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Pond characteristics influence the intraspecific variation in the morphometry of the tadpoles of two species of *Dendropsophus* (Anura: Hylidae) from the Cerrado savanna of northeastern Brazil

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Abstract: Spatial variation in the environment may be an important source of morphological variation in many organisms. Tadpoles are valuable model organisms for studies of morphological variation, and in particular, the evaluation of the relationship between environmental and morphological variables. In heterogeneous environments, such as the temporary ponds found in the Cerrado savanna of central Brazil, understanding how environmental variables influence the morphological variation found in tadpole populations can provide important insights into this phenomenon. The present study thus aimed to (i) describe the morphometric variation in tadpoles found in different populations of *Dendropsophus nanus* and *Dendropsophus minutus* in the Cerrado of southern Maranhão state (Brazil), and (ii) relate this variation in tadpole morphology to the characteristics of the local ponds. Tadpoles were collected from 11 pounds in southern Maranhão, and the morphological space among the different populations was compared using NPMANOVA, separately for each species. The degree of association between the environmental and morphological matrices was then tested using a Stepwise Multiple Linear Regression. The morphological (tadpole morphometry) and environmental (pond characteristics) data matrices were obtained by ordination techniques. Considerable morphometric differences were found among populations in both species. In *D. nanus*, the morphometric variation was correlated with that of the substrate, whereas in *D. minutus*, morphometry was associated with the vegetation found in the pond. Overall, then, the study demonstrated that distinct environmental variables influenced significantly the morphometry of the tadpoles of each frog species.

Key words: anuran larvae, *Dendropsophus minutus*, *Dendropsophus nanus*, Maranhão, morphology, savanna.

INTRODUCTION

Dendropsophus nanus (Boulenger 1889) and *Dendropsophus minutus* (Peters 1872) are anurans of the family Hylidae. Both species are widely distributed in South America (Frost 2016) and are each considered to encompass a complex of species due to the considerable morphological and genetic variation found among populations (Medeiros et al. 2006, Gehara et al. 2014). One way to study this variation is through the detection of potentially independent evolutionary subunits, which can be determined through the quantitative analysis of morphological variation (Reis et al. 2002). A number of *Dendropsophus* species have been described recently (Dias et al. 2017, Motta et al. 2012, Rivera-Correa & Orrico 2013, Orrico et al. 2014) and the number of recognized species in the genus has yet to stabilize (Motta et al. 2012). Given this, the development of studies that show patterns of morphological variation will be an essential first step toward to understand the diversity of this group. Research on the tadpoles of the genus *Dendropsophus* has shown systematic morphological variation among larval stages (Ballen 2018, Abreu et al. 2013), although less evidence has been found of geographic variation in the morphology in the tadpoles of this genus (Marques & Nomura 2018).

Geographic variation among the populations of a given species is an inevitable outcome of the spatial variation in the environment, driven by genetic divergence and/or phenotypic plasticity (Mayr 1977). One of the basic assumptions in morphometric studies is that the morphology of an organism will correlate with its mode of life, such that phenotypes are determined by the variation in environmental factors (Norton et al. 1995). Given this, a second step in morphological research is to correlate morphometric variation with its possible causal factors. The quantitative analysis of covariance between body traits and their potential determining factors may provide important insights into patterns of morphological variation (Bookstein 1991). Systematic phenotypic variation has been observed in a number of studies of tadpoles (Skelly & Werner 1990, Newman 1992, McCollum & Van Buskirk 1996, Smith & Van Buskirk 1995, Stoler & Relyea 2013).

Amphibian larvae present considerable plasticity in response to environmental factors (Van Buskirk & Relyea 1998) and provide excellent models for the study of phenotypic variation (Miner et al. 2005). Van Buskirk (2009) concluded that the morphological variation found in tadpole populations is, in part, a product of the variation in the abiotic filters presented by the environment. In an experimental study (Touchon & Warkentin 2011), for example, the tadpoles of *Dendropsophus ebraccatus* presented morphological variation resulting from a combination of biotic (predation) and abiotic (water temperature) filters, indicating that even small variations in abiotic conditions can influence the expression of different phenotypes. However, most studies of tadpole morphology have been experimental, and have tended to focus on intra-population phenotypic plasticity, reflecting a knowledge gap in terms of field data (Van Buskirk 2009) and inter-population comparisons.

Both *D. nanus* and *D. minutus* are amply distributed in Brazil, and are found in areas of Cerrado savanna, including those in southern Maranhão, a state in northeastern Brazil (Reichle et al. 2004). The Cerrado is the second largest Brazilian biome (Klink & Machado 2005), which encompasses an extensive mosaic of vegetation types, and a high diversity of organisms (Cavalcanti 1999, Silva & Bates 2002). Given the environmental complexity of the Cerrado, and the premise that tadpole morphology is correlated with environmental factors, the present study tests the hypotheses that tadpoles from different populations are morphologically distinct from one another, and that this intraspecific variation is related systematically to specific features of the ponds they inhabit, such as the size of the pond, its substrate, and aquatic vegetation. To test these hypotheses, the external morphology of *D. nanus* and *D. minutus* tadpoles was compared among different populations in the Cerrado savanna of southern Maranhão state, in order to verify the degree of intraspecific variation found in these populations. The observed variation in morphology was analyzed systematically in relation to the variation in pond characteristics (size, substrate, and vegetation) in order to determine the possible relationship between environmental variables and tadpole morphology.

MATERIALS AND METHODS

The tadpoles were collected from 11 ponds in southern Maranhão in the rainy season, that is, in January and February 2012, and in May 2013 (Table I; Figure 1). The ponds were classified as temporary bodies of water of the lentic type, and varied in size (Table I). The study area is located within the Cerrado savanna biome, and the climate of this region is of the Aw type in the Köppen classification system, with a mean annual temperature of 27°C, mean annual rainfall of 1800 mm, and a dry season that typically lasts from May to September (INMET 2018). The study area is characterized by environmental heterogeneity but homogeneous climatic conditions.

The tadpoles were sampled by sweeping the water column and adjacent substrate with a dip net (35 cm diameter, 2 mm mesh) during one hour at each pond. The tadpole specimens collected at each pond were fixed in 10% formaldehyde and later deposited in the herpetology collection of the Federal University of Maranhão. Initial screening select specimens in the Gosner (1960) developmental stages 35–39, which indicated a degree of standardization of the developmental stage for the analyses. Following this screening, 4–10 tadpoles of each species were measured from each pond (Table I).

Table I. Locations, latitude-longitude points (Lat-Long), and the number of *Dendropsophus nanus* and *D. minutus* specimens collected per site for the morphometric analysis. The numbers of the locations correspond to the points plotted in Figure 1.

Locations	Lat-Long	Sample Size		Distance to P01 (Km)
		D. minutus	***D. nanus***	
P01	7° 08’S - 45°21’W	-	10	0
P02	7° 04’S - 45°25’W	-	05	10
P03	7° 02’S - 45°30’W	-	10	19
P04	7° 01’S - 45°30’W	08	-	20
P05	8°26’S - 45°46’W	10	08	152
P06	7°20’S - 45°57’W	10	05	69
P07	7°22’S - 46°29’W	10	-	128
P08	7°21’S - 46°34’W	10	10	135
P09	7°25’S - 46°45’W	04	-	158
P10	7°22’S - 47°20’W	10	07	220
P11	7°21’S - 47°23’W	10	05	225

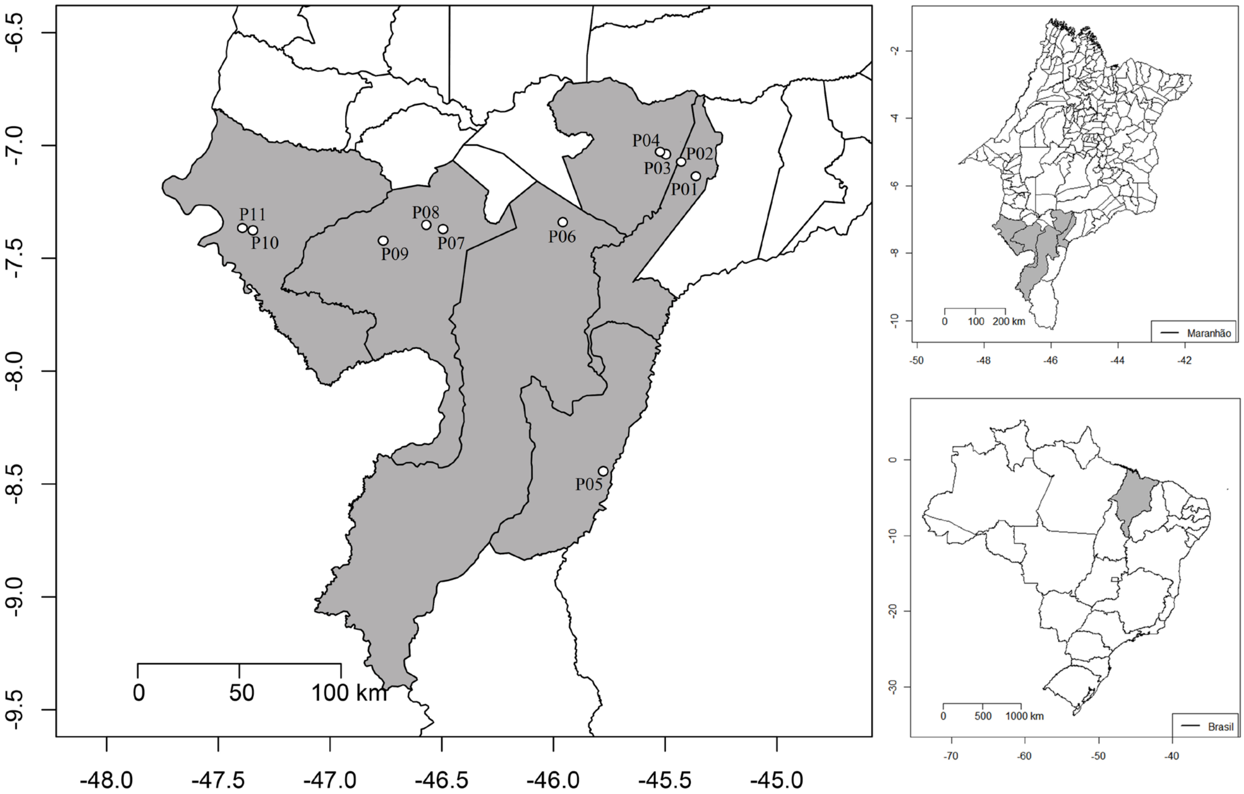

Figure 1. Map showing the locations of the *Dendropsophus nanus* and *Dendropsophus minutus* populations sampled in southern Maranhão, Brazil.

The first step in the morphological analysis was the observation of the specimens under a stereomicroscope, with measurements being taken using the Axion Vision 4.8 software. All measurements were obtained by the same researcher. The following measurements were obtained from each specimen in the lateral view (Figure 2): eye-snout distance (END), body length (BL), tail length (TAL), eye height (EH), body height (BH), tail muscle height (MH), and maximum tail height (TH). These morphometric data were first transformed by the Log10 and Burnaby's method (Hammer et al. 2013) to control for the size effects of tadpole body size, a Canonical Variable Analysis (CVA) was then applied to reduce dimensionality and generate the morphometric matrix. This approach was adopted in the present study in order to (i) summarize the set of morphometric distances into canonical variables that represent the shape of the tadpole; (ii) consider the *a priori* existence of groups, i.e., tadpole populations, and (iii) permit the optimum visualization of the potential differences among populations, through the reduction of the variation within each population (Camussi et al. 1985).

To test the hypothesis that the tadpoles from different populations were morphologically distinct from one another, the scores generated by the first two canonical axes of the CVA were evaluated through the application of a Non-Parametric Multivariate Analysis of Variance (NPMANOVA), followed by a pairwise *post hoc* analysis (pairwise NPMANOVA) between all possible pairs of populations. In the case of the multiple comparisons, a sequential Bonferroni correction was applied to the *p* values.

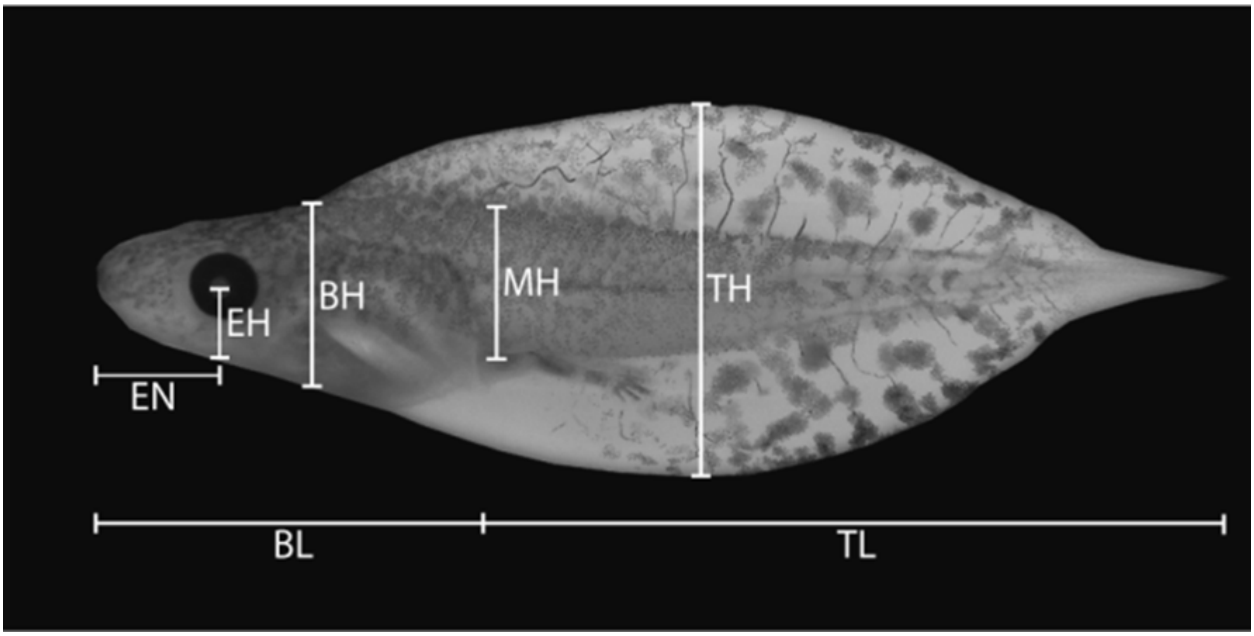

Figure 2. The lateral view of a tadpole, showing the seven linear measurements used for the analysis of morphological variation in the present study: eye-snout distance (END), body length (BL), tail length (TAL), eye height (EH), body height (BH), tail muscle height (MH), and maximum tail height (TH).

A standard set of environmental data was recorded at each pond, providing the following predictive variables: (i) pond size (length, width and depth), (ii) percentage of each substrate type (gravel, sand, clay, mud, and leaf litter), and (iii) percentage of pond vegetation (floating vegetation, herbaceous, shrub and arboreal layers, and bare ground). The latter two variables were visually categorized into classes for analyses: 0% = 0; 1%–20% = 0.1; 21%–40% = 0.3; 41%–60% = 0.5; 61%–80% = 0.7; 81%–100% = 0.9. A Principal Component Analysis (PCA) was applied individually to each predictor variable (pond size, substrate and vegetation cover) to minimize its dimensionality and generate the matrix of environmental data. The Principal Components Analysis (PCA) permits the reduction of a set of variables to a set of indices which, in contrast with the CVA, can be applied in cases where there is no repetition in the data (Johnson & Wichern 1998), as in the environmental data analyzed in the present study, given that each pond is characterized as a whole.

To test the relationship between the morphology of the tadpoles and the pond variables, a Forward Stepwise Multiple Regression was first applied to select the environmental variable that most contributed to the variation found in the morphological data. This approach was adopted here because it permits the analysis of the complete set of variables (Draper & Smith 1981) and reduces the number of multiple comparisons (Zuur et al. 2007). Following Ayres et al. (2007), the selection criterion used here was the correlation coefficient (R^2), which reflects the degree to which a dependent variable (tadpole morphology) is explained by the predictive variable (pond characteristics). In addition, we use the Akaike Information Criterion (AIC) and Adjusted Coefficient of Determination ($R^2_{adjusted}$) as model selection methods (Zuur et al. 2007). Here, the regression analysis was based on the centroids of the first axis of the PCA (environmental data) and CVA (morphometric data), given that these axes encompass the majority of the variation found in the dataset.

Spatial autocorrelation was verified using the Moran index (Legendre & Legendre 1998). There were no quantitative data on predation that allowed an analysis in this work, but field notes indicate that vertebrate predators were absent from all ponds, except P11, while invertebrate predators were present at all sites. All analyses were run in the PAST software (Hammer et al. 2013), with a $p < 0.05$ significance level. The authorization for data collection was obtained by the Biodiversity Authorization and Information System - SISBIO - / Chico Mendes Institute for Biodiversity Conservation - ICMBio license 16716-1 and is in agreement with the Ethics Committee on the Use of Animals CEUA / UFMA protocol 23115.005794 / 2012-58.

RESULTS

The morphometric data on the *Dendropsophus minutus* and *D. nanus* tadpoles are presented in Table II. In *D. minutus*, the first two canonical axes (CV1 and CV2) explained 83.1% of the variation (54.3% and 28.8%, respectively) in body shape among populations (Fig. 3), while in *D. nanus*, the first two canonical axes explained 81.2% (51.6% and 29.6%, respectively) of the variation among populations (Fig. 3).

The morphological variation among the populations of *D. minutus* was analyzed through the visualization of the CVA plot (Fig. 3). The results of the NPMANOVA indicated significant morphometric variation among the *D. minutus* populations (F = 18.45; 10,000 permutations; $p < 0.01$). The post hoc test revealed significant differences ($p < 0.05$; sequential Bonferroni correction) between most pair of populations, except P08 vs. P11 and P09 vs. P06 (Table III). In *D. nanus*, the plots of morphological space also indicated considerable morphological variation among the majority of the populations (Fig. 3), with significant pairwise differences in most cases (NPMANOVA, F = 10.39; 10,000 permutations; $p < 0.05$; sequential Bonferroni correction; Table III). The Moran index did not indicate any spatial autocorrelation in either species (*D. minutus*: -0.0021 to 0.0017; *D. nanus*: -0.0018 to 0.0024; $p < 0.05$ in both cases).

The results of the CVA and PCA for the morphometric and environmental data, respectively, are summarized in Table IV. In *D. minutus*, the morphometric variables that contributed most to the CV1 axis were maximum tail height and tail muscle height (Table IV). In *D. nanus*, the morphometric variables that contributed most to the CV1 axis were the eye-snout distance and maximum tail height (Table IV).

In *D. minutus*, the forward multiple regression analysis indicated that the “Pond Vegetation” was the variable that best explained the morphological variation in the tadpole populations (R^2 = 0. 71; $p = 0.009$; AIC = -29.24; $R^2_{adjusted}$ = 65.7%; Table V). As analyzing the eigenvalues of CPA and CVA (Table IV) and their relationship (Fig. 4), it was observed that ponds with more herbaceous vegetation and less bare ground showed *D. minutus* tadpoles with shallower tail. In *D. nanus*, the forward multiple regression indicated that the “Substrate” was the variable that best explained the morphological variation in the tadpoles (R^2 = 0.50; $p = 0.048$; AIC = 28.10; $R^2_{adjusted}$ = 41.6%; Table V). Analyzing the eigenvalues of CPA and CVA (Table IV) and their relationship (Fig. 4), it was observed that pond substrate with more mud/leaf and less sand/gravel showed *D. nanus* tadpoles with deeper tail.

Table II. **Descriptive statistics (mean in mm ± standard deviation) of the morphometric variables of the *Dendropsophus nanus* and *Dendropsophus minutus* tadpole specimens from southern Maranhão, Brazil. END (eye-snout distance); BL (body length); TL (tail length); EH (eye height); BH (body height); MH (tail muscle height); TH (maximum tail height).**

Locations	END	BL	TL	EP	BH	MH	TH
Dendropsophus nanus							
P01	03.31±0.24	08.00±0.64	18.44±1.53	02.33±0.21	04.40±0.33	02.70±0.23	05.75±0.59
P02	03.04±0.44	06.80±0.55	17.27±2.14	01.99±0.32	03.62±0.31	02.36±0.21	04.79±0.38
P03	03.12±0.19	06.97±0.42	16.90±1.92	02.04±0.19	03.69±0.24	02.21±0.17	04.67±0.44
P05	03.61±0.45	07.67±1.09	17.26±3.43	02.44±0.39	04.03±0.50	02.37±0.35	04.69±0.82
P06	03.34±0.30	07.21±0.98	15.56±1.95	02.21±0.25	03.70±0.52	02.36±0.19	04.02±0.34
P08	03.32±0.18	06.86±0.86	16.51±2.47	02.02±0.20	03.79±0.25	02.28±0.23	03.99±0.09
P10	03.38±0.19	06.87±0.41	15.35±2.17	02.26±0.12	03.64±0.17	02.30±0.15	04.07±0.35
P11	03.61±0.24	07.57±0.65	16.45±2.43	02.42±0.20	04.07±0.37	02.42±0.21	04.62±0.74
Dendropsophus minutus							
P04	02.98±0.37	08.14±1.27	16.28±2.51	02.17±0.40	04.32±0.86	02.91±0.63	06.31±1.01
P05	03.06±0.37	08.52±0.99	16.38±3.27	02.50±0.27	05.20±0.55	02.73±0.40	07.75±1.82
P06	03.59±0.29	09.67±0.69	17.40±1.46	02.68±0.37	05.14±0.53	03.45±0.22	06.80±0.28
P07	03.23±0.17	08.27±0.75	14.33±1.52	02.66±0.27	05.08±0.49	02.59±0.27	06.55±0.85
P08	03.59±0.25	10.76±0.37	19.57±2.27	02.81±0.35	05.69±0.28	03.62±0.25	09.93±0.72
P09	03.38±0.40	09.61±1.10	16.55±1.47	02.76±0.06	05.21±0.30	03.22±0.21	06.56±0.54
P10	03.15±0.27	09.09±0.75	16.41±1.78	02.58±0.38	05.29±0.48	02.70±0.24	08.66±0.63
P11	02.75±0.36	07.84±0.73	14.22±2.44	02.40±0.05	04.74±0.74	02.80±0.32	06.28±1.00

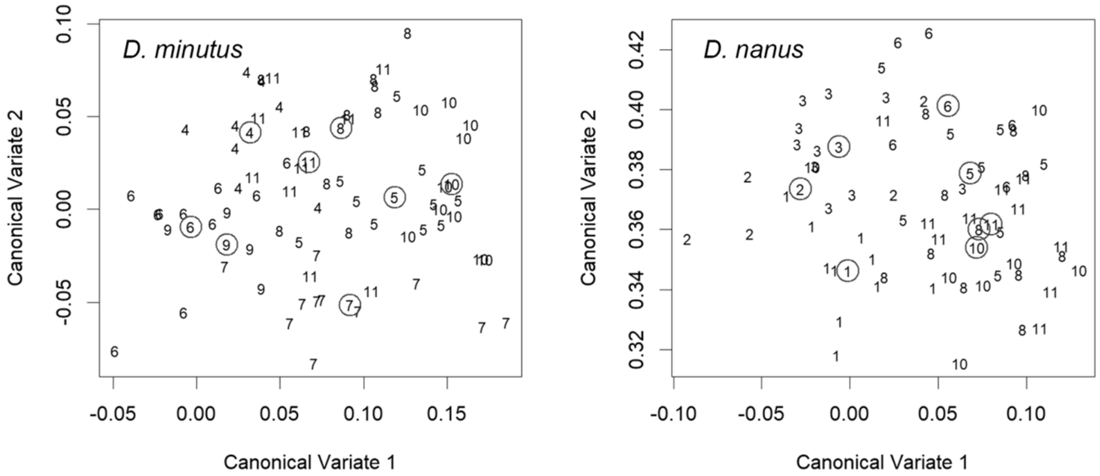

Figure 3. **Morphometric space of the *Dendropsophus nanus* and *Dendropsophus minutus* tadpoles from southern Maranhão, Brazil, obtained from the first two axes of the Analysis of Canonical Variables (CVA). Open circles = centroids.**

Table III. Results obtained from the *post hoc* NPMANOVA. The upper diagonal represents the comparisons between populations of *Dendropsophus minutus*, and the lower diagonal represents the comparisons for *Dendropsophus nanus*. Abbreviations: ** (p <0.01); * (p <0.05); ns (not significant); - (no comparison made).

	P01	**P02**	**P03**	**P04**	**P05**	**P06**	**P07**	**P08**	**P09**	**P10**	**P11**
P01		-	-	-	-	-	-	-	-	-	-
P02	ns		-	-	-	-	-	-	-	-	-
P03	**	ns		-	-	-	-	-	-	-	-
P04	-	-	-		**	**	**	**	**	**	*
P05	**	**	**	-		**	**	**	**	*	**
P06	**	*	**	-	ns		**	**	ns	**	**
P07	-	-	-	-	-	-		**	*	**	**
P08	**	**	**	-	ns	*	-		**	**	ns
P09	-	-	-	-	-	-	-	-		**	**
P10	**	*	**	-	ns	ns	-	ns	-		**
P11	**	**	**	-	ns	*	-	ns	-	ns	

Table IV. Loadings (L) of the first axes of the CVA (morphometric data) and PCA (environmental variables). END (eye-snout distance); BL (body length); TL (tail length); EH (eye height); BH (body height); MH (tail muscle height); TH (maximum tail height).

	D. minutus		***D. nanus***	
	Axis 1	**Axis 2**	**Axis 1**	**Axis 2**
Morphometric Distance				
END	-0.18	-0.59	0.60	0.47
BL	0.05	0.18	-0.08	-0.05
TL	-0.11	0.17	-0.20	0.57
EH	0.02	-0.20	0.29	-0.17
BH	0.34	-0.32	0.19	0.29
MH	-0.60	0.49	0.16	-0.57
TH	0.69	0.45	-0.67	0.13
% Variance	54.30	28.78	51.61	29.65
Pond Size				
Length	0.99	-0.19	1.00	0.05
Width	0.87	025	0.78	-0.62
Depth	0.87	0.49	-0.03	0.34
% Variance	88.31	11.37	96.36	3.64

Table IV. Continuation

Substrate				
Gravel	-0.58	-0.64	-0.55	-0.68
Sand	-0.82	-0.47	-0.82	-0.48
Clay	-0.44	0.68	-0.49	0.66
Mud	0.93	0.11	0.93	-0.14
Leaf Litter	0.74	-0.53	0.87	-0.23
% Variance	60.63	21.91	68.35	15.61
Pond Vegetation				
Floating	-0.53	0.04	0.43	0.17
Herbaceous	0.63	-0.77	0.49	-0.85
Shrub	0.15	0.46	-0.35	0.81
Arboreal	0.23	-0.34	0.28	-0.42
Bare	-0.92	-0.39	-0.97	-0.25
% Variance	57.07	29.74	62.33	29.26

Table V. **Results of forward stepwise multiple regression to environmental and morphology variable.**

Pond Variable	**R^2**	**F**	***p***	**AIC**	**$R^2_{adjusted}$**
Dendropsophus minutus					
Vegetation	70.59%	14.40	0.009	-29.25	65.70%
Vegetation + Size	70.65%	6.02	0.047	-28.53	65.00%
Vegetation + Size + Substrate	79.44%	5.15	0.079	-26.82	57.90%
Dendropsophus nanus					
Substrate	49.95%	5.99	0.049	-28.10	41.60%
Substrate + Vegetation	51.12%	2.61	0.167	-26.40	32.50%
Substrate + Vegetation + Size	55.75%	1.68	0.307	-24.50	16.70%

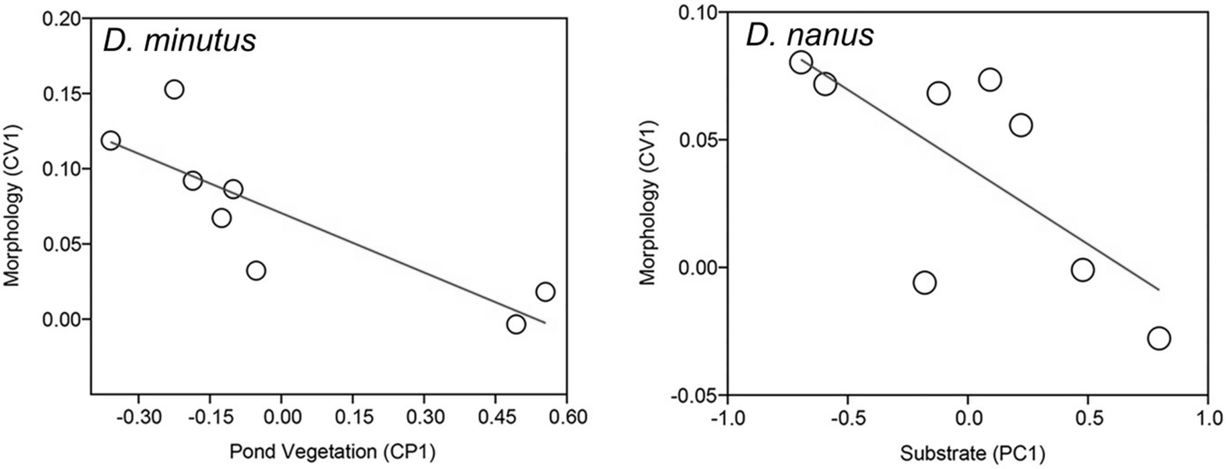

Figure 4. **Relationship between morphometric (scores of first canonical variate) and environmental variables data (scores of first principal component) for *Dendropsophus nanus* and *Dendropsophus minutus* tadpole specimens from southern Maranhão, Brazil.**

DISCUSSION

The present study identified clear morphometric differences in tadpole shape among populations in each study species. Although one potential explanation for this finding may be spatial autocorrelation in the morphometric data, the Moran index did not indicate any significant pattern in either species. Similarly, while body size differences may have influenced the variation among populations, this is also unlikely in the present study, given that all the tadpoles collected were at Gosner (1960) developmental stages 35–39, which would minimize any allometric effects, while the application of the Burnaby correction also eliminated any potential influence of the variation in body size (Wimberger 1992).

Phenotypic variation in natural populations arises from a combination of genetic divergence, natural selection, and phenotypic plasticity (Schluter 2009, Losos et al. 2000). Many studies of tadpoles focus on morphological divergence resulting from phenotypic plasticity within populations (Skelly & Werner 1990, Newman 1992, McCollum & Van Buskirk 1996, Smith & Van Buskirk 1995, Stoler & Relyea 2013). In the present study, the morphometric traits of the *D. minutus* and *D. nanus* tadpoles varied significantly among populations, although the experimental design does not allow for any conclusive analysis of whether this morphological variation is the result of phenotypic plasticity or genetic factors. Some previous studies (Van Buskirk 2009, Van Buskirk & Arioli 2005) have nevertheless concluded that phenotypic plasticity plays a more important role than genetic divergence in the morphological variation found among tadpole populations.

In the present study, significant correlations were found between morphometry and specific environmental variables. In *D. nanus*, morphometry and the substrate were correlated. As the tadpoles of *D. nanus* are found at the bottom of the pond, on the substrate (Bokermann 1963), this correlation may reflect some direct effect of the characteristics of the substrate on the morphology of the tadpoles.

In *D. minutus*, there was a significant correlation between the morphometry of the tadpoles and the vegetation of the pond. As the tadpoles of *D. minutus* are found in the middle water column, the presence of vegetation in the water influences their abundance (Bokermann 1963, Rossa-Feres & Nomura 2006, Kopp & Eterovick 2006). Marques et al. (2018) found a relationship between morphometric and environmental variables in three tadpole species (*Physalaemus cuvieri*, *Scinax fuscomarginatus*, and *Scinax similis*) and concluded that local environmental factors play an important role in morphological shifts and local adaptations. The morphological variation may thus reflect the differential use of environmental conditions, so the correlation found in the present study may reflect morphological changes resulting from the variation in the use of environmental conditions by the tadpoles.

In addition to the spatial resources used by the two species, predation is an important factor determining phenotypic plasticity in tadpoles. While predator abundance was not quantified in the present study, predators were present in all the ponds, and it seems reasonable to suggest that predation does not represent a significant source of intraspecific variation. Relyea (2002) concluded that phenotypic variation in tadpoles may be a response to local environmental conditions, and that predation may be an underlying mechanism. Oyamaguchi et al. (2017) identified a correlation between environmental variables and the morphology of adult *D. minutus* in the Cerrado, and emphasized the role of natural selection in genetic divergence of this species in this biome. Other tadpole studies have indicated a synergistic effect between predation pressure and environmental variables (Relyea & Werner 2000, Van Buskirk et al. 1997, Relyea 2012, Van Buskirk, & Arioli 2005), suggesting that abiotic environmental filters may have an indirect or indirect influence on phenotypic variation, depending on predation levels.

The present study showed clearly that the morphological variation of the tadpoles was related to specific environmental features of the ponds they inhabited. A number of studies have shown an increase in tail height in response to the presence of predators (Relyea & Hoverman 2003, Mccollum & Leimberger 1997, Relyea 2003). Van Buskirk (2009) and Marques & Nomura (2018) concluded that the internal environmental characteristics of the pond may influence tadpole morphology by providing refuge from either predators or competitors. In the present study, the taller tail of *D. minutus* was associated with less herbaceous vegetation in the water, while in *D. nanus*, tail height was associated with less muddy substrates and leaf litter. Given the potential predation in all the ponds, the observed environmental conditions represent greater exposure to predation, which would explain the increase in tail height. There are two explanations here, one is that a taller tail would help avoid attacks to the vital organs, while the other refers to the greater swimming ability of the tadpole, allowing it to escape more efficiently (Van Buskirk & Relyea 1998, Van Buskirk et al. 2003). However, as the locomotory role of tadpole fins is still poorly understood, any conclusion on their contribution to the ecological patterns observed in the present study remain speculative.

The results of the present study provide important insights into the patterns of morphological diversity found in tadpoles, in particular population-level variation in the natural environments of the Neotropical region, and the relationship between specific environmental features and the characteristics of the morphology of the tadpoles, emphasizing the perspective of the ecological guild. The relationships found between tadpole morphology and the pond vegetation, in *D. minutus*, and the substrate, in *D. nanus*, may reflect locomotion patterns in the water column and/or a predator avoidance response. The results of the present study reinforce the need for further research, including experimental approaches and studies that integrate more complex ecological interactions, such as predation and competition, to better elucidate the relationship between tadpole morphometry and the local abiotic variables addressed in this paper.

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