

Sexual dimorphism, female fertility, and diet of *Physalaemus feioi* (Anura: Leptodactylidae) from southeastern Brazil

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Abstract

Sexual dimorphism, female fertility, and diet of *Physalaemus feioi* (Anura: Leptodactylidae) from southeastern Brazil. *Physalaemus feioi* is a South American frog endemic to the Brazilian Atlantic Forest that occurs in the states of Minas Gerais and São Paulo. We evaluated sexual dimorphism, female fertility, and diet of *P. feioi* from two locations in the state of Minas Gerais, including the type locality of the species. We collected 73 individuals (16 adult females and 57 adult males). The number of oocytes of *P. feioi* ($\bar{x} = 1385 \pm 498.13$) was higher than in other species of the genus, such as *P. maculiventris*, *P. signifer*, *P. cuvieri*, and *P. ephippifer*, but it resembles that of *P. centralis*, *P. kroyeri*, and *P. marmoratus*. The prey items with the highest index of importance were Coleoptera (IIR = 24.26) and Formicidae (IIR = 16.46). We found no sex differences regarding the number of ingested prey and the volume of the stomach contents. In summary, *Physalaemus feioi* was similar to other species of the genus regarding sexual dimorphism, fecundity, and feeding habits.

Keywords: Amphibia, Atlantic Forest, Morphology, Natural history, Trophic ecology.

Resumo

Dimorfismo sexual, fertilidade de fêmeas e dieta de *Physalaemus feioi* (Anura: Leptodactylidae) do sudeste do Brasil. *Physalaemus feioi* é uma rã da América do Sul, endêmica da Mata Atlântica, registrada nos estados de Minas Gerais e São Paulo. Avaliamos o dimorfismo sexual, a fertilidade nas fêmeas e dieta dessa espécie em duas localidades do estado de Minas Gerais, incluindo a localidade-tipo da espécie. Coletamos 73 indivíduos (16 fêmeas adultas e 57 machos adultos) por meio de busca

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ativa. O número de oócitos encontrados para *P. feioi* ($\bar{x} = 1385 \pm 498,13$) foi alto quando comparado com outras espécies do gênero como *P. maculiventris*, *P. signifer*, *P. cuvieri* e *P. ephippifer* mas este semelha o número de oócitos de *P. centralis*, *P. kroeyeri* e *P. marmoratus*. As presas com maior índice de importância foram Coleoptera (IIR = 24,26) e Formicidae (IIR = 16,46). Não houve diferenças entre machos e fêmeas quanto ao número de presas ingeridas e nem o volume do conteúdo estomacal. Em resumo, *Physalaemus feioi* teve semelhanças com outras espécies do gênero em relação ao dimorfismo sexual, fecundidade e hábitos alimentares.

Palavras-chave: Amphibia, Ecologia trófica, História natural, Mata Atlântica, Morfologia.

Introduction

Physalaemus Fitzinger, 1826 is a genus of small to medium frogs that occur in South America (Cassini *et al.* 2010, Brasileiro and Haddad 2015, Frost 2021, Leal *et al.* 2021). It was divided into seven groups, based on morphometric, morphological, osteological, and adult patterns (Nascimento *et al.* 2005), but without defined phylogenetic relationships. More recently, Lourenço *et al.* (2015) proposed a phylogeny based on nuclear and mitochondrial DNA sequences and recognized two clades: the *P. signifer* and the *P. cuvieri* clades. The latter is composed of five species groups (Lourenço *et al.* 2015), one of which is the *P. offersii* group, which includes *P. feioi* Cassini, Cruz, and Caramaschi, 2010 together with six other species. Species within this group occur in southeastern and southern states of Brazil (Espírito Santo, Rio de Janeiro, Paraná, Santa Catarina, São Paulo, and Minas Gerais; Nascimento *et al.* 2005, Lourenço *et al.* 2015). *Physalaemus feioi* inhabits the Atlantic Forest of the Serra da Mantiqueira in São Paulo and Minas Gerais states (Figure 1), and is common in gallery forests, around ponds and along creeks and rivulets (Cassini *et al.* 2010, Silva *et al.* 2011, Moura *et al.* 2015). Some aspects of the reproductive biology of the species are known for populations in Piedade de Caratinga (Minas Gerais state), where males vocalize in late spring and summer months (October–February) and females spawn within the same seasons but in a more restricted temporal window (October–December: Silva *et*



Figure 1. Amplexus of *Physalaemus feioi* from Eugê-nópolis, Minas Gerais state, southeastern Brazil.

al. 2011). Like other species in the genus, individuals construct floating foam nests, sometimes communally, as described for individuals from the type locality (Lacerda *et al.* 2009).

Ecological features such as skin patterns and advertisement calls are well known and used in diagnoses of the species (Cassini *et al.* 2010). Other than these features, only phenology and tadpole descriptions are available (Silva *et al.* 2011, Fraga *et al.* 2022). In order to understand the role of a species in an ecosystem, it is necessary to know the diet and trophic connections (Ceron *et al.* 2022a). Data on the natural history and biology of organisms are fundamental to understand the intrinsic characteristics of each species (Stearns 1992), and how ecological and environmental forces

determine their occupation of a given space, including phenology and abiotic factors (Afonso and Eterovick 2007, Schalk and Saenz 2016). Reproductive strategies and investment are essential in determining the conservation status of a species (IUCN 2021). Among these aspects, key characteristics include sexual dimorphism, female fertility, and diet (Vitt and Caldwell 2001). Our objective is to investigate (1) reproductive features, including as sexual dimorphism and female fertility, and (2) the diet of *Physalaemus feioi* from two loctions in Minas Gerais, including the type locality.

Materials and Methods

Data Collection

We collected specimens at night during November and December 2010 by actively searching vocalization and spawning sites (Heyer *et al.* 1994). Four researchers worked four hours per day and collected 26 individuals of *P. feioi* from the type locality, Viçosa municipality (20°45'27.5" S, 42°51'38.7" W, 700 m a.s.l., Datum WGS84), and 47 individuals in Eugenópolis municipality (21°06'06" S, 42°10'59" W, 660 m a.s.l., Datum WGS84). Both areas are in Minas Gerais state, southeastern Brazil, and are separated by a straight-line distance of 66 km. The climate of the region is humid mesothermic, with dry winters (Alvares *et al.* 2013). The type locality has a dam partially surrounded by grass and a secondary semi-deciduous seasonal forest fragment. In Eugenópolis, the collection site is similar and contains a dam bordered by pasture and by a secondary semi-deciduous seasonal forest fragment.

Individuals were killed two hours after collection by application of xylocaine in the ventral region of the body and fixed in 10% formalin. After three days, samples were washed and preserved in 70% Ethanol. We sexed individuals using external sexual characters (e.g., vocal sac and vocal cleft in males) and by dissecting individuals and analyzing their

gonads. All individuals were classified as reproductive adults.

Thirteen variables were measure for each specimen using a 0.01 mm precision digital caliper: snout–vent length (SVL), head length (HL), head width (HW), eye diameter (ED), interorbital distance (IOD), upper eyelid width (UEW), eye–nostril distance (END), nostril–snout distance (NSD), internarial distance (IND), hand length (HAL), thigh length (TL), shank length (SL), and foot length, including the tarsus (FL) (Cassini *et al.* 2010).

Oocyte masses of each female were removed and weighed using a Scientech SA 210 scale with an accuracy of 0.0001 g. We weighed ten oocytes per female and calculated an average weight per female in order to estimate the total number of oocytes in each mass.

Stomachs of all individuals were removed, and their contents were identified to Order and counted, with exception of the insect larvae, which were not identified. Using a stereomicroscope with a millimeter eyepiece and Image Pro Plus software (Media-Cybernetics 2002), the width and length of each intact item were measured.

Specimens were deposited in the Museu de Zoologia João Moojen (MZUFV) of the Universidade Federal de Viçosa, Viçosa municipality, Minas Gerais state, Brazil (Eugenópolis samples: MZUFV 10781–10782, 10796–10801, 10803–10807, 10824–10857; Viçosa samples: MZUFV 10808–10822, 10856–10857, 10966–10976).

Data Analysis

Principal Component Analysis (PCA) was used to test for morphometric sexual dimorphism in *P. feioi*. Once PCA identified the three most explanatory axes (PC1, PC2, and PC3) and a Kruscal-Wallis test was used to test for normality, we performed a MANOVA using the results as response variable and sex as factor. In order to test if bigger females had more oocytes, we ran a liner regression using the female body size (SVL) against the number of oocytes.

To describe the diet of *P. feioi* and determine the importance of each prey item, we used the Pinkas *et al.* (1971) importance index ($IRI = \%F * (\%N + \%V)$), which uses the frequency of occurrence percentage (F%), the numeric percentage (N%) and the volumetric percentage. The prey volume was estimated using the formula for a spheroid (Magnusson *et al.* 2003, Garda *et al.* 2006, Santana and Juncá 2007): $V = 4/3\pi(\text{length}/2)(\text{width}/2)^2$. We did not consider samples with empty stomachs ($N = 9$) in the diet analyses.

Sex differences in diet were assessed using generalized linear models (GLM) with prey number and total prey volume as variables. Response variables were submitted to residue analysis to verify their error distribution (Crawley 2012). All statistical analyzes were done in the R (R Core Team 2022) program with the Vegan package version 2.3-5 (Oksanen *et al.* 2015).

Results

Sexual Dimorphism

We collected 73 individuals, 16 females with mature oocytes externally evident and 57 males. Females were significantly larger than males ($F = 49.15, p < 0.001$). Other variables used for sex discrimination (including SVL) were larger in females: snout–vent length (males: $\bar{x} = 25.55 \pm 1.12$ mm; females: $\bar{x} = 28.58 \pm 1.21$ mm), shank length (males: $\bar{x} = 12.45 \pm 0.56$ mm; females: $\bar{x} = 14.33 \pm 1.51$ mm) and foot length including tarsus (males: $\bar{x} = 17.91 \pm 1.03$ mm; females: $\bar{x} = 19.46 \pm 0.79$ mm) (Table 1). The PCA indicated that 86% of the variance was explained by the three first components (PC1 = 67%, PC2 = 12% and PC3 = 7%), mainly PC1 (Figure 2).

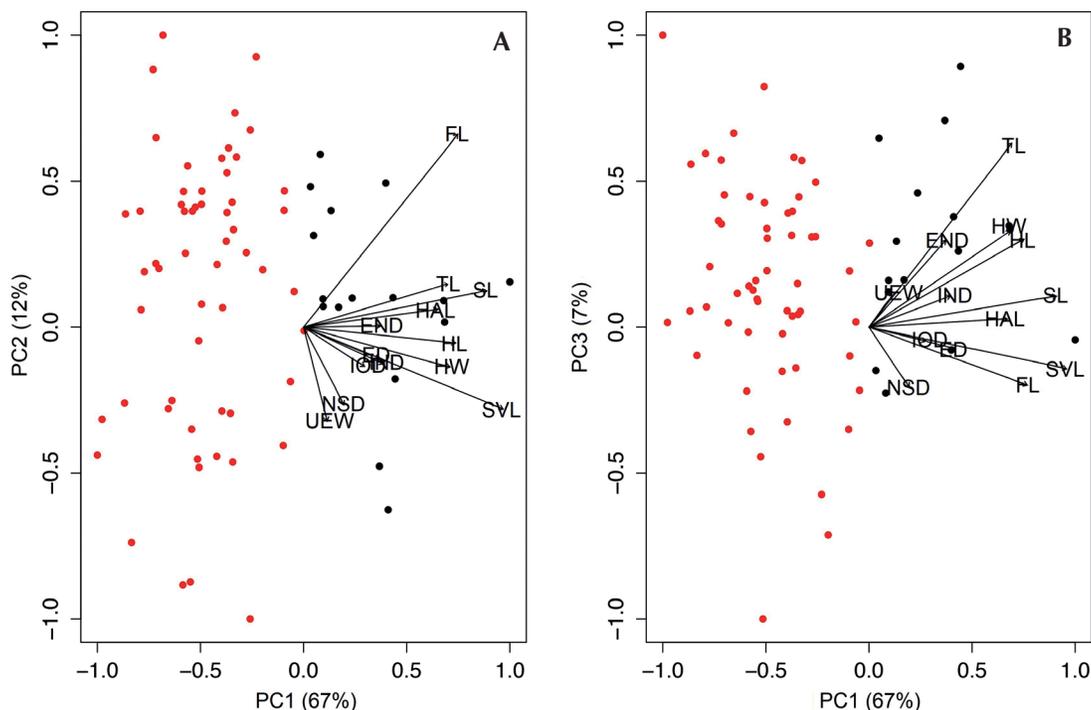


Figure 2. Principal Components Analysis for 13 morphometric characters from adult males (red) and females (black) of *Physalaemus feioi*. Projection of PC1 and PC2 (A), and projection of PC1 and PC3 (B).

Table 1. Morphometric variation of *Physalaemus feioi* from Eugenópolis and Viçosa (Minas Gerais state, Brazil). Values are in mm as mean \pm SD (range). Snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), interorbital distance (IOD), upper eyelid width (UEW), eye-nostril distance (END), nostril-snout distance (NSD), internarial distance (IND) hand length (HAL), thigh length (TL), shank length (SL), foot length including tarsus (FL).

Body measurements	Males (N = 57)	Females (N = 16)
SVL	25.55 \pm 1.12 (23.30–27.92)	28.58 \pm 1.21 (27.10–31.34)
HL	6.96 \pm 0.43 (5.90–7.79)	7.98 \pm 0.50 (6.65–8.60)
HW	6.88 \pm 0.45 (5.66–7.67)	7.80 \pm 0.49 (6.80–8.71)
ED	2.27 \pm 0.28 (1.75–2.85)	2.47 \pm 0.29 (2.12–3.17)
IOD	2.89 \pm 0.30 (2.26–3.52)	3.09 \pm 0.32 (2.52–3.69)
UEW	2.00 \pm .031 (1.23–3.22)	2.11 \pm 0.24 (1.64–2.40)
END	2.56 \pm 0.30 (1.55–3.20)	2.84 \pm 0.27 (2.52–3.36)
NSD	1.33 \pm 0.20 (1.01–1.88)	1.42 \pm 0.10 (1.26–1.62)
IND	2.01 \pm 0.20 (1.59–2.41)	2.25 \pm 0.25 (1.77–2.57)
HAL	7.01 \pm 0.40 (6.18–7.89)	7.72 \pm 0.33 (7.07–8.12)
TL	11.42 \pm 0.68 (9.75–12.69)	12.70 \pm 0.67 (11.28–13.73)
SL	12.45 \pm 0.56 (10.84–13.47)	14.33 \pm 1.51 (13.34–14.72)

Female Fertility

Of the 16 females, eight had developed oviducts and two of these were found in amplexus. The estimated number of oocytes for each female ranged from 585 to 2170 (\bar{x} = 1,385 \pm 498.13; N = 16). We found a positive relationship between SVL and weight (df = 14, F = 16.47, R^2 = 0.5078, p < 0.01; Figure 3A). We did not find a significant relationship between the SVL of females and the number of oocytes (df = 14, F = 1.9, R^2 = 0.056, p = 0.19; Figure 3B).

Diet

Nine individuals (four males and five females) had empty stomachs. The diet was composed mostly of adult and larval insects but also of spiders and snails. We found 279 individual items in 11 prey categories (Table 2). The most numerous categories of prey in the diet

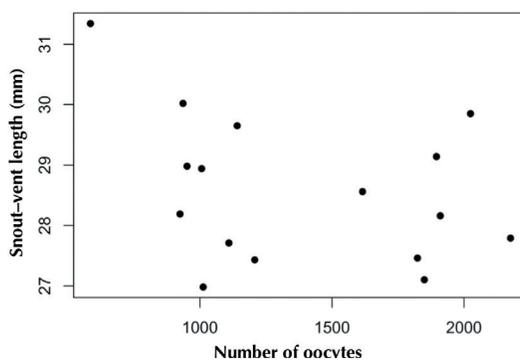


Figure 3. Relationship between size (SVL) and the number of oocytes of *Physalaemus feioi*.

of *P. feioi* were Coleoptera (IRI = 36.37), Formicidae (IRI = 26.77) and unidentified insect larvae (IRI = 17.99). Hymenopterans other than ants and orthopterans occurred only in the Eugenópolis samples, whereas snails were found only in samples from Viçosa. Insect larvae represented the largest volume (39.59%),

Table 2. Diet of *Physalaemus feioi* from Eugenópolis and Viçosa (Minas Gerais state, Brazil). The category “Other” represents remains of arthropods and unidentified material. Number of stomachs containing each category (F); Number of prey; V = volume of prey (mm³). Items with IRI > 10 represented with *.

Prey Category	F	%F	N	%N	V	%V	IRI
ARACHNIDA							
Araneae	8	6.96	8	2.85	4.4	3.56	4.46
INSECTA							
Coleoptera	42	36.52	129	45.91	32.96	26.69	36.37*
Hemiptera	5	4.35	6	2.14	6.7	5.43	3.97
Hymenoptera (Formicidae)	36	31.30	76	27.05	27.11	21.95	26.77*
Hymenoptera (non-Formicidae)	3	2.61	3	1.07	0.46	0.37	1.35
Isoptera	3	2.61	35	12.46	0.16	0.13	5.06
Orthoptera	2	1.74	2	0.71	1.43	1.16	1.20
Unidentified larvae	10	8.70	16	5.69	48.89	39.59	17.99*
GASTROPODA	1	0.87	1	0.36	1.3	1.05	0.76
UNDETERMINED	5	4.35	5	1.78	0.08	0.06	2.06

followed by beetles (26.69%) and ants (21.95%). The numeric frequencies were represented by adult beetles (129 individuals: 45.91%), followed by ants (76 individuals: 27.05%), and termites (35 individuals: 12.46%). Similarly, the first two categories of the occurrence frequencies were adult beetles (36.52%) and ants (31.30%), followed by unidentified insect larvae (2.70%). No significant differences were found between the sexes ($F_{1,59} = 0.19$, $p = 0.66$). We did not find differences in sexes in the number of prey ingested (males: $\bar{x} = 4.39 \pm 3.43$; females: $\bar{x} = 6.10 \pm 9.90$) or the total prey volume (males: $\bar{x} = 2.11 \pm 4.53$ mm³; females: $\bar{x} = 1.46 \pm 1.50$ mm³).

Discussion

The mean number of oocytes in females of *P. feioi* ($\bar{x} = 1385 \pm 498.13$) was large compared to the numbers recorded for other species of *Physalaemus*: *P. maculiventris* (129 eggs; Heyer et al. 1990), *P. signifer* (273 eggs; Wogel et al. 2002), and *P. cuvieri* (474 eggs; Barreto and

Andrade 1995). The mean number of oocytes of *P. feioi* resembled that of *P. kroyeri* (1332 eggs), which has a similar average size (30.2 mm) (Gally and Zina 2013). The number of oocytes in *P. feioi* was smaller than *P. centralis* (1872 eggs), a species with a larger female average size (34.5 mm) (Brasileiro and Martins 2006). It is common to find a positive relationship between the number of eggs and SVL of female anurans, such as in *Leptodactylus natalensis* (Ferreira et al. 2007), *P. centralis* (Brasileiro and Martins 2006), and *P. signifer* (Wogel et al. 2002). However, this relationship was not found for *P. feioi*, indicating that there may be no relationship between the physical status of females of *P. feioi* and the number of oocytes produced.

Although data on prey availability is an important factor in anuran diets (Ceron et al. 2022b), *Physalaemus feioi* is an arthropod feeder, as are most anurans. We found numerous prey categories, even mollusks, in the diet of this species, indicating that it is not a diet specialist (e.g., feeding exclusively on ants). Instead, individuals utilize an abundance of certain

categories in their diet (beetles and ants), particularly those that are abundant in leaf litter in Neotropical environments (Lavelle 1996, Barberena-Arias and Aide 2003, Marinoni and Ganho 2003). Termites and insect larvae were second in the composition of the diet. Other species in the genus [*P. cuvieri* (Santos *et al.* 2004, Silva and Rossa-Feres 2010), *P. cf. cicada* (Santana and Juncá 2007), *P. ephippifer* (Rodrigues and Santos-Costa 2014), *P. biligonigerus* and *P. gracilis* (Oliveira *et al.* 2015), and *P. nattereri* (Silva and Rossa-Feres 2010)] had diets similar to that of *P. feioi*, as did other anurans that feed in leaf litter microhabitats: *Leptodactylus natalensis* (Ferreira *et al.* 2007), *Pristimantis labiosus* (Gutiérrez-Cárdenas *et al.* 2016), *Crossodactylus* spp. and *Hylodes* spp. (Almeida-Gomes *et al.* 2007, Caldart *et al.* 2012). The diet of *P. feioi* differs from several species from Argentina, including *P. albonotatus* from Santa Fe (Falico *et al.* 2012), *P. biligonigerus* from a soybean field in Córdoba (Attademo *et al.* 2007), and *P. santafecinus* from a population near Corrientes (Duré 1998).

Previous reports found sex differences in the numerical and volumetric proportions of the diet and relative importance of the categories of prey consumed by *Leptodactylus fuscus* and *L. mystacinus* (De-Carvalho *et al.* 2008), species with sexual dimorphism. Diets of males and females of *P. feioi* were similar, both qualitatively (categories of prey) and quantitatively (IRI, number of prey ingested, total prey volume), despite the sexual dimorphism detected in body size. A possible cause for the similarity observed might be the spatial distribution of individuals at the collection sites, as both males and females were observed and collected in the same microenvironments (e.g., leaf litter, in the water, on fallen logs in the water). Similarities between diets of males and females have also been observed for other species of anurans, including *Lithobates catesbeianus*, *Physalaemus nattereri*, *P. cuvieri*, *P. ephippifer*, *Leptodactylus podicipinus*, and *Eleutherodactylus planirostris* (Ferreira *et al.* 2007, Silva *et al.* 2009, Silva and

Rossa-Feres 2010, Rodrigues and Santos-Costa 2014).

Our results indicate that *Physalaemus feioi* was similar to other congeneric species in relation to sexual dimorphism, fertility, and diet. Studies that focus on aspects of natural history aid in understanding key requirements affecting conservation strategies for anurans.

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