Ecological aspects of the casque-headed frog *Aparasphenodon brunoi* (Anura, Hylidae) in a Restinga habitat in southeastern Brazil

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Abstract

Ecological aspects of the casque-headed frog *Aparasphenodon brunoi* (Anura, Hylidae) in a Restinga habitat in southeastern Brazil. We describe some aspects of the ecology of *Aparasphenodon brunoi*, a species associated with bromeliads. We comment on the relationships of this species with bromeliad size, microhabitat use, diet and sexual dimorphism. This study was conducted on a Restinga habitat near Presidente Kennedy, state of Espírito Santo, southeastern coast of Brazil. When the animals were found inside the bromeliads, we measure bromeliad and head size of frogs. We analyzed stomach contents and determined the sex and reproductive condition. We found 17 individuals (58.6%) in bromeliad leafs, six (20.7%) in Cactaceae, three (10.3%) in liana and three (10.3%) on trunks. The correlation between head measurements and bromeliad size were high, indicating that animals apparently use bromeliads based on their size, which could be related to the minimization of water loss. The most common prey items were beetles, ants, and insect larvae, suggesting that the species is relatively generalist in prey consumption. *Aparasphenodon brunoi* showed significant sexual size and shape dimorphism with females having larger bodies than males (size) and females having tibia, eye diameter and SVL larger than males (shape), but larger sample size and more detailed ecological and life history data are needed to elucidate the factors that have led to sexual size dimorphism.

Keywords: Anura, Hylidae, *Aparasphenodon brunoi*, casque-headed frog, diet, microhabitat use, bromeliads, sexual dimorphism.

Introduction

Many species of invertebrates and vertebrates use bromeliads for foraging, reproduction and escaping from predators. Among vertebrates, amphibians and reptiles are the most common inhabitants. For example, the lizard *Mabuya macrorhyncha* preys on animals that live between bromeliad leaves of *Neoregelia* (Vrcibradic and Rocha, 1996), and the frog *Phyllodytes luteolus* carries out its entire life cycle inside bromeliads (Teixeira *et al.* 1997, Eterovick 1999).

Aparasphenodon are tree frogs characterized by a strongly ossified skull, which gives them the common name "casque-headed frog" (Pombal 1993). The ossified skull appears to have evolved independently six times in hylids, apparently as an adaptation to similar habitats where water is scarce (Trueb 1970). Several genera of casque-headed frogs occur in South America, including *Osteocephalus, Phrynohyas, Trachycephalus, Corythomantis, Aparasphenodon* and some species of *Scinax* and *Hyla*. *Aparasphenodon* may be closely related to *Corythomantis* (Trueb 1970). The genus *Aparasphenodon* consists of three species, ranging from southern Brazil to the Orinoco river basin in northern Venezuela (Argôlo 2000, Frost 2002). *Aparasphenodon brunoi* Miranda-Ribeiro, 1920 occurs in coastal areas of São Paulo, Rio de Janeiro, Espírito Santo and Bahia states and continental areas of the Parque Estadual do Rio Doce, in Minas Gerais state (Feio *et al.* 1998, Argôlo 2000). The species is relatively common in Restinga habitats, which are white sand dunes partially covered by herbaceous plants and shrubs. This vegetation forms dispersed islands of vegetation (Suguio and Tessler 1984), where the frog is usually found associated to bromeliads. Bromeliads are abundant in the Restinga and occur on many kinds of substrates, including soils with organic material, sandy soils, and tree trunks (Cogliatti-Carvalho *et al.* 2001). The leaves of bromeliads typically form a rosette, within which water accumulates. The shape and size of the bromeliads determine the amount of water that can accumulate (Leme 1984). This frog species is highly associated with bromeliads and reaches 80 mm in snout vent length (SVL) (Feio *et al.* 1998), but little is known about their ecology.

Herein, we describe some aspects of the ecology of *Aparasphenodon brunoi* from a Restinga in the southern Espírito Santo state, southeastern Brazil. We address the following questions: (1) What are the patterns of habitat and microhabitat use? (2) Is there a relationship

Figure 1 - *Aparasphenodon brunoi* from Presidente Kennedy (ES) (sex unknown). Photo: Adrian A. Garda.

between the size of bromeliads and frog size? (3) Is there a significant sexual dimorphism? (4) What are the most important prey items?

Material and Methods

The study was performed in a gradient that varies from a Restinga (21°17'59''S, 40°57'30''W) to a forested area within Restinga (21°17'40''S, 40°57'35''W), near Presidente Kennedy, Espírito Santo state, on the southeastern coast of Brazil, from 20 to 27 September 2001. *Aparasphenodon brunoi* (Figura 1) was collected during the day by the Restinga shrubs and by searching bromeliads. The forest site was visited during the day and night. Individuals were located visually and by their calls. Microhabitat categories, including branch, liana, Cactaceae, trunk, bromeliad leaves, and inside bromeliads were recorded for 29 individuals as the original position that it was found at first sight. The height (in cm) of each individual above the ground was recorded. All frogs were killed in 10% alcohol solution and preserved in 10% formalin. Specimens were deposited in the Coleção Herpetológica da

Universidade de Brasília (CHUNB 15935, 15937–15940, 24369, 24781–24800, 24909–24945).

The following measurements were taken only on frogs found inside the bromeliads and of the correspondent plant which they were associated: snout-vent length (SVL), head length (from the tip of the snout to the commissure of the mouth), head width (at its broadest point) and head height (at its highest point) (N=10) (using a Mitutoyo® digital caliper, to the nearest 0.01 mm), plant height, width between external leaves, and diameter of the bromeliad rosette (using a ruler and a measuring tape). Canonical correlation analysis was used to investigate the relationship between frog size and bromeliad size.

For the analysis of feeding habits we removed the stomachs (N=63) and identified prey items to order. We recorded the length and width (to the nearest 0.01 mm) of intact prey with Mitutoyo® digital calipers, and estimated prey volume (*V*) as an ellipsoid:

$$V = \frac{4}{3} \pi \left(\frac{w}{2}\right)^2 \left(\frac{l}{2}\right),$$

where *w* is prey width and *l* is prey length. We calculated the numeric and volumetric percentages of each prey category for individual frogs and for pooled stomachs. To investigate the relationship between prey size and frog head measurements, we used a canonical correlation analysis with two sets of variables: maximum prey length and width vs. frog head width, height, and length.

We determined sex and reproductive condition of each frog using direct observation of gonads. The females were considered reproductive when their ovaries were extremely convoluted and enlarged. We considered as reproductive males, individuals that have completely evident vocal sac. We recorded for all individuals, collected inside and outside of bromeliads and previously deposited in CHUNB

(N=63), the following morphometric variables: SVL, head width, height, and length; tibia, forearm and foot length; and tympanum and eye diameter. We considered the SVL of the smaller reproductive male and female as the SVL of sexual maturity; all individuals with SVL equal or superior to that ones were considered adults.

We log-transformed (base 10) all morphometric variables prior to analyses to meet the requirements of normality (Zar 1998). To partition the total morphometric variation between size and shape variation, we defined body size as an isometric size variable (Rohlf and 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). Next, we obtained scores from this eigenvector, hereafter called body size, by post-multiplying the *n* by *p* matrix of log-transformed data, where *n* is the number of observations, by the *p* by 1 isometric eigenvector. To remove the effects of body size from the log-transformed variables, we used residuals of regression between body size and each shape variable. To test the null hypothesis of no difference between sexes, we conducted separate analyses of variance on body size (ANOVA) and the shape variables (MANOVA) of adult individuals.

We carried out statistical analyses using SYSTAT 9.0 for Windows with a significance level of 5% to reject null hypotheses. Throughout the text, means appear ± 1 SD. All measures are in mm.

Results

We collected, during the night, a total of 29 active individuals, being 17 (58.6%) in bromeliad leaves, six (20.7%) in cactaceae, three (10.3%) in lianas and three (10.3%) in trunks (Figure 2). The animals were at mean height of 65.7 cm (25-210 cm). During the day, the individuals were found only inside the bromeliads (N=10). In this period their activity

was restricted to emitting call from the interior of bromeliads.

The correlation between the measures of the body and the measures of the bromeliads were high. The first and second canonic variables of the body measures show that the three measures have equal influence in the composition of the canonic variable. The first canonic variable of the bromeliad measurements gave more emphasis in the bromeliad width and height. The first canonical correlation was 0.972, having statistical significance (p=0.022), showing association between the body measures of *Aparasphenodon brunoi* and the bromeliad measurements (Table 1).

We analyzed 85 stomachs and 26% (22) were empty. We found 10 prey categories, being more frequent beetles (56.1%) and ants (16.8%). Considering the number of items per stomach, the diet consisted mainly of beetles (44.0%) and ants (17.1%). By volume, beetles were the most important prey item (60.5%), followed by insect larvae (16.5%) and ants (16.1%) (Table 2). The
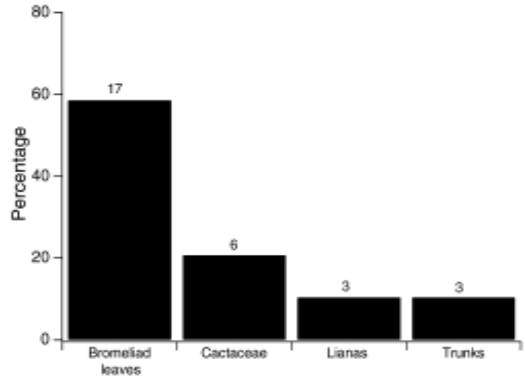

Figure 2 - Frequency distribution of *Aparasphenodon brunoi* according to microhabitat categories. Sample sizes are indicated at the top of the bars.

results based on pooled stomach were similar. Numerically, beetles were most important (38.1%), followed by ants (27.4%), and volumetrically, beetles were dominant (54.0%), followed by insect larvae (20.0%) (Table 2).

Table 1 - Canonical correlation among bromeliad and body measurements of *Aparasphenodon brunoi* (N=10).

Standardized canonical coefficients for the body measurements		
	First canonical variable	Second canonical variable
Snout-vent length	0.837	0.529
Head height	0.814	0.340
Head length	0.887	0.427
Standardized canonical coefficients for the bromeliad measurements		
	First canonical variable	Second canonical variable
Bromeliad height	0.950	−0.234
Bromeliad width	0.964	−0.232
Cup diameter	0.643	0.734
Canonical variables	Canonical correlation	F	p
I	0.972	3.94	0.022
II	0.684	1.22	0.361

Table 2. Diet composition of *Aparasphenodon brunoi* (N= 63).

Prey categories	Occurrence			Stomach Means			Pooled Stomachs			
	f	f%	N	%N	Vol. (mm^3)	%Volume	N	%N	Vol. (mm^3)	%Vol.
Aranae	1	2.44	0.02 ± 0.16	2.5 ± 15.81	819.36 ± 5246.49	4.17 ± 20.41	1	1.19	33593.91	7.79
Coleoptera	23	56.10	0.78 ± 0.88	43.96 ± 43.12	5680 ± 8982.79	60.48 ± 44.40	32	38.10	232915.50	54.03
Formicidae	11	16.83	0.561 ± 1.76	17.08 ± 31.01	798.95 ± 2566.64	16.12 ± 31.70	23	27.38	32756.85	7.60
Gastropoda	2	4.88	0.05 ± 0.22	1.88 ± 8.75	1105.20 ± 7076.72	2.75 ± 13.50	2	2.38	45313.10	10.51
Orthoptera	5	12.20	0.15 ± 0.42	7.44 ± 23.53	–	–	6	7.14	–	–
Isoptera	3	7.32	0.195 ± 0.95	4.64 ± 17.16	–	–	8	9.52	–	–
Insect larvae	4	9.76	0.10 ± 0.30	7.50 ± 24.15	2109.00 ± 8998.80	16.48 ± 37.64	4	4.76	86468.96	20.06
Non identified	6	14.63	0.15 ± 0.36	12.50 ± 31.52	–	–	6	9.52	–	–
Plant material	1	2.44	0.02 ± 0.16	1.25 ± 7.91	–	–	1	1.19	–	–
Insect egg	1	2.44	0.02 ± 0.16	1.25 ± 7.91	–	–	1	1.19	–	–

Table 3. Canonical correlation among prey and head measurements of *Aparasphenodon brunoi* (N=26).

Standardized canonical coefficients for the head measurements		
	First canonical variable	Second canonical variable
Head width	0.003	0.154
Head length	0.099	−0.186
Standardized canonical coefficients for the prey measurements		
	First canonical variable	Second canonical variable
Maximum prey width	0.077	−0.038
Maximum prey length	−0.020	0.077
Canonical variables	Canonical correlation	F	p
I	0.330	0.70	0.594
II	0.094	0.21	0.654

The correlation between the head measurements and the prey measurements were low. The first canonical variable of the head measurements showed that both measures have equal influence on the composition of the canonical variable while the second variable showed a contrast between the head width and height. The first canonical variable of the measurements of the prey represents a contrast between the maximum width and weight of the prey. The first canonical correlation was 0.330 but it has no statistical significance, showing no association between head and prey measurements (Table 3).

Table 4 - Stepwise discriminant analysis of shape variables of *Aparasphenodon brunoi*. Error-rate indicates posterior probability error-rate estimates based on cross-validation.

Step	Variable entered	R^2	Wilk's Lambda	$p <$	Error-rate
1	Adjusted tibia length	0.13	0.87	0.06	0.26
2	Adjusted eye diameter	0.22	0.68	0.009	0.29
3	Adjusted tympanum	0.16	0.57	0.004	0.22

The smallest adult female measured 56.32 mm SVL, whereas the smallest adult male was 48.88 mm. The largest male measured 62.42 mm and the largest female measured 81.24 mm. We found significant differences between sexes in body size (ANOVA $F_{1,25} = 9.743$; $p=0.005$). In addition, we found significant differences between the sexes in shape variables (MANOVA Wilk's Lambda = 0.444; $p=0.032$). To determine which of the shape variables contributed most to sexual dimorphism, we performed a stepwise discriminant analysis (Tabachnick and Fidell 1996). Three shape variables were selected as the most powerful discriminators of the two sexes, correctly classifying 78% of individuals (Table 4). Tibia length was the first variable selected, correctly classifying 74% of individuals, followed by eye and tympanum diameter. To determine whether important variables were excluded from the analysis due to inter-correlation with tibia length, we excluded tibia length and repeated the analysis. Eye diameter was then selected first, correctly classifying 70% of the individuals, followed by SVL and tympanum diameter. We repeated the analysis once more with the exclusion of tibia length and eye diameter and this time only SVL was selected. These results indicate that besides tibia length, eye and tympanum diameter, SVL is also important in stating differences between the sexes, with females having tibia, eye diameter and SVL larger than males (Table 5).

Discussion

Aparasphenodon brunoi shows higher activity during the night, being easily found in the Restinga Forest, outside the bromeliads. However, in most cases, they were found within the bromeliad leaves. Like most vertebrates, anuran diurnal activities are highly affected by requirements of food, mate, and shelter to avoid predation and maintain ideal physiological conditions, because they have a very permeable skin, being highly susceptible to water loss by evaporation (Hodgkison and Hero 2001). Therefore, most anurans are typically nocturnal (Duellman and Trueb 1994). In the case of *A. brunoi*, since bromeliads retains a large amount of water and the air humidity in the forest is usually stable and high, these animals can show diurnal activity, even in the sun (Silva *et al*. 1988). Although unusual, diurnal activities have been reported in many anurans species, such as *Litoria nannotis* (Hodgkison and Hero 2001), dendrobatids (Zug *et al*. 2001), some leptodactylids (Kwet and Di-Bernardo 1999, Zug *et al*. 2001), and others (Duellman and Trueb 1994). In this study, we observed diurnal activity in *A. brunoi* in moisture days, when we noticed some individuals calling from inside bromeliads. However, no individual was observed outside the bromeliads during the day.

We found a significant correlation between frog head and bromeliad size. It has been showed that the phragmatic behavior in *A. brunoi* effectively reduces evaporative water loss (Andrade and Abe 1997). Our data suggest that these animals are selecting bromeliads based on size, which could be an effort to minimize water loss. The annual precipitation in Restinga is high, varying from 1100 to 1500 mm

Table 5 -   Summary statistics of morphometric characters of adult *Aparasphenodon brunoi*. Values indicate mean ± 1 standard deviation. Size-adjusted values (see text) are in parentheses. Values are in mm.

Character	Males (N=14)	Females (N=14)
Body size	3.535 ± 0.078	3.663 ± 0.125
Snout-vent length	55.239 ± 3.794	65.176 ± 8.244
	(-0.010 ± 0.018)	(0.015 ± 0.054)
Tibia length	24.388 ± 1.225	28.459 ± 2.725
	(-0.005 ± 0.014)	(0.016 ± 0.038)
Foot length	32.971 ± 1.832	35.635 ± 9.627
	(0.011 ± 0.013)	(-0.041 ± 0.232)
Head length	19.841 ± 1.125	24.843 ± 9.382
	(-0.011 ± 0.019)	(-0.014 ± 0.084)
Head width	18.221 ± 1.168	20.938 ± 2.158
	(-0.000 ± 0.017)	(-0.010 ± 0.045)
Tympanum diameter	4.135 ± 1.532	4.078 ± 0.281
	(-0.019 ± 0.096)	(-0.006 ± 0.035)
Eye diameter	6.393 ± 0.323	6.736 ± 0.490
	(-0.002 ± 0.025)	(-0.019 ± 0.024)
Forearm length	24.680 ± 1.428	28.446 ± 2.723
	(-0.002 ± 0.019)	(0.009 ± 0.041)

(Louro and Santiago 1984), but the high permeability of sandy soils reduces the water availability. In addition, the shape of bromeliads promotes the accumulation of water and the head adjustment in the bromeliad rosette is important to maintain ideal physiological conditions.

Some groups of amphibians are considered dietary specialists, for example ants are the primary diet item of dendrobatids (Toft 1995). However, this is not the case for other groups. For example, *Rana nigromaculata* (Ranidae) is generalist and eats a high variety of prey items dependent more so with availability than selectivity (Hirai and Matsui 1999). *Rana catesbeiana* and *R. clamitans*, in Michigan, also show a highly diverse diet, eating mainly coleopterans, hemipterans and spiders (Werner *et al*. 1995). In Argentina, the diet of *Pseudis paradoxa* and *Lysapsus limellus* (Hylidae, Pseudinae) primarily consists of other amphibians, beetles, mosquitoes and Osteichthyes fishes, respectively (Duré and Kehr 2001). In a study in the Restinga of Jurubatiba, Rio de Janeiro state, southeastern Brazil, *Aparasphenodon brunoi* ate mainly beetles (Van Sluys *et al*. 2004). In a study in the same area, based on the diets of 21 individuals, *A. brunoi* ate mainly ants, cockroaches and grasshoppers (Teixeira *et al*. 2002). Our study shows that *A. brunoi* has a very diverse diet, eating mainly beetles, insect larvae and ants. Based on proportion of prey use, this species could be considered a generalist; however data on arthropod availability in the study area and the relationship between availability and prey

selectivity are needed to support this statement. Still, it will be necessary more samples throughout the year to avoid the seasonality effects.

Several authors have indicated that relationships between prey and head measurements could be related to resource partitioning between sexes and/or species (Magnusson and Silva 1993, Van Sluys *et al.* 2001). Teixeira *et al.* (2002) did not find any correlation between prey and body size, however they did not use any statistical test to support this evidence. In our study, based on a canonical correlation, no significant relationship between prey and head measurements was found in *Aparasphenodon brunoi*. These relationships are likely more related with resource partitioning among species in an assemblage rather than due to size variation within the same species (Magnusson and Silva 1993).

Three main hypotheses have been proposed to explain the existence of sexual dimorphism in frogs. Several studies have found that when females are larger than males there is a significant relationship between female SVL and clutch size (Kuramoto 1978, Kaplan 1980, Duellman and Trueb 1994). We found statistical differences in body size between sexes in *A. brunoi*, with the females being larger than males; however, we did not collect clutch size data for *A. brunoi*, and we are thus unable to access this hypothesis.

Another hypothesis to explain sexual dimorphism could be related to male-male competition for mates, with larger males benefiting with this kind of dimorphism (Duellman and Trueb 1994). Alternatively, sexual size dimorphism can be a mechanism for reducing intersexual competition for food resources (Magnusson and Silva 1993, Van Sluys *et al.* 2001), where the difference in the head size between sexes leads to a difference in prey size consumed by each sex. However the canonical correlation between head and prey size was not significant. Furthermore, this pattern could be due to resource sharing among species of an assemblage rather than difference

between sexes (Magnusson and Silva 1993). In our study area, there are four other species that use bromeliads in their life cycle (*Hyla albomarginata, Scinax altera, S. cuspidatus* and *Trachycephalus nigromaculatus*) (Teixeira *et al.* 2002), which could interact with and influence *A. brunoi* ecology.

Teixeira *et al.* (2002) stated that sexual size dimorphism occurred in *A. brunoi* with females being larger than males; however their statistical tests do not support this affirmation. Based on univariate and multivariate analyses of variance on a set of morphometric variables we found sexual dimorphism in *A. brunoi*. Nevertheless, a study including a larger sample size and more detailed ecological and life history data are needed to elucidate the factors that have led to sexual size dimorphism.

Acknowledgements

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