

AUTOECOLOGY OF CARVALHO'S SURINAM TOAD (*PIPA CARVALHOI*: ANURA, PIPIDAE) FROM NORTHEASTERN BRAZIL

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Abstract.—Studies on the ecology and natural history of many amphibian species are often limited to data from single populations, revealing the need for new information from different populations. We analyzed the diet composition, sexual dimorphism, and the presence of helminths in Carvalho's Surinam Toad (*Pipa carvalhoi*) from northeastern Brazil. Additionally, we present an updated distribution map for the species. Carvalho's Surinam Toad exhibits a disjunct distribution, with occurrence records concentrated in three areas: (1) north of the São Francisco River in the Caatinga biome; (2) between the Contas and Paraíba do Sul Rivers; and (3) in Cerrado regions in eastern Bahia State. The most important prey items were insect larvae, hemipterans, and ants, indicating a narrow dietary niche breadth. The most significant morphological variables in distinguishing between sexes were foot length, snout-vent length, head length, eye-nostril distance, and forearm length. No helminths were found infecting the species. The diet and body size of individuals are consistent with previous reports; however, the presence of terrestrial prey and the observed sexual dimorphism in certain traits suggest possible terrestrial activity and raise questions about sexual selection involving other characteristics in this species. Lastly, we report the first documented case of predation on the invasive fish Nile Tilapia (*Oreochromis niloticus*) by Carvalho's Surinam Toad.

Key Words.—diet; endoparasites; geographical distribution; natural history; sexual dimorphism.

INTRODUCTION

For centuries, researchers worldwide have aimed to describe various aspects of the natural history of more than two million known species (Bánki et al. 2023; Nanglu et al. 2023). These efforts stem from the understanding that such information is a valuable source of fundamental knowledge about the biology of these organisms, providing a foundation for the development of numerous other studies (Hampton and Wheeler 2012). Knowledge of key ecological attributes, such as diet composition, habitat use, reproductive traits, and distribution, is essential for understanding how species interact with their environment and how environmental changes impact their population dynamics (Benson 2000; Tosa et al. 2021). This information has become increasingly critical in our current era, as climate change and habitat alteration are driving many species toward

extinction globally (Duarte et al. 2012; Liu et al. 2022).

Brazil is a megadiverse country, home to a significant portion of the biodiversity of the world (Lewinsohn and Prado 2005). For anurans alone, 1,141 species have been documented, representing 14.6% of anuran species in the world (Frost 2021; Segalla et al. 2021), with new species frequently being described (Guerra et al. 2020). This impressive diversity can be attributed to the vast territorial expanse and the presence of multiple biomes in Brazil, characterized by large variation in vegetation, climate, and geomorphology, which have facilitated a complex diversification process (Rossa-Feres et al. 2017). Our current knowledge of species existence, however, is not matched by an equally comprehensive understanding of their biology (Silvano and Segalla 2005). Despite considerable progress in addressing this gap, studies on the ecology and natural history

of Brazilian amphibians are often limited to isolated data from single populations (see Camurugi et al. 2017; Protázio et al. 2019; Nascimento et al. 2022). This highlights a significant gap in ecological data across the distribution ranges of many species.

The Carvalho's Surinam Toad (*Pipa carvalhoi*; Miranda-Ribeiro 1937) is in the Pipidae family and is distributed across the Caatinga and Atlantic Forest biomes in northeastern Brazil, occurring in the states of Alagoas, Bahia, Ceará, Espírito Santo, Minas Gerais, Paraíba, Pernambuco, and Sergipe (Santana et al. 2014; Ferreira et al. 2021). Recently, Ferreira et al. (2021) reported a population of this species isolated in Cerrado areas, near transition zones with the Caatinga. Members of Pipidae exhibit a remarkable life history, characterized by an aquatic lifestyle, a flattened body, underwater vocalization, and unique parental care, in which eggs and sometimes even tadpoles develop within the skin of the females (Weygoldt 1976; Büten et al. 1992; Fernandes et al. 2011). Phylogenetic studies have identified three lineages within *P. carvalhoi*, corresponding to the major hydrographic basins of northeastern Brazil: the North-Eastern Atlantic River basin, the São Francisco and East Atlantic River basins, and the South-Eastern Atlantic River basin (Lima et al. 2020; Fouquet et al. 2022). Moreover, morphometric differences observed among the tadpoles of these three lineages (Lima et al. 2020) underscore the need for further studies across different populations of *P. carvalhoi* to better understand the morphological and genetic variation observed across the geographic range of the species.

Ecological information on *P. carvalhoi* has largely focused on its reproductive biology (Weygoldt 1976; Büten et al. 1992; Fernandes et al. 2011), with some sporadic data on its geographic distribution (Santana et al. 2014; Ferreira et al. 2021); however, knowledge of the diet composition of *P. carvalhoi* remains limited. Weygoldt (1976) briefly mentioned the species consumes insect larvae, fish, and even tadpoles while Canedo et al. (2006) analyzed dietary variation among males, females, and juveniles in a population from Ilhéus, located in the southeastern Atlantic Forest of Bahia State. Although valuable, these studies lack ecological data from the Caatinga and regions north of the São Francisco River, limiting our ability to make ecological and behavioral comparisons that could shed light on population or species adaptations across their range.

The Caatinga biome is an exclusively Brazilian semi-arid ecosystem characterized by regular cycles

of drought and concentrated rainfall (Silva et al. 2017). Recent studies indicate that the Caatinga is increasingly experiencing the effects of climate change, leading to more intense and prolonged drought episodes (Marengo et al. 2017). This trend underscores the urgent need to enhance our understanding of the biology of organisms in this ecosystem, particularly anurans, whose reproduction and activity are closely linked to the rainy season. We provide new ecological insights into Carvalho's Surinam Toad from northeastern Brazil. Our goal is to expand knowledge of the ecology of the species, addressing gaps in information, particularly in populations from the northern portion of its range. Specifically, we describe its diet composition, investigate sexual dimorphism, and present an updated map of Carvalho's Surinam Toad occurrence. Additionally, we discuss the incidence of endoparasites in some populations.

MATERIALS AND METHODS

To gather occurrence records of *P. carvalhoi*, we conducted a comprehensive search across multiple sources, covering the entire available time period in each: (1) the Herpetological Collection at the Universidade Federal da Paraíba, Brazil; (2) the SpeciesLink database (specieslink.net); (3) the Brazilian Biodiversity Information System (SiBBR) database; (4) the Global Biodiversity Information Facility (GBIF) database; (5) the iNaturalist platform (inaturalist.org); and (6) the scientific literature. For GBIF data, we used the Occurrences plugin integrated in QGIS 3.16 software. iNaturalist is an open-access platform that provides photographs and occurrence data of various organisms, contributed by the community, and has been increasingly used in scientific research (Forti et al. 2022). When sourcing data from iNaturalist, we filtered for information suitable for scientific research and conducted a visual inspection of the images to confirm species identification. We cross-checked data from all six sources to eliminate redundant records, retaining only those that provided consistent information on occurrence locations. As some records showed little variation in the coordinate values within the same locality, we used the clean points function from the Mappinguari package in R (Caetano et al. 2019) to eliminate records within 5 km of each other.

We analyzed ecological data from *P. carvalhoi* specimens deposited in the Herpetological Collection of the Universidade Federal da Paraíba

(CHUFPB). The specimens were collected in several municipalities in northeastern Brazil (Fig. 1; Appendix Table). To obtain data on diet composition, we removed the stomach of each *P. carvalhoi* individual through an incision in the ventral region and analyzed the food items in a Petri dish under a Leica EZ4 stereomicroscope (Leica Microsystems, Wetzlar, Germany). We identified all items to order, except for ants (classified as Formicidae). Because precise identification was impossible for some items, we considered items that were in an advanced state of digestion as unidentified. We counted the number of stomach contents to determine their numerical frequency ($N\%$). Intact prey items had their length (l) and width (w) measured with digital calipers (accurate to 0.01 mm) to estimate volumetric frequency ($V\%$), using the ellipsoid formula (Vitt and Colli 1994):

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

Subsequently, the frequency of occurrence for each prey category ($F\%$) was calculated as the ratio of the total number of stomachs containing prey category i to the total number of stomachs analyzed. The significance of each item in the diet was then assessed by calculating the Index of Relative Importance (IRI) for each prey category, using the formula provided by Pinkas et al. (1971):

$$IRI = (N\% + V\%) \times F\%.$$

We calculated the diet niche breadth using the inverse of Simpson's Diversity Index (Simpson 1949):

$$B = \frac{1}{\sum_{i=1}^n p_i^2}$$

where p represents the proportion of resource category i used and n is the total number of categories. Values for this index range from 1 (indicating a specialist) to the total number of identified categories (indicating a generalist). We also examined the presence of helminth parasites in 38 individuals from the municipalities of Buíque, Barbalha, and Serra Caiada, located in the states of Pernambuco, Ceará, and Rio Grande do Norte, respectively. This was accomplished through the examination of stomachs, intestines, lungs, livers, and body cavities using a stereomicroscope.

We determined the sex of individuals through dissection and direct observation of the gonads or the presence of eggs on the backs of females. We

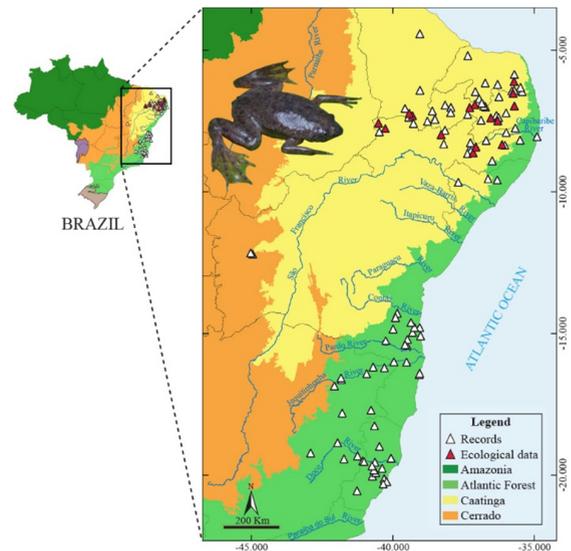


FIGURE 1. Distribution of Carvalho's Surinam Toad (*Pipa carvalhoi*) showing the occurrence records from the Herpetological Collection of Universidade Federal da Paraíba, Specieslink database, Brazilian Biodiversity Information System (SiBBR) database, Global Biodiversity Information Facility (GBIF) database, iNaturalist, and literature, as well as the most important rivers of eastern Brazil. Red triangles represent locations where we obtained diet, morphometric, and parasite data from individuals of the species.

considered individuals for which we could not determine the sex juveniles and we excluded them from the analysis. For the morphometric analysis, we measured 13 variables using digital calipers (precision of 0.01 mm) and a stereomicroscope, following the methodology outlined by Duellman and Trueb (1994). The measured variables included: snout-vent length (SVL), head length (HL), head width (HW), eye-nostril distance (END), interorbital distance (IOD), internarial distance (ID), thigh length (ThL), tibia length (TL), tarsus length (TaL), foot length (FoL), eye diameter (ED), forearm length (FaL), and arm diameter (AD).

The effect size generated by isometric variation was mitigated by creating a body size variable (BS), which accounted for the total partition of variation in size and shape among individuals (Somers 1986). We derived body size values from the equation

$$S = \sum_{i=1}^n p^{-0.5} \cdot x_i$$

where p represents the number of variables (Jolicoeur 1963), multiplied by each observation and then summed over all observations. To remove the effects of body size in our data matrix, we post-multiplied

the $n \times p$ matrix of log₁₀ transformed data by a $p \times p$ symmetric matrix, L , defined as follows (Burnaby 1966):

$$L = I_p - V(V^T V)^{-1} V^T$$

where I_p is a $p \times p$ identity matrix, V is the isometric size eigenvector defined above, and V^T is the transpose of matrix V (Rohlf and Bookstein 1987). The resulting variables were defined as shape variables.

To assess the contribution of each morphometric variable in distinguishing between females and males, we used the Random Forest algorithm (Liaw and Wiener 2002). Random Forest is a machine learning tool that employs decision trees generated through randomization (e.g., bootstrap sampling) to create classifiers and identify optimal splits from these random samples (Breiman 2001). The algorithm uses training data to estimate error rates by predicting outcomes for data not included in the bootstrap samples, referred to as out of bag samples (OOB; Breiman 2001), thereby enhancing the accuracy and reliability of the analysis.

We employed the proximity matrix from the Random Forest model as a measure of similarity between males and females. Additionally, we followed the procedures of Mesquita et al. (2015) to perform a One-way Analysis of Variation (ANOVA) on the body size variable and a Logistic Regression on the shape variable to verify the existence of differences between males and females. We compared the full model (including all shape variables) against a null model (intercept-only) and used a Chi-square Test of the scaled deviance to assess its statistical significance. We used the Akaike Information Criterion (AIC) to select the set of shape variables that best discriminate between males and females. Next, we assessed the misclassification error of the reduced model using 1,000 bootstrap replications of a Linear Discriminant Analysis. For the Random Forest analysis, we used the RandomForest package (Liaw and Wiener 2002), setting the number of trees to 1,000 and the number of variables to try at each split to three. The function createDataPartition from the caret package (Kuhn et al. 2022) was used to create the test/training partition for the Random Forest algorithm. We used the MASS package (Ripley et al. 2025) for Logistic Regression and Linear Discriminant Analyses.

All morphometric variables were logarithmically transformed (\log_{10}) to achieve normality. We assessed the normality of our morphometric data for both univariate (using boxplots and the Shapiro-Wilk

test) and multivariate (using the Henze-Zirkler Test) distributions, employing the MVN package (Korkmaz et al. 2021). We performed the Random Forest algorithm, One-way ANOVA, Logistic Regression, and Linear Discriminant Analysis using R software (R Core Team 2022), conducting all statistical tests at an alpha level of 0.05.

RESULTS

We identified 111 locations with occurrence records of *P. carvalhoi*, revealing a pattern of disjunct distribution (Fig. 1). The occurrence records are concentrated in three main areas: (1) north of the São Francisco River, within the Eastern Northeast Atlantic and São Francisco River basins; (2) between the Contas and Paraíba do Sul Rivers, in the central and southern portions of the East Atlantic basin and the northern portion of the Southeast Atlantic basin; and (3) near the Grande River in a Cerrado area, in western Bahia State, still within the São Francisco River basin. For diet, we analyzed 82 individuals, of which 38 (46.3%) contained food items in their stomachs. We identified 185 prey items distributed across 12 categories (Table 1). Insect eggs were the most numerous prey items, followed by hemipterans and insect larvae. In terms of volume, fish were the most significant item, followed by insect larvae and hemipterans. Insect larvae were the most frequently encountered items, followed by hemipterans and ants. The Relative Importance Index indicated that insect larvae, Hemiptera, and Formicidae were the most important prey categories in the diet of *P. carvalhoi*. The niche breadth analysis revealed low values, suggesting more specialized feeding behavior.

We identified 60 females and 56 males, and we could not determine the sex of 33 individuals. Females exhibited a slightly greater average snout-vent length (SVL) of 45.68 ± 10.47 (standard deviation) mm compared to males, which averaged 42.86 ± 8.34 mm (Table 2), but there were no differences in the body size between males and females ($F_{1,114} = 0.370$, $P = 0.545$). Multidimensional scaling using the similarity matrix produced in the random forest analysis with proximity scores revealed considerable morphological overlap between males and females, with no distinct groups formed (Fig. 2). The OOB was 20.7%, indicating moderate classification error values (Table 3).

We identified a smooth, sharp transition in the Gini and Accuracy indices that would indicate a weak influence of any particular variable, showing that

TABLE 1. Diet composition and Index of Relative Importance of 38 individual Carvalho’s Surinam Toads (*Pipa carvalhoi*) from northeastern Brazilia. Abbreviations are N = number of preys items, N% = numerical frequency, V = volume, V% = volumetric frequency, F = occurrence, F% = frequency of occurrence, IRI = Index of Relative Importance.

Categories	N	N%	V	V%	F	F%	IRI
Arthropoda							
Arachnida							
Acari	1	0.5	0.1	0.0	1	1.2	0.67
Aranae	2	1.1	1.8	0.1	2	2.4	2.82
Hexapoda							
Coleoptera	6	3.2	74.3	3.0	4	4.9	30.63
Diptera	6	3.2	24.5	1.0	6	7.3	31.04
Hemiptera	34	18.4	147.8	6.0	12	14.6	357.32
Lepidoptera	1	0.5	28.3	1.2	1	1.2	2.07
Orthoptera	8	4.3	3.1	0.1	5	6.1	27.15
Insect eggs	71	38.4	147.6	6.0	2	2.4	108.32
Insect larvae	29	15.7	465.8	19.0	18	22.0	761.96
Formicidae	17	9.2	15.1	0.6	10	12.2	119.59
Unidentified	5	2.7	62.5	2.6	5	6.1	32.06
Plant material	4	2.2	61.9	2.5	4	4.9	22.90
Vertebrate							
Fish (<i>Oreochromis niloticus</i>)	1	0.5	1,413.9	57.8	1	1.2	71.13
Numeric breadth				1.63			
Volumetric breadth				1.26			

the variables are not very different from each other. Nevertheless, the variables that had the greatest contribution to the separation between females and males were eye-nostril distance, followed by forearm length and head length (Fig. 2). There were significant differences between males and females in body shape ($\chi^2 = 61.69$, $df = 13$, $P = 0.001$). In contrast, the model selection procedure indicated

that foot length, snout-vent length, head length, eye-nostril distance, and forearm length were the most important variables in distinguishing between sexes (Table 4). The Linear Discriminant function using the five selected variables had a misclassification error of 0.18 based on 100 bootstrap replications (95% confidence interval = 0.10–0.28). Snout-vent length, eye-nostril distance, foot length, and head

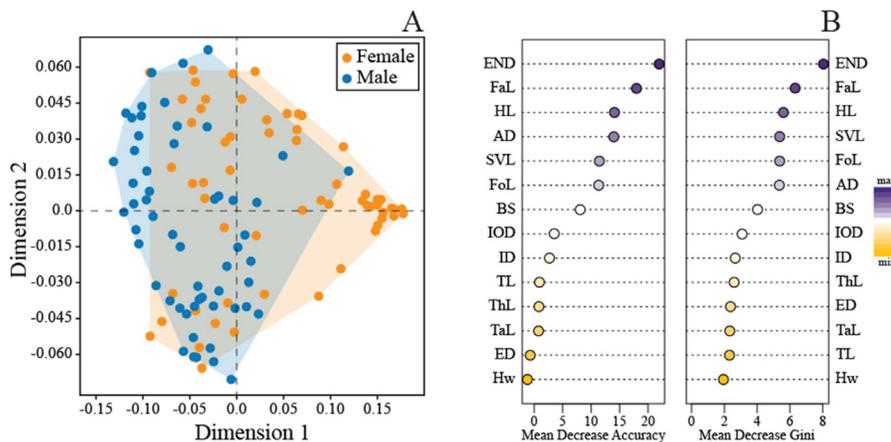


FIGURE 2. Results from Random Forest algorithm used to analyze the existence of morphometric differences between females and males of Carvalho’s Surinam Toad (*Pipa carvalhoi*) from Brazil. (A) First and second dimensions of the multidimensional scaling of the proximity scores. (B) Plots of the decrease in Accuracy and decrease in Gini index showing the variables with more importance (Abbreviations in Materials and Methods).

TABLE 2. Morphometry variables (mm) of 14 measurements of Carvalho’s Surinam Toad (*Pipa carvalhoi*) from the northeast of Brazil. Values above parentheses represent mean ± standard deviation. Values in the parentheses represent the minimum and maximum values. The body size variable was obtained from log-transformed variables.

Variables	Female (n = 60)	Male (n = 56)
Body size variable	3.34 ± 0.33 (2.73–3.97)	3.30 ± 0.32 (2.47–3.81)
Snout-vent length	45.68 ± 10.47 (28.54–70.04)	42.86 ± 8.34 (24.90–63.07)
Head length	10.37 ± 2.09 (6.54–15.30)	9.65 ± 1.81 (5.89–13.02)
Head width	12.47 ± 2.35 (7.95–16.52)	12.04 ± 2.22 (7.18–17.93)
Inter-orbital distance	5.78 ± 1.04 (4.04–8.40)	5.46 ± 0.93 (3.99–7.44)
Eye-nostril distance	3.98 ± 0.82 (2.59–5.78)	3.71 ± 0.73 (2.34–5.33)
Internarial distance	2.18 ± 0.33 (1.48–2.87)	2.08 ± 0.32 (1.50–2.95)
Eye diameter	2.59 ± 0.43 (1.68–3.67)	2.51 ± 0.36 (1.63–3.19)
Thigh length	19.85 ± 4.10 (12.06–30.29)	18.96 ± 3.73 (9.27–25.48)
Tibia length	18.62 ± 4.10 (11.70–27.89)	17.92 ± 3.72 (9.15–25.88)
Foot length	20.56 ± 4.89 (11.61–31.13)	19.37 ± 4.23 (10.43–27.73)
Tarsus length	11.34 ± 2.31 (6.63–16.14)	11.03 ± 2.13 (6.42–15.16)
Forearm length	8.59 ± 2.14 (4.59–14.55)	8.99 ± 2.37 (4.34–13.17)
Arm diameter	2.74 ± 0.99 (1.55–5.08)	3.28 ± 1.17 (1.18–6.86)

length were greater in females, while forearm length was larger in males. Finally, we found no helminths parasitizing *P. carvalhoi*.

DISCUSSION

Our search for *P. carvalhoi* revealed a disjunct distribution pattern consistent with findings in the literature (Santana et al. 2014; Lima et al. 2020; Ferreira et al. 2021; Frost 2021). This distribution is consistent with the genetic variation pattern observed by Lima et al. (2020), who proposed the existence of three distinct lineages of *P. carvalhoi* corresponding to some river basins in eastern Brazil. The species appears to inhabit two disjunct areas in the Caatinga and Atlantic Forest, one northward from the São Francisco River, primarily occupying Caatinga regions with streams and temporary ponds, and one

TABLE 3. Confusion matrix showing the classification performance between females and males of Carvalho’s Surinam Toad (*Pipa carvalhoi*).

	Female	Male	Classification Error
Female	49	11	0.183
Male	13	43	0.232

between the Contas and Paraíba do Sul rivers mostly in Atlantic Forest areas.

The population inhabiting Cerrado areas transitioning with the Caatinga in western Bahia (Ferreira et al. 2021) prompts new considerations regarding the distribution of *P. carvalhoi*. This Cerrado population was found near the Rio Grande, one of the main tributaries of the São Francisco River. Given that the regions bordering the São Francisco River in northern Bahia and central-southern Pernambuco are considered some of the least sampled

TABLE 4. Model selection and importance of shape variables as predictors of sex in *Pipa carvalhoi*. The best model is the final model selected based on the Akaike Information Criterion (AIC). Values represent the coefficients of each variable in the respective model. Importance refers to the relative (%) contribution of each variable, calculated from the absolute values of the coefficients. Asterisks (*) indicate significant coefficients ($P \leq 0.05$). Abbreviations are SVL = snout–vent length, HL = head length, HW = head width, END = eye–nostril distance, IOD = interorbital distance, ID = internarial distance, ThL = thigh length, TL = tibia length, TaL = tarsus length, FoL = foot length, ED = eye diameter, FaL = forearm length, and AD = arm diameter.

	Model			
	Full	Importance	Best	Importance
Intercept	27.8		27.7	
SVL	65.2	5.9	-31.8*	21.2
HL	66.6	6	-31.5*	21
HW	96.8	8.7	–	–
END	63.4	5.7	-30.0*	20
IOD	93.1	8.4	–	–
ID	95.7	8.6	–	–
ThL	97.9	8.8	–	–
TL	76.1	6.8	–	–
TaL	98.8	8.9	–	–
FoL	58.8	5.3	-36.7*	24.5
ED	90.4	8.1	–	–
FaL	114.3	10.3	20.0*	13.4
AD	95.8	8.6	–	–
AIC		127		112.8
χ^2		61.7		59.8
<i>P</i>		< 0.001		< 0.001

areas within the Caatinga (Garda et al. 2017), we do not rule out the possibility that increased research in these regions may uncover additional populations of the species.

The diet of *P. carvalhoi* from Caatinga populations primarily consisted of aquatic insects, although ants were also important in the diet. This finding is consistent with the diet recorded for other populations in the Atlantic Forest, where aquatic dipteran larvae were identified as the most significant food items (Weygoldt 1976; Canedo et al. 2006). Additionally, when comparing the diet of *P. carvalhoi* with that of other congeners, we observe similarities regarding the main food items. Garda et al. (2006) found that dipteran pupae constituted the primary food source for Arrabal's Surinam Toad (*P. arrabali*) in Serra do Cachimbo, located in southwestern Pará State, Amazon. Similarly, Measey and Royero (2005) reported insect larvae as the main food items for a population of the invasive Dwarf Toad (*Pipa parva*) in Venezuela. These similarities suggest a dietary pattern across different species within the genus showing a preference for aquatic insect larvae.

Despite these strong similarities, important differences were also observed and warrant mention. Unlike findings in other studies, hemipterans and ants were of high importance in the diet of *P. carvalhoi* in northeastern Brazil, with hemipterans showing a higher numerical frequency than insect larvae. Furthermore, there are consistent records of tadpole and fish consumption by other species in the genus *Pipa* (Gines 1958; Gascon 1992; Buchacher 1993). According to Gines (1958), the consumption of fingerlings and small fish, alongside insect larvae and aquatic hemiptera, is common within the genus and is associated with the abundance of these organisms in their habitat, suggesting a diet based on the availability of prey items in the environment. We identified a single Tilapia (*Oreochromis niloticus*) fingerling that was preyed upon by *P. carvalhoi*, indicating that fish consumption may be infrequent in this species. New studies on food ecology could further clarify fish consumption by *Pipa carvalhoi*.

In general, anurans are considered opportunistic predators, with their diets varying according to the availability of prey in their habitats (Ceron et al. 2019). Therefore, the dietary pattern of *P. carvalhoi* likely reflects a reliance on the availability of prey items in the environment, which accounts for the significant presence of insect larvae, hemipterans, and ants in its diet. In the aquatic environments of the Caatinga, such as ponds, lakes, and dams, insect

larvae and hemiptera comprise an essential portion of the biotic community, often displaying high abundance, especially during drought periods (Abílio et al. 2007; Abílio et al. 2018). Thus, the prominence of these items in the diet of *P. carvalhoi* may also be indicative of their availability as prey.

On the other hand, the significant presence of ants in the diet of *P. carvalhoi* is unusual and suggests two possibilities: individuals of *P. carvalhoi* may have consumed ants that inadvertently fell onto the surface of the water, as hypothesized by Measey (1998) and Garda et al. (2006) to explain the consumption of terrestrial prey by African Clawed Frogs (*Xenopus laevis*) and *P. arrabali*, respectively; or *P. carvalhoi* may have ventured into terrestrial environments to consume prey that inhabit dry land, as observed by Buchacher (1993) for *P. arrabali*. Reports of terrestrial activity by *P. carvalhoi* have been noted by Meyers and Carvalho (1945), who associated this behavior with foraging activity, and by Fernandes et al. (2011), who observed migration of some individuals of the species between ponds in the Caatinga. Canedo et al. (2006) described a feeding habit primarily based on aquatic prey, however, without any records of terrestrial activity for the species. We did not observe *P. carvalhoi* in the field, which limits our ability to infer about its consumption of terrestrial prey or activity on dry land. Nonetheless, both scenarios seem plausible and highlight the need for direct field observations to clarify this issue.

The snout-vent length of the *P. carvalhoi* individuals in the populations analyzed in our study is consistent with that reported in the literature, which used this measurement as a proxy of body size (Trueb and Cannatella 1986; Lima et al. 2020). Because Trueb and Cannatella (1986) and Lima et al. (2020) examined individuals from locations both north and south of the São Francisco River, their observations support our findings. Moreover, although Lima et al. (2020) did not specifically investigate sexual differences, the absence of morphometric distinctions among the three clades recovered by the authors suggests that these lineages are similar in size across their geographic ranges. Sexual dimorphism can manifest in *Pipa* species through differences in SVL between males and females (Trueb and Massemin 2000; Garda et al. 2006), or variations in the coloration of the gular region (Trueb and Massemin 2000). In the Surinam Toad (*Pipa pipa*), sexual dimorphism is characterized by larger forearm diameter, finger length two, and snout length in males compared to females (Zippel 2006; Alves-Pinto et al.

2014). We found significant differences in snout-vent length, eye-nostril distance, foot length, and head length (with females larger than males), as well as in forearm length (with males larger than females); however, no substantial differences in overall body size were noted, with the isometric variable body size showing no significant difference.

These results indicate that a significant portion of the observed sexual dimorphism in *P. carvalhoi* may be attributed to sexual selection and ecology. Previous studies have described a complex courtship ritual in *P. carvalhoi*, which involves a nuptial dance between males and females during the amplexus and prior to the release of eggs (Weygoldt 1976; Fernandes et al. 2011). Therefore, larger arms may favor amplexus by allowing males to hold a moving female more securely, improving grip and stability during mating (Liao et al. 2012). Similarly, this may help explain the larger foot size in females, ensuring good mobility during the nuptial dance and amplexus. Thus, it is possible that this courtship behavior may play an important role in body-size selection among males and females of this species. Likewise, the relevance of head-related variables is often associated with trophic niche partitioning (e.g., differences in diet and feeding strategies; Khoshnamvand et al. 2018; Goldberg et al. 2024). Thus, it is possible that females feed on a broader range of prey than males, ensuring greater energy availability for producing and developing the clutch.

There is a strong association between the body size of female *Pipa* species and the number of tadpoles and the diameter of the eggs they carry on their backs (Buchacher 1993; Garda et al. 2006). Larger females tend to have greater reproductive success, which may account for their larger size relative to males (Shine 1979). Furthermore, species without free-swimming tadpoles typically have larger eggs and, consequently, fewer eggs compared to species with free-swimming tadpoles (Trueb and Massemín 2000). Notably, within the genus, *P. carvalhoi* is considered the species with females that carry the highest number of eggs, with the smallest egg diameter, and free-swimming tadpoles. This reproductive mode may represent an adaptation to environments prone to intense drying of water bodies (Fernandes et al. 2011). Although *P. carvalhoi* exhibits a directly proportional relationship between female body size and the presence of eggs on their backs (Fernandes et al. 2011), the absence of notable size dimorphism between males and females may reflect a lack of sexual selection favoring larger body sizes and, consequently, different sexual

preferences.

The absence of helminth parasites on individuals in our study is indeed an atypical finding (Bush et al. 1997). Typically, analyses of the parasitic fauna across various anuran species report the presence of helminths (Teles et al. 2018; Mascarenhas et al. 2021). We acknowledge that this unexpected result may stem from limitations in our sampling, which could have been too small or not sufficiently comprehensive to capture the full range of helminths that may infest *P. carvalhoi*. Future research involving additional *P. carvalhoi* populations, both north and south of the São Francisco River, is essential for uncovering new findings regarding helminth presence.

Our study provides valuable insights into the geographic distribution, diet, and morphometric characteristics of *P. carvalhoi* populations located north of the São Francisco River. Notably, our research also marks the first documentation of fish consumption by *P. carvalhoi*, indicating a complex ecological interaction with an invasive fish species. Additional studies focused on the ecological aspects of *P. carvalhoi* populations and lineages along their geographic range, especially in southern regions, would be useful to understand the biology of this species. We believe that these investigations could yield significant information, contributing to a deeper understanding of the genetic lineages recovered by Lima et al. (2020) and strengthened by Fouquet et al. (2022) and their adaptations within different environments.

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APPENDIX

APPENDIX TABLE. Collection sites of Carvalho's Surinam Toad (*Pipa carvalhoi*) individuals used to obtain ecological data. The geographic coordinates represent the centroid of the municipalities.

Municipality	State	Latitude	Longitude	Year of collection
Barbalha	Ceará	-7.3099	-39.3030	undetermined
Crato	Ceará	-7.2303	-39.4122	2011
Araruna	Paraíba	-6.5282	-35.7408	2003
Areia	Paraíba	-6.9667	-35.7000	2011 / 2012
Cabaceiras	Paraíba	-7.4905	-36.2878	1982 / 1983 / 1984
Patos	Paraíba	-7.0244	-37.2797	1989
São Mamede	Paraíba	-6.9263	-37.0969	undetermined
São João do Cariri	Paraíba	-7.3910	-36.5330	2005
Araripina	Pernambuco	-7.5727	-40.5057	2009
Arcoverde	Pernambuco	-8.4180	-37.0580	2009
Buíque	Pernambuco	-8.6230	-37.1560	2013
São Caitano	Pernambuco	-8.3318	-36.1361	1985
Serra Talhada	Pernambuco	-7.9860	-38.2920	2011
Trindade	Pernambuco	-7.7598	-40.2627	2009
Serra Caiada	Rio Grande do Norte	-6.1060	-35.7110	2009



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