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SHORT COMMUNICATION

Diet of *Brachycephalus brunneus* (Anura: Brachycephalidae) in the Atlantic Rainforest of Paraná, southern Brazil

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ABSTRACT. Saddleback toads (Brachycephalidae: *Brachycephalus*) are a remarkable group of highly endemic species from the southern Atlantic Rainforest of Brazil. They are brightly colored, diurnal, minute frogs that live in the leaf litter of high-elevation cloud forests. Little is known about the natural history of these frogs, particularly their diet. In the present study we provide the first published account of a *Brachycephalus* species diet. Specimens of *Brachycephalus brunneus* Ribeiro, Alves, Haddad & Reis, 2005 (N = 20) were collected from two locations in the Serra do Mar mountain range in the state of Paraná, southern Brazil. A total of 137 food items were recorded, with Acari being the most common item (62.4%). This prevalence is much higher than the actual availability of Acari in their environments (37.7% of all items in leaf litter samples) and therefore indicate their preference for this food item.

KEY WORDS. Acari; feeding; foraging; litter frog.

Brachycephalidae currently includes 51 species divided into two genera: *Brachycephalus* Fitzinger, 1826 and *Ischnocnema* Reinhardt & Lütken, 1862. The distribution of the family extends from southeastern Brazil to Paraguay and northeastern Argentina. The genus *Brachycephalus* is composed of 17 species restricted to the Atlantic Rainforest, ranging from the state of Espírito Santo to the state of Paraná (Frost 2011). The species from this genus are notable for displaying several uncommon traits among amphibians. They show diurnal habits and can be observed walking on leaf litter. Some species are restricted to regions of the Serra do Mar mountain range in cloud forests, with elevations ranging from 800 to 1,600 m above sea level (Pombal *et al*. 1998, Ribeiro *et al*. 2005, Alves *et al*. 2006). They have minute body sizes, from 0.8 to 1.8 mm in snout-vent length (SVL). The miniaturization that these species experienced during their evolution also led to a reduction in the number of ankle bones and phalanges in both fore and hind limbs (Hanken & Wake 1993, Yeh 2002, Alves *et al*. 2006, Clemente-Carvalho *et al*. 2008, 2009).

Little is known about the biology of *Brachycephalus* Fitzinger, 1826, particularly with respect to their diets. The only dietary records to date are provided in two unpublished theses, which suggest that the diet of *Brachycephalus* is composed of small invertebrates present in their environment. *Brachycephalus pernix* Pombal, Wistuba & Bornschein, 1998 tends to feed on Acari and parasitic hymenopterans (Chalcidoidea), although seasonal variations might occur, such as the higher frequency of spiders during the winter (E.M. Wistuba pers. comm.). There is also variation in foraging strategies among *Brachycephalus* species: Heyer *et al*. (1990) indicated that *Brachycephalus nodoterga* Miranda-Ribeiro, 1920 is a sit-and-wait predator. On the other hand, *B. pernix* is considered an active predator, as indicated by the overrepresentation of Acari in its diet all year long, as well as the high frequency of larvae during the summer, which would involve their active dislocation to locate them (E.M. Wistuba pers. comm.). The goal of the present study is to investigate the composition of the *Brachycephalus brunneus* Ribeiro, Alves, Haddad & Reis, 2005 diet (0.9-1.20 mm snout-vent length), as well as to analyze its feeding preferences by comparing stomach contents with the availability of food items in the environment.

A total of 20 adult specimens of *B. brunneus* were collected in two locations: Pico Camapuã (25°25′14,5″S, 49°13′41,9″W, 1,200 m a.s.l.) and Pico Caratuva (25º14′22″S, 48º50′09″W, 1,400 m a.s.l.), both located in the municipality of Campina Grande do Sul, Paraná, Brazil. All specimens were deposited in the Herpetological Collection of the Departamento de Zoologia, Universidade Federal do Paraná (DZUP 298 to DZUP 317). Ten specimens from each location were obtained between February 19 and March 24, 2009 (ICMBIO collecting

permit # 22470-1). A 5 x 5 m plot was delimited in each area to search for specimens. The study areas were characterized by a dense understory, low-canopy trees and a thick layer of leaf litter that received little sunlight. The search for specimens was conducted on the litter of the forest floor during the daytime, with aid of a flashlight due to the brown coloration of the species. Captured specimens were anesthetized in 10% ethanol and immediately fixed and kept in 10% formalin for 48 hours, and later transferred to a 70% ethanol solution. Food items were isolated and identified. In order to test for selectivity of food items, their frequency in the diet was compared to that in the environment by obtaining 5 samples of leaf litter in each studied location. Each sample consisted of all the leaf litter in a 50 x 50 cm plot, which was sieved (1 mm^2 mesh size) and screened using a magnifying lens. All items were identified under a stereomicroscope. Invertebrates were classified to order level, according to the classification of Rupert *et al.* (2005).

A total of 137 food items were obtained from the *B. brunneus* specimens, which were compared to 1,022 items identified from the leaf litter (Tab. I). Although Acari were the most abundant item, both in the environment and in stomach contents, they were overrepresented in the *B. brunneus* diet. When both sites are combined, mites accounted for 37.7% of the available food items yet represented 62.4% of the food items. Such difference is highly significant when comparing the frequency of Acari and all the remaining food items combined ($\chi^2 = 22.08$, d.f. = 1, $p < 0.0001$ in Camapuã; $\chi^2 = 10.15$, d.f. = 1, $p = 0.0014$ in Caratuva). If Acari are omitted from the dataset, the abundance of Collembola in the diet is significantly lower than expected by chance ($\chi^2 = 8.06$, d.f. = 1, $p = 0.0045$ in Camapuã; $\chi^2 = 6.33$, d.f. = 1, $p = 0.01$ in Caratuva), whereas ants seem to be consumed according to their abundance in nature ($\chi^2 = 0.31$, d.f. = 1, $p = 0.57$ in Camapuã; $\chi^2 = 2.44$, d.f. = 1, $p = 0.12$ in Caratuva). The results of the present study are in sharp contrast with those obtained for the only two Brachycephalidae species with published data: *Ischnocnema henselii* (Peters, 1870) (females 34.5 mm and males 23 mm SVL) and *Ischnocnema parva* (Girard, 1853) (females 19.1 mm and males 13.2 mm SVL). Ants and isopods, which were infrequent as food items of *B. brunneus*, accounted for more than half of the diet of *I. parva* (Marra *et al.* 2004, Martins *et al.* 2010). Moreover, mites were highly abundant in the habitat of *I. henselli*, yet were avoided by that species (Dietl *et al.* 2009). Such differences underscore the differences between *Brachycephalus* and *Ischnocnema*, in addition to strong their divergence in other life-history traits.

Table I. Dietary items found in the stomach contents of 20 *Bracycephalus brunneus* individuals collected in the Pico Camapuã and Pico Caratuva, municipality of Campina Grande do Sul, Paraná, Brazil, as well as the availability of food items in the environment.

Food item	Camapuã		Caratuva	
	Stomach contents	Availability	Stomach contents	Availability
Acari	43	159	42	226
Formicidae	4	62	2	8
Blatodea	4	20	1	9
Unidentified larva	4	12	6	0
Araneae	3	34	0	35
Isopoda	3	2	3	5
Dermaptera	3	0	0	4
Collembola	2	111	4	129
Gastropoda	1	25	0	10
Hymenoptera (non-formicid)	1	22	2	21
Isoptera	1	19	0	6
Pseudoscorpionida	1	4	0	10
Diptera	1	1	1	1
Plecoptera	1	0	0	4
Coleoptera	0	11	0	7
Diplopoda	0	7	0	19
Orthoptera	0	1	0	9
Diplura	0	1	0	0
Phasmida	0	1	0	0
Homoptera	0	13	2	6
Chilopoda	0	0	0	3
Dipluran larvae	0	0	0	3
Symphyla	0	0	0	2
Unidentified item	0	0	2	0

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