Morphometric variation of *Pipa pipa* (Linnaeus, 1758) (Anura: Pipidae) with notes on diet and gonad development

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Abstract. *Pipa pipa* is an emblematic and endemic aquatic frog from the Amazonian Basin, but much of its biology remains poorly known. In this paper we focused on morphometric variation, diet and gonad examination of *P. pipa*. We measured 23 morphometric variables in 56 individuals of *P. pipa* and tested for sexual dimorphism and for geographic variation using Principal Component Analysis. Data on diet and gonad development were studied in one population. The morphometric study showed that forearm diameter, finger and snout length are sexually dimorphic characteristics, all larger in males. The most abundant item found in the species’ diet was Cyclopoida, whereas Heteroptera represented the group with the highest occurrence in stomachs.

Keywords: Pipidae; sexual dimorphism; diet; morphology; reproduction

Introduction

The frog family Pipidae occurs in Africa and America and includes peculiar aquatic species (Canatella and Trueb, 1988; Duellman and Trueb, 1994; Frost et al., 2006). It is monophyletic and one of the most basal anuran groups (Canatella and Trueb, 1988; Frost et al., 2006, Irisarri et al. 2011). Among the four genera in the family (*Hymenochirus, Pipa, Pseudhymenochirus* and *Xenopus*), *Pipa* is the only genus occurring in Central and South America (Dunn, 1948; Canatella and Trueb, 1988; Frost, 2011), from which four out of seven species occur in Brazil - *P. arrabali* Izecksohn 1976, *P. snethlageae* Müller 1914, *P. carvalhoi* (Miranda-Ribeiro, 1937), and *P. pipa* (Trueb and Cannatella, 1986).

*Pipa pipa*, the largest species in the genus (Dunn, 1948; Rabb, 1961), has distinct external, osteological and behavioural characteristics (Trueb and Massemín 2000). The reproductive behaviour involves a turnover action, during which the couple swims in vertical circles in the water together while the eggs are released and laid on the female back (Rabb and Rabb, 1960; Rabb and Snedigar, 1960; Rabb, 1961). Males are smaller than females and have thicker forearms (Zippel, 2006). The female’s vent is rounder and more turgid, especially during reproductive periods (Rabb and Snedigar, 1960; Rabb, 1961; Zippel, 2006). However, no other characteristics have been investigated regarding sexual dimorphism and those listed are either subjective or observed only during reproductive periods.

Aspects of the biology of *Pipa pipa* other than amplexus behaviour have also been neglected and most information available comes mainly from captive individuals (Zippel, 2006). Literature on diet of the species recorded fish as well as anurans of genus *Leptodactylus* (Deckert, 1917; Duellman, 1978). During foraging, the species uses a combination of inertial suction and forelimb movements, and sometimes prey can be grasped between digits (Carreno and Nishikawa, 2010).

Reproductive biology and diet are relevant traits of natural history that affect survival, define habitats and behaviours (Cuello et al., 2006). The lack of information on the ecology and biology of species is usually the largest obstacle to management projects and conservation of anuran fauna (Greene, 2005; Silvano and Segalla, 2005; Verdaade et al., 2012). The aim of
this study was to identify sexual dimorphism from morphometric characteristics in the species *Pipa pipa* and geographical variation on morphometric variables between populations. We also provide notes on its diet and gonad development.

**Materials and Methods**

We studied 61 individuals of *Pipa pipa* from three different sources: individuals (n=32) collected in January 2007 near the left bank of Abacaxis River, São Sebastião village, Borba municipality, Amazonas state, Brazil (4º18’32” S, 58º 38’11” W) (MZUSP 142744–775); individuals (n=4) collected in April 2010 at the right bank of Madeira river, Abunã village, Porto Velho municipality, Rondônia state, Brazil (9º37’45” S, 65º 27’19” W) (MZUSP 143285–286, 144395–396); and individuals (n=25) from different localities, deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP) collection (MZUSP 1006, 2036, 15903, 16066, 16068, 23062, 23119, 23215, 23374–76, 23795, 37519, 54799–800, 58534–35, 80450, 80454–55, 80474–78).

The sample from São Sebastião village (Abacaxis River) was assembled from a pond approximately six meters long by six meters wide and 30 centimetres deep, in an area susceptible to seasonal flooding. The water was muddy, and leaf litter and mud covered the bottom. The stream where individuals from Abunã village were found was approximately one meter deep and three meters wide, and had similar aspects as the São Sebastião pond. All individuals were euthanized using a lethal dose of anaesthetic, preserved in 10% formalin and stored in 70% ethanol.

**Morphometric studies**

We tested sexual dimorphism in 32 individuals from the São Sebastião sample, in which sex was determined by gonad examination. The other samples were not included in this analysis since gonad examination was not possible. Sexual maturity was determined based on the snout-vent length (SVL) and gonad development. According to Schuette and Ehrl (1987), only individuals larger than 60 mm are considered adults. Although sexual maturity may vary from one population to the other, we still considered this size limit since all individuals were larger than 60 mm with the exception of one, a 36 mm juvenile.

Thirteen measurements were obtained and analysed in this study: snout-vent length (SVL); body height (BOH) taken from dorsal to the ventral side of the body considering an imaginary mid-line between head and urostyle; tibia length (TIL); eye diameter (ED); hand length (HL); fingers 1 to 4 length (F1 – 4 L); foot length (FOL) (Verdade and Rodrigues 2007); mouth (Mt); forearm length (FL) (Márquez-Garcia et al. 2009); forearm diameter (FD) from dorsal to the ventral side in an imaginary mid-line between elbow and hand; toes 1 to 5 length (T1 – 5 L) from base to the tip of each toe; interdigital membranes 1 to 4 length (IM1 – 4 L) from the base to the tip considering an imaginary mid-line between toes; and snout length (SL) from the tip of the mid-line of the jaw to the tip of the snout. We measured variables on the left side of all individuals, with a digital calliper.

To test sexual dimorphism we used variables’ residuals from a regression analysis in order to avoid biases in morphometric comparisons. Levene’s and Shapiro Wilk tests were performed to test normality and residual’s variance. A One-way ANOVA was made with normal and homogeneous data, whereas a Mann-Whitney U test was performed with the nonparametric residuals.

We studied morphological geographic variation using all three samples: from São Sebastião, Abunã and previously specimen deposited at MZUSP, totaling 61 individuals (one young excluded from the original sample) of *Pipa pipa* from 12 different localities. We performed Principal Component Analysis to explore any group formation based on morphometric variation. All variables listed earlier in this study were included in the analysis. Statistics was performed in SPSS 20.0 for Windows.

**Gonad development**

Gonadal studies were restricted to the sample of São Sebastião, since we could not examine internal anatomy from the other populations. Testis length, diameter and volume were measured and compared to male’s weight and size using correlation analysis. Diameter, weight and number of developed ova were measured.

**Diet**

Dietary studies were also restricted to the sample of São Sebastião, since we could not remove stomach contents from the other populations. The approximated time between collection of animals and fixation was of one day. All individuals had their gastric and intestinal contents analysed under a stereoscope microscope. Prey length and diameter were measured, and prey category identified to the level of Order. Degraded items were not considered because they could not be identified.
The frequency of appearance of each prey, as well as the occurrence of stomachs that contained each prey was determined. Prey volume was approximated to the ellipsoid volume, calculated based on the formula of a prolate spheroid $V = \frac{4}{3}\pi \frac{L}{2} \left(\frac{W}{2}\right)^2$, where $V=\text{volume}$, $L=\text{length}$, and $W=\text{width}$.

Results

Morphometric studies

We found 17 males and 15 females in the São Sebastião sample. The One-way ANOVA showed that males and females do not differ on snout-vent length ($p=0.650, F=0.210$), but forearm diameter (FD) ($p=0.00, F=26.833$), finger 2 length ($p=0.00, F=12.522$) and snout length (SL) ($p=0.03, F=10.614$) were significantly different between males and females. SL represented between 3.4 to 4.8% of SVL in females, whereas in males it ranged from 3.8 to 5.4% of SVL.

To investigate the geographic variation in morphometric variables of *Pipa pipa*, data were taken from 12 localities (Figure 1), nine of which were not registered by Vaz-Silva and Andrade (2009), who summarized the distribution of the species. Differences between groups did not clearly split populations. According to the Principal Component Analysis, the

![Figure 1. Geographic distribution of *Pipa pipa* based on literature data (Vaz-Silva and Andrade 2009) and on our data.](image-url)
first component explained 47.98% of the variation, and the second explained 26.93%. SVL (0.846), mouth, (Mt) (0.838) and foot length (FOL) (0.834) were the variables that most contributed to the separation of groups in the score 1, whereas finger 2 length (F2L), finger 1 length (F1L) and finger 4 length (F4L) (0.721; 0.715; 0.712) contributed the most in score 2 (Figure 2).

Gonad development

Testis volume increased positively and proportionally with weight ($r^2 = 0.909$, $p=0.035$) and SVL ($r^2 = 0.807$, $p=0.001$). The two mature females (with developed ova, ready to reproduce; MZUSP 142751, 142768) had large, yellow ova, in which the vegetative and the animal poles had the same colour (Figure 3, Table 1). Inside the ovary, smaller ova were found, and the oviduct occupied a great portion of the visceral cavity. In immature females the ovary was light yellow and long, parallel to the kidneys.

Diet

Only 15 out of the 32 digestive tubes examined contained identifiable items (Table 2). Diet contained mainly arthropods, of which Cyclopoida was more abundant than other items and had the highest frequency (67%), followed by Diptera larvae (7.3%) and Heteroptera (6.3%). Heteroptera showed the highest occurrence in stomachs. Even though decapods were not abundant (3.1%), they represented the higher volume (61.9 mm$^3$), whereas Cyclopoida represented only 8.5 mm$^3$ (Table 2). Skin fragments were found in three stomachs, and their appearance could either indicate these have been from an anuran of the genus *Rhinella* Fitzinger 1926 or from its own individual of *Pipa pipa*, since many amphibians are known to eat their own skin (dermatophagy) (Weldon et al. 1993).

Discussion

Morphometric studies

Forearm diameter and snout length were the only sexually dimorphic characteristics in *Pipa pipa*, both larger in males. Forearm diameter was previously reported in the literature as sexual dimorphic in *Pipa pipa* (Zippel, 2006), but the longer snout is a novelty that may help to externally distinguish sex of individuals. According to Zippel (2006), literature shows that SVL is larger in females, whereas we found no differences between sexes. These contradictory results may be due
to the age of the individuals in the samples studied: the size of the ovule found in females from São Sebastião were smaller than the fully developed ones described in literature (Rabb and Snedigar, 1960), and therefore probably under development (Monnet and Cherry, 2002).

In some anurans males are significantly larger than females, especially when males are territorial and fight each other (Shine, 1979; Woolbright, 1983). In *Pipa pipa* males are extremely territorial and fight frequently. Rabb and Rabb (1963) described the vocalization and physical contact during a fight, in which mainly the head and forearm were used. This might explain the larger forearm diameter in males from São Sebastião. Males with hypertrophied forearms are common among anurans (Duellman and Trueb, 1994), and Liao et al. (2012) showed that forelimbs tend to be stronger in amplexant male specimens of the species *Bufo andrewsi*, helping to keep the grip on the female during amplexus.

Some dimorphic structures may be used in competition for mates between males as a strategy during contests, pushing or holding the contender. However, in some cases the structures may indicate male’s size, strength and fitness without being effectively used in combat (Willemart and Gnaspini, 2003). Snout length is an example of a structure that is not directly used in combat, but that could indicate size superiority.

The morphological variation among samples studied may be suggestive of geographic variation. Nevertheless, considering our limited geographic covering, this could only be confirmed with additional sampling comprising a larger number of individuals per locality.

**Gonad development**

Rabb and Snedigar (1960) found ova with an average diameter of 6 mm in females that spawned between 96 and 273 eggs. Even though the number of ova recorded in the literature is similar to the number we found, their sizes suggest that females from this study were not fully ready for reproduction. Smaller ova as well as the low number of reproductive females in our sample may be explained either by the seasonality (early rainy season, when this species reproduce), or due to young age of the population sample.

**Diet**

According to the results, *Pipa pipa* has an opportunist diet related mainly to the aquatic environment. Literature recorded animals captured in nature to feed on fish - *Erythrinus erythrinus* (Bloch and Schneider...
1801) (Duellman, 1978) and a frog genus *Leptodactylus* Fitzinger 1826 (Deckert, 1917). In captivity it was recorded to feed on different types of items, from bovine meet to copepods (Rabb and Snedigar, 1960; Rabb, 1969; Palmer, 1994).

The little amount of preys found in the sample studied in this work may be a consequence both of the gap time between the animals were collected and euthanized, allowing the preys to be digested; or scarcity of prey items due to the season, since the water level of the ponds may influence the frequency and availability of prey in the habitat, as well as competition among individuals (Deckert, 1917; Duellman, 1978; Palmer, 1994). Nevertheless, composition of *Pipa pipa*’s diet was similar to other species in the same genus: *P. aspera, P. arrabali, and P. carvalhoi* were all recorded to have ingested arthropods, such as nymphs and larvae of insects, and aquatic invertebrates, as well as flying and terrestrial prey (Trueb and Massemin 2000, Canedo et al., 2006; Garda et al. 2006). The latter may indicate either that these fall into the pond or that these species go forage in land (Buchacher, 1993).

**Conclusions**

This study presented some new information about the species *Pipa pipa*, such as new sexually dimorphic morphometric structures, as well as its occurrence in new geographic locations. Furthermore, it added preliminary information about geographic morphometric variation among populations, suggesting further studies with larger samples should be informative. Finally, although seasonality may have interfered in our sample, more information was provided regarding *Pipa pipa*’s diet and gonad characteristics.

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