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Sexual dimorphism in *Lysapsus Bolivianus* (Anura: Hylidae) in an amazonian floodplain

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ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE

SEXUAL DIMORPHISM IN *LYSAPSUS BOLIVIANUS* (ANURA: HYLIDAE) IN AN AMAZONIAN FLOODPLAIN

DIMORFISMO SEXUAL EN LYSAPSUS BOLIVIANUS (ANURA: HYLIDAE) EN UNA LLANURA AMAZÓNICA

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ABSTRACT

Sexual dimorphism (SD) in morphology may be attributed to a variety of ecological pressures, including sexual selection, differential growth rate, age at maturity, longevity, and niche partitioning. This morphological variation between the sexes can help us better understand the evolution of life histories and fill gaps about species/populations from less-explored regions. We investigated sexual dimorphism in morphometric characters of *Lysapsus bolivianus* from a floodplain environment in Eastern Amazon based on 273 specimens collected between 2013 to 2019. Females of *L. bolivianus* attain larger body sizes (Snout-to-Vent Length), but males have proportionally larger heads in length than females. Previous studies indicate that SSD in *L. bolivianus* can be linked to differential growth rate and age to maturity between sexes, whereas differences in head morphometry can be explained by differences in prey volume ingested by males and females. Nevertheless, the species inhabits a highly seasonal environment and bear a high pressure of predation. In this sense, males and females may cope with different selective pressures due to size differences.

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Keywords: morphometry, head length, growth rate, shape dimorphism, eastern Amazon.

RESUMEN

El dimorfismo sexual (SD) en la morfología puede atribuirse a una variedad de presiones ecológicas, incluida la selección sexual, la tasa de crecimiento diferencial, la edad de madurez, la longevidad y la división de nichos. Esta variación morfológica entre sexos puede ayudarnos a comprender mejor la evolución de las historias de vida y llenar vacíos sobre especies/poblaciones de regiones menos exploradas. Investigamos el dimorfismo sexual en caracteres morfométricos de *Lysapsus bolivianus* de un ambiente de llanura aluvial en el este de la Amazonia con base en 273 especímenes recolectados entre 2013 y 2019. Las hembras de *L. bolivianus* alcanzan tamaños corporales más grandes (longitud del hocico a la ventilación), pero los machos tienen tamaños corporales proporcionalmente mayores. cabezas que las hembras. Estudios anteriores indican que la SSD en *L. bolivianus* puede estar relacionada con la tasa de crecimiento diferencial y la edad hasta la madurez entre sexos, mientras que las diferencias en la morfometría de la cabeza pueden explicarse por diferencias en el volumen de presas ingeridas por machos y hembras. Sin embargo, la especie habita en un entorno altamente estacional y soporta una alta presión de depredación. En este sentido, machos y hembras pueden afrontar diferentes presiones selectivas debido a diferencias de tamaño.

Palabras clave: morfometría, longitud de la cabeza, tasa de crecimiento, dimorfismo de forma, Amazonía oriental.

INTRODUCTION

Sexual size dimorphism (SSD) represents the expressed differences in body morphology related to distinct selective pressures acting on each sex that can be attributed to natural or sexual selection (De Lisle and Rowe, 2013; Rudh and Qvarnström, 2013). It has been documented in a wide range of taxa, including reptiles (Cox *et al.*, 2007), birds (Owens and

Hartley, 1998), and mammals (McPherson and Chenoweth, 2012). In anurans, 90% of SSD is female-biased, i.e. females are larger than males in total length, and it is usually associated with fecundity selection (Shine, 1979; Kupfer, 2007). Besides overall size, differences in body morphology may include other body components such as the size of the head and limbs (Berns, 2013). These traits, often overlooked, can reflect shape differences between sexes which may also be the result of selective mechanisms affecting species individually (Monnet & Cherry 2002 Arantes *et al.*, 2015). For example, species inhabiting different environments, or possessing distinct ecological adaptations, may exhibit differing degrees of sexual dimorphism in different body parts and traits (Zajitschek *et al.*, 2020).

Aquatic species have evolved unique morphological adaptations to aquatic lifestyle, such as hydrodynamic bodies, elongated pelvic girdles, and relatively long, muscular hind legs with extensive webbing on the hind feet (Wells, 2007). Particular microhabitats may lead to variation in the degree of SSD in anurans. Females with aquatic lifestyles, for instance, are associated with larger egg clutches, which may substantially exacerbate fecundity selection, resulting in a higher degree of SSD (Juarez, 2023). Similarly, ecological pressures of aquatic lifestyle may be important in driving sexual differences in shape and related morphological traits in anurans.

Despite anurans being one of the key targets of SSD investigations, there is still a shortage of empirical data for many taxa. Although external morphology characterization has been extremely valuable for Paradoxical frogs (Hylidae, Pseudae) systematics (Garda and Cannatella, 2007; Garda *et al.*, 2010), there is still a gap in the extant literature regarding sexual dimorphism for this taxon in less-explored regions. In addition, paradoxical frogs exhibit unique morphological adaptations to cope with their aquatic lifestyle (Turazzini and Gomez, 2023) but very few studies have addressed sex as a factor of intraspecific morphological variation in Pseudae species (genus *Lysapsus* and *Pseudis*). Garda et al. (2007) reported on sexual dimorphism in *L. limellum*, including specimens of *L. bolivianus* from Amapá state treated as synonym of *L. limellum*, and found evidence of sexual dimorphism in body size without a significant difference in shape variables between sexes. In this sense, we check here for morphometric differences among males and females of *L. bolivianus* in a floodplain environment at the eastern portion of the Brazilian Amazon. By addressing this topic, we expect to fill gaps regarding intraspecific phenotypic variation and provide in-depth knowledge about the life-history of this species and its populations.

MATERIALS AND METHODS

The investigation took place in Rio Curiaú Environmental Protection Area (APA Curiaú), north of Macapá city, Amapá state, Brazil. It covers approximately 40% of the 21,700 ha of the Curiaú river basin. Vegetation is predominantly composed of lowland marshes and swamps, called várzea forests, with permanent and temporary floodplains and lakes supporting a rich anuran community (Lima et al. 2017, Baia et al. 2020). We collected specimens at Fazenda Toca da Raposa (00°09'00.7” N, 51°02'18.5” W), one of the floodplain environments of APA Curiaú dominated by herbaceous and shrubs vegetation and surrounding by várzea forest. We surveyed the study site randomly in space by visual and auditory encounter methods (Heyer *et al.*, 1994). We conducted fieldwork once every five months, from February 2013 to October 2019, and each survey lasted 3h (18:00 h-21:00 h). We killed the specimens with a 5% lidocaine topical solution, fixed in 10% formaldehyde and preserved in 70% ethanol to be deposited in the herpetological collection of the Universidade Federal do Amapá (voucher numbers: CECC 538-555, 1042-

1057,2220–2250, 2654–2666, 3352–3371, 3701–3732, 3857–3871, 3929–3957, 4003–4062, 4290–4331).

We measured 273 specimens (114 males and 159 females, see Fig. 1) using a Mitutoyo 500-196-30 digital caliper with 0.01 mm precision and a Carson eFlex MM-840 digital magnifying glass with a millimeter lens for measurements less than 10 mm. We included only adult specimens in the morphometric analysis based on adult snout-to-vent length (Garda et al., 2010). We identified sex by direct observation of the gonads, which we exposed by abdominal dissection, and by secondary sexual characteristics, such as the coloration of the vocal sac in males and the presence of oocytes in females.

We took 13 morphometric measurements based on Garda et al. (2007): snout-vent length (SVL), head length (HL), head width (HW), tympanum diameter (TD), eye diameter (EYDM), interorbital distance (IOD), eye-to-nostril distance (EYNO), snout-to-nostril distance (SND), internarial distance (IND), arm length (AL), forearm length (FAL), thigh length (THL), and tibia length (TL) (Table 1). We applied a cluster and a classification analysis to distinguish between sexes of *L. bolivianus*, respectively: (1) Principal Component Analysis (PCA); and (2) Random Forest (RF) (Breiman, 2001). We conducted these analyses in software R statistical environment version 4.3 (R Core Team 2022) using SVL and 12 morphometric ratios (HL/SVL, HW/SVL, TD/SVL, EYDM/SVL, IOD/SVL, EYNO/SVL, SND/SVL, IND/SVL, AL/SVL, FAL/SVL, THL/SVL, and TL/SVL). Ratios are generally used to distinguishing between morphologically similar species which often differ significantly in body proportions, which can be applied to distinguish morphological differences between sexes.

We performed PCA using the “prcomp” function of the stats package version 4.3.1 using the parameters “scale = T” and “center = T” to scale and center the morphometric variables. We conducted RF with 1000 trees using the "randomForest” function of the randomForest package version 4.7 (Liaw and Wiener, 2002). Random Forest is a machine learning classification algorithm that generates trees from bootstrap samples of a database to aggregate results and classifies samples (specimens) into groups (sex) by choosing the best splits based on a random sample of predictors. To test the hypothesis that the two sexes are statistically distinct in terms of morphometry, we conducted a Multivariate Analysis of Variance (MANOVA) using the first two axes of the PCA. . Additionally, we conducted a univariate approach on SVL and the most important variables (Table 2) indicated by PCA and RF using Analyses of Variance (ANOVA) considering that analysis exhibited a normal distribution of residuals based on Shapiro–Wilk test (Shapiro and Wilk, 1965).

RESULTS

Morphometric multidimensional space occupied by males and females of *L. bolivianus* overlapped substantially for the first two axes of PCA (Fig. 2a). The variables that contributed most to the PC1 were, SVL and HL/SVL. In PC2 the biggest loadings were TL/SVL and HW/SVL (Table 2). MANOVA test does not indicate a robust difference between sexes for the first two axes of PCA (Pillai trace = 0.27, degrees of freedom (d.f.) = 2 and 270, $p < 0.0001$), with a low Pillai’s trace (<0.5) indicating a weak effect of sex on the morphometric variation of individuals.

The capacity to reliably classify morphometrics to the correct sex was moderated, as assessed by the classification error in the confusion matrix of Random Forest (Fig. 2c). In males, 10% of individuals were misclassified. In comparison, the model exhibited 22% of incorrect predictions for females. Based on Mean Decrease Accuracy, SVL and HL/SVL were the variables that most contributed to the model’s accuracy. Similarly, Mean Decrease Gini indicated that SVL has the largest contribution to the correct split between sexes, while HL/SVL also contributed to the split rule, but to a lesser extent (Fig. 2b).

Our univariate approach confirmed significant differences in morphometric variables analyzed separately, females were significantly larger than males in SVL (ANOVA, $F_{1,271}$=247.3, P<0.0001; Fig. 3), while males have a proportionally larger head length ratio (HL/SVL) (ANOVA, $F_{1,271}$=53.8, P<0.0001, Fig. 4).

DISCUSSION

Similar to the findings of Garda et al. (2007) and Sa-Oliveira et al. (2020), , our results indicated that females of *L. bolivianus* from different populations in Amapá state are larger than males in SVL. These findings agree with previous studies of sexual size dimorphism in *L. limellum* in the Brazilian Pantanal (Prado and Uetanabaro, 2000) and *L. laevis* in the Brazilian Amazon (Vaz-Silva *et al.*, 2005). Such a pattern of sexual dimorphism has been the root of the hypothesis involving SSD in anurans and sexual selection (Shine 1979). Woolbright (1983) hypothesized that larger female size is an adaptation to increasing clutch size capacity and the chances of offspring survival. For *L. limellum*, a positive relationship was found between female SVL and the number of deposited eggs (Prado and Uetanabaro, 2000), but the same was not observed by analyzing the number of eggs in ovaries and ovisacs (Garda *et al.*, 2007). Nevertheless, a comprehensive investigation of female fecundity is necessary to confirm such a correlation in *L. bolivianus*.

Alternative explanations for a female-biased SSD are differential growth or age at maturity, which can be explained by a higher male mortality rate or an earlier male maturation (Trivers, 1974; Monnet and Cherry, 2002). *Lysapsus bolivianus* from APA Curiaú is characterized by rapid growth to maturity with males growing faster than females (Sa-Oliveira *et al.*, 2020). However, both sexes have similar longevity and predation is one of the main causes of mortality in this population. Therefore, the differential growth to maturity between sexes associated to mortality can also explain a female-biased SSD observed in *L. bolivianus*. Moreover, the allometric relationships between size and shape can also be influenced by selective pressures acting differently upon each sex (Melo-Moreira *et al.*, 2021).

Some studies report sexual dimorphism in body shape, in which the size of a given morphometric attribute is proportionally different between sexes. For instance, males of *Leptodactylus fuscus* bear broader heads with longer and spatulate-shaped snouts in comparison to females due to their burrowing behavior (Melo-Moreira *et al.,* 2021). Likewise in the Neotropical Savanna, where males of *Rhinella rubescens* were found to have narrower heads, females of *R. schneideri* retained higher heads, likely associated to niche partitioning among sexes as head size is correlated to diet (Arantes *et al.,* 2015). However, the causes of sexual dimorphism in body shape are hard to track, given the impact of several life-history traits on the evolution of sexual dimorphism in anurans (Shine, 1989; Han and Fu, 2013). Contrary to the findings of Garda et al. (2007) for *L. limellum*, which found no evidence of shape dimorphism, the data gathered here suggest males may have proportionally larger heads in length. A previous study with the same population of *L. bolivianus* detected difference in prey volume ingested by males and females (Furtado and Costa-Campos, 2020). However, evidence is poor to support the formulation of a definitive conclusion relating diet with sexual dimorphism in head morphology in anurans. In addition, the time and conditions at which aquatic species reach maturity are crucial in determining survival, development, and growth between sexes have the potential to influence dimorphism (Zhang and Lu, 2013). Studies conducted on *L. bolivianus* in the same population reported marked seasonal fluctuations in water levels at the same study site, as well as environmental instability, which can affect sexes differently (Sa-Oliveira et al., 2020).

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TABLES AND FIGURES

Table 1. Summary of morphometric variables measured for *Lysapsus bolivianus* according to sex.

Variables	*Lysapsus bolivianus*	
	Males=168	Females=122
Snout-vent length (SVL)	14.01 - 20.92 (17.5 ± 1,13)	16.4 - 23.48 (19.7 ± 1.40)
Head length (HL)	3,98 - 8,93 (5,81 ± 0,76)	4.26 - 7.91 (5.86 ± 0.63)
Head width (HW)	4.24 - 7.35 (6.01 ± 0,58)	5.41 - 8.14 (6.86 ± 0.64)
Diameter of tympanum (DT)	0.94 - 2.14 (1.45 ± 0.22)	0.89 - 2.28 (1.55 ± 0.26)
Eye diameter (EYDM)	1.12 - 6.58 (2.27 ± 0.46)	1.71 - 3.22 (2.39 ± 0.26)
Interorbital distance (IOD)	1.13 - 3.8 (2.37 ± 0.75)	1.2 - 3.91 (2,49 ± 0.81)
Eye-to-nostril distance (EYNO)	0.8 - 4.14 (1.52 ± 0.32)	0.74 - 3.02 (1,66 ± 0.30)
Snout-nostril distance (SND)	0.25 -1.87 (0.96 ± 0.23)	0.43 - 1.54) (1.01 ± 0.20)
Internarial distance (IND)	0,71 - 2,27 (1.17 ± 0.22)	0.74 - 6.95 (1.23 ± 0.54)
Arm length (AL)	1.28 - 8.85 (2.64 ± 0.8)	0.65 - 4.96 (2.77 ± 0.74)
Forearm length (FAL)	1.5 - 4.27 (2.75 ± 0.5)	1.77 - 4.55 (2.96 ± 0.46)
Thigh length (THL)	6.52 - 12.55 (9.76 ± 1.07)	7.47 - 13.41 (10.7 ± 1.12)
Tibia length (TL)	6.98 -13.3 (10.3 ± 1,07)	7.97 - 13.71 (11,5 ± 1.13)

Table 2. Contribution of each variable to the first two axes of the PCAs in multidimensional morphometric space to explain the variation between males and females of *Lysapsus bolivianus* from APA do Curiaú, Amapá state, Brazil. Bold numbers highlight the variables with the highest contribution values for each Principal Component (PC).

Morphometric measurements	**PC1**	**PC2**
Snout-vent length (SVL)	**-0.43964**	0.052614
Head length ratio (HL/SVL)	**0.396395**	-0.09906
Head width ratio (HW/SVL)	0.141651	**-0.45538**
Diameter of tympanum ration (TD/SVL)	0.203381	-0.15397
Eye diameter ratio (EYDM/SVL)	0.257746	-0.21955
Interorbital distance ratio (IOD/SVL)	**0.343854**	**0.416092**
Eye-to-nostril distance ratio (EYNO/SVL)	0.195645	0.107765
Snout-nostril distance ratio (SND/SVL)	0.260847	0.053582
Internarial distance ratio (IND/SVL)	0.310285	0.07756
Arm length ratio (AL/SVL)	0.236180	0.354423
Forearm length ratio (FAL/SVL)	**0.331883**	0.076313
Thigh length ratio (THL/SVL)	0.177411	**-0.3811**
Tibia length ratio (TL/SVL)	0.059096	**-0.48768**
Explanation	19.3%	18.4%

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Figure 1. Adult males (A) and females (B) of *Lysapsus bolivianus* collected at Rio Curiaú Environmental Protection Area (APA Curiaú), north of Macapá city, Amapá state, Brazil.

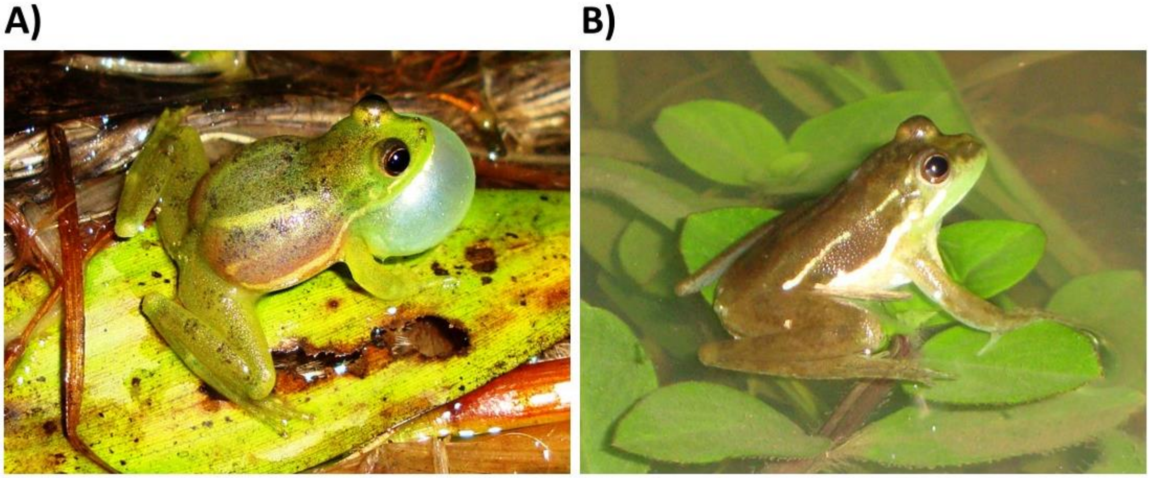

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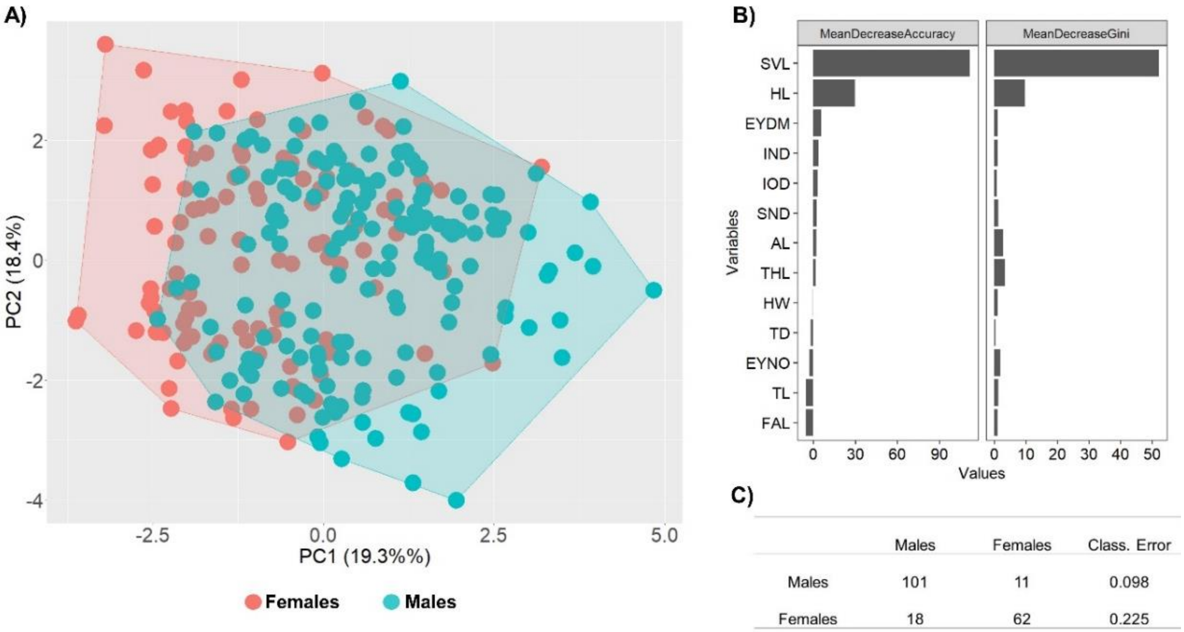
Figure 2. Cluster analysis based on morphometric variables for males and females *Lysapsus bolivianus*. A) Plot of the first and second multidimensional axis of the Principal Component Analysis, B) charts of variable importance scores of Random Forest and C)

confusion matrix showing the classification error of the individuals generated by RandomForest.



	Males	Females	Class. Error
Males	101	11	0.098
Females	18	62	0.225

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Figure 3. Boxplot representing snout-to-vent length (SVL). In females (F), SVL is greater than in males (M), and values were significantly different.

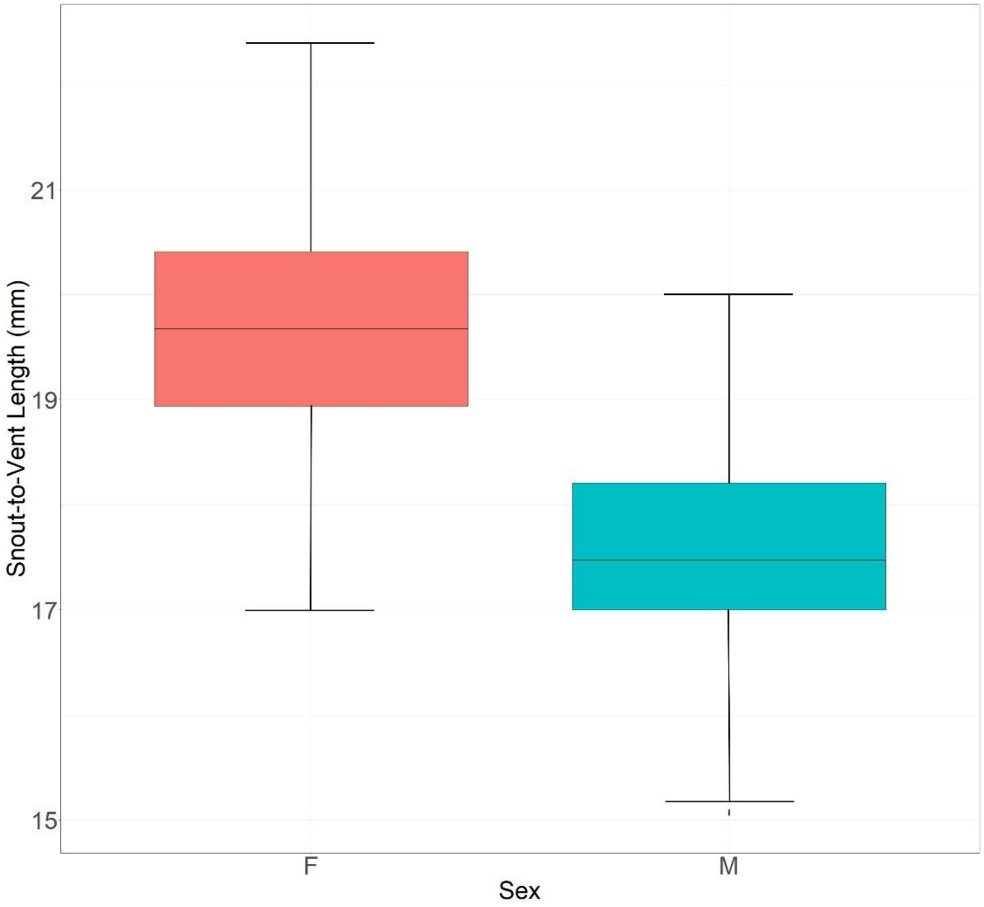

Figure 4. Boxplot representing head length ratio (head length/snout-to-vent length). In males (M), HL ratio is greater than in females (F), and values were significantly different.

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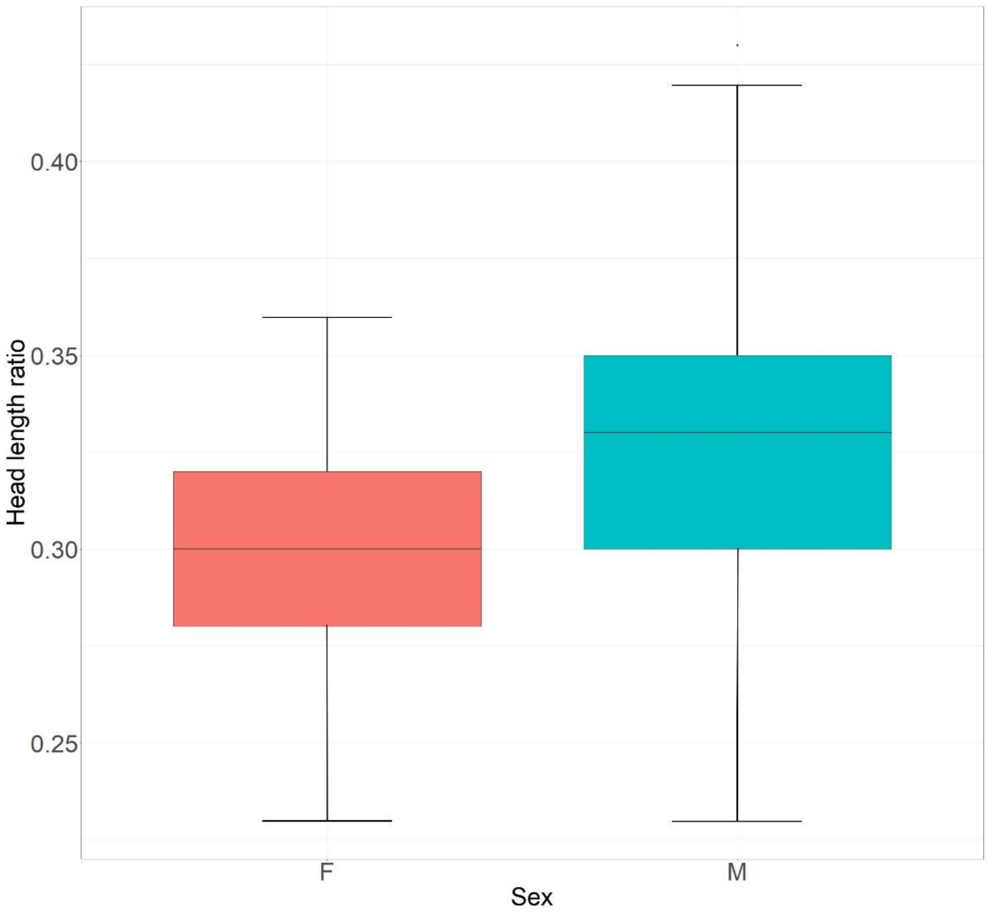

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