoologia de Vertebrados, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (acronym VESALQ), but mites were deposited in the mite reference collection of the Departamento de Entomologia e Acarologia of the same institution.

Results

We analyzed 72 individuals of *C. leucosticta* (47 males and 25 females). Only one stomach was empty, which was excluded from the analysis. We identified 1,981 food items distributed in 13 prey categories (Table 1). The diet was composed solely of arthropods, mainly ants, mites and collembolans. Formicidae was the more abundant and frequent prey category. Furthermore, we found a high diversity of ants in the diet (16 genera from seven subfamilies), Myrmicinae being the most abundant and frequent ant subfamily, mostly represented by the genera *Pheidole* and *Solenopsis* (Table 2).

The second main prey category was Acari, predominantly represented by the suborder Oribatida, with 33 individuals (Table 3). Other prey categories appeared in low abundances (one to five individuals). Crustaceans and hemipterans were identified only in males, while non-ant hymenopterans and carabid larva were found only in females. The presence of plant material in the stomachs was interpreted as accidental ingestion.

There was no statistical difference between males and females regarding diet composition ($t = 0.5022$, $p > 0.05$).

Discussion

In this study, only one stomach (1.4%) was empty. The proportion of empty stomachs is highly variable in *Chiasmocleis* species whose diet was determined, varying from 19.3% in *C. capixaba* (Van Sluys et al. 2006) to 82.3% in *C. ventrimaculata* (Schluter & Salas 1991).

Our results suggest that *Chiasmocleis leucosticta* is an ant and mite specialist anuran (sensu Toft 1981), as many other Neotropical [*C. antipes*, *C. bassleri* (Duellman 1978), *C. ventrimaculata* (Schluter & Salas 1991), *C. capixaba* (Van Sluys et al. 2006), *C. albopunctata* (Araújo et al. 2009), *Hamptophryne boliviana* (Schluter & Salas 1991), *Elachistocleis ovalis* (Solé et al. 2002), and *E. bicolor* (Berazategui et al. 2007, Araújo et al. 2009)], and Australian [e.g., *Cophyxalus* spp. (Williams et al. 2006, Hoskin & Aland 2011)] microhylids. Data on arthropod availability at Reserva

Table 1. Food items of the diet of the microhylid frog *Chiasmocleis leucosticta*. N = total number of prey by category; F = frequency of prey categories.

Prey categories	Males (n = 47)		Females (n = 24)		
	N	F	N	F	
ARACHNIDA					
Acari	34	0.447	18	0.542	
Araneae	7	0.149	2	0.083	
CRUSTACEA	3	0.064	—	—	
HEXAPODA					
Collembola	16	0.191	8	0.208	
Diptera	1	0.021	2	0.083	
Hemiptera					
	Heteroptera	2	0.043	—	—
	Sternorrhyncha	8	0.149	2	0.083
Hymenoptera					
	Formicidae	498	0.830	328	0.917
	Remains of Formicidae (number of ant heads)	740	0.894	272	0.833
	Other (no-ant)	—	—	4	0.167
Insect larvae					
	Carabeidae	—	—	1	0.042
	Diptera	6	0.064	2	0.042
	undetermined	1	0.021	1	0.042
PLANT MATERIAL		0.213		0.250	
UNIDENTIFIED		18	0.255	7	
Total	1334		647		

Table 2. Subfamilies and genera of Formicidae found in the diet of the microhylid frog *Chiasmocleis leucosticta*. N = total number of ants; F = frequency of ants.

Subfamilies	Genera	Males (n = 47)		Females (n = 24)	
		N	F	N	F
Cerapachyinae		6	0.064	1	0.042
	<i>Acanthostichus</i>	4	0.043	1	0.042
	Undetermined	2	0.021	–	–
Ecitoninae		3	0.043	75	0.167
	<i>Labidus</i>	3	0.043	75	0.167
Ectatomminae		50	0.340	40	0.333
	<i>Gnamptogenys</i>	50	0.340	40	0.333
Formicinae		43	0.085	47	0.250
	<i>Brachemyrmex</i>	–	–	17	0.021
	<i>Paratrechina</i>	9	0.043	2	0.042
	Undetermined	34	0.043	28	0.167
Heteroponerinae		4	0.064	3	0.125
	<i>Heteroponera</i>	4	0.064	3	0.125
Myrmicinae		372	0.596	139	0.667
	<i>Acanthognathus</i>	–	–	1	0.042
	<i>Adelomyrmex</i>	1	0.021	–	–
	<i>Basiceros</i>	2	0.043	–	–
	<i>Crematogaster</i>	–	–	10	0.042
	<i>Cyphomyrmex</i>	5	0.042	–	–
	<i>Pheidole</i>	306	0.383	86	0.500
	<i>Solenopsis</i>	40	0.234	40	0.250
	<i>Strumigenys</i>	4	0.064	–	–
	<i>Wasmania</i>	5	0.021	–	–
	Undetermined	9	0.128	2	0.042
Ponerinae		18	0.213	15	0.167
	<i>Odontomachus</i>	1	0.021	3	0.042
	<i>Hypoponera</i>	16	0.191	12	0.125
	Undetermined	1	0.021	–	–
Unidentified		1	0.021	1	0.042

Table 3. Mites found in the diet of the microhylid frog *Chiasmocleis leucosticta*. N = total number of mites; F = frequency of mites.

	Males (n = 47)		Females (n = 24)	
	N	F	N	F
Mesostigmata	4	0.064	7	0.250
Laelapidae	–	–	2	0.042
Macrochelidae	–	–	2	0.083
Podocinidae	4	0.064	1	0.042
Rhodacaridae	–	–	1	0.042
Veigaiidae	–	–	1	0.042
Oribatida	25	0.298	8	0.250
Prostigmata	–	–	1	0.042
Smarididae	–	–	1	0.042
Unidentified	5	0.106	2	0.083

Florestal do Morro Grande indicate that Formicidae was not the most abundant item along the year neither in number of individuals nor in biomass (Bovendorp 2013), which suggests that the prevalence of ants in the diet of *C. leucosticta* in this study is related to prey selection by *C. leucosticta*.

Myrmicinae ants dominated the diet of *C. leucosticta*, which can be explained by the higher diversity of genera and habits (arboreal, soil and leaf litter inhabitants, and some associated with plants, fungus and other ants) of this taxon when compared with other ant subfamilies (Fernández 2003). All Myrmicinae genera found in our study include leaf litter or epigeal ants, except *Crematogaster*, an arboreal genus that can be associated with plants and other ants (Fernández 2003). *Crematogaster* ants were

found only in one stomach, suggesting the predation event occurred when those were foraging in the leaf litter. Among Myrmicinae, *Pheidole* was the most speciose genus, followed by *Solenopsis*; *Pheidole* is the most common genus in the Neotropics and very abundant locally, comprising up to 70 species by locality, while *Solenopsis* includes about 90 species (Fernández 2003). These two genera were also the most abundant in the diet of the microhylid *Elachistocleis ovalis* (Solé et al. 2002).

Considering males and females together, Formicinae and Ectatomminae were the second most abundant food category in our study. Formicinae ants are characterized by the production of formic acid and by the presence of venom gland; *Brachemyrmex* is a common genus in the soil and leaf litter, and *Paratrechina* is a cosmopolitan genus in natural and disturbed habitats (Fernández 2003). Solé et al. (2002) found that although the genus *Paratrechina* is present in the diet of *E. ovalis*, in field experiments the frogs adopted defensive postures when these ants were offered to them as potential prey. The relatively high consumption of Formicinae in this study (10 frogs) indicates that this behavior may not be present in *C. leucosticta*.

The only Ectatomminae ants our frogs ingested belong to the genus *Gnamptogenys*, represented in the Neotropics by 81 species of predatory ants inhabiting the forest floor or the vegetation (Fernández 2003). Ecitoninae ants are the famous legionary ants, nomad predators that live in dense colonies, alternating stationary and migratory stages; ants in the genus *Labidus* impose heavy impact on invertebrate populations (Fernández 2003). Six out the nine ant genera consumed by the microhylid *Elachistocleis ovalis* (Solé et al. 2002) were also consumed by *C. leucosticta* (*Cyphomyrmex*, *Pheidole*, *Solenopsis*, *Wasmania*, *Gnamptogenys*, and *Hypoponera*).

Mites have been rarely reported as food items to anurans. Duellman (1978) did not find mites in the diet of *Chiasmocleis anatis*, *C. bassleri* and *C. ventrimaculata*, while Schluter & Salas (1991) reported two individuals *C. ventrimaculata* feeding on mites at night (but did not find them in the stomachs). The importance of mites in the diet of *C. leucosticta* may be explained by the fact that this group of organisms is the most abundant in the forest floor, especially the Oribatida (Franklin et al. 2001), which suggests that its presence could be due to the great abundance rather than a food preference. Mites of this group are mostly known for being mainly saprophagous and/or fungivorous, thus being more numerous in soil covered with abundant litter (Norton & Behan-Pelletier 2009). All other mite families found in this study are known mainly for their predatory habits, being typically soil inhabitants (Carrillo et al. 2015).

Some *Chiasmocleis* species are considered important mite predators in leaf litter communities, the level of acarophagy being negatively correlated to the anuran body size (Simon and Toft 1991). *Chiasmocleis leucosticta* is a small (male SVL = 18.3 ± 2.80 mm; female SVL = 23.5 ± 1.72 mm) fossorial frog that forages in the leaf litter, being included in Class 2 of Simon & Toft (1991), which could explain the presence and the relative abundance of mites in its diet. Mites were also well represented in the diet of the Amazonian microhylid *Sincopa antenori* (Simon & Toft 1991) and in the Atlantic forest microhylid *Chiasmocleis capixaba* (Van Sluys et al. 2006). To our knowledge this is the first work to identify mites to the family level in the diet of a Microhylidae frog.

Some dendrobatid frogs are able to remove alkaloids from their diets (especially from ants, bees, millipedes and mites) and turn them into chemical defenses (Saporito et al. 2004). Myrmicinae ants have several alkaloids, such as pyrrolidine, pyrrolizidine, piperidine, indolizidine, and quinolizidine decahydroquinoline (Daly et al. 1994a, 2000). Similarly, pumiliotoxins were also found in *Brachemyrmex* and *Paratrechina* ants (Saporito et al. 2004). Since most microhylids are ant-specialists, some studies have investigated whether microhylids are able to remove alkaloids from the diet. Berazategui et al. (2007) pointed out that the high consumption of Myrmicinae suggests that *Elachistocleis bicolor* is able to capture alkaloids. However, as toxicity is generally associated to diurnal activity and aposematism, the adaptive nature of this character in *E. bicolor*, a cryptic and nocturnal species, remains unclear. Additionally, Mebs et al. (2010) were unable to detect alkaloids in the skin of *Elachistocleis* sp. from Bolivia, but have detected them in the dendrobatid *Ameerega picta*, concluding that “myrmecophagy provides dendrobatids with alkaloids, which they sequester and store in their skin, but an alkaloid sequestering system is absent in the microhylid *Elachistocleis* sp.” This system of alkaloid sequestration was previously described for dendrobatids by Daly et al. (1994b), and it is probably absent in ant-specialized microhylids.

Some studies have been conducted to verify the presence of alkaloids in mites. Saporito et al. (2007) isolated more than 80 alkaloids from oribatid mites, and half of them are also present in the dendrobatid *Oophaga pumilio*. Oribatid mites are the main source of alkaloids to Dendrobatidae and Eleutherodactylidae (Raspotnig et al. 2011). For the same reason discussed above for ants, microhylids seem to be unable to sequester alkaloids from their mite prey.

Author Contributions

Mariana S. Lopes: conceptualization, methodology, laboratory work (dissections, ant determination), data analysis, validation, writing.

Ricardo S. Bovendorp: methodology, fieldwork (anuran collection), funding acquisition, validation, writing.

Gilberto José de Moraes: methodology, laboratory work (mite determination), resources, validation, writing.

Alexandre Reis Percequillo: methodology, funding acquisition, resources, project administration, supervision, validation, writing.

Jaime Bertoluci: conceptualization, methodology, funding acquisition, resources, project administration, supervision, validation, writing.

Conflicts of interest

The authors have declared that no conflicts of interest exist.

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