Diet and microhabitat use in *Bolitoglossa nicefori* (Caudata: Plethodontidae)

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TITULO:

Dieta y uso del microhábitat de *Bolitoglossa nicefori* en Piedecuesta (Caudata; Plethodontidae) *

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PALABRAS CLAVES:

Salamandras, arbóreas, hojarasca, ítem de presa, Formicidae, Araceae

CONTENIDO:

La salamandra *Bolitoglossa nicefori* es una salamandras sin pulmones con desarrollo directo de juveniles en huevos colocados en la tierra, esta salamandra terrestre es conocida únicamente por su descripción original. En este estudio reportamos el descubrimiento de una población grande de *Bolitoglossa nicefori* analizando la dieta y el uso del microhábitat, y comparando esta información con lo conocido para otras especies de *Bolitoglossa*.
El contenido de 89 estómagos fue analizado así como la altura de la percha, tipo de vegetación y el tipo de sustrato fue registrado. La dieta de esta población consiste de 13 ítems; con hormigas y coleópteros con un 87% de las presas ingeridas por diferentes edades y tipos de sexos. Las hormigas son el recurso alimenticio con mayor disponibilidad en el sitio de estudio, sugiriendo que el consumo de presas esta relacionado con la disponibilidad. Esta salamandra ocupa los sitios arbóreos y terrestres donde las hormigas son el recurso más abundante. Las hormigas representan el 58% de la dieta de *B. nicefori* (la presa mas ingerida pertenece al genero *Atta sp*). No se encontraron diferencias intra e inter específicas en la dieta. El tamaño de las presas fue correlacionado significativamente con el tamaño del individuo y con el tamaño de la boca.
Hembras maduras no reproductivas, machos y juveniles fueron encontrados perchando en el rango de 0 – 60 cms sobre hojas de Araceae que son las predominantes en el sitio de estudio.

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* Trabajo de grado

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TITLE:

Diet and microhabitat use in *Bolitoglossa nicefori* (Caudata: Plethodontidae)[*]

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KEY WORDS:

Salamander, arboreal, leaf litter, prey items, Formicidae, Araceae

CONTEND:

The salamander *Bolitoglossa nicefori* is lungless salamander have direct development of young within terrestrially laid eggs, is a terrestrial salamander only known for its original description. This study reports the discovery of a large population of *Bolitoglossa nicefori*, analyses its diet and microhabitat use, and compares this information with that is known for other *Bolitoglossa* species. The contents of 89 stomachs were analyzed and perch height, type of vegetation and substrate type were registered. The diet of this population consists of 13 items; with ants and coleopterans accounting for 87% of food ingested by the different age and sex classes. Ants were the most abundant dietary resource in the zone, suggesting that prey availability is related to prey consumption. This species inhabits both arboreal and terrestrial sites where ants are the most abundant resource. Ants represent 58% of the diet (the prey most ingested was of genus *Atta sp*). Intra and inter sexual differences in diet were not found. Prey sizes were significantly correlated with body and mouth sizes, suggesting diet is constrained by prey size. Mature non reproductive females, males and juveniles were found perching from 0-60 cm above the ground on leaves of Araceae bushes that predominate in the study area, whereas reproductive (vitellogenic and gravid) females were always found within the leaf litter.

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5. Introduction

The Neotropical genus *Bolitoglossa* (Plethodontidae) contains more than 80 species, being the largest and most widely distributed genus of salamanders (García-Paris et al. 2000, Parra-Olea et al. 2004). In spite of this great diversity, detailed information concerning the natural history of these tropical salamanders is available for only a few species: *B. subpalmata* (Vial 1966, 1968); *B. rostrata (*Houck 1977); *B. colonnea* (Bruce 1997); *B. mexicana* and *B. rufescens* (Anderson and Mathis 1999); *B. pesrubra and B. cerroensis* (Mead and Boback 2002) and *B. occidentalis* and *B. rostrata* (Chan 2003). Diet and microhabitat preferences have been studied only in even fewer species; e. g. *B. subpalmata* (Vial 1968), *B. persubra* and *B. cerroensis* (Mead and Boback 2002) in Costa Rica, and *B. mexicana* and *B. rufescens* in Mexico (Anderson and Mathis 1999). For these populations, the dominant prey category was Formicidae, followed by Coleoptera, and Collembola, and other prey items of minor importance; also, for these populations a significant positive relationship was found among body size (standard length, SL) and the size and volume of the consumed prey. Individuals of these populations were active when collected on the surface of leaves, plants stems, on the forest floor, and on tree trunks; Vial (1968) also collected individuals in bromeliads and under rocks.

Bolitoglossa nicefori Brame and Wake 1963 is a large species from the northern part of the Colombian Cordillera Oriental. It was grouped in the *Bolitoglossa (Eladinea) adspersa* group by Parra-Olea et al. (2004). The species is only known from the data registered in its original description based upon a population from San Gil municipality, and no aspects of its natural history are known. The present study reports the discovery of a relatively large population of this species and some aspects of its natural history (diet composition and microhabitat use) and compares the information with reports on other species of the genus.

6. Materials and methods

The population studied is located in Vereda Las Amarillas, Microcuenca La Venta, Municipality of Piedecuesta, Santander, Colombia, South America (N: 06° 58" 10.6" and W: 73° 1" 17.5", 1400 – 2000 m altitude). The habitat is a highly disturbed tropical dry forest. The mean annual rainfall is 1160 mm and mean annual temperature is 17.36°C. The rainfall regime is bimodal, with one peak in April-May and a second peak in October. November to March and July to August are the months with the lowest rainfall.

Salamanders were captured by visual encounters during two nights of censuring (19:00 to 01:00 h) each month between January and December of 2004, covering different areas inside the forest. The sampling was standardized as 2 persons / 6 hours each night. The specimens were collected by hand and placed into individually labeled bags. Salamanders with standard length (SL) of less than 20 mm were considered juveniles (according to the histological evidence of male and female gonads from specimens of the same population; Monares et al*.*, unpublish. data). We recorded SL and mouth width (MW) according to Bovero et al*.* (2003). The following variables were registered from each collected individual: perch height, type of vegetation and substrate type (fern, moss, forest floor, leaf litter, and leaves). The juveniles were set free after registering their size. Sub-adult and adult salamanders were immediately euthanized with 10% ethanol and fixed in 10% formalin for 15 days to interrupt the digestive processes and prey decomposition. Then, the specimens were transferred to 70% ethanol and deposited in the herpetological collection of the Museo de Historia Natural, Universidad Industrial de Santander (UIS-A). Leaf litter arthropods were collected with an insect net and Berlesse funnel at the study location to quantify food resource availability and richness.

Digestive tracts of every individual were removed and their contents preserved in 70% ethanol. Taxonomic determinations of prey were made using the keys by Borror et al. (1989). An index of relative importance was calculated to evaluate the contribution of each category to this population's diet (following Pinkas et al. 1971). The relationship of the prey items and their contribution with the diversity of the diet was measured with the Shannon-Wiener diversity index. The number, percentage, frequency, weight and prey length were recorded. We tested correlations between salamander SL and MW and prey size and volume (using the ovoid spheroid formula, Caldwell and Vitt 1999) to see whether prey size depended on salamander size. Chi-square tests and G tests were used to investigate whether significant statistical differences existed in the use of microhabitat and perch heights.

Diet/Prey	Numerical Importance	Frequency	Prey Volume	% IRI
Acarinae	20	8	30,7591	93,95
Formicidae	299	61	1610,118	15109,77
Coleoptera	99	49	1005,348	5497,08
Collembola	19	13	166,5956	260,13
Diptera (larvae)	36	23	378,5666	1018,22
Diptera (adult)	8	7	5,2239	32,89
Araneida	18	10	181,1447	268,97
Hymenoptera	8	7	91,2565	73,76
Blattaria	1	1	0,7372	0,85
Pseudoescorpion	3	3	5,9429	5,44
Dermaptera	1	1	4,361	1,22
Hemiptera	1	1	9,4248	2,82
Orthoptera	3	3	10,1994	8,63
Total	516	84	3499,677	1055165

Table I. Diet composition in a population of *Bolitoglossa nicefori*. Numerical Importance (number of prey items in relation to the total number of items found in the species), frequency (percentage of prey item number in relation to total number of items found in the species), prey volume (total volume of prey category in all individuals examined), and percentage of relative importance [IRI = % FO (% V + % N), FO, percentage of stomachs containing a specific item, V volumetric importance, and N, Numerical importance].

7. Results

Eighty four stomachs (39 males and 45 females) were dissected, revealing 516 prey items identified to order and grouped into 13 categories (Table I). The diet was composed of insects and other arthropods such as spiders and mites. Although there were vegetable remnants, they were not considered as a diet resource because only 3 out of 84 stomachs contained this item. Our results indicated a diet composed of 13 prey categories, each contributing in different ways to the overall diet of the individuals. According to the Index of Relative Importance (IRI), the most important food category was Formicidae (all genera 58 %; with *Atta* spp. the most represented), followed by Coleoptera (19.2 %) and Diptera larvae (7 %). The categories with a low IRI value possibly reflect an occasional ingestion due to the abundance of such a resource in the study area. The high percentage of ants in the diet was related with a high availability of this resource in the sampling places (Table II).

Prey	Leaf litter	Perch
Formicidae	43,86	50,61
Atta spp	5,26	39,61
Other genera	38,6	11
Acarinae	31,58	0
Coleoptera	3,51	8,54
Araneida	7,02	5,49
Blattaria	0	11,59
Orthoptera	0	9,76
Colembolla	3,51	7,32
Diptera (larvae)	10,53	0
Diptera (adults)	0	6,7
Total	100	100

Table II. Richness of arthropods in the study site. Ants were the most abundant prey in the litter and in the higher perch sites.

Both sexes ate in a similar way. Prey composition of each sex was not significantly different ($U_{1,\ 13}$ = 592; P = 0.31). There also were no significant differences between sexes in the numeric importance ($U_{1,\ 13}$ = 70, P = 0.78), frequency in stomachs ($U_{1,\ 13}$ = 80.5; P = 0.83), volume of ingested prey ($U_{1,\ 13}$ = 84, P = 0.98) and in the index of relative importance ($U_{1,\ 13}$ = 80; P = 0.82). The adult population of *B. nicefori* showed a positive relationship between SL and MW, and size and volume of consumed preys, with a significant correlation between SL and prey size (r = 0.28; P = 0.015; N = 75); SL and prey volume (r = 0.24; P = 0.039; N = 75) and between MW and prey size (r = 0.27; P = 0.02; N = 75) and MW and prey volume (r = 0.24; P = 0.021; N = 75); indicating that larger salamanders with wider mouths ingested larger prey.

Most of the specimens were collected on herbaceous plants. However, preovulatory (individuals with large yolked follicles) and gravid females were collected exclusively within the leaf litter between January - March of 2004. There was no significant difference between sexes in perch use ($X^2_{(0.05\text{-}\ 4)}$ = 9.41; $P \geq$ 0.05). There was a significant difference in substrate use (ferns, floor, moss, leaves and leaf litter) within the population ($X^2_{(0.05\text{-}4)}$ = 147.1; $P \leq$ 0.001; N = 89), with leaves being the most used substrate. The leaf type used as a perch also exhibited significant differences ($X^2_{(0.05\text{-}4)}$ = 9.405; $P \geq$ 0.05; N = 89), with Araceae´s leaves being the most used; there were notsignificant differences between sexes ($X^2_{(0.05,\ 4)}$ = 2.46; $P \geq$ 0.05; Males N = 39, Females N = 45). Perch height was significantly different in the population ($X^2_{(0.05,\ 4)}$ = 55.63; $P \leq$ 0.001; N = 89), with individuals encountered in the 0 – 29, 9 cm and 30 – 59, 9 cm ranges (see Figure 1); there were no significant differences in perch height between the sexes ($X^2_{(0.05,\ 4)}$ = 3.06; $P \geq$ 0.50; males N = 39, females N = 45).

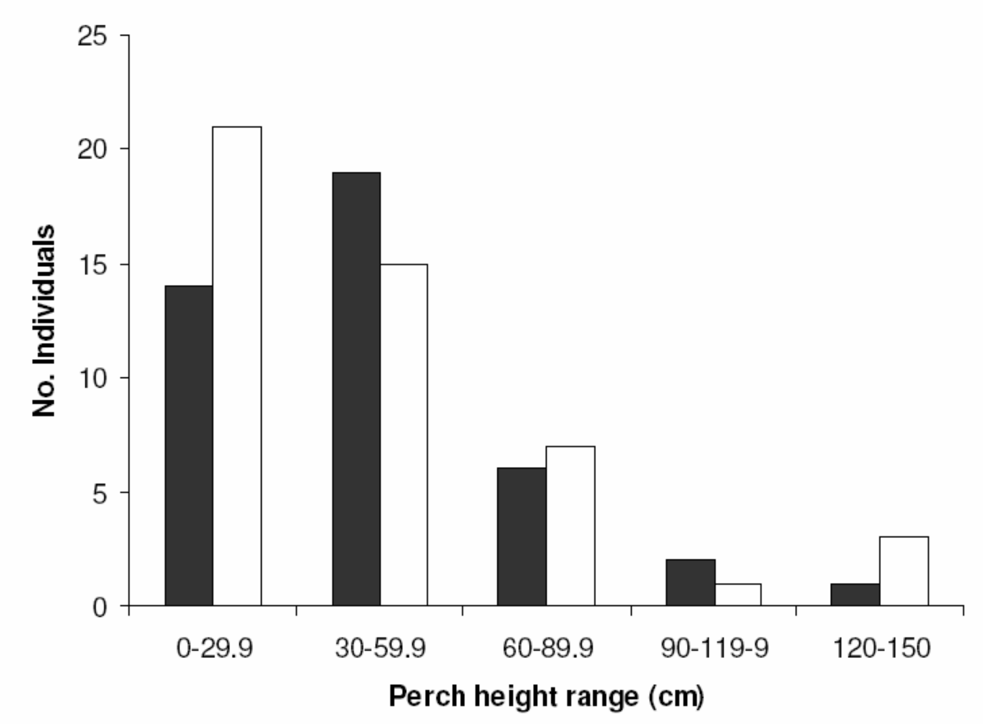

Fig. 1. Perch height used by both sexes of *Bolitoglossa nicefori.* Males, black bars; females, white bars. Inter-sexually differences in perch height range are presumed to be associated with the search for humidity and Tº conditions in reproductive females.

8. Discussion:

Bolitoglossa nicefori feeds mainly on ants; however, it has a wide spectrum of dietary items. Ants were the most abundant dietary resource in the zone, suggesting that prey availability is related to prey consumption. This species inhabits both arboreal and terrestrial sites where ants are the most abundant resource. However, a wider generalist diet also is suggested because it ingests several other prey items. In fact, the diet of *B. nicefori* suggests two foraging modes; active foraging (feeding on mites and larvae) and sit and wait foraging (feeding on ants and coleopterans).

Diet studies for other *Bolitoglossa* species have shown variable results. Some species have low diversity indices in consumed prey species, suggesting that only a few prey types are consumed or at least that the greatest number of individuals belong to a single prey category. Anderson and Mathis (1999) and McCranie et al. (1996) found that ants were the most important prey item for *B. mexicana* and *B. rufescens*, (*H'* = 1.58 and *H'* = 1.16, Table III) in Veracruz, Mexico and *B. dofleni* in Guatemala, respectively. This low diversity index and high percentage of ants is similar to that found for *B. nicefori* (*H'* = 0.98, Table III) in our study. In contrast, *Bolitoglossa cerroensis* and *B. pesrubra* feed on a wider variety of preys, with no single prey taxon being dominant in their diets (*H'* = 2.11 and *H'* = 1.97, Mead and Boback, 2002). For these species Colleoptera, Diptera, Collembola, and Acarina were the most important prey.

	S	H'	E	ln(S)	ln(E)
B. cerroensis	12	2.11	0.687	2.48	-0.37
B. pesrubra	10	1.97	0.717	2.30	-0.33
B. mexicana	14	1.58	0.346	2.63	-1.05
B. rufuscens	17	1.16	0.187	2.83	-1.67
B. nicefori	13	0.98	0.21	2.56	-1.56

Table III. Shannon-Wiener index and relative contributions of richness and evenness to the diet of *B. nicefori*. Data for *B. cerroensis y B. pesrubra* are from Mead and Boback (2002); for *B. mexicana* and *B. rufuscens* are from Anderson y Mathis (1999). Abbreviations are S = Number of prey taxa, H' = diversity index, y E =evenness.

Vial (1968), Wake (1987), García-Paris et al. (2000) and Walton (2005) suggested that external factors (seasonal prey abundances and presence or absence of predators) and intrinsic factors (ecological and morphological tolerance) related to body size influenced the election of the types of prey consumed, as well as microhabitats used for foraging. These relationships also were reported in the populations of *B. mexicana* and *B. rufescens* (Anderson and Mathis, 1999) and for *B. cerroensis* and *B. pesrubra* (Mead and Boback, 2002), showing that in salamanders, as in all other amphibians, the type and size of prey is explained by morphological constraints and by variation in the spectrum of prey types available for consumption (Vial, 1968; Harper and Guynn, 1999; Lima and Magnusson, 2000; Walton, 2005).

Bolitoglossa nicefori exhibits both terrestrial and arboreal behaviors that are related to temperature and humidity conditions during the daily cycle. At night, the salamanders perch on leaves with thick and strong stems, especially on plants from the Araceae family. This offers them an easier climb up to the leaf surface where there is ample room for free movement related to prey capture. It also provides a substrate for mating during the reproductive season (as observed by us on three occasions). The prevalence of this family of plants in the study area has increased due to its constant sowing. During the day, the salamanders hide within the leaf litter or deep within the axils of the leaves.

There was no relationship between perch height and body size in *B. nicefori.* Mead and Boback (2002) also found no significant differences in the relationship between mass no LS and perch height for *B. cerroensis* and *B. pesrubra* in Costa Rica. However, there were differences for microhabitat use by reproductive females during January – March, when they were found exclusively buried in the leaf litter. This behavior seems to be related to their inability to ascend perches due to the increase in their corporal mass while gravid, and possibly also to predator avoidance.

The utilization of prey items and microhabitat resources in *B. nicefori* is determined by their availability and by reproductive condition in the case of females. In the same sense, Vial (1968) and Houck (1977) suggested that *Bolitoglossa* species prefer to perch on large wide leaves with flat surfaces (e.g Araceae, bromeliads) because they have high mobility and may better benefit from available thermal and humidity conditions. *B. nicefori* showed significant differences in the use of specific types of leaves and plants in this study. This selection appeared to be related to the abundance of resources in the area (type and abundance of plants). Thus, as in other salamanders, particular microhabitat features determine variation in preferences of populations in different areas (Wake and Lynch, 1976; Mead and Boback 2002; Walton, 2005).

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