UNIVERSIDADE DE SÃO PAULO

FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA COMPARADA

“Sexual dimorphism in digit lengths in South American lizards: relationships with environment and embryonic development”

“Dimorfismo sexual no comprimento dos dígitos em lagartos sul-americanos: relações com o ambiente e o desenvolvimento embrionário”

Ana Paula Cipriano

Dissertação apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte das exigências para obtenção do título de Mestre em Ciências, obtido no Programa de Pós-Graduação em Biologia Comparada

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Orientadora: Profª Drª Tiana Kohlsdorf

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Profª Drª Tiana Kohlsdorf

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“All of us in the academy and in the culture as a whole are called to renew our minds if we are to transform educational institutions - and society - so that the way we live, teach and work can reflect our joy in cultural diversity, our passion for justice and our love of freedom”.

&

“One of the most vital ways we sustain ourselves is by building communities of resistance, places where we know we are not alone”.

Bell Hooks

RESUMO

CIPRIANO, A.P. Dimorfismo sexual no comprimento dos dígitos em lagartos sul-americanos: relações com o ambiente e o desenvolvimento embrionário. Dissertação (mestrado) - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2023.

O dimorfismo sexual na razão digital foi amplamente investigado em humanos. Tradicionalmente, o comprimento do segundo dígito (dedo indicador) e do quarto dígito (dedo anelar) são calculados em uma razão (2D:4D), na qual os homens possuem o DIV maior do que as mulheres. O dimorfismo no comprimento dos dígitos está associado aos níveis de hormônios esteroides durante o desenvolvimento embrionário e provavelmente não se altera durante a vida adulta, sendo estabelecido no período pré-natal. Embora a relação entre os dígitos e a dinâmica hormonal seja considerada consistente, a associação com parâmetros que possam afetar os níveis de esteroides, como climáticos e reprodutivos, ainda é inexplorada. Diversos estudos com Tetrapoda indicam que o dimorfismo no comprimento dos dígitos é diferente entre as espécies. É possível que todos os dígitos sejam sensíveis aos hormônios esteroides e o dimorfismo possa variar mesmo entre *manus* e *pes* na mesma espécie. Nosso estudo compilou dados morfológicos, climáticos e reprodutivos para explorar o dimorfismo sexual no comprimento dos dígitos em lagartos sul-americanos dos gêneros *Liolaemus* e *Tropidurus*. Os resultados indicam que o dimorfismo não é conservado e todos os dígitos podem ser dimórficos quando comparamos diferentes espécies e populações de lagartos. Além disso, o modo reprodutivo pode estar desempenhando um papel no dimorfismo devido às distintas condições de desenvolvimento embrionário, e lagartos vivíparos podem ter padrões de dígitos diferentes quando comparados aos ovíparos. Nosso estudo também analisou os efeitos de diferentes regimes térmicos durante o desenvolvimento embrionário. Os neonatos investigados são monomórficos para todas as características fenotípicas estudadas, independentemente da temperatura de incubação. Por outro lado, os receptores de andrógenos no Dígito IV são diferentes entre machos e fêmeas desde o nascimento, indicando o papel das interações hormonais no estabelecimento da morfologia ao longo da ontogenia. Nesse contexto, este estudo forneceu uma perspectiva diferente para investigar a função dos parâmetros climáticos, da dinâmica do desenvolvimento e de como o dimorfismo no comprimento dos dígitos é estabelecido em diferentes níveis de organização biológica. Como principais contribuições, exploramos pela primeira vez o papel dos parâmetros ambientais e reprodutivos no dimorfismo sexual no comprimento dos dígitos e indicamos os prováveis efeitos de receptores de esteroides no estabelecimento de traços fenotípicos em lagartos.

Palavras-chave: Comprimento dos dígitos, Dimorfismo sexual, Hormônios esteroides, Desenvolvimento Embrionário, Squamata.

ABSTRACT

CIPRIANO, A.P. Sexual dimorphism in digit lengths in South American lizards: relationships with environment and embryonic development. Dissertação (mestrado) - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2023.

The sexual dimorphism in digit ratio was extensively investigated in humans. Traditionally, the second digit (index finger) and the fourth digit (ring finger) lengths are calculated in a ratio (2D:4D), in which men have higher DIV than women. The dimorphism in digit lengths is associated with the levels of steroid hormones during the embryonic development and likely does not change during adulthood, being established prenatally. Although the relationship between digit ratio and hormonal dynamics is considered consistent, the association with parameters that might affect the steroid levels, such as climatic and reproductive ones, is still unexplored. Several studies with Tetrapoda show that the dimorphism in digit lengths is different amidst species. It is possible that all digits may have the sensitivity to steroid hormones and the dimorphism may vary even between *manus* and *pes* in the same species. Our study compiled morphological, climatic, and reproductive databases to explore the sexual dimorphism in digit lengths in South American lizards of the genera *Liolaemus* and *Tropidurus*. Results indicate that the dimorphism is not conserved and all digits can be dimorphic when comparing different lizards species and populations. Furthermore, the reproductive mode can be playing a role in the dimorphism due to the distinct developmental conditions, and viviparous lizards can have different digits patterns when compared to oviparous ones. Our study also analyzed the effects of different thermal regimes during embryonic development. The neonates investigated are monomorphic for all the phenotypic traits studied, regardless of incubation temperature. On the other hand, the androgen receptors in Digit IV are already different between male and female since hatch, indicating the role of hormonal interactions in shaping the morphology along ontogeny. In this context, this study provided a different perspective to investigate the role of climate parameters, developmental dynamics, and how the dimorphism in digit lengths is established in different levels of biological organization. As our main contributions, we explored for the first time the role of environmental and reproductive parameters in the sexual dimorphism in digit lengths and indicated the probable effects of steroid receptors in phenotypic traits establishment in lizards.

Keywords: Digit lengths, Sexual dimorphism, Steroid hormones, Embryonic development, Squamata.

SUMMARY

GENERAL INTRODUCTION

Differences in digit ratios between males and females were first investigated in humans (Manning et al. 1998). In this species, the length of the second digit (index finger; DII) is usually similar between men and women, while that of the fourth digit (ring finger; DIV) sometimes is sexually dimorphic (Manning et al. 1998). By dividing the lengths of these two digits, the 2D:4D ratio is estimated. Often men have higher DIV than women, and the first studies suggested that the dimorphism in digit ratios would relate to the levels of steroid hormones, mostly testosterone, during the embryonic development (Manning et al. 1998). Early studies observed that men with higher levels of testosterone have longer DIV and, consequently, lower 2D:4D ratio (Manning et al. 1998), a crucial finding for subsequent studies testing for associations between the 2D:4D ratio and a multitude of parameters, including health, athletic performance, and even complex traits such as behavior (Manning 2002). Hormones act regulating different morphological traits, and the digit lengths and associated ratios became a proxy of testosterone and estrogen levels during embryonic development (Manning et al. 1998, 2002), being considered good predictors for studies investigating aspects associated to endocrine regulation. After 1998, when Manning and collaborators shed light to the theme, the number of papers investigating the 2D:4D ratio hugely increased (Lutchmaya et al. 2004, Romano et al. 2005, Gobrogge et al. 2008, Talarovičová et al. 2009, Hönekopp and Watson 2010, Muller et al. 2011, Manning et al. 2014, Ribeiro et al. 2016, Manning and Fink 2018, 2020, Nagy et al. 2019).

Relationships between sexual dimorphism in digit ratios and other morphological traits regulated by testosterone and estrogen levels have been extensively investigated in humans (Manning et al. 1998, Manning 2002, Ribeiro et al. 2016, Manning and Fink 2018). For example, the condition known as Congenital Adrenal Hyperplasia (CAH) results in higher levels of embryonic androgens, and studies show that the individuals with CAH have masculinized 2D:4D ratio (i.e. a higher DIV; see Hönekopp and Watson 2010). On the other hand, conditions that lead to reduced levels of androgen during fetal development represent situations in which the digit ratio is, on average, feminized (see Klinefelter syndrome: Manning et al. 2013). Moreover, the 2D:4D ratio seems negatively related to physical fitness and performance in soccer, skiing, and a diversity of other sports (Manning and Taylor 2001, Manning 2002, Hönekopp et al. 2006). The correlation with diseases has been also highly explored (Manning 2002, Manning and Fink 2018). The digit ratio is positively associated to coronary heart disease and breast cancer, for example (Zhenghao et al. 2012), and recent studies suggest a positive association between the 2D:4D ratios and the most severe cases of COVID-19 (Manning and Fink 2020). The 2D:4D ratio in humans may be also associated with characteristics of personality and behavior (Manning 2002, Buchholz et al. 2019). A lower 2D:4D in men seems to be related to higher sociability and a larger social network, for example (Buchholz et al. 2019). Finally, the 2D:4D ratio also seems negatively related to sperm number and other reproductive functions in humans (Manning et al. 1998, Auger and Eustache 2011). Therefore, these studies indicate digit ratios as a reliable proxy for steroid dynamics during embryonic development and point out that digits should be analyzed as a way of understanding a tendency to develop some conditions.

In this context, Zheng and Cohn (2011) investigated the several mechanisms that are involved in the regulation of digit lengths and the embryonic stage in which the dimorphism is established. Using mice as their biological system, the authors show that androgen and estrogen receptors are higher in DIV compared to DII. Furthermore, digits' elongation is modulated by steroid dynamics during a narrow window in embryonic development in mice (i.e. stage 17 out of 21). The androgen and estrogen also play a role regulating the genes that are involved in the chondrocyte proliferation, which combined to the steroid receptors and levels, modulate a longer DIV in males (Zheng and Cohn 2011). The Zheng and Cohn work was fundamental to validate the DIV as the dimorphic one, responding in a higher magnitude to sex hormones regulation during development, and providing experimental base to consider the 2D:4D as a proxy of hormonal exposure in the early ontogenetic stages.

Results with humans and mice indicate that the sexual dimorphism in digit lengths is established during prenatal development and does not change after birth (Manning et al. 1998, 2002, Zheng and Cohn 2011). Investigations focusing on two-year-old children and adults show that individuals were already dimorphic since childhood, and authors did not identify differences in digit ratios associated to age (Manning et al. 1998, 2002). Similar to the results from Zheng and Cohn (2011) with mice, it is probable that the digits become dimorphic in humans also in a narrow stage during development, by week 13 (Manning 2002). The pioneer work published by Manning in 1998 was the first to suggest associations between embryonic testosterone in males and a longer DIV (Manning et al. 1998). On the other hand, relationships between digit ratio and steroid hormones after puberty remain unclear, and a possible role of hormones in digit elongation during adulthood is not completely discarded (see Manning et al. 2014). Steroid hormones may modulate embryos digits through the amount of receptors and, during adulthood, testosterone and estrogen might eventually reactivate some processes through interactions between hormonal signaling and receptors dynamics (Manning et al. 2014).

Other factors likely involved in sexual dimorphism in digits may involve the signaling cascade of *Hox* genes, especially two groups - *Hoxa* and *Hoxd* - which regulate the development of urogenital system and the digits (Kondo et al. 1997). Studies addressing the 'hand-foot-genital' syndrome describe abnormal development of Digit II and Digit V and irregularities in the reproductive system, highlighting the association between these two developmental processes (Mortlock and Innis 1997, Peichel et al. 1997). Due to this, Manning (2002) hypothesized that 2D:4D ratios would be similar among tetrapod lineages, considering the conserved *Hox* patterns in vertebrates (see Kondo et al. 1997) and a strong association between digit development and gonadal dynamics. However, recent studies indicate a great diversity in digit ratios among tetrapods, especially regarding the identity of the dimorphic digits (e.g. Burley and Foster 2004, Tobler et al. 2012, Lofeu et al. 2017, 2020, Kazimirski et al. 2019). For example, in zebra finches the 2D:4D pattern is the opposite of that described for humans and mice, being smaller in females than males (Burley and Foster 2004). In these animals, the digit ratios are also smaller in the first eggs to be laid, which suggests an effect from less androgen levels related to the egg laying order (Burley and Foster 2004). Manipulation of testosterone in the eggs of *Phasianus colchius*, for instance, results in larger 2D:3D digit ratios in females, but does not affect males (Romano et al. 2005). Finally, in salamanders there is a high diversity of patterns in the 2D:4D ratios, with some species exhibiting a similar trend to that described for humans, being larger in females for *Mesotriton alpestris, Lissotriton montandoni* and *Liossotriton vulgaris*, while other species seem monomorphic for this trait, as *Triturus cristatus* (Kaczmarski et al. 2015).

The hypothesis of evolutionary flexibility of sexual dimorphism in digit lengths has been organized into a theoretical model by Lofeu and collaborators (2017, 2020), based on data regarding the patterns and identity of dimorphic digits in lineages from different tetrapod clades. Exploring the 2D:4D ratio in *Leptodactylus* frogs, the authors identified that in the species *L. podicipinus* the digit that is dimorphic is DII, instead of DIV, resulting in a male-biased 2D:4D, while in *L. fuscus* the digits seem to be monomorphic (Lofeu et al. 2017). Testosterone treatments in the monomorphic *L. fuscus* resulted in tadpoles with a male-biased 2D:4D digit ratio in the experimental group, and the digit responding to the testosterone treatment was DII (Lofeu et al. 2017). Sexual dimorphism in the digit lengths of *Leptodactylus* frogs seem correlated with body size, and tadpoles treated with testosterone also become smaller (Lofeu et al. 2017). The model proposed from this study suggests that the evolution of the sexual dimorphism initially originates in some digits due to increased tissue sensitivity to steroids, admitting that all digits have this potential (Lofeu et al. 2017). Subsequently, phenotypic integration with other traits also sensitive to steroid levels may be established due to the hormonal pleiotropy (Lofeu et al. 2017, 2020). The phenotypic integration between digit lengths and other traits, including body size, may therefore be fitness-independent, and vary according to hormone sensitivity by specific digits and the hormonal dynamics (Figure 1, see also Hau 2007; Lofeu et al. 2017).

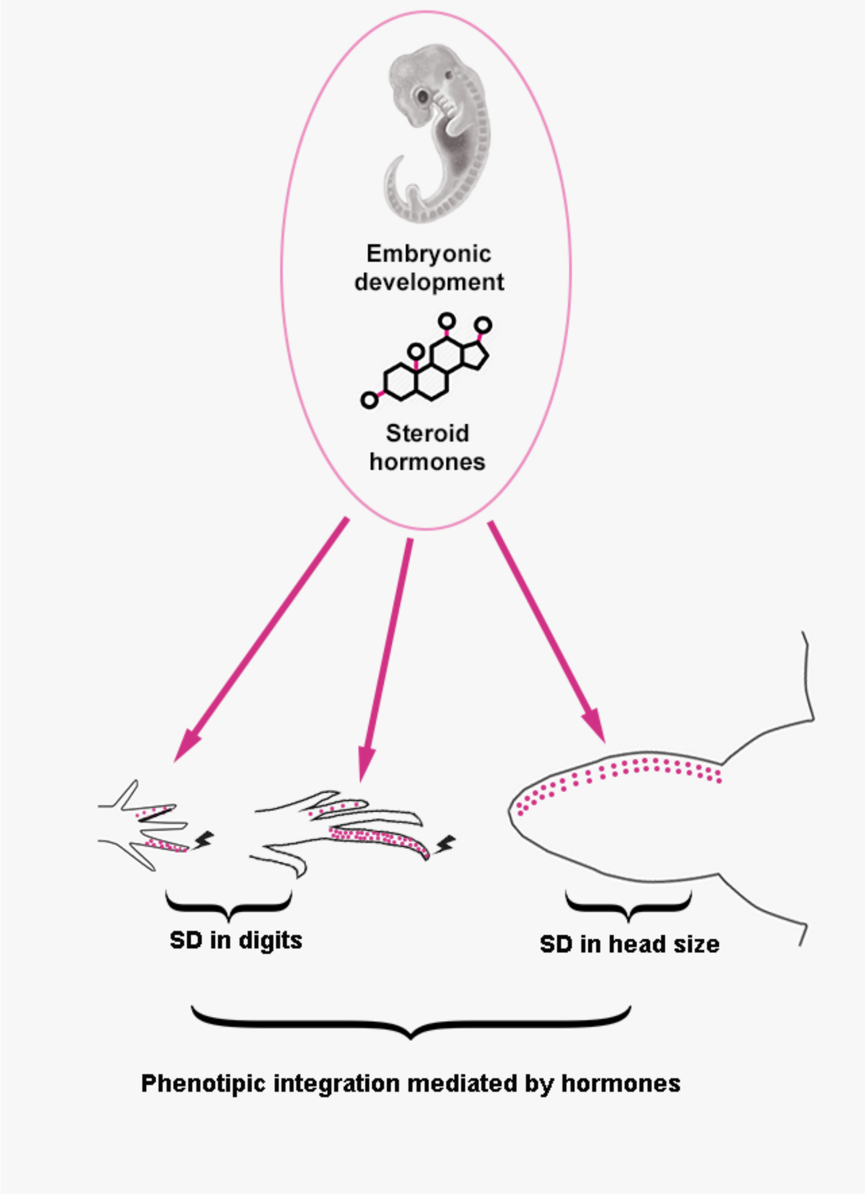


Figure 1. Representation of the model proposed by Lofeu et al (2017, 2020): steroid hormones during embryonic development modulate several traits, and phenotypic integration might occur mediated by this interaction. The small rays indicate digits that acquired sensitivity to hormones, and this can be very labile among species or populations. The abbreviation SD corresponds to sexual dimorphism.

The relationship between sexual dimorphism in digit ratios and the hormonal dynamics during development seems consistent in the literature (Manning et al. 1998, Manning 2002, Romano et al. 2005, Tobler et al. 2011, Zheng and Cohn 2011, Lofeu et al. 2017, Manning and Fink 2018), but it remains unclear if other parameters, including the climatic conditions where embryos develop, influence steroid levels and affect such association. The patterns of sexual dimorphism in 2D:4D in humans seem to differ among populations (Manning et al. 2000, Manning 2002). Differences in digit patterns among localities have also been identified in birds (e.g. *Taeniopygia guttata*: [Burley and Foster 2004, Forstmeier 2005]) and lizards (e.g. *Anolis carolinensis:* [Chang et al. 2006, Lombardo and Thorpe 2008]; *Tropidurus montanus:* [Lofeu et al. 2020, Miranda et al. 2021]). These examples highlight the potential of investigating patterns of sexual dimorphism along environmental gradients.

Although the majority of studies on sexual dimorphism in the digits focus on ratios between digits, investigating the digit length itself allows identification of which specific digits acquired sensitivity to the steroid hormones (see Kazimirski et al. 2019, Lofeu et al. 2020). Ratios between digits likely mask which digit is dimorphic, challenging discussions regarding variation in patterns and identity of dimorphic digits involving different species or populations. For example, the digit ratios in *Ciconia ciconia* birds do not differ between males and females, but DII, DIII and DIV are larger in the males, a pattern only identified when digits are evaluated separately (Kazimirski et al. 2019). As aforementioned, Lofeu and collaborators (2020) described great diversity in the identity of dimorphic digits in tropidurid lizards using digit lengths. Therefore, the use of digit lengths can be a relevant source of information for studies addressing relationships between steroids signaling during development and environmental conditions.

The present dissertation addresses these topics using integrative approaches that combine information of sexual dimorphism in digit lengths with information related to climate, geographical distribution along environmental gradients, and developmental dynamics of processes involved in digit elongation in lizards. The document is structured in two chapters that investigate relationships between sexual dimorphism in digit lengths and environmental parameters in lizards using different taxonomic, geographic, and ontogenetic scales. The first chapter presents results from *Liolaemus* lizards distributed along an altitudinal gradient, also evaluating relationships regarding the reproductive mode (i.e oviparous-viviparous continuum). The second chapter evaluates sexual dimorphism in digit lengths at intraspecific level by comparing lizards of *Tropidurus catalanensis* from different localities in the Brazilian central axis. In this second chapter, we manipulated incubation temperatures to evaluate sexual dimorphism in digit lengths of *T. catalanensis* neonates, also quantifying androgen receptors in the digits of neonates to investigate possible mechanisms regulating tissue responses to circulating steroids. Together, these two chapters may contribute significantly to elucidate how patterns of sexual dimorphism in digit lengths evolve in lizards, highlighting associations with the environment and candidate mechanisms modulating sexual dimorphism in this trait.

CHAPTER 1

Sexual dimorphism in digit lengths in *Liolaemus* lizards: relationships with altitudinal gradients and reproductive modes

ABSTRACT

Morphological patterns often evolve in association with environmental parameters, being the altitudinal gradient a particularly interesting source of environmental variation less often addressed in this context. In Tetrapoda, digit lengths (and associated ratios) are traits likely modulated by steroid hormones during embryonic development in several lineages, and hormone levels might be sensitive to environmental variation, including temperature and precipitation. Therefore, climatic patterns associated to altitudinal gradients may comprise environmental differences that modulate digit lengths through effects on hormone levels during development. Steroid hormones act in a systemic way, so their pleiotropic effects can modulate several developmental processes, integrating digit lengths with other phenotypic traits, such as body and head proportions. Developmental environments are determined by the condition where the embryos are incubated, and some lizard lineages comprise species with different reproductive modes, which may result in embryos developing under varied environmental conditions. In this study, we evaluated patterns of sexual dimorphism in the digit lengths of *Liolaemus* lizards distributed along elevational gradients and with different reproductive modes. We hypothesize that dimorphic patterns in digit lengths differ according to the reproductive mode and also in association with variation in climatic parameters in distinct elevations. We analyzed digit lengths in males and females of 46 *Liolaemus* species widely distributed, testing for associations with temperature, precipitation, reproductive modes, elevation, and body and head proportions. Results suggest that dimorphic patterns of digit lengths are not conserved among *Liolaemus* species, and the identity of the dimorphic digits varies even between closely related species. Patterns in digit lengths are not associated with environmental parameters in *Liolaemus*, but the reproductive mode seems to play a role in the establishment of this trait. We also found evidence of phenotypic integration between dimorphism in DIII from *pes*, body, and head proportions. Our study suggests some flexibility in the identity of the digits that become dimorphic in *Liolaemus* lizards, corroborating the hypothesis of labile processes establishing sexual dimorphism in the digits of this genus. The establishment of sexually dimorphic traits seems to be modulated by complex interactions between hormonal signaling and receptor regulation, which likely differ according to the species, the environment used, and the reproductive mode.

Keywords: Digit lengths, Sexual dimorphism, Altitudinal gradients, Reproductive modes, *Liolaemus*.

1. THEORETICAL CONTEXTUALIZATION

Species distributed along environmental gradients may present similar phenotypic patterns due to the sensitivity of developmental pathways to specific environmental parameters (Liefting et al. 2009, Oostra et al. 2011). Altitudinal gradients are a special source of environmental variation, comprising differences in parameters such as temperature and precipitation that likely affect physiological and developmental processes that ultimately establish body shape and size (Fischer et al. 2011, Keller et al. 2013, Slavenko et al. 2021). Mountain ranges are often claimed to represent 'sky islands' due to their potential to isolate animals in different zones that congregate diverse climate conditions (Mccormack et al. 2009). For example, the Andes mountain range, in South America, has a remarkable species diversity and, since it surpasses 6.000 meters of elevation, some animals must cope with several environmental fluctuations along this range (Herzog et al. 2011, Hazzi et al. 2018). Studies investigating phenotypic profiles in species that inhabit high altitudes described interesting patterns in body size (Leaché et al. 2010, Üzüm et al. 2018, Ortega et al. 2019, Slavenko et al. 2021), coloration (Moreno Azócar et al. 2020), enzyme activities (Dosek et al. 2007) and endocrine dynamics (Ortega et al. 2019). Investigation of phenotypic patterns related to environment parameters in Andean species may contribute for a better understanding of processes that are sensitive to the climatic variation observed along an altitudinal gradient.

Altitudinal gradients comprise differences in temperature and precipitation, two of the most important environmental factors explaining morphological differences among species distributed in localities that differ in climatic conditions (Iraeta et al. 2006, Ortega et al. 2019). As altitude increases, temperatures tend to decrease, with a corresponding reduction in atmospheric pressure and available oxygen (Barry 1992). Furthermore, precipitation dynamics usually changes with elevation, with high altitude regions experiencing lower precipitation levels, although not always expressing a linear relationship with altitude (Barry 1992). The cold temperatures and harsh environments observed in the mountains likely impose significant challenges for animals, especially ectotherms (Slavenko et al. 2021), and may affect physiological and developmental processes that ultimately influence the morphological patterns observed (Oostra et al. 2011, Reguera et al. 2014).

Among the physiological processes likely affected by environmental differences derived from altitudinal gradients, the endocrine signaling deserves special attention because hormones act in different ontogenetic stages and may affect several traits simultaneously. Steroid hormones, such as testosterone and estrogen, play important roles in the development and regulation of reproductive tissues and behaviors (Wingfield et al. 1987, Romeo 2003, Martínez-Juárez and Moreno-Mendoza 2019), and their dynamics may be influenced by environmental factors that include temperature, humidity and photoperiod (Schwabl 1996, Husak et al. 2020, Martínez-Juárez and Moreno-Mendoza 2019). The effects of environmental conditions on steroid levels may significantly impact the animal phenotype (Martínez-Juárez and Moreno-Mendoza 2019). For example, in some Tetrapoda species, differences in temperature together with hormone levels may modulate sex determination or the timing of reproductive events (Ball and Ketterson 2008, Ding et al. 2012, Rosmalen et al. 2021). In addition, differences in the hormonal dynamics may also affect muscles and bones that ultimately define morphological profiles, establishing sexually dimorphic patterns in specific traits (Brown 2008). Digit lengths (and associated ratios) are of particular interest in this context due to recent studies claiming this trait as a good proxy for hormone levels during development (see Lofeu et al., 2017, 2020). Associations between digit lengths and steroid levels have been suggested for several tetrapod lineages (e.g. Manning et al. 1998, Zheng and Cohn 2011, Lofeu et al., 2017, 2020), and this relationship is likely sensitive to environmental parameters (see Husak et al. 2021).

The idea of using digit lengths as a proxy for prenatal levels of circulating hormones was first developed from studies focusing on humans that identified correlations between the ratio of digits II and IV (2D:4D ratio) and several phenotypic traits that are also modulated by steroid hormones (see Manning et al. 1998, 2014, Ribeiro et al. 2016). It is important to note that the identity of the digit sensitive to prenatal hormone levels, and therefore sexually dimorphic, seems to be evolutionary labile and differ among lineages such as birds (Romano et al. 2005, Leoni et al. 2008, Cain et al. 2013), anurans (Beaty et al. 2016, Lofeu et al. 2017) and lizards (Tobler et al. 2012, Lofeu et al. 2017, Miranda et al. 2021), not being restricted to the Digit IV described for humans. A hypothetical model for the evolution of sexual dimorphism in digit lengths has been proposed by Lofeu et al. in 2017 and suggests that the initial sensibility of a given tissue to hormones might originate from changes in concentrations of steroids receptors independently of the hormone levels circulating in the embryo, being eventually favored by selection depending on the functional implications of dimorphic patterns.

Sexual dimorphism in digit ratios correlates with differences between males and females in morphological, reproductive, and behavioral traits (e.g. Manning et al. 1998, Lutchmaya et al. 2004, Manning and Fink 2018), an observation that paved the way for a hypothesis of phenotypic integration due to pleiotropic action modulated by steroid hormones (see Lofeu et al. 2017, 2021). According to this hypothesis, circulating levels of steroid hormones may integrate developmental responses in different tissues and structures that are sensitive to specific hormones (Lofeu et al. 2017, Cox 2020). Such ‘hormonal pleiotropy’ would be analogous to an “one-to-many” relationship, in which hormonal receptors and their respective ligands modulate the establishment of morphological traits that are functionally dissociated (see Cox 2020 and Lofeu et al. 2020). Accordingly, sexually dimorphic traits would be integrated through their sensitivity to steroids regardless of a functional relationship or a shared developmental pathway (Sanger et al. 2012, Lofeu et al. 2017, Cox 2020). The relationship between prenatal levels of circulating hormones and the sensitivity of specific tissues to steroids may be influenced by external parameters related to the developmental environment, such as incubation temperature or relative humidity in the nests, as thermal performance curves have been described for the endocrine system (Bradshaw 2003, Angilletta 2009).

Possible effects of the external developmental environment (e.g. incubation temperature or relative humidity in the nests) on the interaction between circulating hormones and developing traits might be particularly relevant for vertebrate ectotherms, especially considering the diversity of reproductive modes in some lineages. For example, the clade Squamata encompass at least three different reproductive modes that considerably differ in terms of the developmental environment provided for the embryos. Some lizards develop embryos inside an egg structure (oviparous species), while others represent the opposite condition of a placental-like development established inside the female’s body (viviparous species), and a continuum between these two extremes can be observed in several lineages (Blackburn 1994, 2000, Crocco et al. 2008, Rodríguez-Díaz and Braña 2011). The evolution of viviparity seems particularly relevant during occupation of cold environments by reptiles, including colonization of high elevations and latitudes (Shine 2004, Fernández et al. 2017, Cruz et al. 2022). In viviparous species, developmental conditions inside the female’s body might be buffered from external climatic fluctuations, representing a more stable developmental environment for the embryos (Cei et al. 2003, Fernández et al. 2017). On the other hand, embryos in viviparous species likely face longer exposure to maternal effects, including hormones that circulate in the female, due to such prolonged development within the mother (Woodhead et al. 2018), and maternal and between-siblings hormone transfers may affect phenotypic traits including digit lengths (see van Anders et al. 2006, Tapp et al. 2011, Auger et al. 2013).

Given the wide diversity of reproductive modes among species distributed in environmental gradients reported in several Squamata lineages, lizards represent an ideal biological system to investigate environmental and reproductive correlates of sexually dimorphic traits established during prenatal development. The genus *Liolaemus*, in particular, is a diversified lizard group distributed along altitudinal gradients of South America (Schulte et al. 2004, Esquerré et al. 2019, 2022). The genus comprises more than 250 described species (Esquerré et al. 2019) that occupy diverse habitats, from coastal environments to deserts and high-altitude localities in the Andes (e.g. Rocha 1999, Carothers et al. 2001, Esquerré et al. 2019), which implies exposure to remarkable environmental fluctuations in some *Liolaemus* species (Marquet et al. 1989, Carothers et al. 2001, Rodríguez-Serrano et al. 2009, Esquerré et al. 2019). Furthermore, the clade encompasses a similar number of species that are oviparous and viviparous, being the latter associated with cold climates (Schulte et al. 2000, Esquerré et al. 2019). We use *Liolaemus* lizards in this chapter to investigate relationships between dimorphic patterns in digit lengths and A) environmental parameters, B) reproductive modes and C) body and head proportions. We assembled a database composed by morphological measurements, climatic parameters, and a reproductive classification to test the following hypotheses: 1) *Liolaemus* lizards do not show conserved patterns of dimorphic digits, so we expect to identify different dimorphic digits between *manus* and *pes* and among species; 2) patterns of sexual dimorphism in digits are related to environmental parameters such as temperature and precipitation, due to the expected effects of these factors on physiological processes; 3) dimorphic patterns in digit lengths differ between viviparous and oviparous species; 4) dimorphic digits are related to other phenotypic traits that are also modulated by steroid hormones, such as head and body proportions.

2. MATERIAL AND METHODS

2.1 LIZARDS MORPHOLOGICAL MEASUREMENTS

We compiled a morphological database composed by measurements obtained in males and females of *Liolaemus* lizards available at *Museo Nacional de Historia Natural*, *Colección de Flora y Fauna Profesor Patricio Sánchez Reyes* in Santiago - Chile, and in the *Museo de La Plata*, in La Plata - Argentina. The specimens were sexed based on identification of cloacal differences between males and females (i.e. the cloacal shape and the presence of anal pores, see Torres-Pérez et al. 2003), and by evaluating the gonads morphology whenever necessary. We analyzed 629 individuals from 46 *Liolaemus* species (total of 306 females and 323 males; see Supplementary Material for details). All morphological measurements were obtained by the same person (APC), always on the left side of animals, using a digital caliper (Mitutoyo Inc. ± 0.01 mm). The morphological variables were chosen based on current literature (Van Damme et al. 2015, Barros 2016, Lofeu et al. 2020) and are structured into four categories (Figure 1):

1. Body size: snout-vent length and trunk length.

2. Body proportions: lengths of humerus, radius, femur, and tibia; length and height of pelvic and scapular girdles.

3. Head proportions: length, height, and width of the head.

4. Autopodium: lengths of the digits I, II, III, IV e V from *manus* and *pes*.

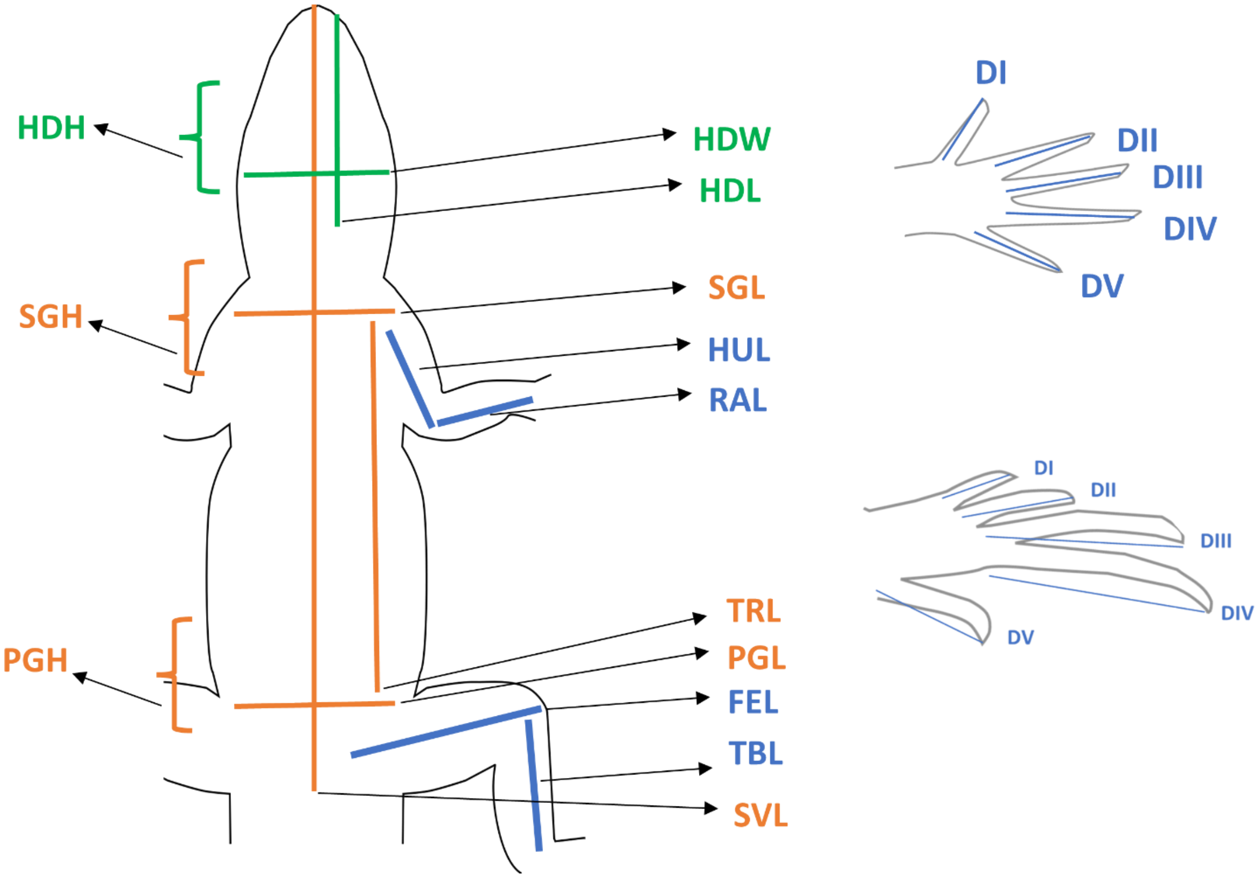


Figure 1. Morphological measurements for digits (right), and body (in orange), limbs (in blue), and head (in green) traits. Abbreviations correspond to: HDH = head height, HDW = head width, HDL = head length; SGH = shoulder girdle height, SGL = shoulder girdle length, PHG = pelvic girdle height, PGL = pelvic girdle length, TRL = trunk length, SVL = snout-vent length; HUL = humerus length, RAL = radio length, FEL = femur length, TBL = tibia length, D = digit.

2.2 CLASSIFICATION OF REPRODUCTIVE MODE AND ENVIRONMENTAL DATA

We classified species in viviparous or oviparous based on literature information regarding the reproductive mode (Esquerré et al. 2019, Figure 2). We also compiled an environmental database comprising information for altitude, mean temperature of wettest quarter, and precipitation seasonality (Coefficient of Variation). These parameters were selected based on previous studies suggesting a relationships between steroid hormones and precipitation seasonality (Husak et al. 2021) and also between temperature and reproductive season (Ramírez Pinilla 1991, 1992, Verrastro and Rauber 2013). We included the altitudinal gradient as a factor due to morphological correlations with altitude described in lizards (Shine 2004, Esquerré et al. 2019, Slavenko et al. 2021). In the museum collections, we recorded the latitudinal and longitudinal points for each location in which the specimens measured were collected, and then extracted the climate database from WorldClim. We used the bioclimatic variables 8 and 15 from WorldClim, with the resolution 2.5. The species localities and the classification of reproductive modes are synthesized in Table 1 for the 46 *Liolaemus* species studied here.

Table 1. *Liolaemus* species studied, with associated location and reproductive mode (o = oviparous, v = viviparous).

Species	**Location**	**Reproductive mode**
Liolaemus chiliensis	Nuble CL	o
Liolaemus fuscus	Nuble CL	o
Liolaemus gravenhorstii	Santiago CL	v
Liolaemus lutzae	Cabo Frio BR	o
Liolaemus nigroviridis	Santiago CL	v
Liolaemus occipitalis	Gravatai BR	o
Liolaemus alticolor	Putri CL	v
Liolaemus andinus	El Loa CL	v
Liolaemus atacamensis	Chanaral CL	o
Liolaemus bellii	Santiago CL	v
Liolaemus bibronii	Neuquen AR	o
Liolaemus carlosgarini	Talca CL	v
Liolaemus constanzae	El Loa CL	o
Liolaemus curicensis	Conchaga CL	o
Liolaemus darwinii	La Batea AR	o
Liolaemus elongatus	Chubut AR	v
Liolaemus fitzgeraldi	Valparaiso CL	v
Liolaemus fitzingerii	Santa Cruz AR	o
Liolaemus hellmichi	Antofagasta CL	o
Liolaemus kingii	Chubut AR	v
Liolaemus koslowskyi	Catamarca AR	v
Liolaemus lemniscatus	Santiago CL	o
Liolaemus lorenzmuelleri	Elqui CL	o
Liolaemus magellanicus	Chubut AR	v
Liolaemus monticola	Marga CL	o
Liolaemus nigromaculatus	Chanaral CL	o
Liolaemus nitidus	Limari CL	o
Liolaemus ornatus	Potosi BO	v
Liolaemus paulinae	El Loa CL	v

Liolaemus pictus	Neuquen AR	v
Liolaemus platei	Elqui CL	o
Liolaemus poconchilensis	Arica CL	v
Liolaemus pseudolemniscatus	Limari CL	o
Liolaemus ramonensis	Santiago CL	v
Liolaemus schroederi	Santiago CL	v
Liolaemus tenuis	Santiago CL	o
Liolaemus valdesianus	Cordillera CL	v
Liolaemus wiegmannii	San Luis AR	o
Liolaemus zapallarensis	Valparaiso CL	o
Liolaemus lineomaculatus	Chubut AR	v
Liolaemus multicolor	Jujuy AR	v
Liolaemus loboi	Neuquen AR	o
Liolaemus audituvelatus	Copiapo CL	v
Liolaemus moradoensis	Cordillera CL	v
Liolaemus buergeri	Maule CL	v
Liolaemus velosoi	Barranquila CL	o

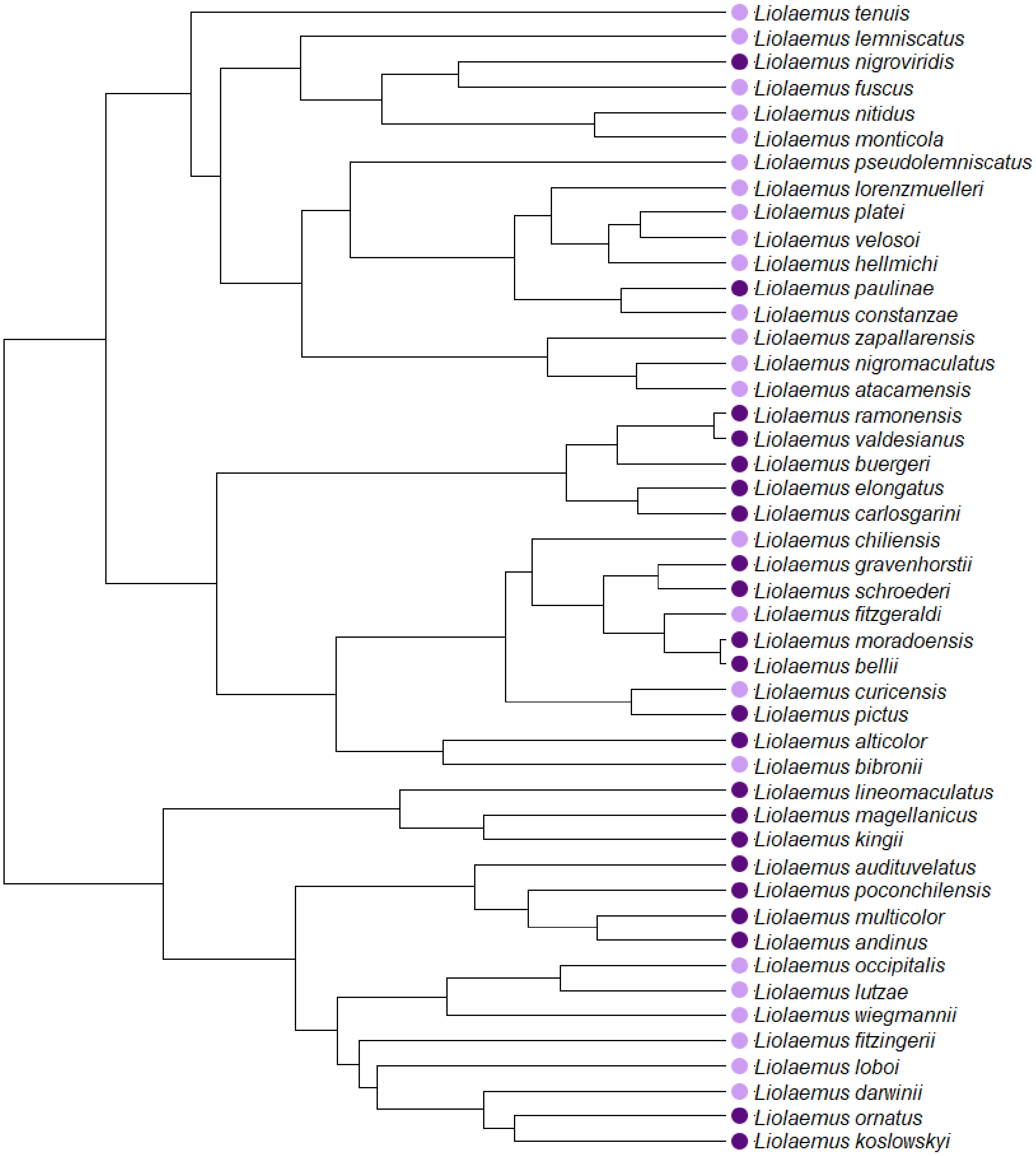


Figure 2. Phylogenetic tree representing the *Liolaemus* species studied here, classified as viviparous (dark purple) or oviparous (light purple).

2.3 DATA ANALYZES

Statistical analyses were performed in the R environment, version 4.2.0. All morphological measurements were log10 transformed and corrected by SVL before subsequent analyses. For each species, we did regressions of the log-transformed traits against log SVL and then extracted the residuals (Shingleton 2010). Data were tested for normality and homogeneity using Shapiro-wilk and Levene tests, and some morphological traits did not match the premises for parametric approaches. We performed Mann-Whitney tests considering the length of the digit in relation to the sex to identify the dimorphic digits within each *Liolaemus* species. We also implemented a Random Forest (RF) analysis using the package randomForest to evaluate differences between males and females in the digit lengths of the *manus* and *pes* in each species (see Breiman 2001). The RF is a machine-learning approach that can be used for morphological classification. In this work, the RF analyzes summarized results produced by 1000 trees, creating a consensus that congregates classification matches in males or females. The function plotProximity (package rfPermute) allows a graphic visualization of the RF results using multidimensional scaling. The Accuracy and Gini index in the RF analysis demonstrate which variables are more important to correctly classify specimens as males or females. We selected the digits ranked as the most important for the sex classification in *Liolaemus* species to perform analyses testing for correlations with environmental parameters, reproductive modes, and other phenotypic traits.

To test for associations with the environmental and reproductive factors, a Phylogenetic Generalized Least Squares (PGLS) analysis was performed using the function gls (package nlme; Pinheiro et al. 2018). The models were implemented using the most relevant digits selected from the RF analysis. For each of the seven models proposed, a PGLS analysis with variable lambda was implemented. The Akaike criteria was used to select the best-fit model for each digit, assuming AIC differences higher 2 as statistically significant. The PGLS analyses were implemented using Sexual Dimorphism Indexes (SDI) of digit lengths and also the residuals from digits regressed on SVL for males and females separately (see Corl et al. 2010, Gomes and Kohlsdorf 2011). We calculated the SDI with the aim of inferring the magnitude of sex differences in the digits evaluated. To calculate the SDI, we divided the male values by those of the females in male-biased species and used the formula [2-female/male)] in species where digit lengths are longer in females than in males (Corl et al. 2010, Gomes and Kohlsdorf 2011). We added 1 to the residuals from the size correction to obtain positive values to standardize the SDI (see Gomes and Kohlsdorf 2011). We also used SDI to test for associations between digit lengths and other morphological traits, implementing PGLS analyzes as described by Lofeu et al. (2020). All phylogenetic analyzes used a topology based on Esquerré et al. (2019; see Figure 2).

3. RESULTS

We identified sexual dimorphism in digits of 26 out of 46 *Liolaemus* species (Mann-Whitney tests, Figure 3), and the identity of dimorphic digits differed among species (Tables 2 and 3). In some species, such as *Liolaemus lutzae*, *Liolaemus nigroviridis*, and *Liolaemus poconchilensis,* digits were dimorphic only in the *manus*, while in *Liolaemus chiliensis, Liolaemus occipitalis, Liolaemus atacamensis, Liolaemus fitzgeraldi, Liolaemus lemniscatus, Liolaemus magellanicus, Liolaemus ornatus, Liolaemus pictus, Liolaemus schroederi, Liolaemus tenuis, Liolaemus valdesianus,* and *Liolaemus lineomaculatus* the dimorphic digits were concentrated in the *pes.* The patterns of sexual dimorphism in digit lengths are not conserved among *Liolaemus,* and sexual dimorphism in body, head and limb proportions also varied among species (Supplementary Material).

Table 2. Results of Mann-Whitney tests implemented to identify differences between sexes in digit lengths in the *manus* of *Liolaemus* species; significant results ($p < 0.05$) are highlighted in bold.

Species	**DI**		**DII**		**DIII**		**DIV**		**DV**	
	U	**p**	**U**	**P**	**U**	**p**	**U**	**p**	**U**	**p**
Liolaemus chiliensis	41	0.529	24	0.052	32	0.190	26	0.075	42	0.579
Liolaemus fuscus	**8**	**0.043**	14	0.228	9	0.059	9	0.059	22	0.852
Liolaemus gravenhorstii	5	0.082	5	0.082	**2**	**0.017**	5	0.082	7	0.177
Liolaemus lutzae	**12**	**0.042**	19	0.210	21	0.299	29	0.837	**7**	**0.008**
Liolaemus nigroviridis	15	0.699	**1**	**0.004**	**6**	**0.006**	8	0.132	11	0.310
Liolaemus occipitalis	14	0.589	8	0.132	7	0.093	9	0.180	8	0.132
Liolaemus alticolor	10	0.429	10	0.429	11	0.537	4	0.052	**3**	**0.030**
Liolaemus andinus	21	0.898	**7**	**0.042**	**6**	**0.029**	**2**	**0.004**	10	0.112
Liolaemus atacamensis	21	0.463	19	0.336	27	0.955	24	0.694	28	1.000
Liolaemus bellii	22	0.230	30	0.669	24	0.315	29	0.601	31	0.740
Liolaemus bibronii	35	0.592	23	0.120	41	0.967	38	0.773	30	0.340
Liolaemus carlosgarini	17	0.724	19	0.943	12	0.284	9	0.127	18	0.833
Liolaemus constanzae	22	0.428	23	0.492	29	0.958	20	0.313	29	0.958
Liolaemus curicensis	40	0.720	33	0.356	22	0.065	30	0.243	33	0.356
Liolaemus darwinii	17	0.142	20	0.252	26	0.606	28	0.758	27	0.681
Liolaemus elongatus	20	0.620	12	0.128	22	0.805	21	0.710	14	0.209
Liolaemus fitzgeraldi	4	0.052	12	0.662	5	0.082	10	0.429	6	0.126

Liolaemus fitzingerii	16	0.345	15	0.282	22	0.852	17	0.414	11	0.108
Liolaemus hellmichi	14	0.639	13	0.530	17	1.000	16	0.876	17	1.000
Liolaemus kingii	**11**	**0.042**	26	0.713	**9**	**0.022**	**7**	**0.011**	**4**	**0.003**
Liolaemus koslowskyi	34	0.261	45	0.766	44	0.710	49	1.000	29	0.131
Liolaemus lemniscatus	41	0.840	43	0.968	37	0.600	41	0.840	35	0.492
Liolaemus lorenzmuelleri	14	0.931	13	0.792	11	0.537	15	1.000	13	0.792
Liolaemus magellanicus	26	0.867	20	0.397	17	0.232	16	0.189	21	0.463
Liolaemus monticola	10	0.081	**8**	**0.043**	17	0.414	20	0.662	16	0.345
Liolaemus nigromaculatus	13	0.295	15	0.445	15	0.445	16	0.534	15	0.445
Liolaemus nitidus	10	0.050	25	0.864	13	0.113	23	0.689	**6**	**0.012**
Liolaemus ornatus	21	0.755	17	0.414	13	0.181	12	0.142	13	0.181
Liolaemus paulinae	22	0.200	30	0.606	30	0.606	32	0.743	30	0.606
Liolaemus pictus	29	0.360	36	0.729	37	0.829	27	0.274	36	0.762
Liolaemus platei	29	0.601	28	0.536	20	0.161	**13**	**0.033**	26	0.417
Liolaemus poconchilensis	**5**	**0.041**	6	0.065	12	0.394	8	0.132	13	0.485
Liolaemus pseudolemniscatus	6	0.073	**5**	**0.048**	6	0.073	6	0.073	11	0.343
Liolaemus ramonensis	14	0.931	15	1.000	13	0.792	11	0.537	9	0.329
Liolaemus schroederi	10	0.429	4	0.052	7	0.177	4	0.052	13	0.792
Liolaemus tenuis	24	0.470	19	0.210	16	0.114	13	0.055	30	0.918
Liolaemus valdesianus	15	0.755	12	0.432	12	0.432	6	0.073	11	0.343
Liolaemus wiegmannii	18	0.833	14	0.435	**6**	**0.045**	**5**	**0.030**	15	0.524
Liolaemus zapallarensis	21	0.463	25	0.779	20	0.397	27	0.955	12	0.072
Liolaemus lineomaculatus	10	0.527	12	0.788	14	1.000	10	0.527	11	0.648
Liolaemus multicolor	2	0.143	6	0.786	6	0.786	6	0.786	7	1.000
Liolaemus loboi	2	0.114	5	0.486	5	0.486	7	0.886	7	0.886
Liolaemus audituvelatus	6	0.548	8	0.905	2	0.095	**0**	**0.024**	3	0.167
Liolaemus moradoensis	6	1.000	4	0.629	4	0.629	5	0.857	3	0.400
Liolaemus buergeri	8	0.905	4	0.262	7	0.714	7	0.714	8	0.905
Liolaemus velosoi	4	0.114	9	0.610	6	0.257	3	0.067	8	0.476

Table 3. Results of Mann-Whitney tests implemented to identify differences between sexes in digit lengths in the *pes* of *Liolaemus* species; significant results (p <0.05) are highlighted in bold.

Species	**DI**		**DII**		**DIII**		**DIV**		**DV**	
	U	**P**	**U**	**P**	**U**	**P**	**U**	**p**	**U**	**P**
Liolaemus chiliensis	33	0.218	**21**	**0.029**	**16**	**0.009**	25	0.063	**2**	**<.001**
Liolaemus fuscus	22	0.852	21	0.755	21	0.755	**7**	**0.029**	12	0.142
Liolaemus gravenhorstii	**0**	**0.004**	5	0.082	4	0.052	**2**	**0.017**	**3**	**0.030**
Liolaemus lutzae	19	0.210	29	0.837	30	0.918	30	0.918	21	0.299
Liolaemus nigroviridis	14	0.589	13	0.485	12	0.394	17	0.937	10	0.240
Liolaemus occipitalis	9	0.180	17	0.937	**2**	**0.009**	**2**	**0.009**	7	0.093
Liolaemus alticolor	6	0.126	**0**	**0.004**	10	0.429	**3**	**0.030**	**3**	**0.030**
Liolaemus andinus	**4**	**0.012**	**5**	**0.019**	**4**	**0.012**	**4**	**0.012**	**2**	**0.004**
Liolaemus atacamensis	27	0.955	23	0.613	**7**	**0.014**	17	0.232	22	0.536
Liolaemus bellii	16	0.070	28	0.536	28	0.536	30	0.669	22	0.230
Liolaemus bibronii	36	0.650	39	0.837	35	0.592	30	0.340	33	0.482
Liolaemus carlosgarini	13	0.354	13	0.354	13	0.354	10	0.171	8	0.093
Liolaemus constanzae	12	0.056	17	0.181	24	0.562	26	0.713	25	0.635
Liolaemus curicensis	44	0.968	30	0.243	24	0.095	21	0.053	29	0.211
Liolaemus darwinii	26	0.606	20	0.252	29	0.837	24	0.470	27	0.681
Liolaemus elongatus	22	0.805	24	1.000	23	0.902	16	0.318	19	0.535
Liolaemus fitzgeraldi	8	0.247	**2**	**0.017**	4	0.052	**1**	**0.009**	7	0.177
Liolaemus fitzingerii	22	0.852	20	0.662	20	0.662	18	0.491	16	0.345
Liolaemus hellmichi	16	0.876	13	0.530	13	0.530	14	0.639	14	0.639
Liolaemus kingii	**10**	**0.031**	15	0.118	**9**	**0.022**	12	0.056	15	0.118
Liolaemus koslowskyi	40	0.503	42	0.603	33	0.230	25	0.067	47	0.882
Liolaemus lemniscatus	30	0.272	32	0.351	**19**	**0.041**	**19**	**0.041**	29	0.238
Liolaemus lorenzmuelleri	13	0.792	14	0.931	11	0.537	12	0.662	12	0.662
Liolaemus magellanicus	22	0.536	**10**	**0.040**	**8**	**0.021**	**6**	**0.009**	21	0.463
Liolaemus monticola	9	0.059	16	0.354	16	0.354	18	0.491	**8**	**0.043**
Liolaemus nigromaculatus	16	0.534	20	0.945	18	0.731	18	0.731	13	0.295
Liolaemus nitidus	18	0.328	22	0.607	21	0.529	15	0.181	**0**	**<.001**
Liolaemus ornatus	**8**	**0.043**	15	0.282	**2**	**0.003**	10	0.081	20	0.662
Liolaemus paulinae	28	0.481	31	0.673	35	0.963	30	0.606	16	0.059
Liolaemus pictus	26	0.237	**14**	**0.021**	21	0.101	30	0.408	27	0.247
Liolaemus platei	18	0.109	**1**	**<.001**	**9**	**0.010**	**1**	**<.001**	**9**	**0.010**

Liolaemus poconchilensis	10	0.240	12	0.394	8	0.132	7	0.093	12	0.940
Liolaemus pseudolemniscatus	**4**	**0.030**	**2**	**0.010**	**4**	**0.030**	**1**	**0.005**	**4**	**0.030**
Liolaemus ramonensis	10	0.429	14	0.931	11	0.537	12	0.662	15	1.000
Liolaemus schroederi	**3**	**0.030**	8	0.247	4	0.052	8	0.247	9	0.329
Liolaemus tenuis	**7**	**0.008**	**7**	**0.008**	**6**	**0.005**	**6**	**0.005**	27	0.681
Liolaemus valdesianus	**1**	**0.005**	14	0.639	16	0.876	15	0.755	14	0.639
Liolaemus wiegmannii	8	0.093	14	0.435	**0**	**0.002**	**4**	**0.019**	9	0.127
Liolaemus zapallarensis	24	0.694	27	0.955	25	0.779	27	0.955	12	0.072
Liolaemus lineomaculatus	7	0.230	5	0.109	**0**	**0.006**	**3**	**0.042**	**2**	**0.024**
Liolaemus multicolor	5	0.571	6	0.786	6	0.786	5	0.571	4	0.393
Liolaemus loboi	5	0.486	7	0.886	4	0.343	8	1.000	8	1.000
Liolaemus audituvelatus	8	0.905	**0**	**0.024**	**0**	**0.024**	6	0.548	8	0.905
Liolaemus moradoensis	2	0.229	1	0.114	1	0.114	1	0.114	3	0.400
Liolaemus buergeri	5	0.381	7	0.714	6	0.548	3	0.167	7	0.714
Liolaemus velosoi	9	0.610	7	0.352	8	0.476	6	0.257	5	0.171

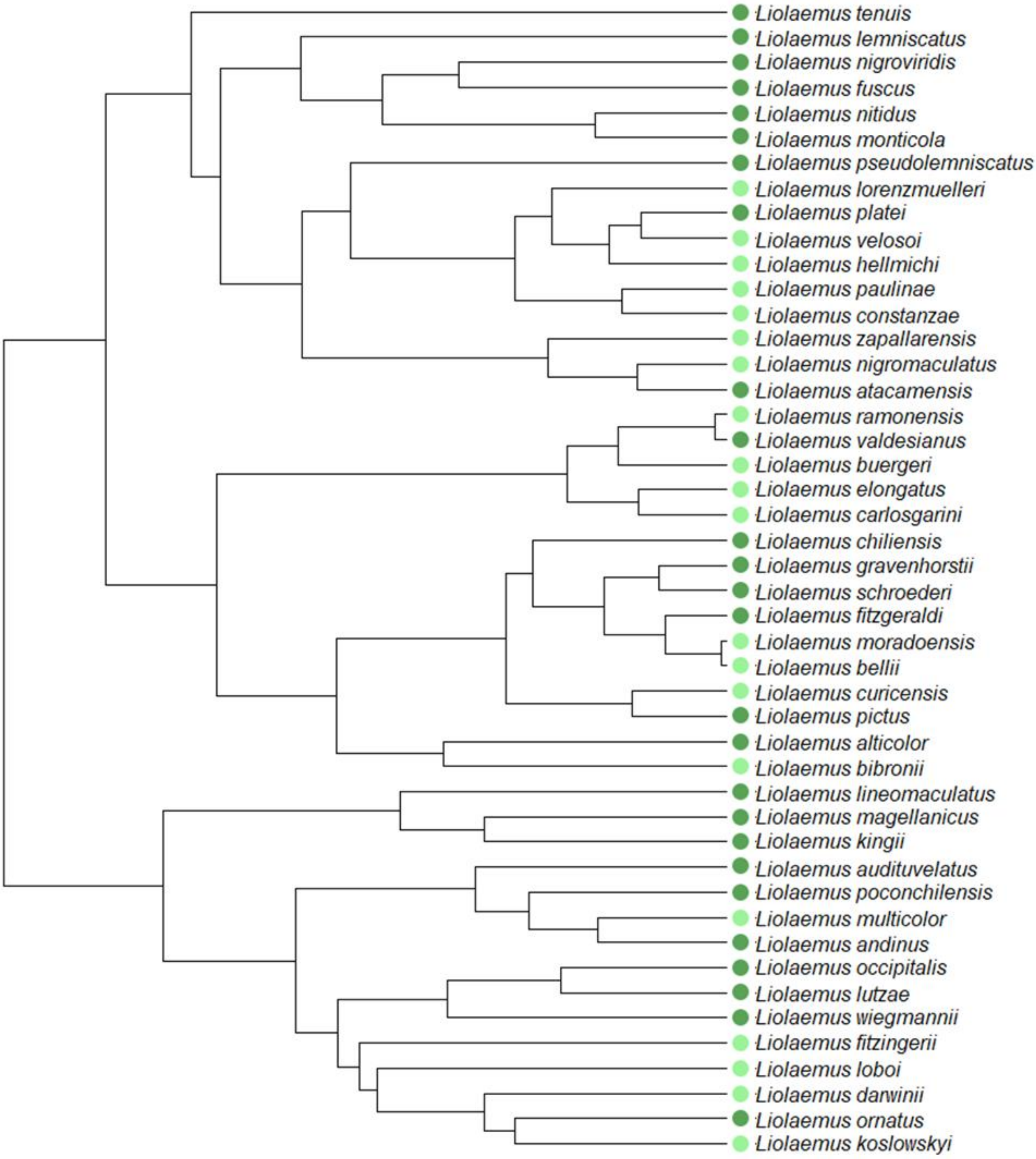


Figure 3. Topology of *Liolaemus* species coded for the presence of sexual dimorphism in the digits (dark green) or monomorphic patterns in digit lengths (light green).

We used results from the RF analyzes to identify in each species which digits from the *manus* and *pes* contribute to correctly classify individuals in males or females based on their morphology. The DIV and DIII from *pes* contributed for the classification between sexes in most *Liolaemus* species, as described by the Accuracy and Gini index. Also, the error rate differed among species (Table 4). In species with higher error rates, it was possible to identify digits that did not discriminate males from females (Figure 4).

Table 4. Confusion matrix for the RF results using *Liolaemus* species; comparisons based on the phenotypic profile of males and females using 1000 tree permutations.

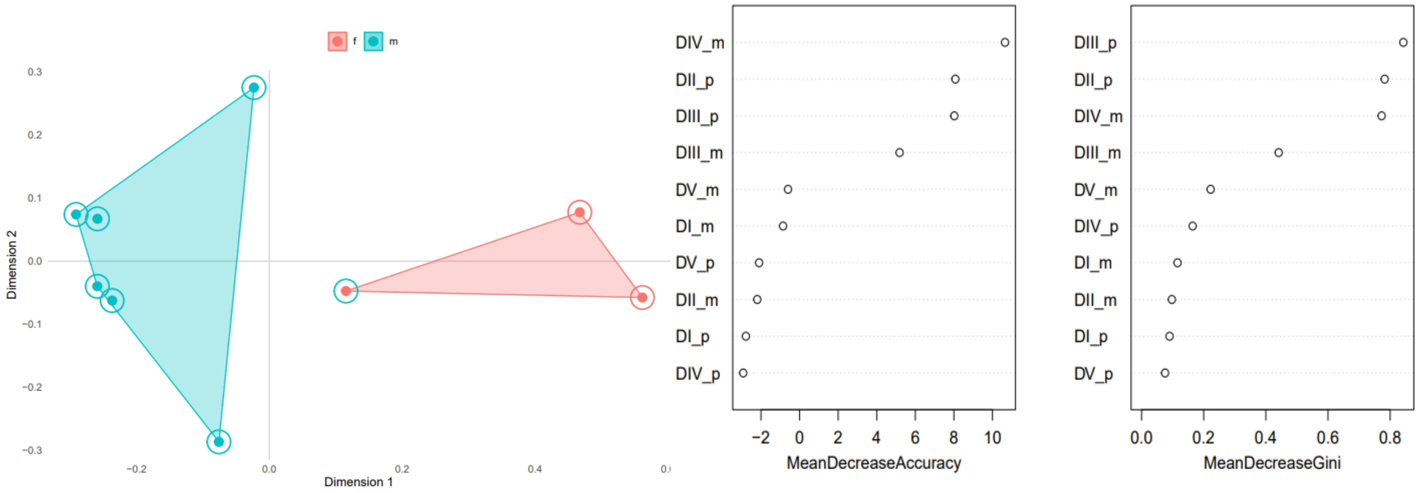
Species	General average error		Female	Male	Classification error
Liolaemus chiliensis	15.00%	**Female**	9	1	10.00%
Liolaemus chiliensis		**Male**	2	8	20.00%
Liolaemus fuscus	35.71%	**Female**	3	3	50.00%
Liolaemus fuscus		**Male**	2	6	25.00%
Liolaemus gravenhorstii	27.27%	**Female**	5	1	16.66%
Liolaemus gravenhorstii		**Male**	2	3	40.00%
Liolaemus lutzae	35.50%	**Female**	6	3	33.33%
Liolaemus lutzae		**Male**	3	4	42.85%
Liolaemus nigroviridis	33.33%	**Female**	3	3	50.00%
Liolaemus nigroviridis		**Male**	1	5	16.66%
Liolaemus occipitalis	41.67%	**Female**	4	2	33.30%
Liolaemus occipitalis		**Male**	3	3	50.00%
Liolaemus alticolor	18.18%	**Female**	4	1	20.00%
Liolaemus alticolor		**Male**	1	5	16.60%
Liolaemus andinus	21.43%	**Female**	8	1	11.11%
Liolaemus andinus		**Male**	2	3	40.00%
Liolaemus atacamensis	53.33%	**Female**	3	4	57.14%
Liolaemus atacamensis		**Male**	4	4	50.00%
Liolaemus bellii	41.18%	**Female**	3	4	57.14%
Liolaemus bellii		**Male**	3	7	30.00%
Liolaemus bibronii	47.37%	**Female**	0	7	100.00%
Liolaemus bibronii		**Male**	2	10	16.66%
Liolaemus carlosgarini	38.46%	**Female**	5	3	37.50%
Liolaemus carlosgarini		**Male**	2	3	40.00%
Liolaemus constanzae	56.25%	**Female**	0	6	100.00%
Liolaemus constanzae		**Male**	3	7	30.00%
Liolaemus curicensis	52.63%	**Female**	5	5	50.00%
Liolaemus curicensis		**Male**	5	4	55.55%

Liolaemus darwinii	50.00%	**Female**	3	4	57.14%
Liolaemus darwinii		**Male**	4	5	44.44%
Liolaemus elongatus	78.57%	**Female**	2	5	71.42%
Liolaemus elongatus		**Male**	6	1	85.71%
Liolaemus fitzgeraldi	36.36%	**Female**	4	2	33.33%
Liolaemus fitzgeraldi		**Male**	2	3	40.00%
Liolaemus fitzingerii	71.43%	**Female**	1	5	83.33%
Liolaemus fitzingerii		**Male**	5	3	62.5%
Liolaemus hellmichi	75.00%	**Female**	0	5	100.00%
Liolaemus hellmichi		**Male**	4	3	57.14%
Liolaemus kingii	25.00%	**Female**	4	2	33.33%
Liolaemus kingii		**Male**	2	8	20.00%
Liolaemus koslowskyi	50.00%	**Female**	7	4	36.36%
Liolaemus koslowskyi		**Male**	6	3	66.66%
Liolaemus lemniscatus	57.89%	**Female**	7	4	36.36%
Liolaemus lemniscatus		**Male**	7	1	85.50%
Liolaemus lorenzmuelleri	63.64%	**Female**	1	5	83.33%
Liolaemus lorenzmuelleri		**Male**	2	3	40.00%
Liolaemus magellanicus	26.67%	**Female**	7	1	12.50%
Liolaemus magellanicus		**Male**	3	4	42.85%
Liolaemus monticola	35.71%	**Female**	3	3	50.00%
Liolaemus monticola		**Male**	2	6	25.00%
Liolaemus nigromaculatus	69.23%	**Female**	0	6	100.00%
Liolaemus nigromaculatus		**Male**	3	4	42.85%
Liolaemus nitidus	13.33%	**Female**	4	2	33.33%
Liolaemus nitidus		**Male**	0	9	0.00%
Liolaemus ornatus	42.86%	**Female**	3	3	50.00%
Liolaemus ornatus		**Male**	3	5	37.50%
Liolaemus paulinae	41.18%	**Female**	4	4	50.00%
Liolaemus paulinae		**Male**	3	6	33.33%
Liolaemus pictus	33.33%	**Female**	7	3	30.00%
Liolaemus pictus		**Male**	3	5	37.50%
Liolaemus platei	11.76%	**Female**	9	1	10.00%
Liolaemus platei		**Male**	1	6	14.28%

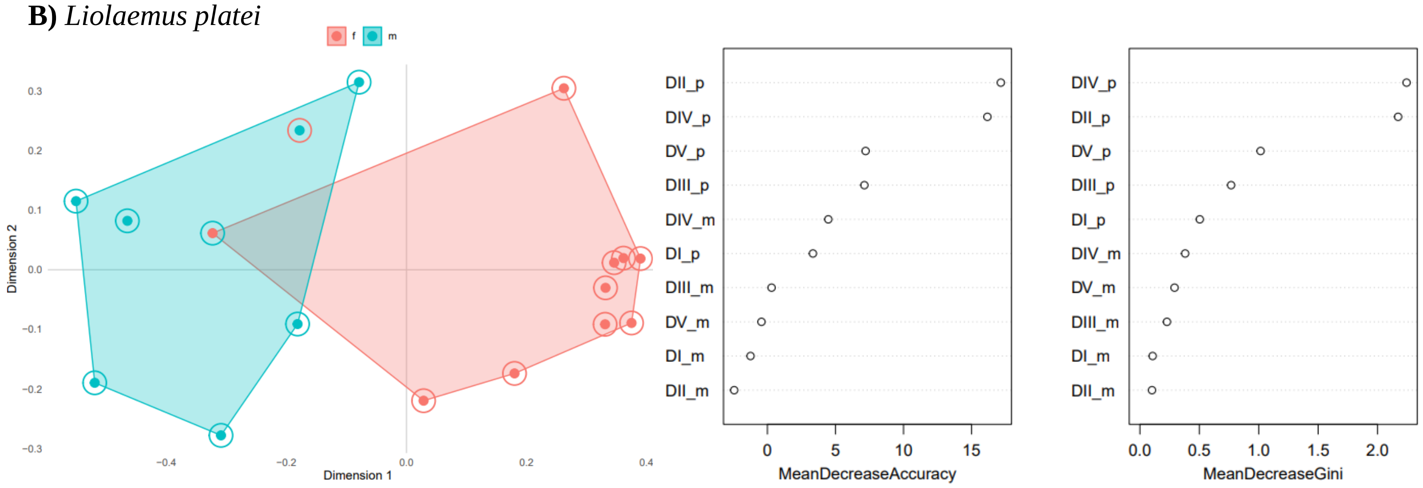
Liolaemus poconchilensis	33.33%	**Female**	4	2	33.33%
Liolaemus poconchilensis		**Male**	2	4	33.33%
Liolaemus pseudolemniscatus	16.67%	**Female**	3	2	40.00%
Liolaemus pseudolemniscatus		**Male**	0	7	00.00%
Liolaemus ramonensis	72.73%	**Female**	2	4	66.66%
Liolaemus ramonensis		**Male**	4	1	80.00%
Liolaemus schroederi	36.36%	**Female**	4	2	33.33%
Liolaemus schroederi		**Male**	2	3	40.00%
Liolaemus tenuis	18.75%	**Female**	8	1	11.11%
Liolaemus tenuis		**Male**	2	5	28.57%
Liolaemus valdesianus	33.33%	**Female**	2	3	60.00%
Liolaemus valdesianus		**Male**	1	6	14.28%
Liolaemus wiegmannii	23.08%	**Female**	7	1	12.50%
Liolaemus wiegmannii		**Male**	2	3	40.00%
Liolaemus zapallarensis	33.33%	**Female**	5	2	28.57%
Liolaemus zapallarensis		**Male**	3	5	37.50%
Liolaemus lineomaculatus	27.27%	**Female**	7	0	00.00%
Liolaemus lineomaculatus		**Male**	3	1	75.00%
Liolaemus multicolor	75.00%	**Female**	1	2	66.66%
Liolaemus multicolor		**Male**	4	1	80.00%
Liolaemus loboi	62.50%	**Female**	1	3	75.00%
Liolaemus loboi		**Male**	2	2	50.00%
Liolaemus audituvelatus	11.11%	**Female**	2	1	33.33%
Liolaemus audituvelatus		**Male**	0	6	00.00%
Liolaemus moradoensis	57.14%	**Female**	1	2	66.66%
Liolaemus moradoensis		**Male**	2	2	50.00%
Liolaemus buergeri	55.56%	**Female**	0	3	100.00%
Liolaemus buergeri		**Male**	2	4	33.33%
Liolaemus velosoi	40.00%	**Female**	1	3	75.00%
Liolaemus velosoi		**Male**	1	5	16.66%

In figure 4, we exemplified for 6 *Liolaemus* species the RF graphics and classification indexes, some associated to low error rates that illustrate species in which the specimens were correctly classified in relation to the sex, and others associated to high error rates, in which digits are not good predictors of sex.

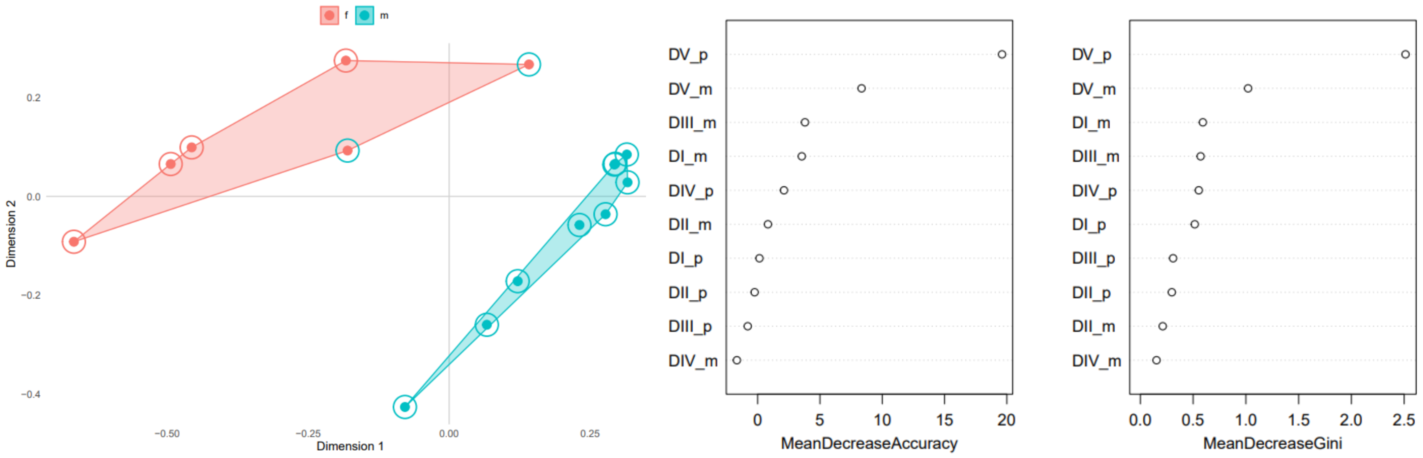
A) *Liolaemus audituvelatus*



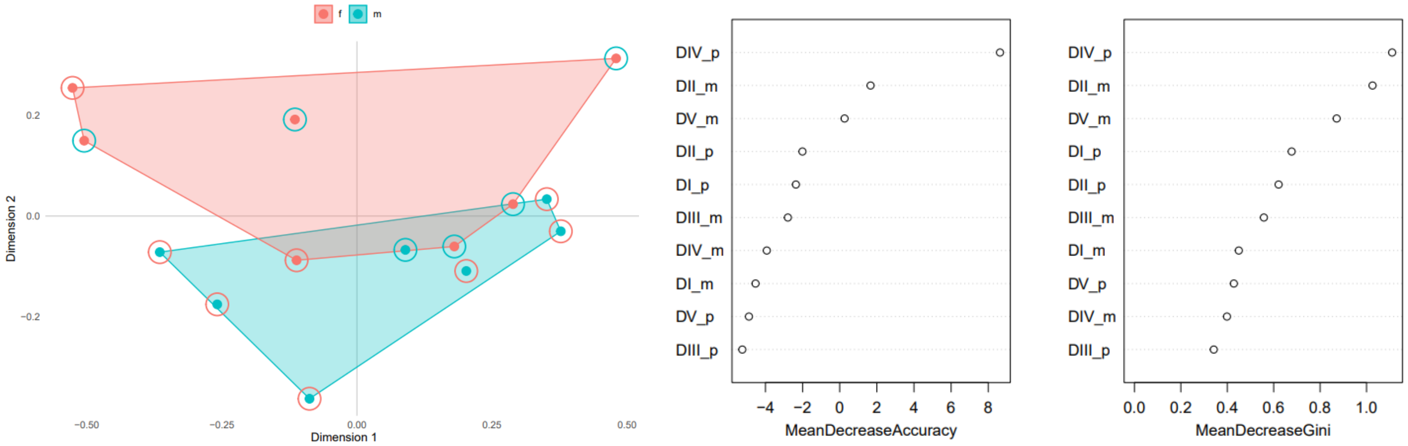
B) *Liolaemus platei*



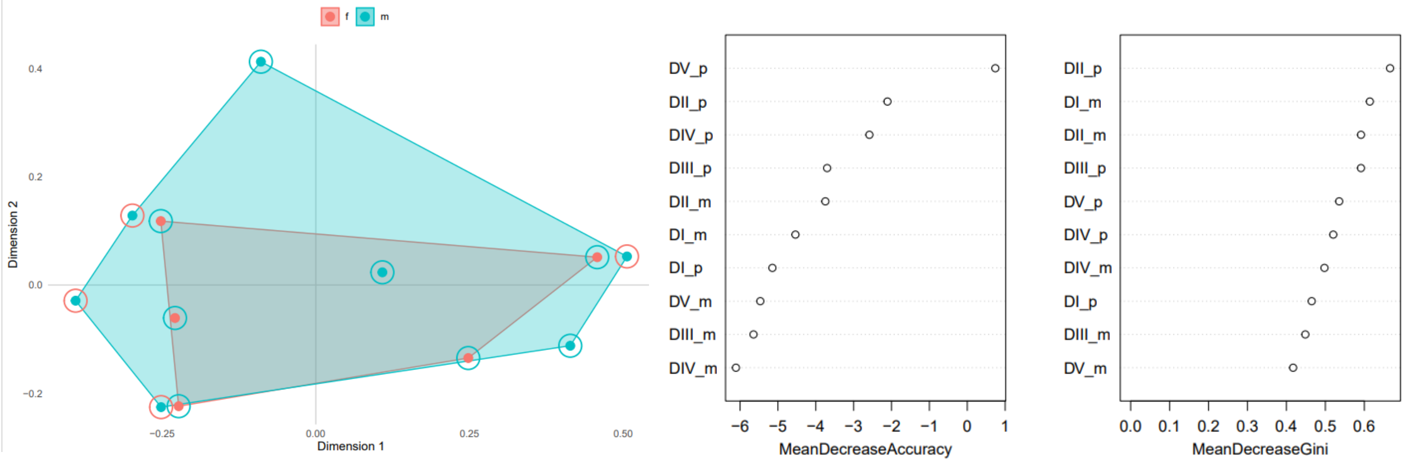
C) *Liolaemus nitidus*



D) *Liolaemus elongatus*



E) *Liolaemus hellmichi*



F) *Liolaemus multicolor*

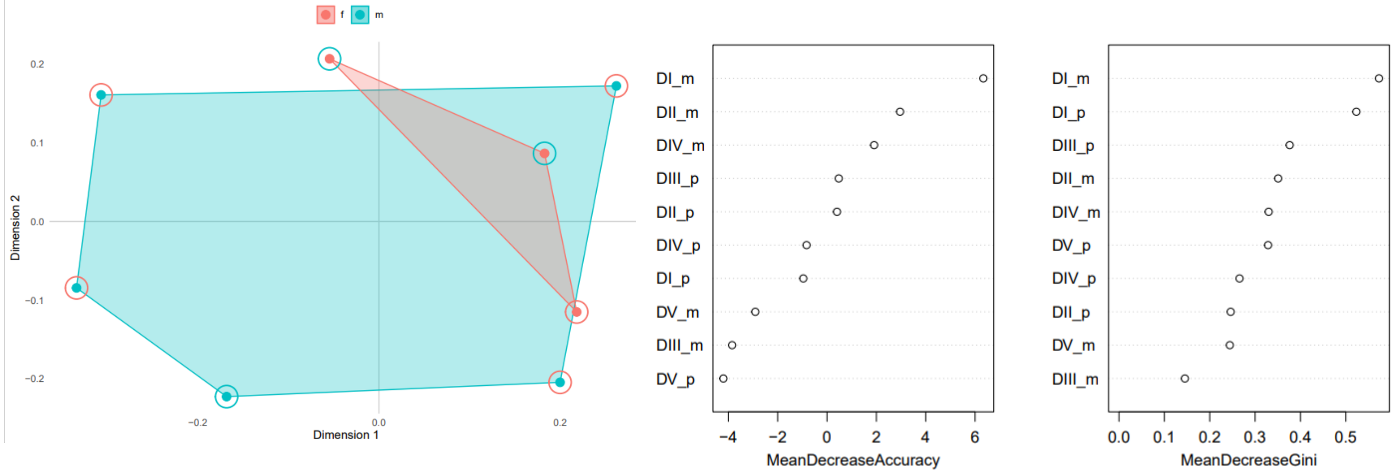


Figure 4. Relationships between the two first dimensions retained by the Random Forest analysis in six species of *Liolaemus* lizards (right) and associated ranks of Accuracy and Gini classifications illustrating the importance of each morphological trait for overall classification as male or female. Males are represented in blue and females in pink, circles around each point represent the sex classification: if circles and dots are of the same color, the classification is accurate. Abbreviations correspond to: D = digit, m = *manus*, p = *pes*. A) *Liolaemus audituvelatus*, B) *Liolaemus platei*, C) *Liolaemus nitidus*, D) *Liolaemus elongatus*, E) *Liolaemus hellmichi*, F) *Liolaemus multicolor*.

We implemented seven PGLS models for the digits retrieved from the RF analyses as the most relevant predictors for sex classification to test for environmental and reproductive associations. The SDI analyses of digit lengths are presented in tables 5 and 6. We observed similar AIC values among models using SDI, being the reproductive model the best fit for DIII in the *pes* (Table 5) and the altitude model the best fit for DIV in the *pes* (Table 6).

Table 5. Results of PGLS analyses using the Sexual Dimorphism Index (SDI) for Digit III in the *pes*, with best-fit models selected from AIC values indicated in bold.

Digit	Model	AIC	Delta AIC	Log-likelihood	Parameters p
DIII *pes*	**Reproductive**	**-198.2050**	**0.0000**	**103.1025**	**0.349**
	Altitude	**-197.8137**	**0.3913**	**102.9069**	**0.480**
	Temperature	**-197.6648**	**0.5402**	**102.8324**	**0.547**
	Precipitation	**-197.2903**	**0.9147**	**102.6452**	**0.961**
	Altitude + Reproductive	**-197.2336**	**2.0000**	**103.6168**	**0.474 / 0.247**
	Temperature + Precipitation	-195.6658	2.5392	102.8329	0.552 / 0.975
	Altitude + Reproductive + Temperature + Precipitation	-193.5147	4.6903	103.7574	0.483 / 0.258 /0.699 / 0.754

Table 6. Results of PGLS analyses using the Sexual Dimorphism Index (SDI) for Digit IV in the *pes*, with best-fit models selected from AIC values indicated in bold.

Digit	Model	AIC	Delta AIC	Log-likelihood	Parameters p
DIV pes	**Altitude**	**-209.0548**	**0.0000**	**108.5274**	**0.389**
	Temperature	**-208.7459**	**0.3089**	**108.3730**	**0.714**
	Precipitation	**-208.4208**	**0.6340**	**108.2104**	**0.506**
	Reproductive	**-208.3367**	**0.7181**	**108.1683**	**0.814**
	Altitude + Reproductive	**-207.0548**	**2.0000**	**108.5274**	**0.394 / 0.998**
	Temperature + Precipitation	-206.9074	2.1474	108.4537	0.511 / 0.699
	Altitude + Reproductive + Temperature + Precipitation	-203.8853	5.1695	108.9426	0.401 / 0.999 /0.813 / 0.411

The PGLS analyses using residuals of digit lengths (size-corrected for SVL) in males suggested the reproductive model as having the lowest AIC value for DIII (Table 7) and DIV (Table 8).

Table 7. Results of PGLS using size-corrected digit lengths in males for Digit III in the *pes;* best-fit model selected based on AIC values is indicated in bold.

Digit	Model	AIC	Delta AIC	Log-likelihood	Parameters p
DIII *pes*	**Reproductive**	**-148.4109**	**0.0000**	**78.2054**	**0.367**
	Temperature	-141.0230	7.3879	74.5110	0.058
	Precipitation	-140.5410	7.8699	74.2104	0.224
	Altitude	-133.2910	15.1199	70.6420	0.173
	Temperature + Precipitation	-124.6249	23.7860	67.31246	0.060 / 0.224
	Altitude + Reproductive	-124.4281	23.9828	67.2140	0.255 / 0.598
	Altitude + Reproductive + Temperature + Precipitation	-91.1564	57.2545	52.5782	0.725 / 0.702 / 0.112 / 0.242

Table 8. Results PGLS using size-corrected digit lengths in males for Digit IV in the *pes;* best-fit model selected based on AIC values is indicated in bold.

Digit	Modelo	AIC	Delta AIC	Log-likelihood	Parameters *p*
DIV *pes*	**Reproductive**	**-151.5337**	**0.0000**	**79.7668**	**0.997**
	Temperature	-147.2068	4.3269	77.6034	0.017
	Precipitation	-143.8947	7.6390	75.9473	0.358
	Altitude	-136.8195	14.7142	72.4097	0.280
	Temperature + Precipitation	-129.7508	21.7829	69.8754	0.019 / 0.446
	Altitude + Reproductive	-127.6183	23.9154	68.8091	0.302 / 0.993
	Altitude + Reproductive + Temperature + Precipitation	-97.4551	54.0786	55.7270	0.279 / 0.590 / 0.010 / 0.197

The PGLS analyses using residuals of digit lengths (size-corrected for SVL) also suggested the reproductive model as having the lowest AIC value for DIII (Table 9) and DIV (Table 10) in females. The DIII was the only selected model with significative associations (p = 0.001), the reproductive mode parameter for the DIII in females had a lambda of -0.528 and a R^2 of -0.075. In general, the DIII is slightly longer in viviparous species (Figure 5).

Table 9. Results of PGLS using size-corrected digit lengths in females for Digit III in the *pes;* best-fit model selected based on AIC values is indicated in bold.

Digit	**Model**	**AIC**	**Delta AIC**	**Log-likelihood**	**Parameters *p***
DIII *pes*	**Reproductive**	**-141.3951**	**0.0000**	**74.6970**	**0.001**
	Precipitation	-136.9875	4.4076	72.4937	0.157
	Temperature	-135.9995	5.9560	71.9997	0.026
	Altitude	-129.4249	11.9702	68.7124	0.086
	Temperature + Precipitation	-122.5650	18.8301	66.2824	0.029 / 0.143
	Altitude + Reproductive	-118.5711	22.8240	64.2855	0.787
	Altitude + Reproductive + Temperature + Precipitation	-86.7126	54.6825	50.3563	0.459 / 0.889 / 0.214 / 0.606

Table 10. Results of PGLS using size-corrected digit lengths in females for Digit IV in the *pes;* best-fit model selected based on AIC values is indicated in bold.

Digit	**Modelo**	**AIC**	**Delta**	**Log-likelihood**	**Parameters *p***
DIV *pes*	**Reproductive**	**-115.6322**	**0.0000**	**61.8160**	**0.378**
	Temperature	-106.0847	9.5475	57.0423	0.125
	Precipitation	-105.1180	10.5142	56.5590	0.898
	Altitude	-98.6081	17.0241	53.3041	0.353
	Altitude + Reproductive	-91.5433	24.0889	50.7716	0.579 / 0.479
	Temperature + Precipitation	-89.1811	26.4511	49.5905	0.218 / 0.914
	Altitude + Reproductive + Temperature + Precipitation	-57.5349	58.0973	35.7674	0.866 / 0.549 /0.425 / 0.835

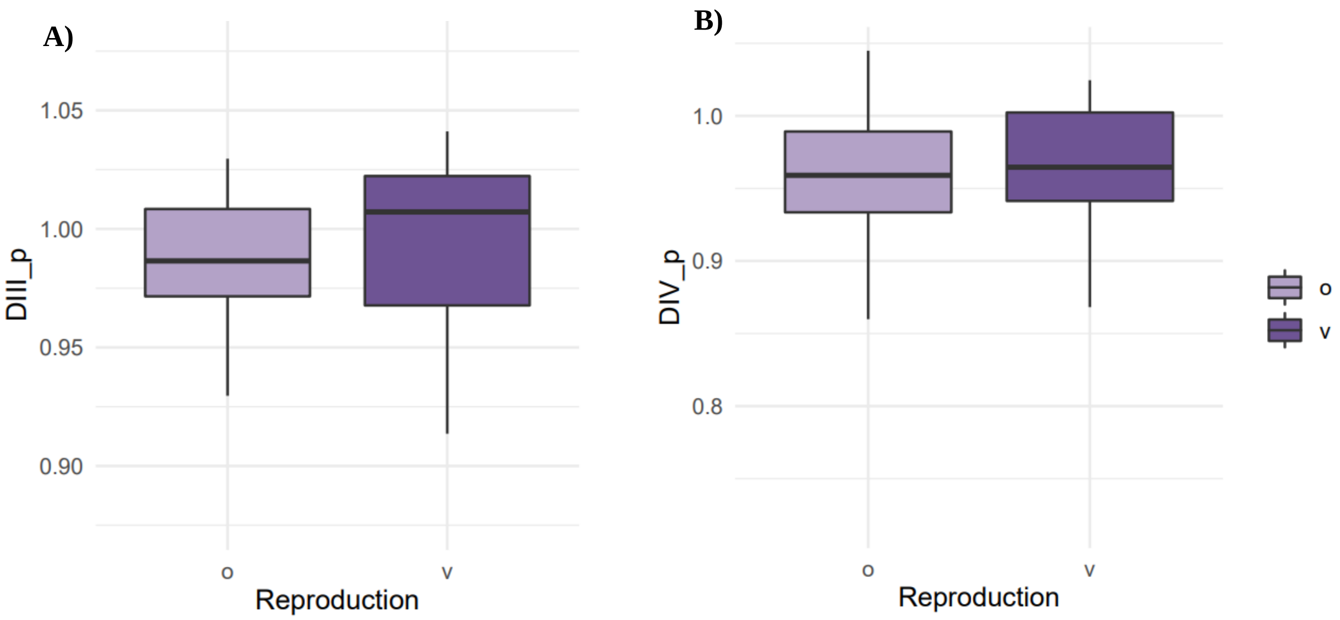


Figure 5. Box plot representing the DIII A) and DIV B) from *pes* in females from the *Liolaemus* species. o = oviparous, v = viviparous.

We evaluated the hypothesis of phenotypic integration mediated by hormones through phylogenetic regressions between digit lengths (DIII and DIV from *pes*), body size (SVL) and head proportions. The SDI of DIII in the *pes* was negatively related with SVL and positively associated with head length and head width (Table 11, Figures 6 and 7).

Table 11. Results of phylogenetic regressions, significant associations ($p < 0.05$) indicated in bold. Abbreviations correspond to: SVL = snout-vent length, HDL = head length, HDW = head width, HDH = head height, D = digit.

Morphological traits	**DIII *pes***			**DIV *pes***		
	F	**R²**	**p**	**F**	**R²**	**p**
SVL	**-4.784**	**0.347**	**<.001**	-1.941	0.081	0.058
HDL	**2.380**	**0.105**	**0.021**	1.755	0.059	0.086
HDW	**2.061**	**0.093**	**0.045**	1.759	0.067	0.856
HDH	0.521	0.007	0.604	0.117	0.001	0.906

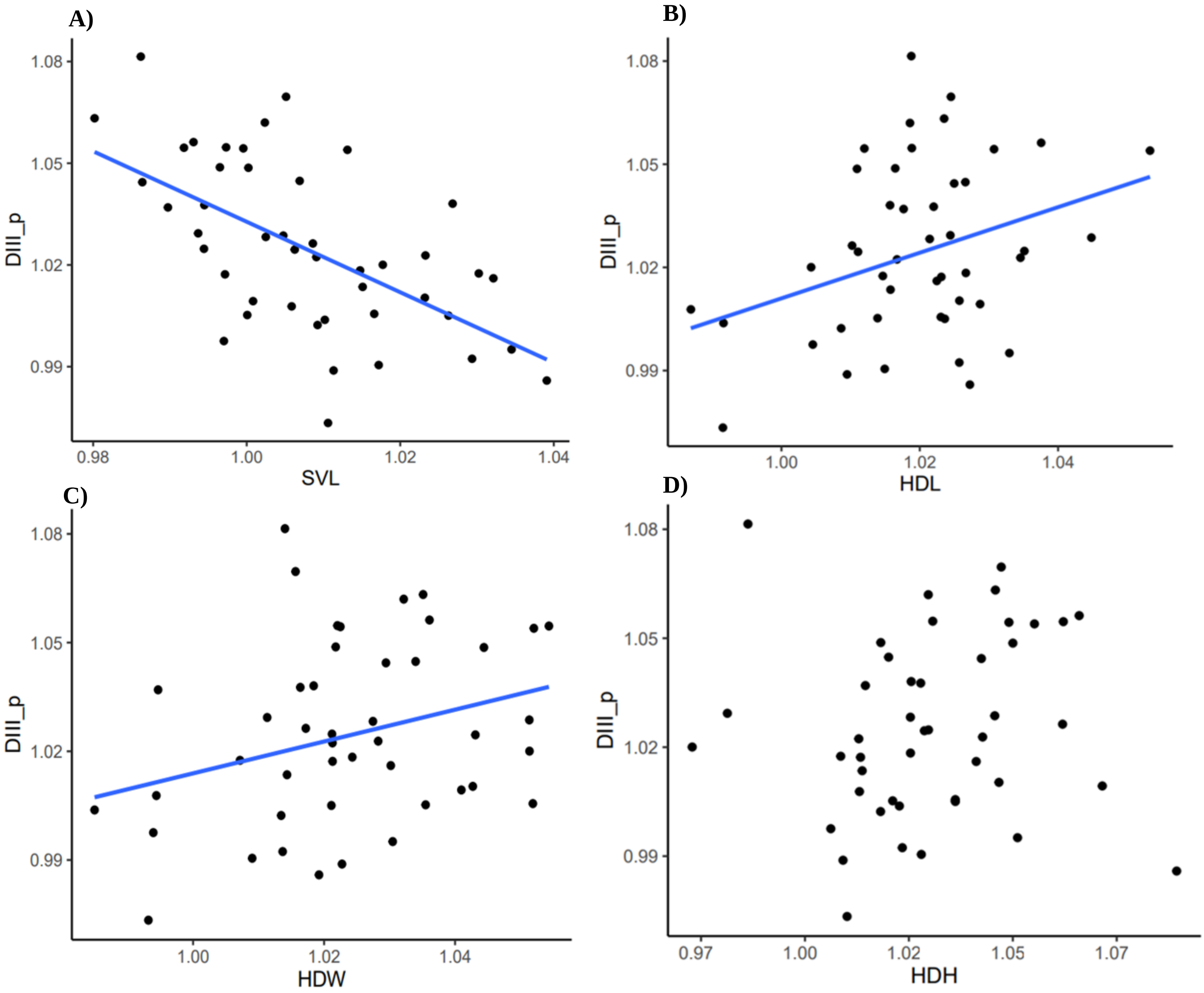


Figure 6. Relationship between SDI in the DIII from *pes* and body and head proportions. Abbreviations correspond to: SVL = snout-vent length, HDL = head length, HDW = head width, HDH = head height, D = digit.

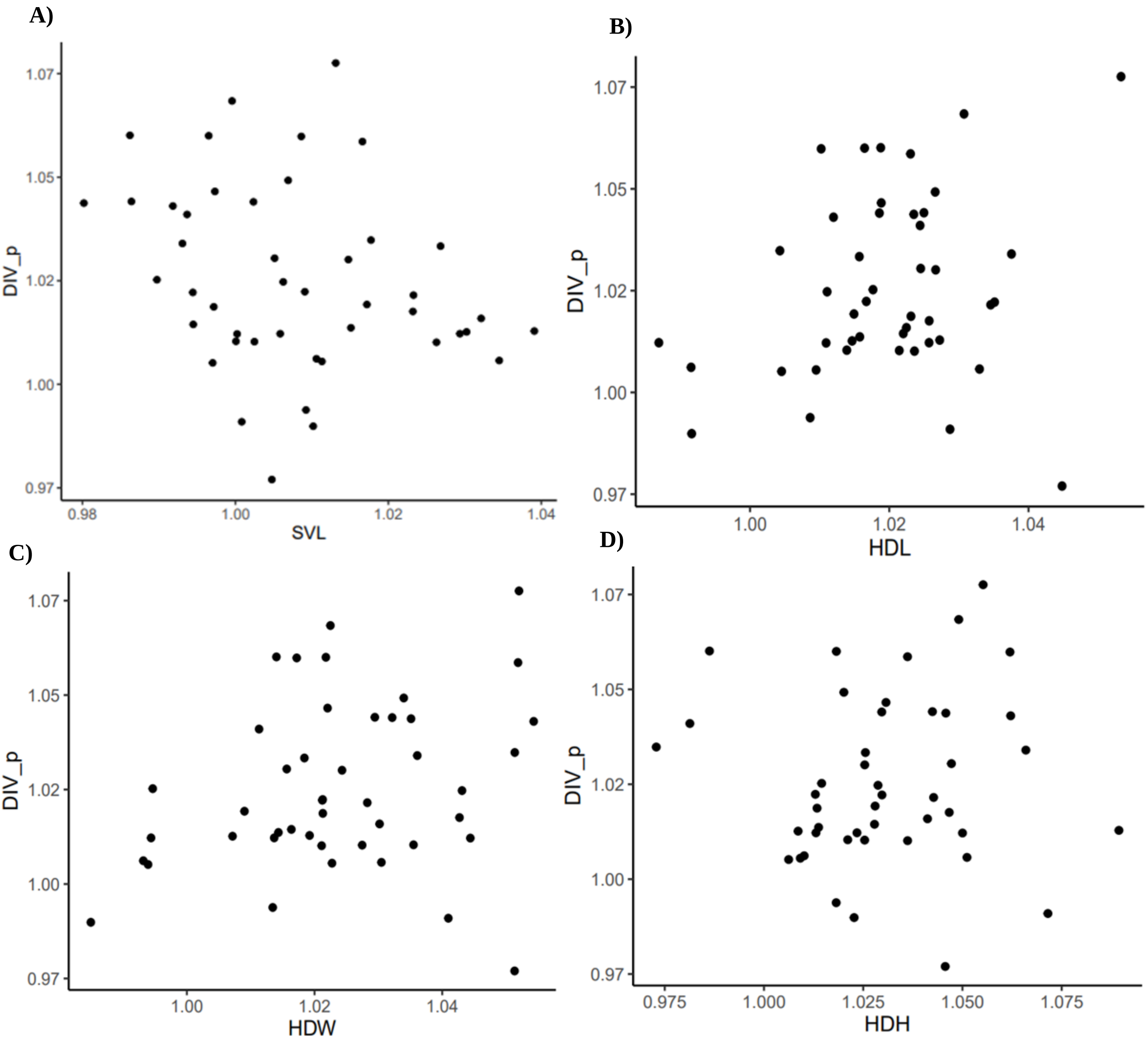


Figure 7. Relationship between SDI in the DV from pes and body and head proportions. Abbreviations correspond to: SVL = snout-vent length, HDL = head length, HDW = head width, HDH = head height, D = digit.

4. DISCUSSION

Our findings for *Liolaemus* lizards agree with studies performed in different lineages of Tetrapoda that identified sexual dimorphism in other digits besides DIV (Rubolini et al. 2006, Tobler et al. 2011, Van Damme et al. 2015, Lofeu et al. 2017, 2020, Kaczmarski et al. 2021). The identity of the dimorphic digits may not be conserved even among closely-related species, suggesting some evolutionary flexibility in which digits are sensitive to circulating hormones and therefore become different between males and females (Lofeu et al. 2020). Our results corroborate the theoretical model that suggests that each digit may independently acquire the initial sensitivity to circulating steroids (Lofeu et al. 2020). We also identified associations between the magnitude of the sexual dimorphism in some digits (e.g. DIII from *pes*) and that of head and body proportions, corroborating the hypothesis of phenotypic integration mediated by steroid hormones during development (Lofeu et al. 2017, 2020). Finally, our study innovates by describing that evolution of digit lengths in *Liolaemus* lizards is not associated with climate but relates with differences in reproductive modes. This result is particularly interesting because females of viviparous species might better modulate incubation conditions, which is not possible after laying the eggs, and therefore differences in the developmental environments between viviparous and oviparous species might define hormonal dynamics throughout incubation and influence morphological traits in developing lizards.

The databased used in this study resulted in similar proportions of *Liolaemus* species that are dimorphic or monomorphic for digit lengths. The identity of the dimorphic digits is considerably diverse within this genus, which corroborates the model proposed by Lofeu et al. (2017, 2020) and may also relate to the complexity of steroid hormones signaling (Hau 2007, Zheng and Cohn 2011, Cox 2020). Even though most studies on digit lengths focus only on the effects of hormonal levels, the tissue responsiveness to hormones is an important component of this complex equation (Zheng and Cohn 2011, Cox 2020). The 'Evolutionary Potential Hypothesis' defined by Hau (2007) postulates that different components in the steroid cascade can change independently. Accordingly, factors including gene regulation for hormonal levels and the concentration of steroid receptors in the digits may simultaneously play central roles modulating sexual dimorphism in the digits (Forstmeier et al. 2010, Zheng and Cohn 2011, Cox 2020). The establishment of sexually dimorphic traits, including digit lengths, may be modulated by complex interactions between hormonal signaling and the receptors dynamics (Hau 2007, Cox 2020). We expected that environmental parameters related to climate could influence such interactions, but our analyses suggest that the reproductive mode might be a major factor establishing the developmental environment of *Liolaemus* lizards.

The hypothesis that patterns of sexual dimorphism in digits would evolve in association with environmental parameters including temperature and precipitation derives from studies describing relationships of several morphological traits, such as coloration and body, head, and limb sizes, with altitudinal gradients in lizards (Iraeta et al. 2006, Reguera et al. 2014, Lu et al. 2018, Üzüm et al. 2018, Ortega et al. 2019, Moreno Azócar et al. 2020, Slavenko et al. 2021). For example, individuals of *Phoenicolacerta laevis* exhibit smaller body sizes at high altitudes, while *Podarcis liolepsis* lizards that occupy highlands are darker and characterized by larger body and head when compared to individuals from the same species found at lower altitudes (Üzüm et al. 2018, Ortega et al. 2019). Some authors interpret that such phenotypic traits may be associated with variation in environmental conditions as well as genetic factors (Reguera et al. 2014, Ortega et al. 2019). In *Phrynochephalus vlangalii* lizards, individuals from high elevation sites grow faster comparing to lower elevation animals, although common garden experiments in this species showed individuals from different elevations grow at similar rates, pointing out plastic components during the development of *P. vlangalii* (Lu et al. 2018). Finally, another study that deserves attention implemented interspecific analysis using more than 70 skink species and suggested elevation as a robust predictor of morphological traits (Slavenko et al. 2021): skinks from elevated regions were characterized by smaller bodies and shorter limbs (Slavenko et al. 2021). We innovate here by incorporating the sexual dimorphism component into evaluations of relationships between digits and the climate. In *Liolaemus*, we did not identify significant associations with climate either when using Sexual Dimorphism Indexes or when testing for digit lengths separately in males and females. Although testosterone levels might vary with climate (Husak et al. 2021), androgen receptors in the digits may not have the same responsiveness, weakening the hypothesis of direct interactions with environmental factors (Hau 2007, Cox 2020). Moreover, phenotypic effects of altitudinal may diverge among species (Üzüm et al. 2018, Ortega et al. 2019), and parameters such as temperature and humidity might also be differentially modulated in the developmental environments depending on the way embryos are incubated (i.e. inside the mother or eggs in the nests).

Distinct reproductive modes (e.g. viviparous or oviparous) likely impose differences regarding the influence of overall climatic conditions on the developmental environments embryos grow and differentiate. Oviparous species lay their eggs in nests, so the embryos are exposed to several climate conditions that will not be regulated after the female lays the eggs (Qualls and Shine 1998, Iraeta et al. 2006). In these species, it is expected that the hatching success is associated with the nest and environmental conditions throughout embryonic development. On the other hand, in viviparous species the female can be considered the developmental environment, being able to thermoregulate and retain the embryo according to external conditions (Shine 2004, Fernández et al. 2017, Pérez et al. 2019, Cruz et al. 2022). Moreover, in these species the transference of hormones from the mother to the embryos, and also between siblings, can affect the development of morphological traits, particularly in scenarios where female embryos are developing alongside males (see van Anders et al. 2006, Tapp et al. 2011, Auger et al. 2013). Although in some viviparous lizards, such as the *Woodworthia* gecko, that are dimorphic in the ratio 2D:3D, neonates are monomorphic and the sex of the siblings does not affect digit lengths (Woodhead et al. 2018). Our study suggests that reproductive mode is a major factor related to evolution of sexual dimorphism in digits of *Liolaemus* lizards.

Sex-differences in digit lengths are expected to be associated with other sexually-dimorphic phenotypic traits due to pleiotropic effects of hormones (Lofeu et al. 2017, Cox 2020), and we identified that in *Liolaemus* the Sexual Dimorphism Index of DIII in the *pes* is significantly associated with other morphological traits. With the snout-vent length, the association is negative and with head length and width, positive. Differences in the type of digit associations involving body size or the head might reflect disparities in the timing these traits become dimorphic during ontogeny (see Cain et al. 2013, Sanger et al. 2014). For example, in *Junco hyemalis* birds digit lengths and body size are negatively correlated in first-year males but then express a positive relationships in the second-year males (Cain et al. 2013). It is interesting to note that DIV is the most frequent digit being dimorphic among *Liolaemus* species but is not associated with dimorphism in other phenotypic traits, a result that challenges the assumption that dimorphism in digit lengths would predict steroid effects in the sex-differentiation of other phenotypic traits.

The present study comprised the largest species sampling used to evaluate sex differences in digit lengths in Squamata, and identified, for the first time to our knowledge, that the reproductive mode plays a major role in the evolution of dimorphic patterns in the digits of *Liolaemus* species. The results reiterate the importance of investigations focusing on the hormone-receptor complex considering the developmental environment modulated by the reproductive mode. Information of the timing when digits become dimorphic is essential to understand how these processes interact with environmental factors, and it is important to note that for most lizard species it is even unknown whether the digits are already dimorphic at birth (see Tobler et al. 2011, Woodhead et al. 2018). Even though the study of sexual dimorphism in digit lengths is advanced in mammals, especially humans, several questions in the field remain unanswered, and broad taxonomic studies are essential for understanding the dynamics involved in digit development and differentiation between sexes. In this chapter, the sexual dimorphism in digit lengths was investigated in the interface with environmental parameters and reproductive mode for the very first time, and the results encourage further research using different taxa, more refined datasets and different scales (e.g. a populational approach).

CHAPTER 2

Sexual dimorphism in digit lengths in *Tropidurus catalanensis*: relationships with climate and embryonic development

ABSTRACT

The phenotypic differences among populations can increase in scenarios with distinct climatic parameters that animals living along environmental gradients likely face. Particularly, digit length is a morphological characteristic that can be sexually dimorphic and are modulated by steroid hormones during embryonic development. Although the relationship between climate and hormones is expected, the associations between digit lengths and environmental conditions are still obscure. The traditional literature in digit lengths states that the sexual dimorphism is established prenatally and does not change after birth, enabling associations with embryonic hormone levels. Therefore, investigating intraspecific differences in digit lengths can elucidate environmental associations in different ontogenetic scales. The present work aimed to understand whether *Tropidurus catalanensis* lizards distributed in different locations along Brazilian central axis show differences in dimorphic patterns of digit lengths, and if these patterns can be associated with climatic conditions. We also added to this investigation the experimental approach with manipulations of thermal regimes during *T. catalanensis* embryonic development. Measures of digit length were obtained in adults males and females from 13 localities available in Brazilian herpetological collections. For one locality (São Simão - SP), we analyzed the morphology of neonates from both sexes incubated in two different temperatures and quantified the androgen receptors in digits, to explore the mechanisms involved in the sexual dimorphism in digit lengths. Results suggest that the sexual dimorphism in digit lengths varies even among localities in *T. catalanensis.* Our results corroborate the hypothesis that the differences in digit lengths between sexes can be established in all digits, being different even between *manus* and *pes* in *T. catalanensis*. In addition, dimorphic digits are associated with environmental parameters, indicating that the interaction between climate and hormone levels can play a role in digits establishment. Interestingly, the neonates were not dimorphic at birth, even though the androgen receptors are different between males and females neonates in DIV from *pes*, one of the dimorphic digits in adults from São Simão. This chapter outcomes indicate that the diversity in sexual dimorphism in digit lengths may be regulated by a complex interaction with steroid hormones, that varies according to environmental conditions. The association with climate was investigated in the context of the identity of the dimorphic digit amidst localities for the first time. The dimorphism in androgen receptors for neonates also improve our knowledge on the mechanisms modulating the establishment of differences between digit lengths in males and females. The integrative approaches implemented in this work bring a new perspective to understand the patterns of sex dimorphism in digit lengths in a lizard widely distributed in the central region of Brazil.

Keywords: Digit lengths, Sexual dimorphism, Environmental gradients, Embryonic development, *Tropidurus*.

1. THEORETICAL CONTEXTUALIZATION

The amount of morphological variation expressed in a given population may be increased when animals face different environmental conditions (Quaus and Shine 2006, Crispo and Chapman 2010, Jaffe et al. 2016, Bonini-Campos et al. 2019, Lofeu et al. 2021). Variation in morphological, behavioral, and physiological traits induced by environmental signals can be defined as phenotypic plasticity (West-Eberhard 2003, Laland et al. 2015, Levis and Pfennig 2020), an intrinsic property of biological systems that may be involved in adaptative processes but can also correspond to variation that is neutral to fitness and vary depending on external conditions (Ghalambor et al. 2007, Gibbin et al. 2017). In general, the capacity of animals to exhibit differences in phenotypic traits according to the environment is a fundamental concept to be explored in the process of understanding how animals interact with their surroundings.

Intraspecific studies focusing on morphological differences explore different types of associations with the environment. Animals raised in different developmental environments regarding the temperature (e.g. Booth 2018, Fraimout et al. 2018), humidity (e.g. Lorenzon et al. 2001, Richter et al. 2012), presence of predators (e.g. Relyea 2001), foraging sites (e.g. Bonini-Campos et al. 2019, Ammresh et al. 2023), and structural complexity (e.g. Losos et al. 2000) often differ in the morphological traits revealed. For example, African cichlid fishes present distinct levels of phenotypic plasticity in the gills and brains depending on the oxygen availability (Crispo and Chapman 2010). The expansion to novel environments also induce phenotypic changes in house finches, which result from a reorganization of pre-existing morphophysiological structures (Badyaev 2009). The environmental conditions may induce a complex interplay between sex-specific phenotypic traits and developmental plasticity in populations that are adjusting to new areas (Badyaev 2009), resulting in alternative phenotypes that differ between sexes in shape or size.

Differences in body size have been investigated in several vertebrate lineages, often with latitudinal gradients (see Ashton and Feldman 2003, Watt et al. 2010), and morphological patterns of other phenotypic traits have been also described in association with environmental parameters, such as head and limb proportions (see Jaffe et al. 2016). In this context, an important factor that can modulate traits development and is sensitive to environmental changes is hormonal dynamics. Hormones integrate multiple information during the ontogeny (Potau et al. 1999, Hau 2007, Sanger et al. 2014). Sex steroids, in special, are interpreted as a strong link between the environment and the phenotype because they regulate processes of growth and differentiation, besides modulating physiological and behavioral differences between sexes (Lerner and Mason 2001, Cox et al. 2009, Huyghe et al. 2010). Relationships between hormones and climate have been more frequently evaluated in the context of sex determination in species in which this process is not exclusively genetic (Crews 1996, Elf 2003). For example, males offspring of marine turtles are produced at higher rates in cold environments which reflects interactions between sex hormones and temperature during embryonic development (Ackerman 1997). Testosterone is one of the most studied steroids and circulating levels of this hormone seem to be modulated by environmental parameters in a variety of species and populations (Husak et al. 2021). In species of tropical birds and amphibians, there is a significative variation in the concentration of circulating testosterone from higher to lower latitudes (Moore et al. 2002, Goymann et al. 2004, Garamszegi et al. 2008, Hau et al. 2008, 2010, Eikenaar et al. 2012), and environmental temperature and precipitation seasonality seem to be the most important factors modulating testosterone levels among vertebrates (Husak et al. 2021). Given that hormone levels may be affected by environmental parameters, the distribution of phenotypic patterns along environmental gradients may, therefore, express some responses of specific ontogenetic process modulated by hormones.

The interplay between temperature and morphological traits during embryonic development has been well studied in different species of ectothermic vertebrates (Angilletta et al. 2000, 2004, Quaus and Shine 2006, Monasterio et al. 2011, Noble et al. 2018). Temperature affects physiological processes that are related to cell proliferation, differentiation, and metabolism (e.g. fishes: Santoro 2014, lizards: Amiel et al. 2017). Developing tissues may also be affected by circulating hormones, which levels are often influenced by environmental conditions, explaining the associations between incubation regimes and phenotypic profiles (Ding et al. 2012, Martínez-Juárez and Moreno-Mendoza 2019). Variations in the steroid dynamics during embryonic development affect processes of digit growth and elongation in mammals (Manning et al. 1998, 2014, Zheng and Cohn 2011), being the basis for sexual dimorphism in this morphological trait. In mammals, the dimorphic patterns in digit lengths do not seem to change after birth (Manning et al. 1998, Lutchmaya et al. 2004, Manning 2011, Zheng and Cohn 2011). Furthermore, sex-differences in the length of Digit IV occur in a narrow developmental window in mice (i.e. day 17 out of 21$^{)}$, and higher concentrations of androgen and estrogen receptors in Digit IV, which are differentially expressed between males and females, seem to modulate the digit elongation process (Zheng and Cohn 2011). Although this study has been crucial to validate digit lengths as a proxy for steroid dynamics during embryonic development (Zheng and Cohn 2011), it remains unknown if other lineages also exhibit differential concentrations of steroid receptors between males and females in dimorphic digits.

Relationships between digit lengths and other phenotypic traits, including morphology, behavior, performance and general health, have been explored in different mammals species (Manning et al. 1998, Manning 2002, Paul et al. 2006, Ribeiro et al. 2016, Bunevicius 2018), but environmental parameters are often relegated in this discussion. Testosterone seems to play a major role in sex differentiation of digit lengths during mammal development (Zheng and Cohn 2011, Huber et al. 2017), and it is expected that environmental parameters affect the levels of circulating hormones, so that variation among populations in the dimorphic patterns of digit lengths are expected. In fact, a considerable variation in the dimorphic patterns of digit lengths has been described in birds (e.g. *Taeniopygia guttata*: dimorphic [Burley and Foster 2004], monomorphic [Forstmeier 2005]), and lizards (e.g. *Anolis carolinensis*: dimorphic [Chang et al. 2006] monomorphic [Lombardo and Thorpe 2008]*; Tropidurus montanus:* dimorphic [Lofeu et al. 2020], monomorphic [Miranda et al. 2021]).

Species of Squamata, in particular, are ideal for studies addressing possible relationships between sexually dimorphic traits and environmental parameters. This group is very diversified ecologically and morphologically (see Pough et al. 2001, Pyron et al. 2013), and a remarkable variation in the identity of the dimorphic digits has been observed in lizards (Tobler et al. 2011, Van Damme et al. 2015, Woodhead et al. 2018, Lofeu et al. 2020), including description of species that are monomorphic for this trait (e.g. Lombardo and Thorpe 2008, Lofeu et al. 2020, Miranda et al. 2021). In lizards, sexual dimorphism in digit lengths may be associated with microhabitat usage (e.g. Gomes and Kohlsdorf 2011), performance (e.g. Tobler et al. 2012, Van Damme et al. 2015) and coloration (e.g. Tobler et al. 2011), and also relate with other morphological traits including head size, trunk length and limb proportions (Lofeu et al. 2020). The Tropiduridae lizard family (Iguania) stands out for a considerable diversity in the identity of the digits that are dimorphic, including differences between *manus* and *pes* in some species (Lofeu et al. 2020). Although patterns of sexual dimorphism in digit lengths have been well characterized in Tropiduridae, relationships with environmental gradients remain unexplored, especially in an intraspecific perspective (see Lofeu et al. 2020, Miranda et al. 2021).

The evolutionary flexibility of the identity of the digit dimorphic has been addressed in a model discussing the origin and evolution of steroid sensibility during digit development (Lofeu et al. 2017, 2020). According to this theoretical model, differences in the concentrations of steroid receptors could affect the process of digit growth and elongation, so that any digit could acquire increased sensibility to hormones, which initially would be neutral to fitness (Lofeu et al. 2017, 2020). Studies using *Anolis carolinensis* lizards described two peaks of testosterone during embryonic development, one provided by the mother near the 8th day after fecundation, and the second one produced by the embryo near the 24th day after fecundation (Lovern and Wade 2003). Digits usually start to develop at day 11, and are still elongating during this second testosterone peak, being potentially influenced by the combination of steroid dynamics established by these peaks (Lovern and Wade 2003, Sanger et al. 2008). Considering the suggested role of testosterone in digit elongation through androgen receptors and the expected effects of environmental parameters on this relationship, we explore the topic in this chapter using *Tropidurus catalanensis* lizards. This species is distributed along a wide latitudinal gradient that congregates different climatic conditions (Kunz and Borges-Martins 2013, De Sena 2015). First, we tested for sexual dimorphism in digit lengths, body, head, and limb proportions in *T. catalanensis* from different localities and evaluated possible associations between dimorphic patterns in digits and climate. Then, we incubated eggs of *T. catalanensis* in different incubation temperatures; the clutches were obtained from one locality identified as having adults sexually dimorphic for digit lengths. Finally, we compared the concentrations of androgen receptors between male and female neonates of *T. catalanensis* from this same locality. We hypothesized that 1) the identity of sexual dimorphism in digits of *T. catalanensis* differs among localities; 2) the patterns of digit lengths are associated with temperature and precipitation along the geographical gradient of study, considering the expected effects of these environmental parameters on the endocrine system; 3) the incubation temperature during embryonic development affects the patterns of sexual dimorphism identified in the digits of neonates; 4) the distribution of androgen receptors in neonates digits differ according to the sex and the identity of the dimorphic digit in the population.

2. MATERIAL AND METHODS

We complied a database that congregates two units with specific goals and approaches: 1) morphological measurements of adult males and females of *T. catalanensis* lizards from different localities available in scientific collections, combined with climatic data from the localities sampled; 2) morphological measurements in neonates incubated in different temperatures and the intensity of fluorescence of testosterone receptors in the digits of neonates quantified using immunohistochemistry.

2.1 ADULTS: MORPHOLOGICAL DATABASE

All morphological measurements were obtained by the same person (APC) on the left side of the animals, using a digital caliper (Mitutoyo Inc. ± 0.01 mm). A repeatability test was performed to calculate variation among measurements. To standardize the database regarding asymmetries, digits were measured on both sides in individuals from one locality (Manning et al. 1998). Only adult males and females of *T. catalanensis* larger than 60mm were considered (Kunz and Borges-Martins 2013). The morphological traits measured were selected based on current literature (Barros 2016, Lofeu et al. 2020, Van Damme et al. 2015; see also Chapter 1 of the current dissertation), and can be structured into four categories:

1. Body size: snout-vent length and trunk length.
2. Body proportions: lengths of humerus, radius, femur, and tibia; length and height of pelvic and scapular girdles.
3. Head proportions: length, height, and width of the head.
4. Autopodium: lengths of Digits I, II, III, IV e V from *manus* and *pes*.

The database comprised specimens available at two Brazilian Herpetological Collections: *Museu de Zoologia da USP - MZUSP* and *Coleção Herpetológica de Ribeirão Preto - CHRP*. To describe the area sampled for this study, it is important to approach the distribution of *T. catalanensis* in South America. The initial descriptions for the occurrence of *T. catalanensis* Gudynas & Skuk 1983 were revalidated by Kunz and Borges-Martins (2013) and suggested that these lizards are distributed in Northwestern Uruguay, northeastern Argentina, southern Paraguay, and southern Brazil. In Brazil, *T. catalanensis* is present in the southwestern of São Paulo and the southeastern of Mato Grosso do Sul. Recent studies provided a wider distribution of *T. catalanensis*, with the northernmost point for this species being considered Lajeado in Tocantins (De Sena 2015). Therefore, this species occurs along the region of the Brazilian Central Axis and is present in several localities along this latitudinal gradient (but see De Sena, 2015 and Kunz Borges-Martins, 2013), as illustrated by Figure S1 in the Supplementary Material (extracted from De Sena 2015). In this study, we measured a total of 329 adults of *T. catalanensis*, being 164 males and 165 females, from 13 localities (Table 1 and Figure 1), as follows: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

Table 1. Localities represented in the morphological database using adult males and females of *T. catalanensis*. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

Locality	Males	Females
AC	7	7
AR	13	12
AU	15	19
BA	11	11
BR	12	9
CG	10	15
LA	17	18
LU	12	9
PI	15	12
RP	11	7
SS	13	15
TA	20	20
UN	8	11
Total	164	165

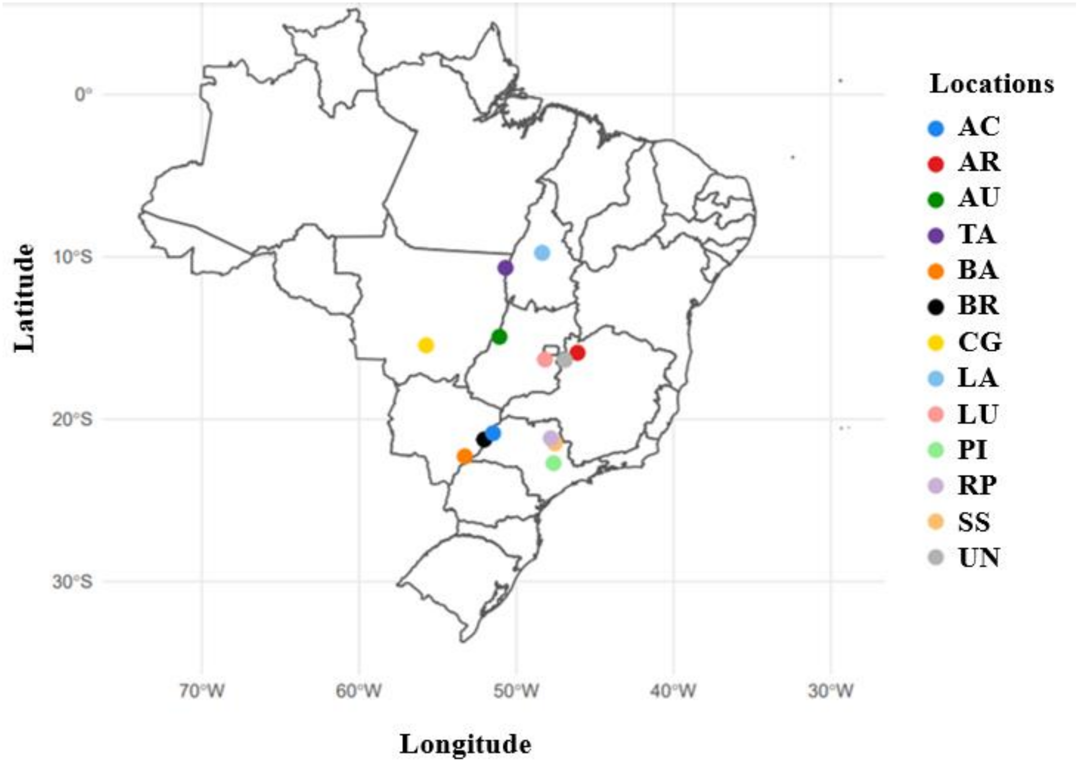


Figure 1. Geographic points representing the studied localities for *T. catalanensis* lizards. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

2.2 ADULTS: ENVIRONMENTAL DATABASE

The environmental database comprised information of 1) Mean Temperature of Wettest Quarter (Temperature) and 2) Precipitation Seasonality, in a latitudinal gradient of *T. catalanensis* distribution. The climate database was extracted from WorldClim using the same approach described in Chapter 1.

2.3 NEONATES: HUSBANDRY OF GRAVID FEMALES AND EGG INCUBATION

Pregnant females of *T. catalanensis* were collected by lasso in the locality of São Simão (SS) during the reproductive season of this species, in the years 2020/2021 and 2021/2022. Females were maintained at the Animal Facility (Biotério de Vertebrados Silvestres - FFCLRP/USP) associated to the Laboratory of Integrative Biology and Evolution. The terraria consisted of plastic boxes (40 x 40 x 60 cm) with a 15cm moist vermiculite layer and bricks to provide refuge for the lizards. Incandescent and UVA/UVB lamps were placed at the top of the plastic box and a 12h light: dark cycle was established in each terrarium. Females were fed with cockroaches and mealworms and water was offered *ad libitum*.

During the year 2020/2021 we obtained 16 clutches which resulted in 48 eggs. In the season 2021/2022, we incubated 36 eggs from 12 clutches. Each clutch was identified immediately after oviposition, and eggs were then individually incubated in small plastic boxes (7.5 x 5.0 cm), to avoid hormone transference among embryos (Braña 2008). We equally divided the eggs in two incubation temperatures, 24ºC and 30ºC, which are suitable for development and allow high hatching success (see Rossigalli-Costa et al. 2021). The eggs were maintained in moist vermiculite at 150kpa and daily inspected to verify their viability (Steele et al. 2018). Neonates usually hatched at stage 42 and incubation period lasted around 75 days (30ºC) or 135 days (24ºC) (see also Py-Daniel et al. 2017, Rossigalli-Costa et al. 2021). The SISBIO/ICMBio authorized *T. catalanensis* lizard captures (permits 58029-3 and 33335-2), and the CEUA-USP approved animal maintenance (process: 21.1.49.59.2).

2.4 NEONATES: HATCHING OUTCOMES AND PHENOTYPES

Duration of the incubation period, hatching success and neonate morphology were recorded for the two developmental conditions. After hatching, lizards were maintained in terraria in similar conditions described for the females. To standardize size in the neonates for subsequent analyses we obtained the following morphological measurements no longer than 48 hours after hatching:

Caliper:

1. Body size: snout-vent length and trunk length.
2. Body proportions: lengths of humerus, radius, femur, and tibia; length and height of pelvic and scapular girdles.
3. Head proportions: length, height, and width of head.

X-ray:

4. Autopodium: lengths of the Digits I, II, III, IV and V from *manus* and *pes*.

The radiographies were taken in the x-ray machine Faxitron LX-60, and measurements were obtained in the Image J software (version 1.8.0) by two researchers (APC and DF). We considered possible effects of measurement bias and performed a repeatability test (Supplementary Material). After being euthanized with an anesthetic overdose of Thiopental® injected into the abdominal cavity (concentration from 60 to 100mg/Kg), the neonates were morphologically sexed based on the anatomy of the reproductive system (see Delssin et al. 2019).

2.5 NEONATES: IMMUNOHISTOCHEMISTRY FOR QUANTIFICATION OF ANDROGEN RECEPTORS

We applied an immunohistochemistry protocol to verify the presence and quantify the androgen receptors in Digit III and Digit IV from the left *pes* of male and female neonates of *T. catalanensis*. These digits were chosen because Digit IV from the *pes* is dimorphic in adults from the locality of São Simão, and the Digit III is monomorphic in this population (see Results section). After the neonates were euthanatized, the *pes* were removed and fixed in 4% paraformaldehyde (PFA) diluted in 0.2 M phosphate buffer (PB) for 24 hours at 4°C. Subsequently, the *pes* was immersed in a 30% sucrose solution for at least 48 h at 4°C, dipped in 2-methylbutane at -20°C, frozen and fixed in Tissue-Plus (Fisher Healthcare™ O.C.T. Compound, CA, USA). Serial sections (40 µm) of Digit III and Digit IV were obtained in triplicates using a cryostat microtome (CM1860 - Ag Protect; Leica, Wetzlar, Germany).

The slices were initially washed three times with phosphate buffered saline (PBS, 0.01 M, pH 7.4) for 5 minutes, followed by an antigenic recovery process, where slices were incubated for 30 minutes in a citrate buffer (ScyTek, Utah, USA) at 70°C, then cooled to room temperature and washed three times with PBS for 5 minutes. The sections were incubated in 1% hydrogen peroxide solution for 3 minutes, washed three times with PBS and then incubated for 1 hour in a solution of PBS with 0.3% triton X-100 (Sigma-Aldrich, USA) and 10% horse serum solution (Sigma-Aldrich, USA) at room temperature to prevent non-specific binding, followed by 48 hours of incubation with rabbit anti-androgen receptor antibody (1:200; ab133273, Abcam) diluted in T-PBS (0.3% Triton-PBS, pH 7.4) solution with 5% horse serum at room temperature with constant agitation. Then, the slices were washed three times with PBS and incubated for 2 hours with secondary goat anti-rabbit IgG antibody (h&l), conjugated to Alexa Flour 594 (1:200, Jackson ImmunoResearch, USA) in T-PBS solution with 5% horse serum at room temperature, on a shaker. After that, the slices were washed three times with PBS. Finally, the slices were mounted on gelatinized sheets, dried, and covered with a coverslip to be examined by fluorescence microscope. We also included a negative control (without primary antibody) to evaluate the specificity of the immunohistochemistry protocol. In addition, we simultaneously performed immunohistochemistry in the rat testis, a tissue well known to express androgen receptor-positive labeling (positive control).

2.5 DATA ANALYZES

We first evaluated measurements error by measuring ten times the same structures in four *T. catalanensis* specimens and test for repeatability using the CalcRepeability function (package EvolQG). Then all variables in the morphological database were log10 transformed. For each locality, we regressed the log-transformed traits against log SVL and extracted the residuals (Shingleton 2010), which were used in subsequent analyses. We evaluated normality and homogeneity in the adults and neonates databases using Shapiro-wilk and Levene tests. Given that some morphological traits did not attend the premises for parametric tests, alternative approaches were implemented. All statistical analyses were performed in the R environment, version 4.2.0.

2.5.1 Adults

To test for sexual dimorphism in morphological traits of adults from different localities, we implemented a machine learning approach (Random Forest - RF) for each locality, which evaluates if the specimens can be correctly classified as males or females based on their phenotypic profile. These analyzes were performed as described in Chapter 1 of the present dissertation. The function randomForest (RF; package randomForest) builds classification trees to classify, gathers results, and identifies the classes established. The function plotProximity (package rfPermute) generates a graphic visualization of RF. In addition, we also used Mann-Whitney tests to identify sexual dimorphism in each morphological trait separately in the 13 localities sampled.

We also tested for associations with the climatic parameters, using generalized linear models (GLM) for each specific digit from *manus* and *pes* as a function of temperature and precipitation. The climatic database was obtained with the package raster, using the WorldClim information for the specific coordinates representing the 13 areas for *T. catalanensis* lizards distribution. The GLM analyses were performed using Sexual Dimorphism Indexes (SDI) of digit lengths and also the residuals from digits regressed on SVL for males and females. The SDI represents the magnitude of differences between the sexes and was calculated as explained in chapter 1 (see Corl et al. 2010, Gomes and Kohlsdorf

2011, Lofeu et al. 2020). The best-fit models were selected based on AIC values.

2.5.2 Neonates

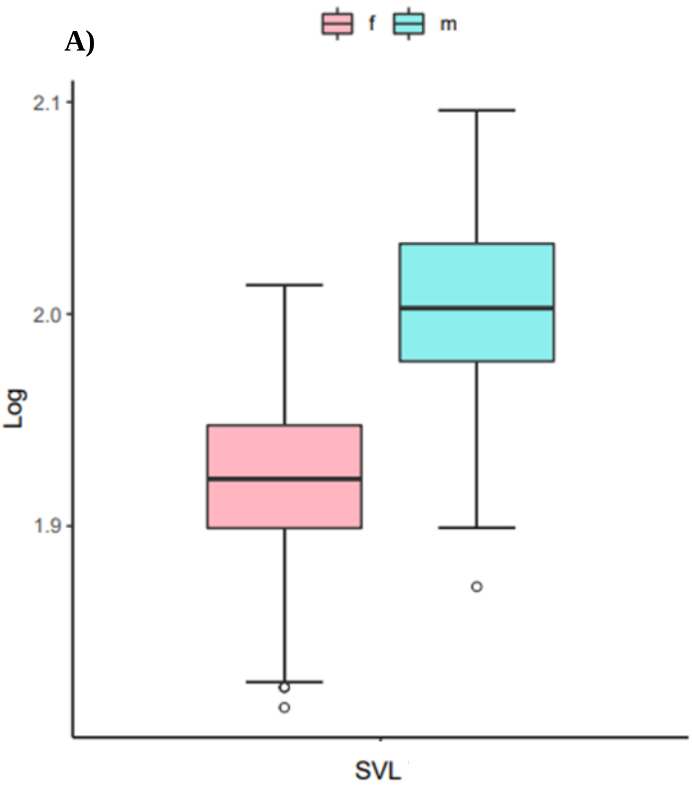
The analysis performed using the morphological database for the neonates was similar to that applied for the adults. First, we tested for possible effects of year (2020/2021 and 2021/2022) on the morphology of the neonates in each incubation temperature using Mann-Whitney tests. Given that we identified year effects in some of the traits, we performed subsequent analyses grouping individuals from the two years and also separately for each year (this second approach only when evaluating each trait separately). We first implemented a RF test to investigate if individuals are correctly classified based on the morphology. We performed this test separately for each incubation temperature. Then, we performed Mann-Whitney tests to evaluate sexual dimorphism in each phenotypic trait for the two incubation temperatures.

For the analysis of data from immunohistochemistry, we captured photomicrographs of the digits using a fluorescence microscope (Zeiss, Axio Image Z2, Baden-Württemberg, Germany) with 40x objective in the LAS image acquisition program. The analysis was based on the intensity of fluorescent light emitted in the central region of the digits. The adjustment of contrast and brightness were the same for all animals. To avoid background interference, the background intensity was subtracted from the fluorescent intensity obtained in the central region of the digits. The quantification of fluorescent light was performed by the mean gray value analysis using the ImageJ program. Comparisons based on Analysis of Variance for two-factor comparisons (two-way ANOVA) with *Post-hoc* multiple comparisons were performed using Tukey’s test. The immunohistochemistry data were normally distributed and attained the homogeneity of variance criteria.

3. RESULTS

3.1 ADULTS: PHENOTYPIC PROFILES AND CLIMATIC ASSOCIATIONS

The repeatability values ranged from 0.922 to 0.988 for the digits measurements in *T. catalanensis*. For head and body proportions, values ranged from 0.938 to 0.998. The measurement variation was considered minimal, as the values associated to the repeatability were near 1 (Harper 1994). The symmetry between left and right sides was confirmed with a paired *t-test* (Supplementary Material), and all the measurements were obtained on the left side of the specimens. Overall, the lengths of the digits were larger in males than in females of *T. catalanensis*, a pattern also observed for body size, limbs, and head proportions (Figure 2; Supplementary Material).



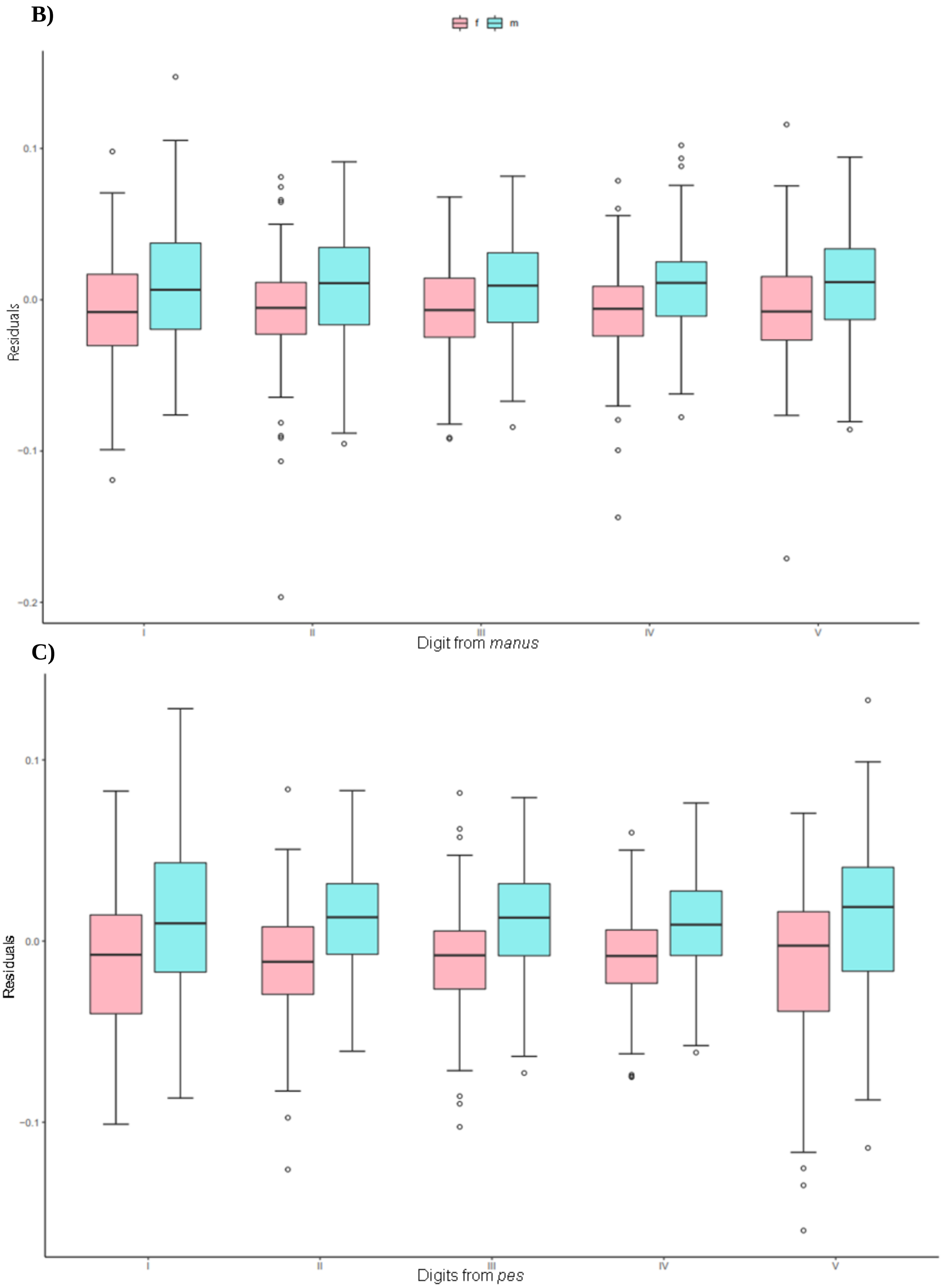


Figure 2. Residuals from morphological traits regressed on SVL (total of 329 specimens for males (M, in blue) and females (F, in pink) of *T. catalanensis*. A) body size (Log10 of snout-vent length [SVL]), B) digits in manus, C) digits in pes.

The results from the Random Forest analyses indicate the relevance of each morphological trait for discriminating males and females of *T. catalanensis*. The first analysis was performed with the complete database for *T. catalanensis* adults (i.e. 23 morphological traits and specimens from the 13 localities together) and encompassed an average error of only 5.75% for sex classification based on morphological traits (Table 2). The snout-vent length, head width, head length and DII from *pes* contributed more for the classification in males and females (Accuracy and Gini index in Figure 3). The analyses performed separately for each locality provided different results, as error rates and variable contributions varied among localities (Table 3, Figure 4). The lowest average errors in the male-female classifications were observed in the localities of TA (2.50%) and PI (3.70%), while the highest errors were observed in AC (57.14%) and BR (28.57%). For almost all localities, the SVL and the digits from *manus* and *pes* were the most representative traits to discriminate males from females of *T. catalanensis* (Figure 4).

Table 2. Confusion matrix for the RF results using *T. catalanensis*; comparisons based on the phenotypic profile of males and females using 1000 tree permutations.

	Female	**Male**	**Classification**
Female	155	10	6.06%
Male	8	156	4.87%

Table 3. Confusion matrix for the RF results using *T. catalanensis* specimens; comparisons based on the phenotypic profile of males and females using 1000 tree permutations. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

Locality		**Female**	**Male**	**Classification error**
AC	**Female**	3	4	57.14%
AC	**Male**	4	3	57.14%
AR	**Female**	10	2	16.66%
AR	**Male**	2	11	15.38%
AU	**Female**	15	4	21.05%
AU	**Male**	2	13	13.33%
BA	**Female**	10	1	9.09%
BA	**Male**	1	10	9.09%

BR	**Female**	5	4	44.44%
BR	**Male**	2	10	16.66%
CG	**Female**	13	2	13.33%
CG	**Male**	3	7	30.00%
LA	**Female**	16	2	11.11%
LA	**Male**	3	14	17.64%
LU	**Female**	4	5	55.55%
LU	**Male**	1	11	8.33%
PI	**Female**	12	0	0.00%
PI	**Male**	1	14	6.66%
RP	**Female**	4	3	42.85%
RP	**Male**	1	10	9.09%
SS	**Female**	12	3	20.00%
SS	**Male**	4	9	30.76%
TA	**Female**	19	1	5.00%
TA	**Male**	0	20	0.00%
UN	**Female**	9	2	18.18%
UN	**Male**	3	5	37.50%

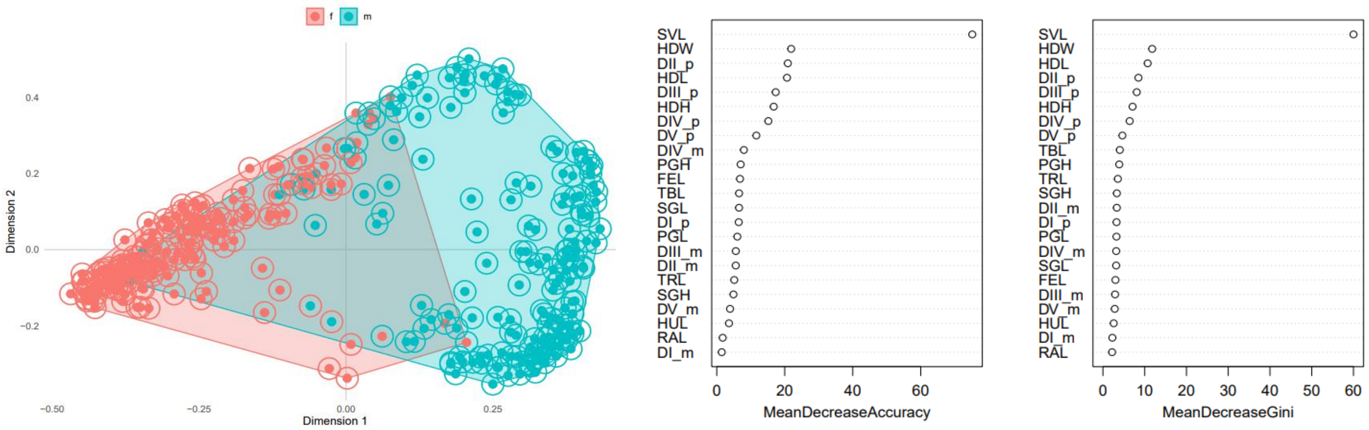
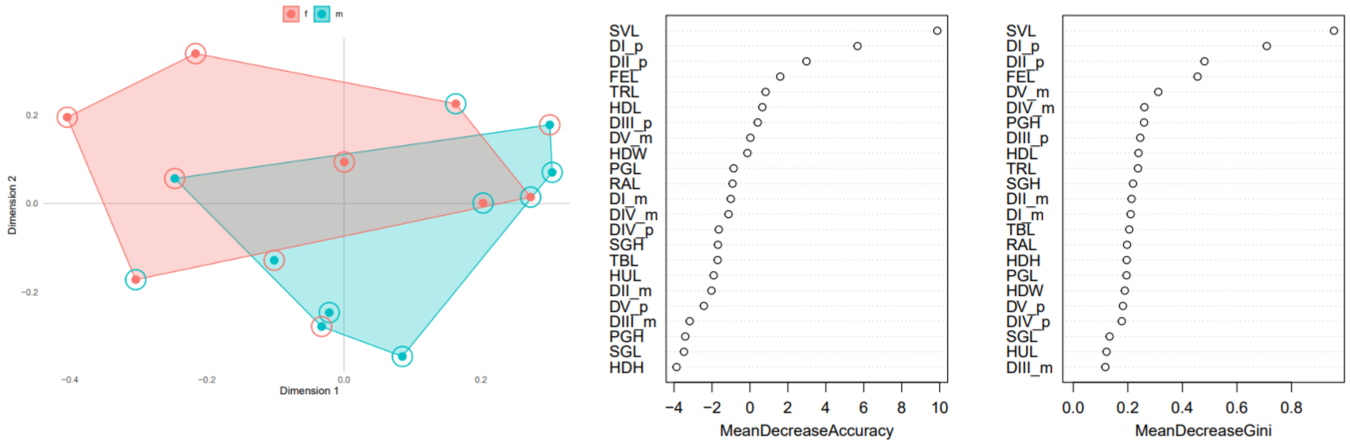
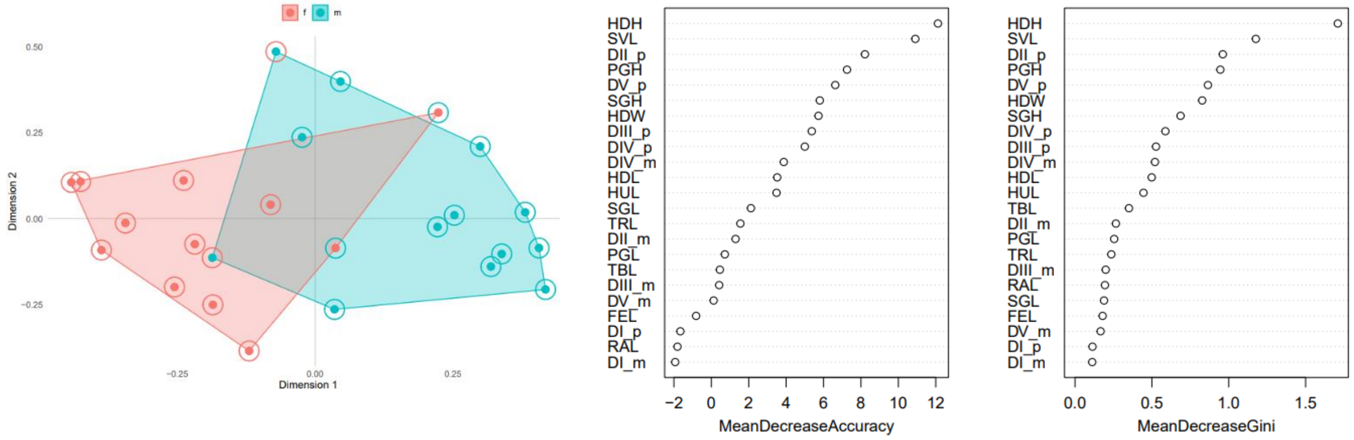


Figure 3. Relationships between the two first dimensions retained by the Random Forest analysis using the *T. catalanensis* from all localities together, and associated ranks of Accuracy and Gini classifications illustrating the relevance of each morphological trait for overall classification as male or female. Males are represented in blue and females in pink, circles around each point represent the sex classification: if circles and dots are of the same color, the classification is accurate. Abbreviations correspond to: HDH = head height, HDW = head width, HDL = head length, SGH = shoulder girdle height, SGL = shoulder girdle length, PGH = pelvic girdle height, PGL = pelvic girdle length, TRL = trunk length, SVL = snout-vent length, HUL = humerus length, RAL = radio length, FEL = femur length, TBL = tibia length, D = digit, m = *manus*, p = *pes*.

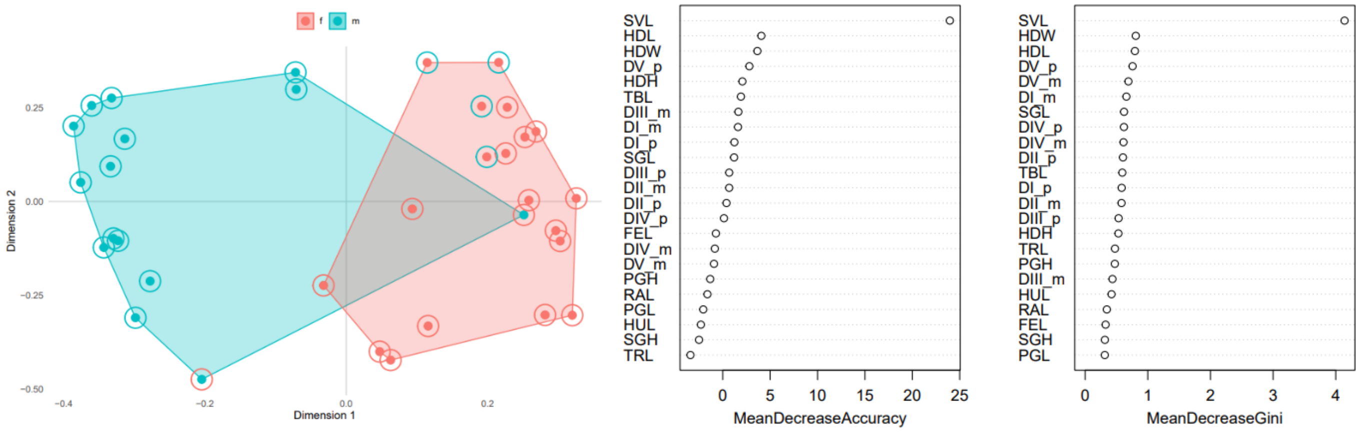
A) Alfredo de Castilho



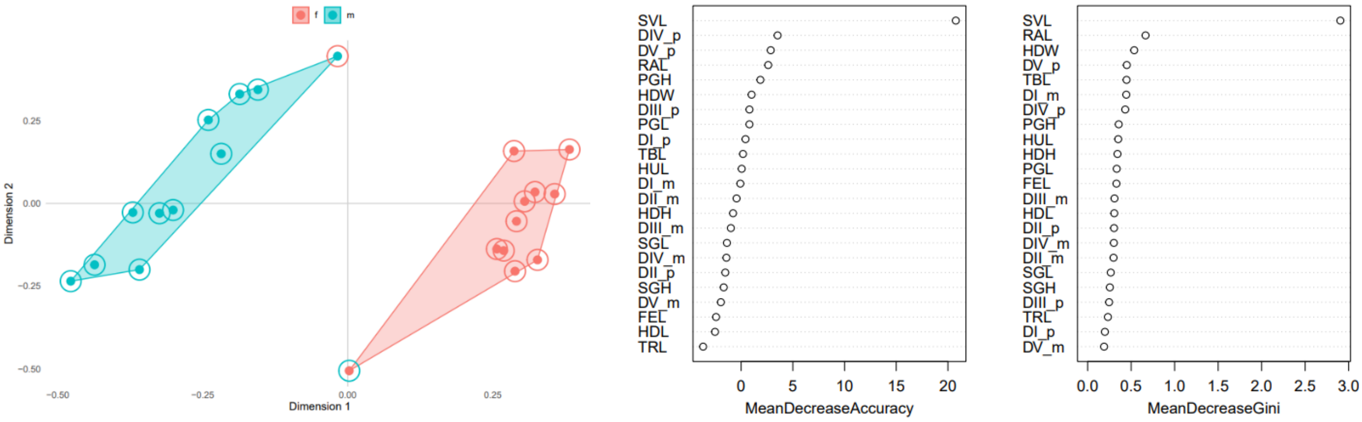
B) Arinos



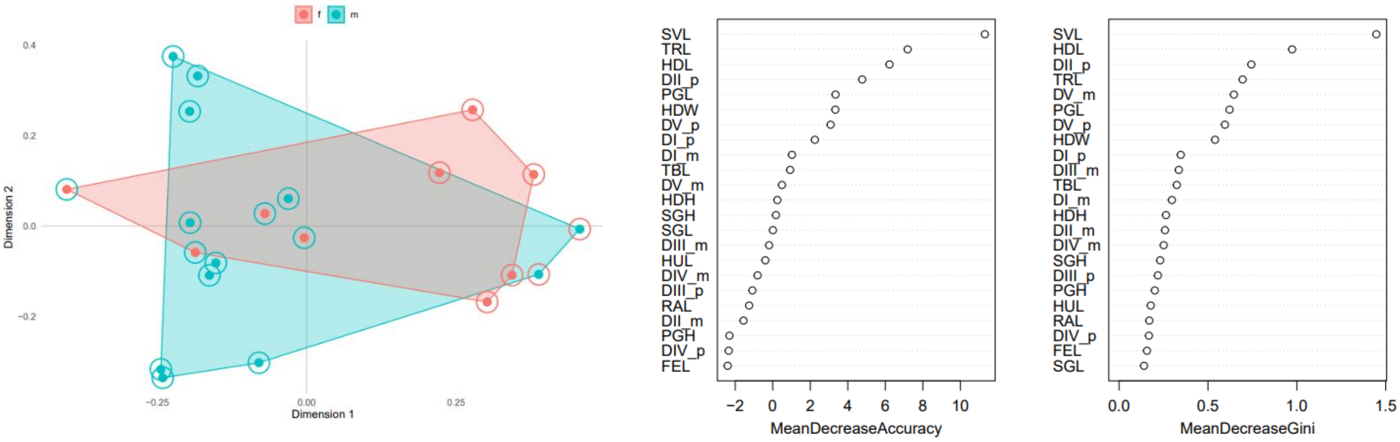
C) Aruanã



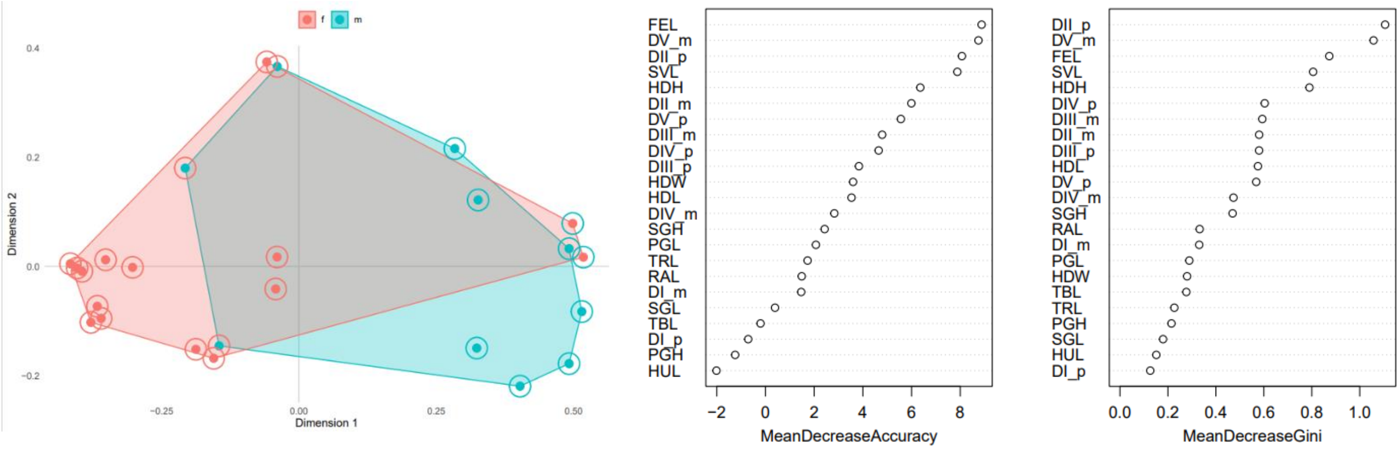
D) Batayporá



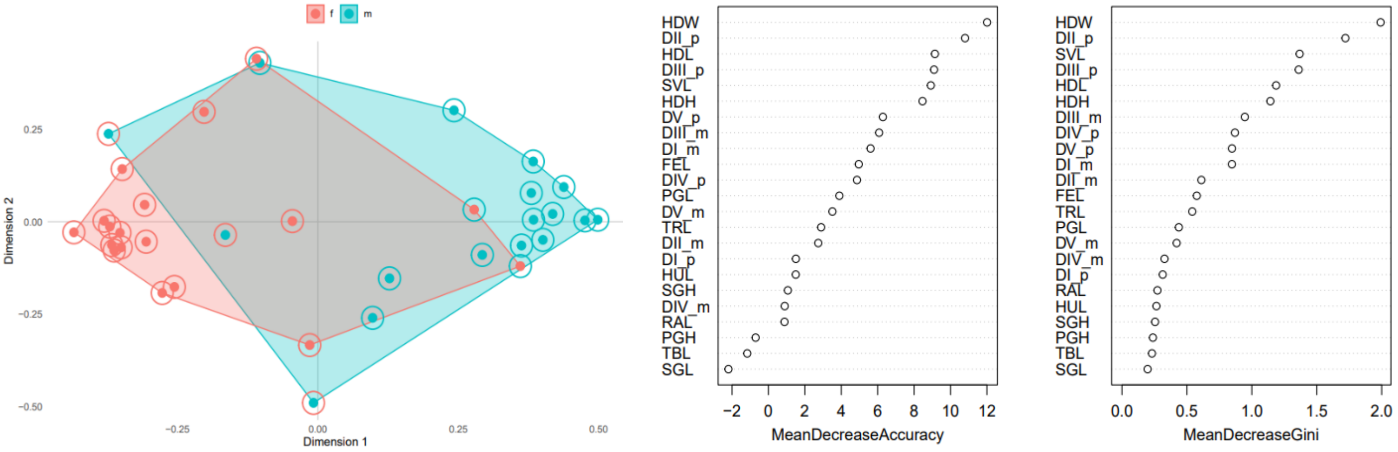
E) Brasilândia



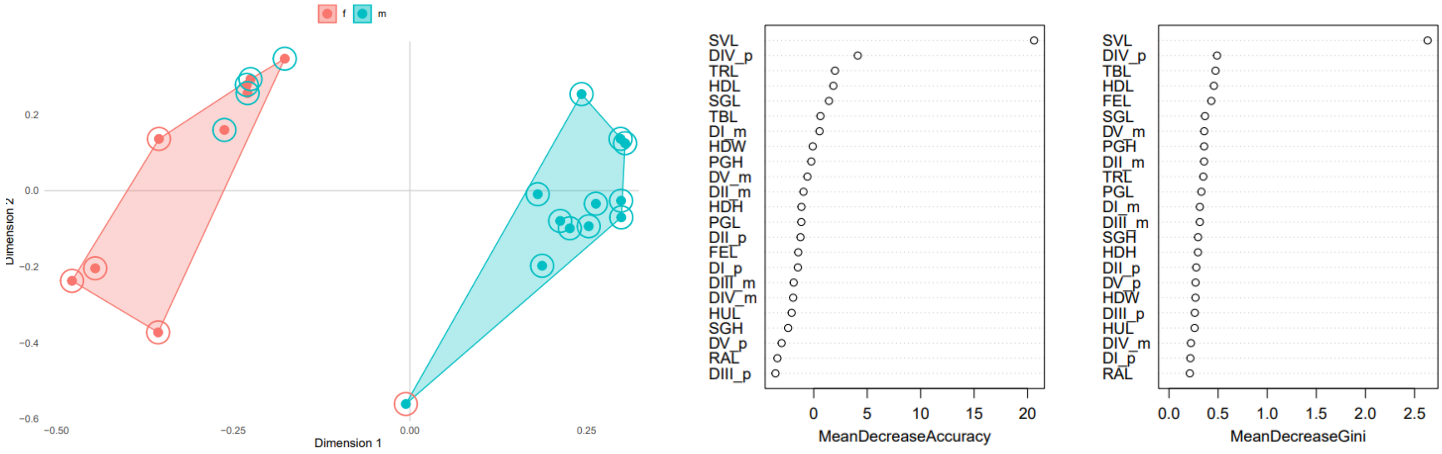
F) Chapada dos Guimarães



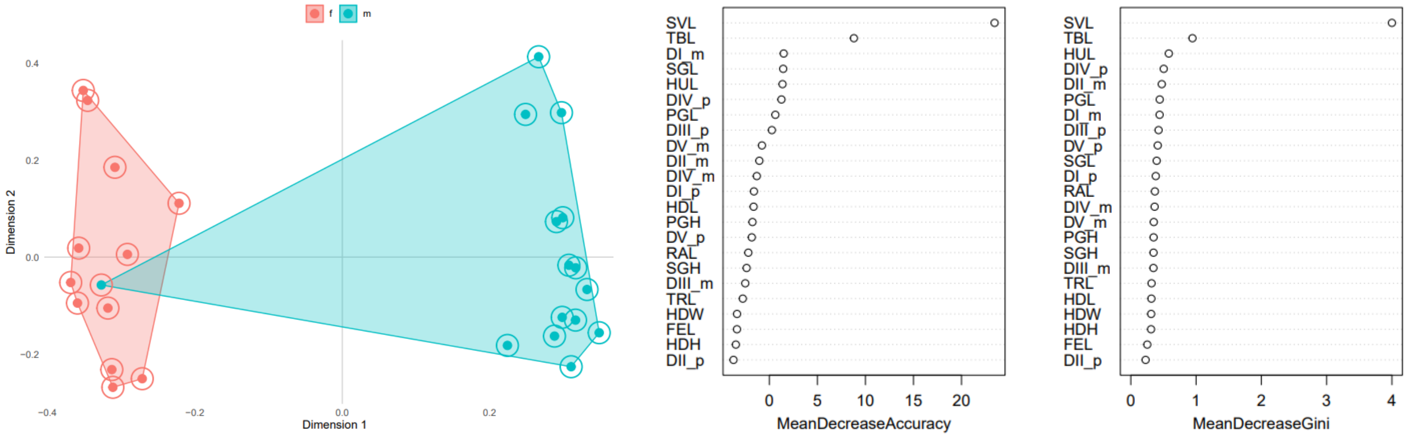
G) Lajeado



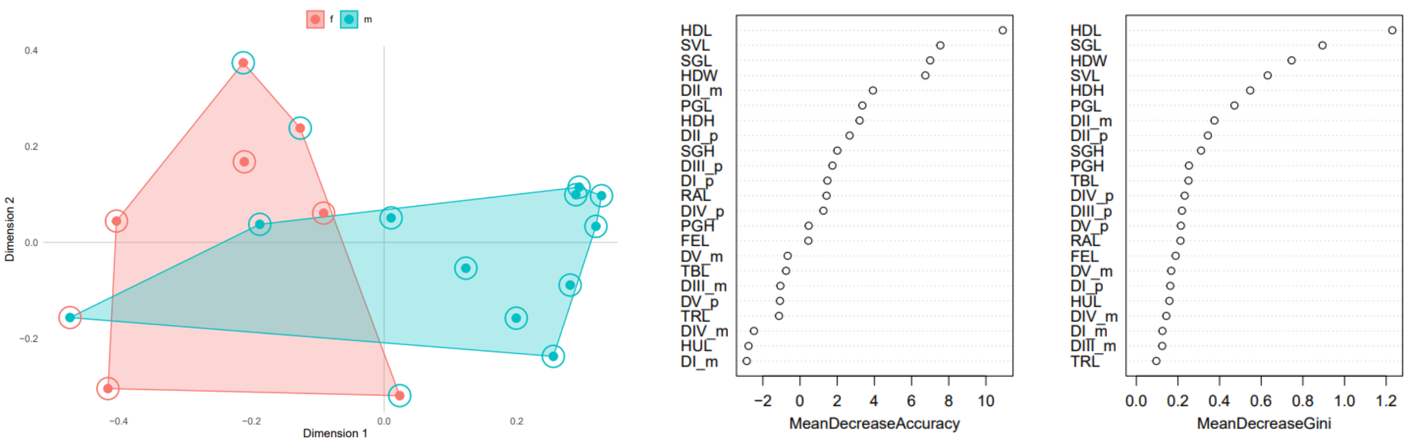
H) Luziânia



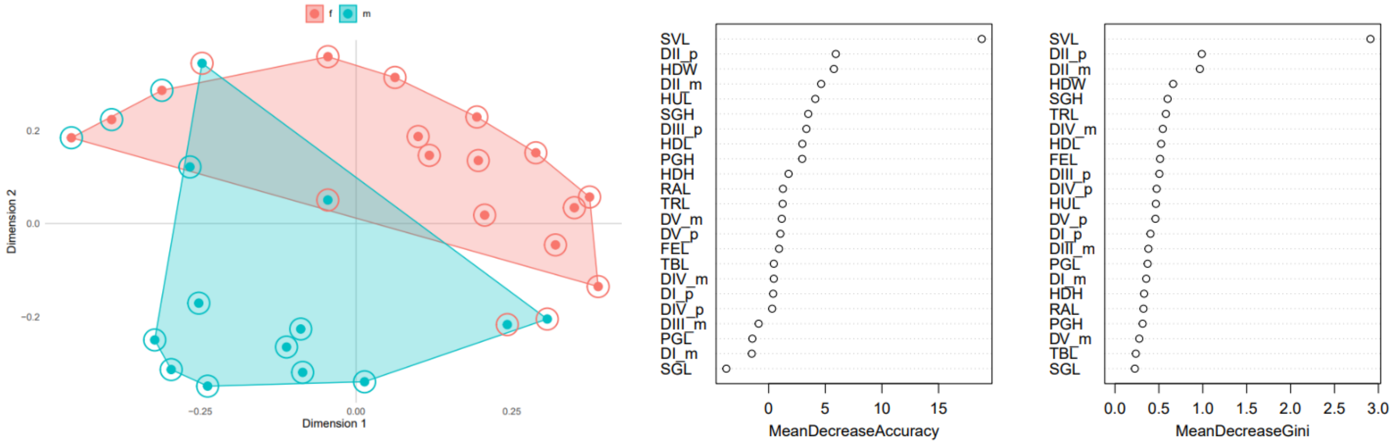
I) Piracicaba



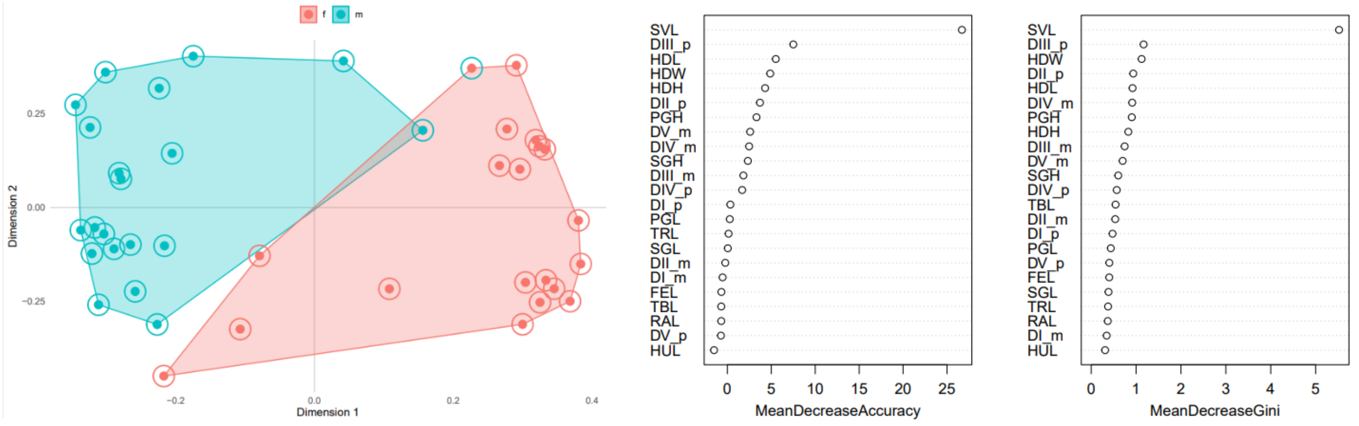
J) Ribeirão Preto



K) São Simão



L) Tapirapé



M) Unaí

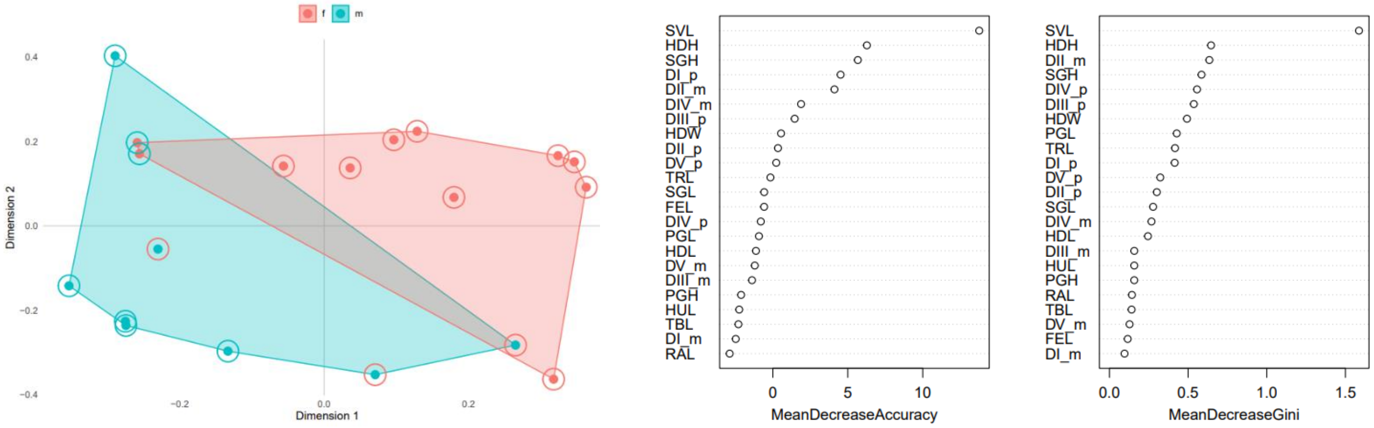


Figure 4. Relationships between the two first dimensions retained by the Random Forest analysis using individuals from each locality of the *T. catalanensis* database, and associated ranks of Accuracy and Gini classifications illustrating the importance of each morphological trait for overall classification as male or female. Males are represented in blue and females in pink, circles around each point represent the sex classification: if circles and dots are of the same color, the classification is accurate. Abbreviations correspond to: HDH = head height, HDW = head width, HDL = head length, SGH = shoulder girdle height, SGL = shoulder girdle length, PGH = pelvic girdle height, PGL = pelvic girdle length, TRL = trunk length, SVL = snout-vent length, HUL = humerus length, RAL = radio length, FEL = femur length, TBL = tibia length, D = digit, m = manus, p = pes. Localities: A) AC = Alfredo de Castilho (SP), B) AR = Arinos (MG), C) AU = Aruanã (GO), D) BA = Batayporá (MS), E) BR = Brasilândia (MS), F) CG = Chapada dos Guimarães (MT), G) LA = Lajeado (TO), H) LU = Luziânia (GO), I) PI = Piracicaba (SP), J) RP = Ribeirão Preto (SP), K) SS = São Simão (SP), L) TA = Tapirapé (MT) and M) UN = Unaí (MG).

After investigating the contribution of morphological traits together for sex discrimination (i.e. RF), we analyzed sexual dimorphism in *T. catalanensis* separately for each trait, using Mann-Whitney tests. Analyses performed using the entire database (i.e. all 13 localities together) suggested that all digits from *manus* and *pes* are dimorphic in *T. catalanensis* (Table 4). Mann-Whitney tests implemented separately for each locality, however, suggest diversity in the identity of the dimorphic digits along the species distribution (Table 5 for *manus* and Table 6 for *pes*; example for DIV in Figure 5). Except for two localities (AC and BA) where *T. catalanensis* seems to be monomorphic for digit lengths, in all the 11 remaining localities we identified sexual dimorphism in at least one digit, although patterns differed among areas (Figure 6). In some localities, such as BR, PI, RP, and TA, only the digits from *pes* were dimorphic (Tables 5 and 6), while in others (e.g. AU, CG, LA, LU, SS, and UN) the digits from *manus* and *pes* differ between males and females (Tables 5 and 6). We have also identified variation in the sexual dimorphism of body traits, especially head proportions, among localities (Supplementary Material).

Table 4. Results of Mann-Whitney analyses testing for differences between sexes in the digit lengths of the *manus* and *pes* of *T. catalanensis* considering all localities together (total of 329 specimens)*;* significant results ($p < 0.05$) are highlighted in bold.

	Digits	**U**	**p**
Manus	DI	**11**	**0.005**
	DII	**10**	**< .001**
	DIII	**9**	**< .001**
	DIV	**9**	**< .001**
	DV	**10**	**< .001**
Pes	DI	**9**	**< .001**
	DII	**7**	**< .001**
	DIII	**7**	**< .001**
	DIV	**7**	**< .001**
	DV	**8**	**< .001**

Table 5. Results of Mann-Whitney analyses testing for differences between sexes in the digit lengths of the *manus* of *T. catalanensis* separated by locality*;* significant results ($p < 0.05$) are highlighted in bold. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

Locality	**DI**		**DII**		**DIII**		**DIV**		**DV**	
	U	**P**	**U**	**p**	**U**	**p**	**U**	**p**	**U**	**p**
AC	19	0.535	13	0.165	23	0.902	24	1.000	23	0.902
AR	69	0.650	49	0.123	48	0.110	47	0.098	63	0.437
AU	**52**	**0.001**	107	0.228	**74**	**0.017**	92	0.083	**84**	**0.043**
BA	53	0.652	52	0.606	59	0.949	56	0.797	49	0.478
BR	47	0.651	53	0.972	32	0.129	32	0.129	31	0.111
CG	40	0.055	**18**	**< .001**	**20**	**0.001**	**21**	**0.002**	**11**	**< .001**
LA	**73**	**0.007**	**46**	**<.001**	**48**	**<.001**	94	0.053	105	0.118
LU	**7**	**< .001**	**22**	**0.023**	**22**	**0.023**	**18**	**0.009**	**26**	**0.049**
PI	79	0.614	89	0.981	73	0.427	76	0.516	84	0.792
RP	30	0.479	20	0.104	36	0.860	27	0.328	31	0.536
SS	96	0.964	61	0.098	58	0.072	63	0.118	**44**	**0.013**
TA	155	0.231	175	0.512	173	0.478	139	0.102	170	0.429

UN	40	0.778	**13**	**0.009**	21	0.062	43	0.968	**17**	**0.026**

Table 6. Results of Mann-Whitney analyses testing for differences between sexes in the digit lengths of the *pes* in *T. catalanensis* separated by locality*;* significant results (p <0.05) are highlighted in bold. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

Locality	**DI**		**DII**		**DIII**		**DIV**		**DV**	
	U	**p**	**U**	**p**	**U**	**p**	**U**	**p**	**U**	**p**
AC	13	0.165	17	0.383	15	0.259	22	0.805	20	0.620
AR	57	0.270	47	0.098	**39**	**0.035**	36	**0.022**	**17**	**<.001**
AU	**79**	**0.027**	**81**	**0.033**	**7**	**0.025**	96	0.111	**57**	**0.002**
BA	47	0.401	50	0.519	50	0.519	46	0.365	46	0.365
BR	51	0.862	**22**	**0.023**	42	0.422	46	0.602	**20**	**0.015**
CG	**19**	**0.001**	**4**	**< .001**	**0**	**< .001**	**8**	**< .001**	**10**	**< .001**
LA	**67**	**0.004**	**35**	**< .001**	**45**	**<.001**	**67**	**0.004**	**49**	**< .001**
LU	51	0.862	**20**	**0.015**	**11**	**0.001**	**4**	**< .001**	46	0.602
PI	77	0.548	84	0.792	**48**	**0.041**	**45**	**0.028**	**35**	**0.006**
RP	26	0.285	**16**	**0.044**	31	0.536	21	0.126	25	0.246
SS	**38**	**0.005**	**50**	**0.029**	94	0.892	**37**	**0.004**	**39**	**0.006**
TA	**121**	**0.033**	**126**	**0.046**	**102**	**0.007**	**126**	**0.046**	173	0.478
UN	**8**	**0.002**	**18**	**0.033**	**9**	**0.003**	**9**	**0.003**	**2**	**<.001**

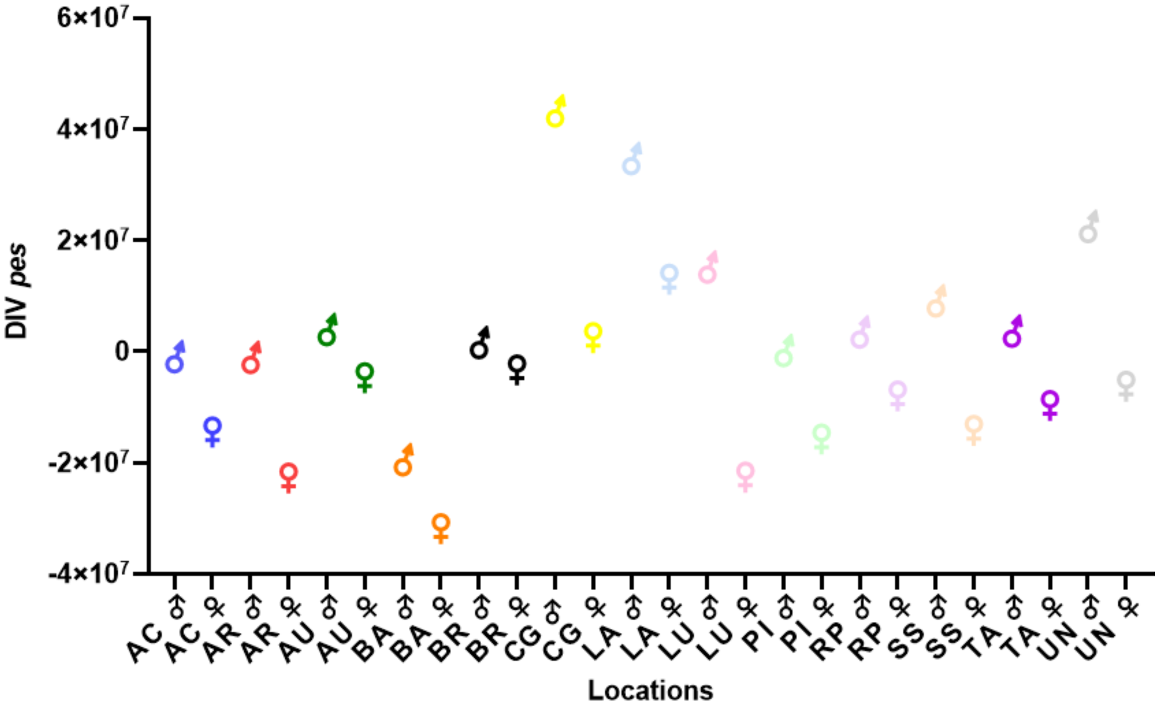


Figure 5. Residuals from DIV in the *pes* regressed by SVL for each locality sampled. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

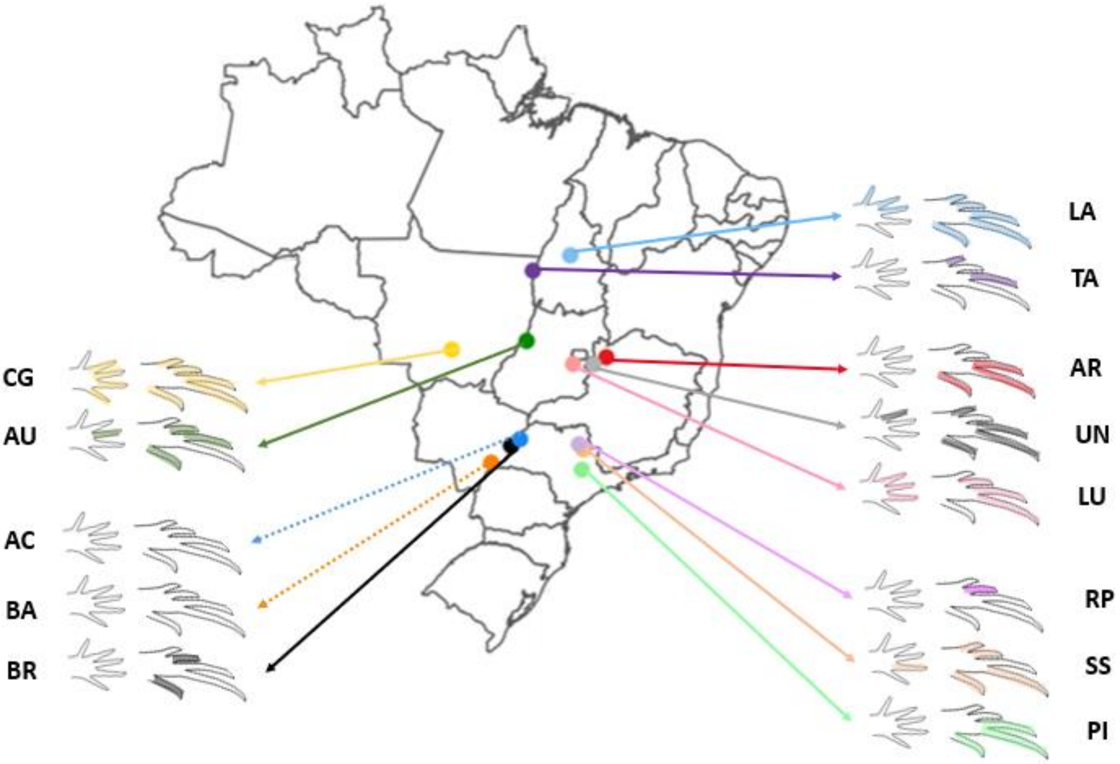


Figure 6. Representation of the dimorphic digits in different localities (represented by distinct colors) along the distribution of *T. catalanensis* lizards; digits sexually dimorphic indicated by the color representing the locality. Solid arrows indicate localities where we identified sexual dimorphism in the digits, and dotted arrows correspond to localities where individuals are monomorphic for digit lengths. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

The models testing for relationships between the SDI and climatic parameters presented very similar AIC values and did not suggest significant associations between the magnitude of the sexual dimorphism and temperature or precipitation (Table 7).

Table 7. Generalized linear models testing for associations between the Sexual Dimorphism Index (SDI) for digits from the *manus* and the *pes* of *T. catalanensis* and climatic parameters.

Autopodium	**Model**	**AIC**	***p value***
Manus	DI ~ temperature	-63.06	0.500
	DI ~ precipitation	-62.63	0.737
	DI ~ temperature + precipitation	-61.29	0.490 / 0.684
	DII ~ precipitation	-61.97	0.588
	DII ~ temperature	-61.82	0.674
	DII ~ temperature + precipitation	-60.27	0.636 / 0.566
	DIII ~ temperature	-69.86	0.811
	DIII ~ temperature + precipitation	-69.48	0.709 / 0.276
	DIII ~ precipitation	-61.97	0.588
	DIV ~ precipitation	-75.39	0.847
	DIV ~ temperature	-75.37	0.875
	DIV ~ temperature + precipitation	-73.43	0.863 / 839
	DV ~ temperature	-70.97	0.749
	DV ~ precipitation	-70.94	0.776
	DV ~ temperature + precipitation	-69.10	0.733 / 0.756
Pes	DI ~ precipitation	-69.38	0.319
	DI ~ temperature	-68.34	0.694
	DI ~ temperature + precipitation	-67.48	0.784 / 0.360
	DII ~ precipitation	-68.10	0.509
	DII ~ temperature	-67.58	0.909
	DII ~ temperature + precipitation	-66.10	0.972 / 0.536
	DIII ~ precipitation	-68.15	0.285
	DIII ~ temperature	-66.74	0.958
	DIII ~ temperature + precipitation	-66.16	0.942 / 0.308

DIV ~ temperature + precipitation	-77.98	0.051 / 0.102
DIV ~ temperature	-76.35	0.958
DIV ~ precipitation	-74.77	0.285
DV ~ precipitation	-65.84	0.145
DV ~ temperature + precipitation	-63.88	0.858 / 0.162
DV ~ temperature	-63.21	0.958

We also evaluated associations between digit lengths and environmental parameters separately for each sex, and identified different results in the GLM analyses when compared to the tests performed using the SDI. In females, the DI from *manus* and the digits I and IV from *pes* were associated with precipitation and temperature (Table 8), while in males the relative lengths of DI, DII, DIII and DIV from the *manus* and DI and DIV from the *pes* seem associated with temperature and precipitation (Table 9). In both sexes, digit lengths from the *manus* and the *pes* usually tend to be longer in localities having higher temperatures and/or increased precipitation (Figures 7 and 8).

Table 8. Generalized linear models testing for associations in females of *T. catalanensis* between the residuals of digits (*manus* and *pes*) regressed on SVL climatic parameters; significant results ($p < 0.05$) highlighted in bold.

Autopodium	**Model**	**AIC**	***p value***
Manus	**DI ~ precipitation**	**-616.3**	**0.022**
	DI ~ temperature + precipitation	**-614.4**	**0.696 / 0.032**
	DI ~ temperature	-611.7	0.345
	DII ~ precipitation	-647.8	0.099
	DII ~ temperature + precipitation	-645.9	0.957
	DII ~ temperature	-645.0	0.713 / 0.092
	DIII ~ temperature	-707.3	0.245
	DIII ~ precipitation	-706.0	0.771
	DIII ~ temperature + precipitation	-705.7	0.203 / 0.549
	DIV ~ precipitation	-684.5	0.420
	DIV ~ temperature + precipitation	-683.8	0.255 / 0.152
	DIV ~ temperature	-683.7	0.420

	DV ~ precipitation	-629.2	0.089
	DV ~ temperature + precipitation	-627.2	0.912 / 0.106
	DV ~ temperature	-626.5	0.598
Pes	**DI ~ temperature**	**-615.1**	**0.021**
	DI ~ temperature + precipitation	**-613.3**	**0.018 / 0.652**
	DI ~ precipitation	-609.7	0.889
	DII ~ precipitation	-695.5	0.322
	DII ~ temperature	-694.9	0.530
	DII ~ temperature + precipitation	-693.7	0.694 / 0.390
	DIII ~ precipitation	-722.6	0.452
	DIII ~ temperature	-722.3	0.585
	DIII ~ temperature + precipitation	-721.2	0.450 / 0.360
	DIV ~ temperature	-771.0	0.585
	DIV ~ temperature + precipitation	**-769.5**	**0.006 / 0.445**
	DIV ~ precipitation	-763.9	0.143
	DV ~ precipitation	-562.3	0.194
	DV ~ temperature + precipitation	-561.9	0.207 / 0.116
	DV ~ temperature	-561.4	0.585

Table 9. Generalized linear models testing for associations in males of *T. catalanensis* between the residuals of digits (*manus* and *pes*) regressed on SVL and climatic parameters; significant results ($p < 0.05$) are highlighted in bold.

Autopodium	Model	AIC	*p value*
Manus	**DI ~ precipitation**	**-596.5**	**0.008**
	DI ~ temperature + precipitation	**-594.8**	**0.601 / 0.007**
	DI ~ temperature	-589.4	0.948
	DII ~ temperature + precipitation	**-630.0**	**0.126 / 0.003**
	DII ~ precipitation	**-629.6**	**0.007**
	DII ~ temperature	-623.0	0.377
	DIII ~ temperature + precipitation	**-677.6**	**0.026 / 0.011**
	DIII ~ precipitation	**-674.6**	**0.035**
	DIII ~ temperature	-673.0	0.092

	DIV ~ temperature + precipitation	**-681.6**	**0.137 / 0.042**
	DIV ~ precipitation	-681.4	0.079
	DIV ~ temperature	-679.4	0.289
Pes	DV ~ precipitation	-621.2	0.674 / 0.064
	DV ~ temperature + precipitation	-619.4	0.674 / 0.064
	DV ~ temperature	-617.9	0.986
	DI ~ temperature	**-576.5**	**0.001**
	DI ~ temperature + precipitation	**-574.6**	**0.002 / 0.755**
	DI ~ precipitation	-567.5	0.347
	DII ~ precipitation	-699.2	0.852
	DII ~ temperature	-699.1	0.926 / 0.872
	DII ~ temperature + precipitation	-697.2	0.926 / 0.872
	DIII ~ temperature	-704.8	0.228
	DIII ~ precipitation	-704.5	0.287
	DIII ~ temperature + precipitation	-703.5	0.318 / 0.410
	DIV ~ precipitation	**-733.4**	**0.004**
	DIV ~ temperature + precipitation	**-731.4**	**0.833 / 0.004**
	DIV ~ temperature	-725.2	0.679
	DV ~ temperature	-572.2	0.383
	DV ~ precipitation	-571.5	0.727
	DV ~ temperature + precipitation	-570.2	0.416 / 0.870

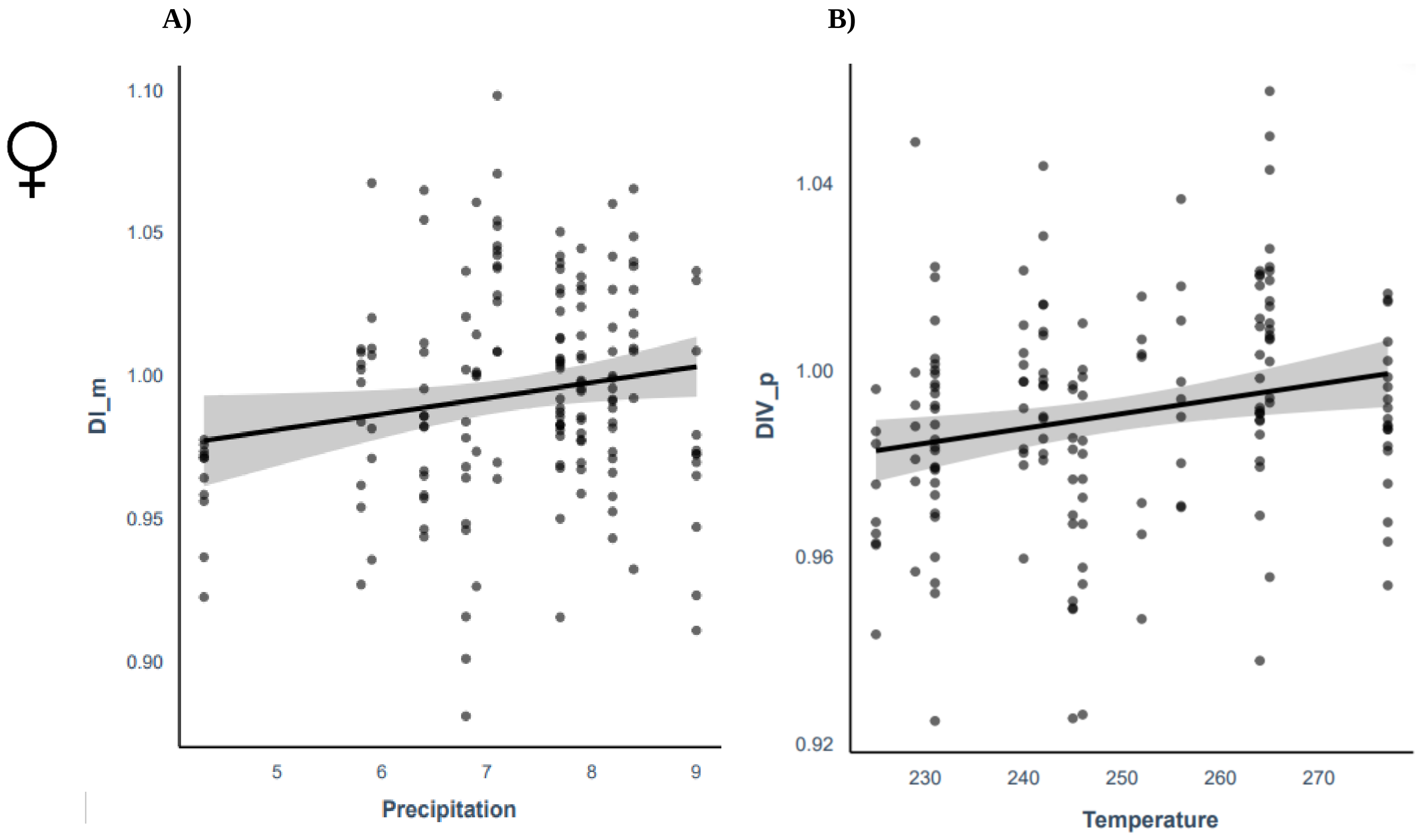


Figure 7. Residuals of digit lengths regressed on SVL for females of *T. catalanensis*. A) Associations between DI from *manus* and precipitation. B) Associations between DIV from the *pes* and temperature. Abbreviations correspond to: *manus* = m, *pes* = p, Digit = D. The Precipitation corresponds to the coefficient of variation; Temperature values are multiplied by 10.

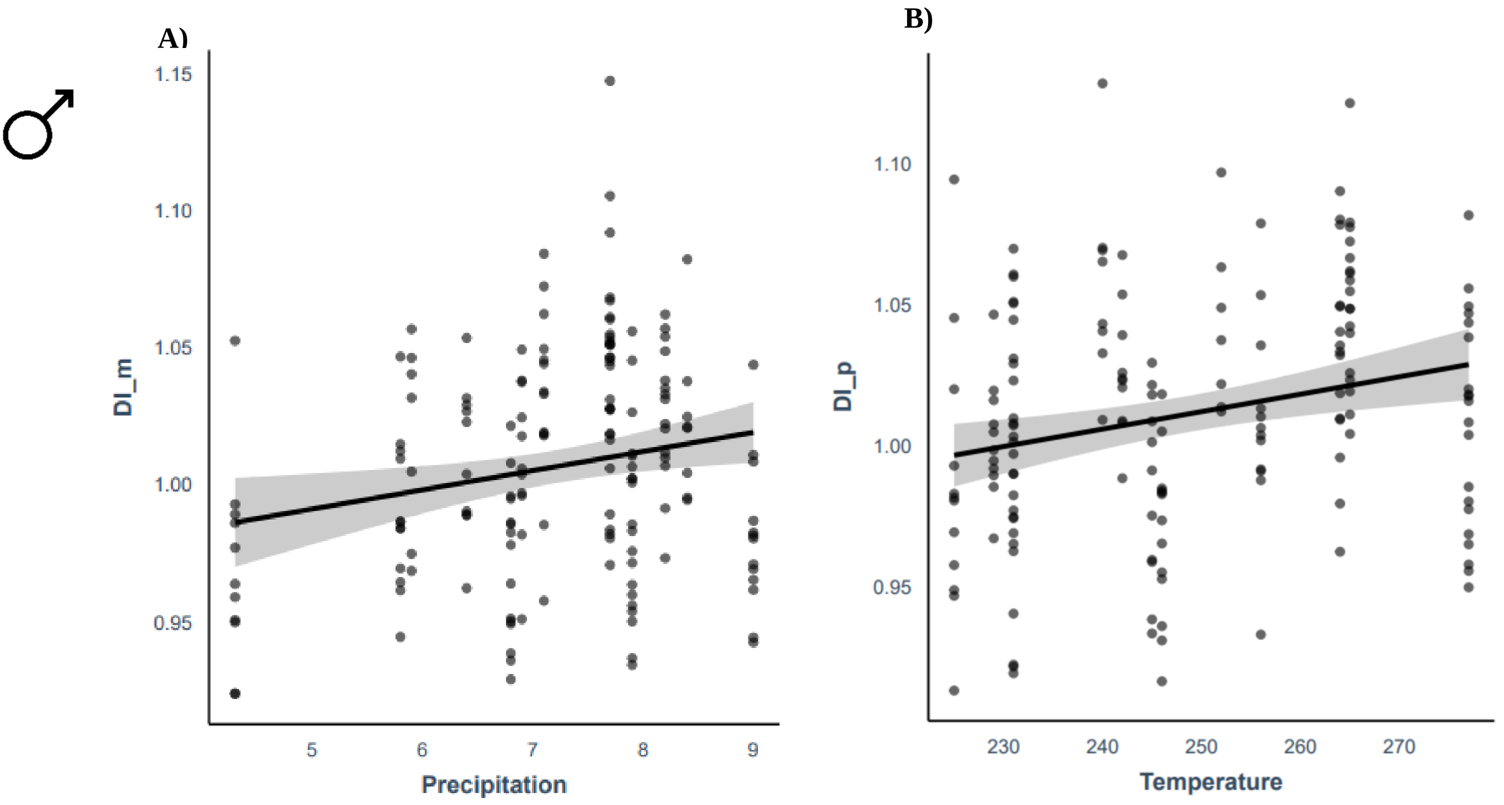


Figure 8. Residuals of digit lengths regressed on SVL for males of *T. catalanensis*. A) Associations between DI from *manus* and precipitation. B) Associations between DI from the *pes* and temperature. Abbreviations correspond to: *manus* = m, *pes* = p, Digit = D. The Precipitation corresponds to the coefficient of variation; Temperature values are multiplied by 10.

3.2 NEONATES: HATCHING OUTCOMES AND PHENOTYPIC PROFILES

The hatching success and days until birth in each temperature were very similar between the two years sampled (Table 10). In both years only three embryos died due to fungi contamination, and sex ratio was also consistent with similar proportions of males and females (Table 11). Anatomical sex identification was very difficult in some neonates due to problems during the process of tissue preservation, and some x-ray images were also not clear enough for measuring digits, so we excluded these neonates from the analyzes. Therefore, we evaluated a total of 80 neonates in tests considering only incubation temperatures regardless of sex and used a total of 59 neonates in analyses that separated individuals by sex; 12 neonates were used in the immunohistochemistry assays.

Table 10. Total of eggs, hatching success, days until birth and neonates SVL (snout-vent length) in each incubation temperature for the two years we collected gravid females for this study.

Hatching outcomes	**2021**		**2022**	
	24ºC	**30ºC**	**24ºC**	**30ºC**
Number of eggs	24	24	21	18
Hatched eggs	24	22	20	18
Hatching Rate	100.00%	91.60%	95.20%	100.00%
Number of days	138	77	132	72
SVL	28.9	29.4	29.5	30.2

Table 11. Percentage of males and females of *T. catalanensis* hatched in each incubation temperature, combining data from the two years we incubated the eggs.

Sex ratio	**Females**	**Males**
24ºC	46.15%	53.84%
30ºC	48.50%	51.50%

Results from the Random Forest analyses suggest an overlap among neonates in most morphological traits (Figure 9) regardless of incubation temperature, and we observed high error rates for correct classification of neonates as males or females based on morphological traits, with an average error of 66.67% for neonates from the 30ºC condition and 50.00% error for animals incubated at 24ºC (Table 12). The phenotypic traits that mostly contributed for correct sex classification differed between incubation temperatures (Table 12 and Figure 9):

animals from the 24ºC condition were classified as males or females mostly based on DI from the *pes*, and DIII, DI, DII from the *manus*, in addition to femur length (Figure 9A), while in those from 30ºC, the traits pelvic girdle height and humerus length, DV and DI from the *pes*, and femur length were the most expressive traits for sex classification (Figure 9B).

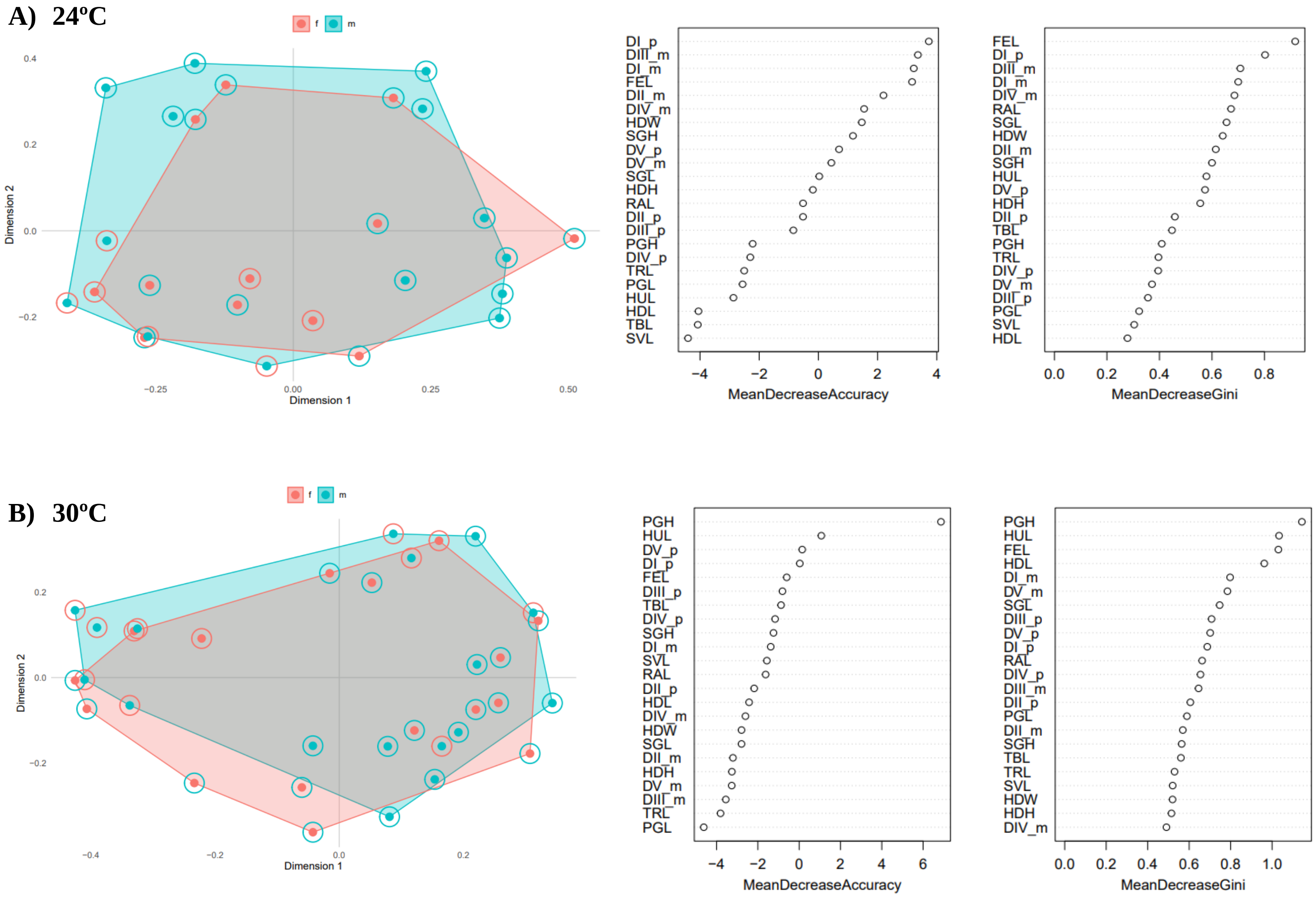


Figure 9. Relationships between the two first dimensions retained by the Random Forest analysis using neonates of *T. catalanensis* from two incubation temperatures, with associated ranks of Accuracy and Gini classifications illustrating the importance of each morphological trait for correct sex classification. Males are represented in blue and females in pink, circles around each point represent the sex classification: if circles and dots are of the same color, the classification is accurate. Abbreviations correspond to: HDH = head height, HDW = head width, HDL = head length, SGH = shoulder girdle height, SGL = shoulder girdle length, PGH = pelvic girdle height, PGL = pelvic girdle length, TRL = trunk length, SVL = snout-vent length, HUL = humerus length, RAL = radio length, FEL = femur length, TBL = tibia length, D = digit, m = manus, p = pes. A) Animals incubated in 24ºC, B) Animals incubated in 30ºC.

Table 12. Confusion matrix for neonates of *T. catalanensis* incubated at 24ºC and 30ºC; comparisons based on the phenotypic profile of males and females with a Random Forest analysis. Tree permutations = 1000.

Temperature		Female	Male	Classification error
30º C	**Female**	3	13	81.25%
30º C	**Male**	9	8	52.94%
24º C	**Female**	3	9	75.00%
24º C	**Male**	4	10	28.57%

We identified significant effects of the year for two digits (DV in the *manus* and DI in the *pes,* Supplementary Material), and therefore we implemented Mann-Whitney tests using the whole database (i.e. 2021 and 2022 neonates together), and the same analysis separating data from each year (see Supplementary Material). The results are similar using these two approaches, and we did not identify sexual dimorphism in the digits at birth, regardless of the year and the incubation temperature (Tables 13 and 14).

Table 13. Results of Mann-Whitney analysis testing for differences between sexes in digit lengths in the *manus* and the *pes* of *T. catalanensis* neonates incubated at 24ºC.

24ºC			
Autopodium	**Digit**	**U**	**p**
Manus	DI	52	0.106
	DII	66	0.374
	DIII	50	0.085
	DIV	58	0.193
	DV	66	0.374
Pes	DI	73	0.595
	DII	72	0.560
	DIII	68	0.432
	DIV	66	0.374
	DV	52	0.106

Table 14. Results of Mann-Whitney analysis testing for differences between sexes in digit lengths in the *manus* and the *pes* of *T. catalanensis* neonates incubated at 30ºC.

30ºC			
Autopodium	**Digit**	**U**	**p**
Manus	DI	94	0.136
	DII	128	0.790
	DIII	131	0.873
	DIV	124	0.683
	DV	135	0.986
Pes	DI	133	0.929
	DII	125	0.709
	DIII	127	0.763
	DIV	131	0.873
	DV	133	0.929

Given that digit lengths did not differ between males and females at birth, we also tested for differences between neonates from different incubating temperatures, using Mann-Whitney and grouping males and females from each developmental condition. These analyses suggested differences in the relative lengths (residuals) of DII and DIII from the *manus* according to the incubating temperature, and neonates from 24ºC exhibited relatively longer digits than those from 30ºC (Table 15 and Figure 10).

Table 15. Results of Mann-Whitney analysis testing for differences in the digit lengths of the *manus* and the *pes* between neonates of *T. catalanensis* incubated at distinct temperatures; significant results (p <0.05) are highlighted in bold.

Autopodium	**Digit**	**U**	**p**
Manus	DI	746	0.622
	DII	**426**	**< .001**
	DIII	**553**	**0.018**
	DIV	594	0.050
	DV	619	0.086
	DI	720	0.457
	DII	639	0.127

Pes	DIII	747	0.628
	DIV	667	0.210
	DV	762	0.734

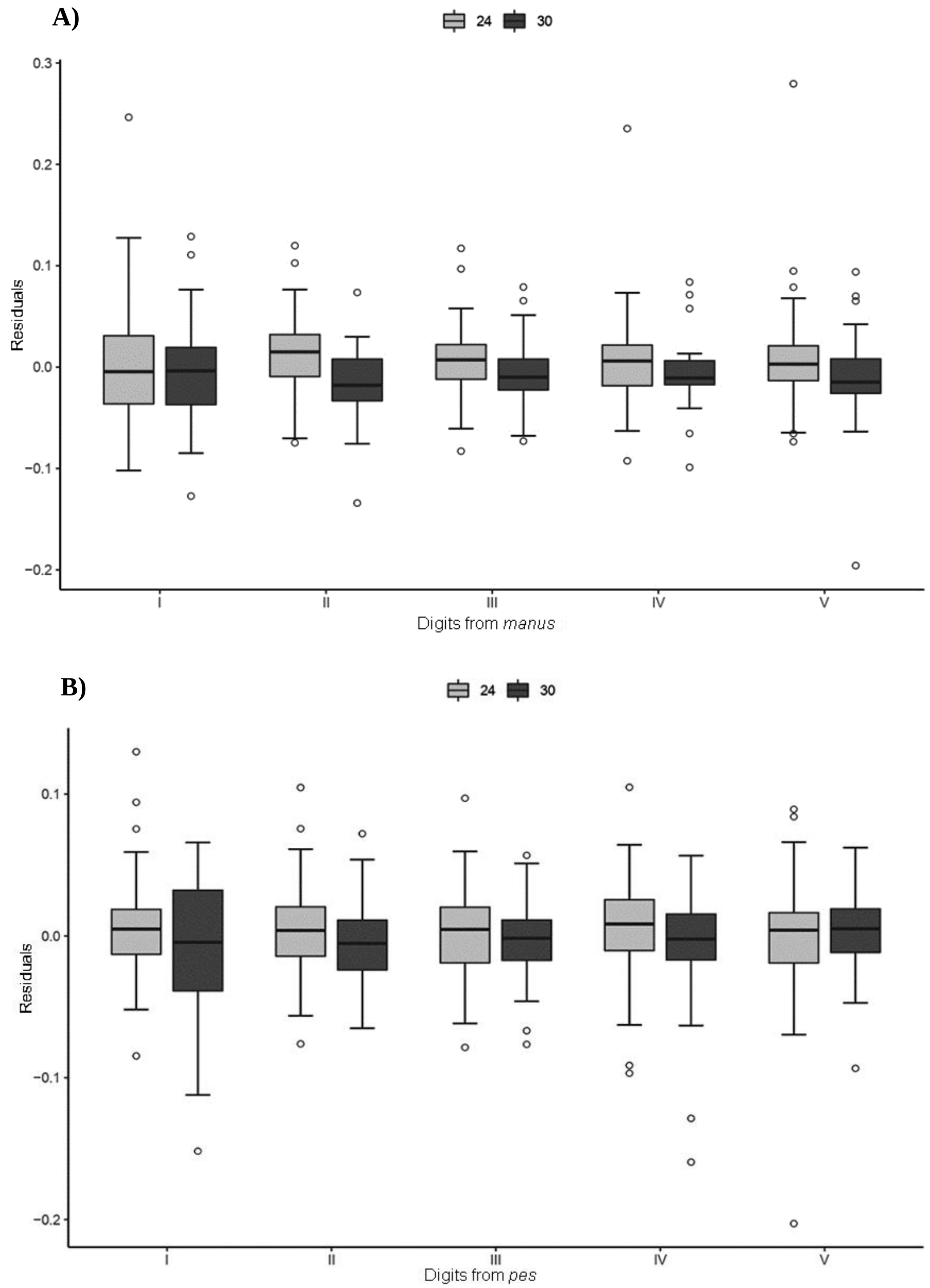


Figure 10. Residuals from digit lengths regressed on SVL in neonates of *T. catalanensis* according to the incubation temperature, 24ºC and 30ºC. A) DI-V in *manus* B) DI-V in *pes*.

3.3 IMMUNOHISTOCHEMISTRY FOR ANDROGEN RECEPTOR

The immunohistochemistry analyzes to quantify the androgen receptor in Digits III and IV from *pes* of male and female neonates of *T. catalanensis* suggested differences in the presence of testosterone receptors in these digits. In DIV, which is dimorphic in the adults sampled in São Simão, the males exhibited higher fluorescence intensity than the females (Figures 11 and 12, Table 16). On the other hand, in the monomorphic digit (i.e. DIII), quantification of the androgen receptors was similar between sexes (Figure 11 and Table 16).

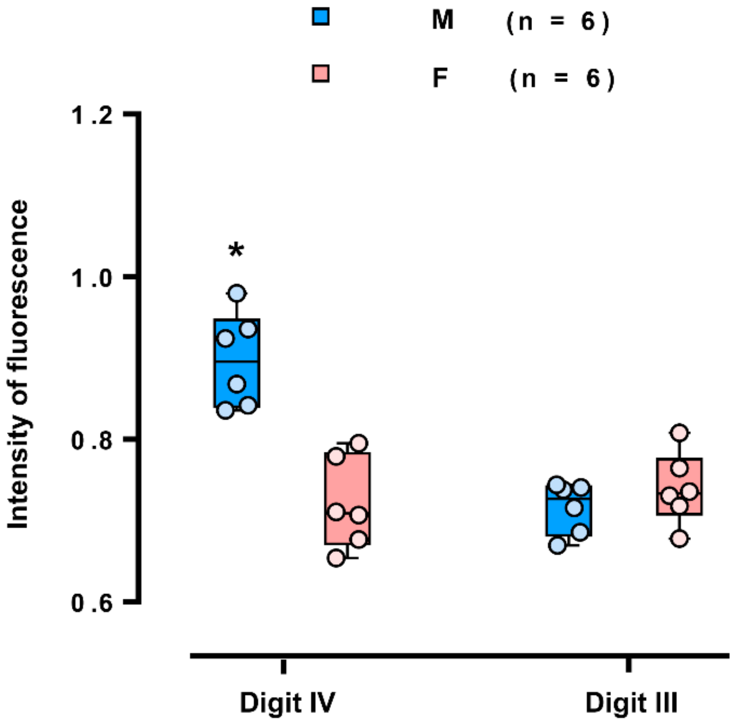


Figure 11. Fluorescence intensity in Digits IV and III from the *pes* of *T. catalanensis* neonates*;* females (F) in pink, males (M) in blue.

Table 16. Comparison from the analyzes of Pairwise Multiple Comparison Procedures (Tukey Test) for the androgen receptors in *T. catalanensis* neonates; significant differences ($p < 0.05$) highlighted in bold.

Comparison	q	p
DIII x DIV: males	**9.201**	**<.001**
DIII x DIV: females	0.953	0.508
DIII: male x female	1.189	0.411
DIV: male x female	**8.965**	**<.001**

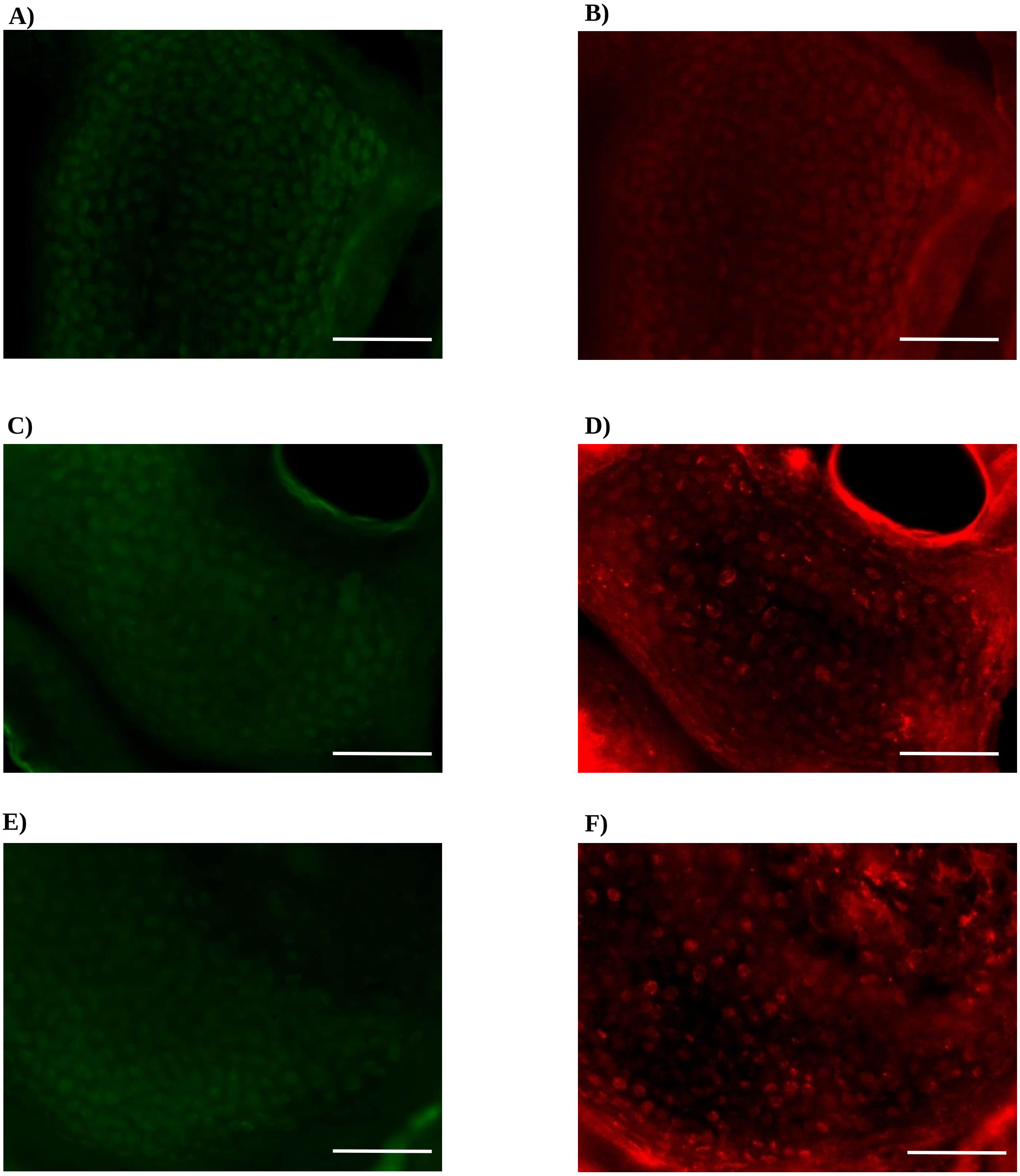


Figure 12. Immunofluorescence for androgen receptor in the Digit IV of male and female neonates. Representative photomicrographs in a transverse plane of the digits were acquired under 40x objective through the filter for light spectrum 488 (green) and 594 (red) to confirm labeling specificity. A, B) Negative staining (without primary antibody) in male lizard. C, D) Female and E, F) Male lizard with positive staining. Scale bar = 50 µm.

4. DISCUSSION

Investigating the relationship between phenotypic traits modulated by steroid hormones and environmental parameters at different ontogenetic periods contributes for a productive discussion with implications for several fields in Biology. The present chapter addressed sexual dimorphism in digit lengths and other phenotypic traits (body and head dimensions) among adults of *T. catalanensis* distributed in different localities of Central Brazil and also between neonates incubated at different developmental temperatures. We identified a great diversity among localities in the identity of the digits that are dimorphic. Specifically, analyses performed considering all the 13 localities sampled together suggested sexual dimorphism in all digits, while analyses performed separately for each locality revealed significantly differences in the patterns of dimorphic digits in *T. catalanensis*, corroborating the hypothesis of evolutionary flexibility in the identify of digits that become dimorphic in tetrapods (see Lofeu et al. 2017, 2020).

Contrary to our expectations, we identified that neonates from a dimorphic population are monomorphic at birth regardless of incubation temperatures, but males and females already differ in the presence of androgen receptors in the digit that will become dimorphic later in ontogeny (DIV), corroborating the model addressing the origin of hormone sensitivity in the digits (Lofeu et al. 2017, 2020). Finally, incubation at lower temperatures (24ºC) resulted in neonates with longer digits in the *manus* (DII and DIII), regardless of sex, which points possible effects of environmental conditions in developmental processes. Differences between adults and juveniles in dimorphic patterns of digits have already been described in lizards (Tobler et al. 2011, Woodhead et al. 2018), and may express variation in hormone levels or receptors concentrations during ontogeny (Hau 2007, Zheng and Cohn 2011). The present study innovates by combining population approaches and ontogenetic scales to evaluate patterns of sexual dimorphism in digit lengths and highlights the complexity of processes of phenotypic diversification that are modulated by hormone dynamics and likely sensitive to environmental conditions along the ontogeny.

The sensitivity of specific tissues to hormones during embryonic development may result in sexually dimorphic patterns in several traits, including digit lengths (see Zheng and Cohn 2011, Huber et al. 2017). Most experimental studies addressing the effect of sex hormones in digit lengths manipulated hormonal levels and evaluated effects on digit lengths (e.g. Tobler et al. 2011, Lofeu et al. 2017), and current knowledge suggests that gene regulation and the amount of steroid receptors in specific tissues also play a role modulating digit growth (Zheng and Cohn 2011, Huber et al. 2017). The DIV in mice has a relatively higher amount of sex hormone receptors when compared to DII, which explains the differences in digit ratios between males and females of this species (Zheng and Cohn 2011). Observations regarding the diversity of dimorphic patterns in digit lengths nurtured the theoretical model suggesting that the initial origin of sexual dimorphism in digit lengths may not depend of the hormonal levels, residing mostly on increased concentrations of steroids receptors (Lofeu et al. 2017, 2020). Here we show that dimorphic patterns in digit lengths may vary even within the same species, with differences in the identity of the dimorphic digits among geographically close localities (e.g. São Simão and Ribeirão Preto are less than 60 km distant). In this context, animals distributed along environmental gradients often face different climatic conditions. Since temperature and precipitation parameters may interact with steroid hormones during development, eventually affecting the phenotype (see Martínez-Juárez and Moreno-Mendoza 2019), exploring relationships between climate and sexual dimorphism in digit lengths is very exciting. We addressed this topic in *T. catalanensis* adults from 13 localities distributed in different Brazilian regions of Brazilian and did not identify associations between the magnitude of sexual dimorphism (SDI) in digit lengths and environmental parameters, although relationships between digit lengths and the climate are detectable when males and females are evaluated separately. Digit lengths from both autopodia seem to be associated with climate, increasing with temperature and precipitation, although the identity of the digits responding to climate seems to differ between males and females.

Studies addressing relationships between geographical ranges and morphological diversity frequently focus on the role climate but also gene expression during development (e.g. Thorpe et al. 2005, Jaffe et al. 2016). In *Anolis*, common garden experiments revealed that toe, limb, and head proportions that are divergent among lizards distributed in different areas are maintained when animals are raised in common garden, highlighting the genetic role of morphological patterns in these lizards (Thorpe et al. 2005). We identified associations between morphology and climatic parameters (temperature and precipitation), but the mechanisms underlying variation among populations in the sexual dimorphism in digit lengths remains obscure. Manning and collaborators (2003) explored relationships of androgen receptor genes and identified that a high number of CAG in its alleles are associated with insensitivity to testosterone. The authors suggest that the levels of embryonic testosterone and the structure of the androgen receptor genes modulate patterns of digit ratio (Manning 2002, Manning et al. 2003), probably regulating populational differences. However, other studies failed to find evidence for the relationships between CAG repeats and the 2D:4D ratio in humans, and the genetic correlations with sexual dimorphism in digits deserve further investigation (Hönekopp 2013, Warrington et al. 2018).

Another important point that requires attention in the study of dimorphism in digit lengths is when digits become dimorphic. Even in mammals, such as humans and mice, that are used as references in digit lengths dimorphism, there are contrasting results, in which the identity of the dimorphic digits may not reflect hormonal levels during development (Yan et al. 2008, Galis et al. 2010, Richards et al. 2021). In humans, evidence shows that the prenatal sex steroids affect digit elongation, and the 2D:4D ratio in children is correlated with embryonic levels of testosterone and estradiol during the second trimester of development (Lutchmaya et al. 2004). However, Richards and collaborators (2021) failed to replicate these results, and this hypothesis that digit lengths reflect sex steroids levels during pregnancy is still ambiguous. In *Hoplodactylus* maculatus geckos, juveniles' digits were not dimorphic at birth, however the adults of the same species presented differences between sexes in digit ratio (Woodhead et al. 2018). Likewise, in *Ctenophorus* pictus lizards, the patterns of digit ratio differ between neonates and adults, being discussed the role of sexual maturation hormones in digits elongation (Tobler et al. 2011).

Androgen levels after sexual maturation may modulate the digit lengths, especially if tissues are already dimorphic regarding the hormone receptors like in *T. catalanensis* neonates in this work. During the embryonic development of *T. catalanensis*, all digit condensations become visible at stage 34, and at stage 38 the interdigital webbing is already retreated, and the claws start to differentiate (Py-Daniel et al. 2017). Interestingly, it is also at stage 34 that gonadal differential starts, and this process continues until the embryo hatches at stage 42 (Delssin et al. 2019). Steroids likely modulate sex differences throughout gonadal differentiation in embryos by regulating tissue development and probably gene expression (Lovern and Wade 2003, Zheng and Cohn 2011). Furthermore, the increased concentrations of steroid receptors in specific digits, such as Digit IV in mice (Zheng and Cohn 2011) and also in lizards (this study) may result in different tissue sensitivity to circulating hormones. The dynamics of hormonal peaks in embryos of *Anolis* lizards also shed light on the potential of testosterone produced by the embryo in modulating the digits elongation, as their stages may coincides during development (Lovern and Wade 2003). Nevertheless, in lizards, it is might possible that the testosterone peaks in adulthood contribute for sexual dimorphism in several traits.

The testosterone levels contribute for secondary sexual characteristics in lizards (Emerson 2000), and the dynamics of this hormone may affect size, coloration, aggressiveness and immunity in several species (Moore and Marler 1987, Cox et al. 2008, 2009). In this context, some *Anolis* species become sexually dimorphic in early developmental stages, while others only express differences between males and females after sexual maturation (Sanger et al. 2014). Facial length, for example, may become sexually dimorphic through differences in the estrogen pathway that accelerate growth in adult males (Sanger et al. 2014). The estrogen and androgen signaling is associated with sex differences also in the 2D:4D digit ratio in mice (Zheng and Cohn 2011), and likely establishes differences in dimorphic phenotypic traits at different stages along the ontogeny (Sanger et al. 2014). In our study, we observed that neonates of *T. catalanensis* from São Simão are monomorphic for digit lengths, while adults from the same locality are dimorphic for this trait; it is interesting to note that these neonates are already sexually dimorphic in the concentration of steroid receptors in the digit that will differentiate between males and females later in ontogeny.

The current literature in the evolution of traits regulated by testosterone discusses the 'Evolutionary potential hypothesis', according to which elements composing the testosterone signaling cascade could change independently (Hau 2007). According to this hypothesis, relationships between morphological traits and the testosterone would also be evolutionarily flexible and be represented by a multitude of factors that congregate many pathways beyond circulating levels of testosterone (Hau 2007). The distribution of steroid receptors, the action of cofactors that can interact with the 'hormone-receptor' complex, the conversion that exists among hormones, and other downstream apparatus might be actually modulating tissue development (Hau 2007, Zheng and Cohn 2011), and the testosterone-signaling processes and associated effects could vary among animals living in different environments (Hau 2007). The diversity among localities in digit lengths described in our study may result from interactions between testosterone-signaling processes and climate parameters throughout *T. catalanensis* ontogeny. Moreover, previous studies addressing the phenotypic profiles in *T. catalanensis* neonates from São Simão described that neonates incubated at 30ºC are larger than those from 24ºC (Rossigalli-Costa et al. 2021), a trend not recovered in this study. We did not identify sexual dimorphism in neonates of *T. catalanensis* for any phenotypic trait, regardless of incubation temperature, a result in agreement with Prado-Prandini (2022) study using animals from the same locality.

To summarize, the results obtained in this study using *T. catalanensis* indicate that the diversity in patterns of sexual dimorphism in digit lengths is present among localities. The relationship with environmental conditions, probably reflecting steroid hormones signaling processes, was explored in the context of the identity of dimorphic digits in adults and neonates and represents an important advance for understanding phenotypic diversification modulated by sex hormones during ontogeny. We expand the discussion regarding the diversity of sexually dimorphic patterns in *Tropidurus* (see Lofeu at al. 2020) by formally testing for differences among localities. Data related to sex differences in steroid receptors in the DIV of *Tropidurus* neonates provides the first evidence for this mechanism in groups external to mammals. This chapter has a great potential to pave future directions to investigate the evolution of phenotypic traits modulated by this complex and fascinating network that congregates steroid hormones signaling and climatic conditions along environmental gradients.

CONCLUSION

This dissertation explored associations between the sexual dimorphism in digit lengths and environmental parameters in lizards, also considering the effect of reproductive modes in the case of *Liolaemus*. We combined different taxonomic, geographic, and ontogenetic scales in the chapters, in order to provide a comprehensive and integrative perspective for the topic. Classical studies in this field discussed effects of steroids in digit development and also described associations between sexual dimorphism in digit lengths and several other morphological and behavioral traits. Important additional factors likely associated with steroid dynamics and the dimorphism in digit lengths and associated ratios remained relegated, including the influence of environmental parameters and reproductive modes in this relationship. In this dissertation, we address these factors also providing, for the first time in lizards to our knowledge, information about a mechanism based on androgen receptors that may underlie the acquisition of steroids sensitivity by specific digits.

The first chapter innovates by showing an association between dimorphic patterns in the digit lengths of *Liolaemus* lizards and reproductive modes, a factor so far not considered in discussions on this topic. These results pave the way for further investigation focusing on the embryonic development of *Liolaemus* lizards to understand the dynamics and mechanisms involved in the evolution of sexual dimorphism in digits. The relationship between sexually dimorphic traits and reproductive modes is especially interesting to be investigated in *Liolaemus* because this genus congregates a similar number of viviparous and oviparous species.

The second chapter of this dissertation also explored relationships between the sexual dimorphism in digit lengths and environmental parameters but used an intraspecific scale that compared individuals of *T. catalanensis* lizards from different localities distributed in the central region of Brazil. The chapter also investigated possible influence of incubation temperature during embryonic development and described that neonates of *T. catalanensis* from a sexually dimorphic population are monomorphic for digit lengths. We described differences in androgen receptors in the neonates between the digits that remain monomorphic and those that will become dimorphic in *T. catalanensis* later during ontogeny.

In conclusion, the present dissertation explored the relationships of sexual dimorphism in digit lengths in lizards with unexplored factors related to climate and reproductive mode. We also evaluated possible effects of incubation temperature in sexually dimorphic traits using neonate lizards, and postulated a mechanism based on differences in the androgen receptors among digits to explain how digits become dimorphic along the ontogeny of *T. catalanensis* lizards. Results highlight the importance of steroid receptors during the evolution of sexual dimorphism in digit lengths, providing a mechanism that may explain the great diversity of patterns and identity of digits that become dimorphic in lizards.

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SUPPLEMENTARY MATERIAL – CHAPTER 1

Table S12. Localities represented in the morphological database using adult males and females of *Liolaemus* sp.

Species	Females	Males
Liolaemus chiliensis	10	10
Liolaemus fuscus	6	8
Liolaemus gravenhorstii	6	5
Liolaemus lutzae	9	7
Liolaemus nigroviridis	6	6
Liolaemus occipitalis	6	6
Liolaemus alticolor	5	6
Liolaemus andinus	9	5
Liolaemus atacamensis	7	8
Liolaemus bellii	7	10
Liolaemus bibronii	7	11
Liolaemus carlosgarini	8	5
Liolaemus constanzae	6	10
Liolaemus curicensis	10	9
Liolaemus darwinii	7	9
Liolaemus elongatus	7	7
Liolaemus fitzgeraldi	6	5
Liolaemus fitzingerii	6	8
Liolaemus hellmichi	5	7
Liolaemus kingii	6	10
Liolaemus koslowskyi	11	9
Liolaemus lemniscatus	11	8
Liolaemus lorenzmuelleri	6	5
Liolaemus magellanicus	8	7
Liolaemus monticola	6	8
Liolaemus nigromaculatus	6	7
Liolaemus nitidus	6	9
Liolaemus ornatus	6	8
Liolaemus paulinae	8	9
Liolaemus pictus	10	8

Liolaemus platei	10	7
Liolaemus poconchilensis	6	6
Liolaemus pseudolemniscatus	5	7
Liolaemus ramonensis	6	5
Liolaemus schroederi	6	5
Liolaemus tenuis	9	7
Liolaemus valdesianus	5	7
Liolaemus wiegmannii	8	5
Liolaemus zapallarensis	7	8
Liolaemus lineomaculatus	7	4
Liolaemus multicolor	3	5
Liolaemus loboi	4	4
Liolaemus audituvelatus	3	6
Liolaemus moradoensis	3	4
Liolaemus buergeri	3	6
Liolaemus velosoi	4	6

Table S13. Representation of the studies with dimorphism in digits in lizards in the current literature and in this work with *Liolaemus*.

Groups	**Dimorphic**	**Ratio *manus***	**Bias *manus***	**Ratio *pes***	**Bias *pes***	**Reference**
Iguanidae, Polychrotidae, Tropiduridae	yes	2D:4D	male	2D:4D	male	Gomes & Kohlsdorf 2011
Woodworthia	yes			2D:3D	male	Woodhead et al. 2018
Ctenophorus pictus	yes			3D:4D	female	Tobler et al. 2011
Podarcis melisellensis	yes	2D:4D, 2D:3D, 3D:4D		2D:4D,2D:3D, 3D:4D	male	Van Damme et al. 2015
Podarcis siculus	yes			2D:4D, 2D:3D	male	Van Damme et al. 2015
Anolis carolinensis	yes	2D:4D				Chang et al. 2006
Anolis carolinensis	no					Lombardo & Thorpe 2008
Lacerta vivipara	no					Branã, 2008
Podarcis muralis	yes	2D:4D, 2D:3D			male	Rubolini et al. 2006
Mabuya planifrons	yes	2D:3D			female	Rubolini et al. 2006
Anolis humilis	yes	2D:4D	female	2D:4D	male	Direnzo & Stynoski 2012
Anolis limifrons	yes	2D:4D	female			Direnzo & Stynoski 2012

Acanthodactylus blanfordi	yes	2D:4D	male	2D:4D	male	Heidari 2021
Lacerta agilis	yes	2D:3D, 2D:4D, 3D:4D	male	2D:3D, 2D:4D	male	Kaczmarski et al. 2020
Tropidurus montanus	no					Miranda et al. 2021
Tropidurus torquatus	no					Miranda et al. 2021
Eurolophosaurus nanuzae	yes	2D:4D	female			Miranda et al. 2021
Eurolophosaurus divaricatus	no					Lofeu et al. 2020
Eurolophosaurus nanuzae	yes	III, V	male			Lofeu et al. 2020
Tropidurus cocorobensis	no					Lofeu et al. 2020
Tropidurus erythrocephalus	no					Lofeu et al. 2020
Tropidurus hispidus	no					Lofeu et al. 2020
Tropidurus imbituba	yes	III, V	male	II	male	Lofeu et al. 2020
Tropidurus montanus	yes	I	male	I	male	Lofeu et al. 2020
Tropidurus oreadicus	no					Lofeu et al. 2020
Tropidurus pinima	yes			I, III, IV	female	Lofeu et al. 2020
Tropidurus psammonastes	yes	IV	female			Lofeu et al. 2020
Tropidurus semitaeniatus	no					Lofeu et al. 2020
Liolaemus chiliensis	no					
Liolaemus fuscus	no					
Liolaemus gravenhorstii	no					
Liolaemus lutzae	yes			2D:4D	male	
Liolaemus nigroviridis	no					
Liolaemus occipitalis	yes			3D:4D	male	
Liolaemus alticolor	yes			2D:3D	male	
Liolaemus andinus	no					
Liolaemus atacamensis	yes	2D:4D, 2D:3D	male	3D:4D	male	
Liolaemus bellii	no					
Liolaemus bibronii	no					
Liolaemus carlosgarini	yes	2D:4D, 2D:3D	female			
Liolaemus constanzae	yes	3D:4D	female	2D:4D, 2D:3D	male	
Liolaemus curicensis	no					
Liolaemus darwinii	no					
Liolaemus elongatus	yes			3D:4D	male	
Liolaemus fitzgeraldi	no					

Liolaemus fitzingerii	no				
Liolaemus hellmichi	no				
Liolaemus kingii	yes	2D:4D, 2D:3D	female		
Liolaemus koslowskyi	no				
Liolaemus lemniscatus	no				
Liolaemus lorenzmuelleri	yes	2D:3D	male	3D:4D	male
Liolaemus magellanicus	no				
Liolaemus monticola	no				
Liolaemus nigromaculatus	no				
Liolaemus nitidus	yes			3D:4D	male
Liolaemus ornatus	no				
Liolaemus paulinae	no				
Liolaemus pictus	yes			2D:4D, 2D:3D	male
Liolaemus platei	no				
Liolaemus poconchilensis	yes	2D:3D	male		
Liolaemus pseudolemniscatus	no				
Liolaemus ramonensis	no				
Liolaemus schroederi	no				
Liolaemus tenuis	no				
Liolaemus valdesianus	no				
Liolaemus wiegmannii	yes			2D:4D, 2D:3D	female
Liolaemus zapallarensis	yes				
Liolaemus lineomaculatus	no			2D:4D	male
Liolaemus multicolor	no				
Liolaemus loboi	yes	3D:4D	female	3D:4D	female
Liolaemus audituvelatus	yes			2D:4D, 3D:4D	male
Liolaemus moradoensis	no				
Liolaemus buergeri	no				
Liolaemus velosoi	no				

Table S14. Results of Mann-Whitney tests implemented to identify differences between sexes in body traits of *Liolaemus*; significant results (p <0.05) are highlighted in bold.

Species	**SVL**		**TRL**		**SGL**		**SGH**		**PGL**		**PGH**	
	U	**p**	**U**	**p**	**U**	**p**	**U**	**p**	**U**	**p**	**U**	**p**
Liolaemus chiliensis	29	0.123	**9**	**0.001**	36	0.315	24	0.052	25	0.063	35	0.280
Liolaemus fuscus	**4**	**0.008**	**0**	**<.001**	12	0.142	20	0.662	18	0.491	16	0.345
Liolaemus gravenhorstii	4	0.052	5	0.082	15	1.000	12	0.662	14	0.931	6	0.126
Liolaemus lutzae	**2**	**<.001**	**12**	**0.042**	31	1.000	25	0.536	**8**	**0.012**	28	0.758
Liolaemus nigroviridis	**0**	**0.002**	**0**	**0.002**	8	0.132	13	0.485	16	0.818	16	0.818
Liolaemus occipitalis	16	0.818	11	0.310	17	0.937	12	0.394	8	0.132	13	0.485
Liolaemus alticolor	8	0.247	6	0.126	12	0.662	14	0.931	12	0.662	6	0.126
Liolaemus andinus	19	0.699	**6**	**0.029**	18	0.606	14	0.298	18	0.606	9	0.083
Liolaemus atacamensis	**0**	**<.001**	**5**	**0.006**	14	0.121	22	0.536	12	0.072	25	0.779
Liolaemus bellii	**11**	**0.019**	**5**	**0.002**	31	0.740	25	0.364	24	0.315	28	0.536
Liolaemus bibronii	**11**	**0.007**	22	0.100	34	0.536	25	0.167	34	0.536	37	0.711
Liolaemus carlosgarini	16	0.622	**1**	**0.003**	11	0.222	17	0.724	7	0.065	15	0.524
Liolaemus constanzae	**3**	**0.002**	**9**	**0.022**	20	0.313	21	0.368	14	0.093	**8**	**0.016**
Liolaemus curicensis	**7**	**<.001**	25	0.113	23	0.079	45	1.000	44	0.968	42	0.842
Liolaemus darwinii	**9**	**0.016**	30	0.918	28	0.758	23	0.408	15	0.091	19	0.210
Liolaemus elongatus	10	0.165	21	0.710	10	0.073	16	0.318	20	0.620	23	0.902
Liolaemus fitzgeraldi	10	0.429	14	0.931	**1**	**0.009**	7	0.177	**2**	**0.017**	12	0.662
Liolaemus fitzingerii	23	0.950	**8**	**0.043**	23	0.950	21	0.755	19	0.573	23	0.950
Liolaemus hellmichi	**5**	**0.048**	7	0.106	12	0.432	16	0.876	17	1.000	13	0.530
Liolaemus kingii	25	0.625	**6**	**0.007**	20	0.313	27	0.792	15	0.118	24	0.562
Liolaemus koslowskyi	38	0.412	**6**	**<.001**	**17**	**0.012**	31	0.175	**23**	**0.046**	39	0.456
Liolaemus lemniscatus	35	0.492	**10**	**0.004**	39	0.717	25	0.129	41	0.840	25	0.129
Liolaemus lorenzmuelleri	**3**	**0.030**	**2**	**0.017**	14	0.931	9	0.329	6	0.126	7	0.177
Liolaemus magellanicus	28	1.000	**5**	**0.006**	**18**	**0.281**	**25**	**0.779**	28	1.000	15	0.152
Liolaemus monticola	**5**	**0.013**	22	0.852	15	0.282	12	0.142	23	0.950	15	0.282
Liolaemus nigromaculatus	**2**	**0.005**	13	0.295	15	0.445	15	0.445	20	0.945	9	0.101
Liolaemus nitidus	17.5	0.288	17	0.272	24	0.776	24	0.776	21	0.529	**9**	**0.036**
Liolaemus ornatus	19	0.573	11	0.108	23	0.950	15	0.282	19	0.573	18	0.491
Liolaemus paulinae	25	0.321	**14**	**0.036**	34	0.888	24	0.277	31	0.673	36	1.000
Liolaemus pictus	38	0.897	18	0.055	30	0.408	37	0.829	28	0.315	29	0.360
Liolaemus platei	34	0.962	24	0.315	24	0.315	24	0.315	31	0.740	34	0.962

Liolaemus poconchilensis	16	0.818	7	0.093	15	0.699	14	0.589	18	1.000	8	0.132
Liolaemus pseudolemniscatus	**5**	**0.048**	**5**	**0.048**	15	0.755	14	0.639	17	1.000	**4**	**0.030**
Liolaemus ramonensis	8	0.247	**3**	**0.030**	14	0.931	12	0.662	8	0.247	10	0.429
Liolaemus schroederi	**9**	**0.329**	13	0.792	7	0.177	14	0.931	13	0.792	13	0.792
Liolaemus tenuis	24	0.470	**0**	**<.001**	16	0.114	24	0.470	19	0.210	**8**	**0.012**
Liolaemus valdesianus	12	0.432	**5**	**0.048**	17	1.000	14	0.639	15	0.755	14	0.639
Liolaemus wiegmannii	**5**	**0.030**	**4**	**0.019**	16	0.622	**5**	**0.030**	10	0.171	12	0.284
Liolaemus zapallarensis	**4**	**0.004**	**2**	**0.001**	25	0.779	16	0.189	24	0.694	**10**	**0.040**
Liolaemus lineomaculatus	13	0.927	**0**	**0.006**	13	0.927	14	1.000	10	0.527	11	0.648
Liolaemus multicolor	7	1.000	3	0.250	5	0.571	4	0.393	4	0.393	6	0.786
Liolaemus loboi	6	0.686	7	0.886	4	0.343	4	0.343	2	0.114	7	0.886
Liolaemus audituvelatus	9	1.000	**1**	**0.048**	8	0.905	**0**	**0.024**	**1**	**0.048**	2	0.095
Liolaemus moradoensis	5	0.857	6.000	1.000	3	0.400	5	0.857	4	0.629	6	1.000
Liolaemus buergeri	7	0.714	2	0.095	3	0.167	**0**	**0.024**	6	0.548	7	0.714
Liolaemus velosoi	7	0.352	11	0.914	12	1.000	7	0.352	9	0.610	8	0.476

Table S15. Results of Mann-Whitney tests implemented to identify differences between sexes in limb traits of *Liolaemus*; significant results (p <0.05) are highlighted in bold.

Species	**HUL**		**RAL**		**FEL**		**TBL**	
	U	**p**	**U**	**p**	**U**	**p**	**U**	**p**
Liolaemus chiliensis	25	0.063	43	0.631	35	0.280	29	0.123
Liolaemus fuscus	**7**	**0.029**	19	0.573	9	0.059	16	0.345
Liolaemus gravenhorstii	**2**	**0.017**	8	0.247	8	0.247	6	0.126
Liolaemus lutzae	**5**	**0.003**	17	0.142	23	0.408	24	0.470
Liolaemus nigroviridis	16	0.818	13	0.485	16	0.818	17	0.937
Liolaemus occipitalis	11	0.310	9	0.180	11	0.310	**4**	**0.026**
Liolaemus alticolor	15	1.000	11	0.537	4	0.052	11	0.537
Liolaemus andinus	19	0.699	**2**	**0.004**	14	0.298	**1**	**0.002**
Liolaemus atacamensis	20	0.397	19	0.336	18	0.281	20	0.397
Liolaemus bellii	34	0.962	32	0.813	27	0.475	**11**	**0.019**
Liolaemus bibronii	30	0.340	38	0.773	19	0.056	27	0.227
Liolaemus carlosgarini	17	0.724	20	1.000	16	0.622	12	0.284
Liolaemus constanzae	19	0.263	15	0.118	21	0.368	27	0.792

Liolaemus curicensis	44	0.968	40	0.720	**20**	**0.043**	24	0.095
Liolaemus darwinii	24	0.470	21	0.299	21	0.299	25	0.536
Liolaemus elongatus	21	0.710	21	0.710	18	0.456	19	0.535
Liolaemus fitzgeraldi	8	0.247	14	0.931	14	0.931	10	0.429
Liolaemus fitzingerii	21	0.755	18	0.491	22	0.852	17	0.414
Liolaemus hellmichi	16	0.876	16	0.876	**2**	**0.010**	9	0.202
Liolaemus kingii	30	1.000	21	0.368	28	0.875	17	0.181
Liolaemus koslowskyi	32	0.201	49	1.000	**16**	**0.010**	32	0.201
Liolaemus lemniscatus	23	0.091	36	0.545	**17**	**0.026**	21	0.062
Liolaemus lorenzmuelleri	9	0.329	14	0.931	6	0.126	14	0.931
Liolaemus magellanicus	18	0.281	**7**	**0.014**	**1**	**<.001**	**7**	**0.014**
Liolaemus monticola	21	0.755	**2**	**0.003**	23	0.950	18	0.491
Liolaemus nigromaculatus	14	0.366	21	1	12	0.234	20	0.945
Liolaemus nitidus	23	0.689	18	0.328	20	0.456	18	0.328
Liolaemus ornatus	20	0.662	**8**	**0.043**	**8**	**0.043**	10	0.081
Liolaemus paulinae	19	0.114	28	0.481	23	0.236	25	0.321
Liolaemus pictus	39	0.965	40	1.000	34	0.633	32	0.515
Liolaemus platei	15	0.055	21	0.193	19	0.133	21	0.193
Liolaemus poconchilensis	17	0.937	16	0.818	8	0.132	**2**	**0.009**
Liolaemus pseudolemniscatus	10	0.268	**1**	**0.005**	**4**	**0.03**	**0**	**0.003**
Liolaemus ramonensis	**3**	**0.030**	15	1.000	12	0.662	6	0.126
Liolaemus schroederi	5	0.082	14	0.931	15	1.000	15	1.000
Liolaemus tenuis	**4**	**0.002**	**6**	**0.005**	15	0.091	**7**	**0.008**
Liolaemus valdesianus	10	0.268	14	0.639	11	0.343	14	0.639
Liolaemus wiegmannii	14	0.435	20	1.000	16	0.622	17	0.724
Liolaemus zapallarensis	15	0.152	21	0.463	15	0.152	19	0.336
Liolaemus lineomaculatus	6	0.164	6	0.164	8	0.315	9	0.412
Liolaemus multicolor	6	0.786	3	0.250	5	0.571	2	0.143
Liolaemus loboi	5	0.486	7	0.886	3	0.200	8	1.000
Liolaemus audituvelatus	**1**	**0.048**	5	0.381	6	0.548	2	0.095
Liolaemus moradoensis	3	0.400	1	0.114	4	0.629	4	0.629
Liolaemus buergeri	7	0.714	**1**	**0.048**	9	1.000	**1**	**0.048**
Liolaemus velosoi	12	1.000	11	0.914	7	0.352	9	0.610

Table S16. Results of Mann-Whitney tests implemented to identify differences between sexes in head traits of *Liolaemus*; significant results (p <0.05) are highlighted in bold.

Species	**HDL**		**HDH**		**HDW**	
	U	**p**	**U**	**p**	**U**	**p**
Liolaemus chiliensis	**18**	**0.015**	49	0.971	40	0.481
Liolaemus fuscus	**3**	**0.005**	13	0.181	**0**	**<.001**
Liolaemus gravenhorstii	6	0.126	7	0.177	**1**	**0.009**
Liolaemus lutzae	20	0.252	22	0.351	28	0.758
Liolaemus nigroviridis	**2**	**0.009**	**0**	**0.002**	8	0.132
Liolaemus occipitalis	**5**	**0.041**	**4**	**0.026**	**3**	**0.015**
Liolaemus alticolor	8	0.247	**0**	**0.004**	10	0.429
Liolaemus andinus	**4**	**0.012**	12	0.190	**6**	**0.029**
Liolaemus atacamensis	**1**	**<.001**	**6**	**0.009**	**7**	**0.014**
Liolaemus bellii	17	0.088	23	0.270	20	0.161
Liolaemus bibronii	25	0.167	**14**	**0.017**	29	0.299
Liolaemus carlosgarini	8	0.093	19	0.943	12	0.284
Liolaemus constanzae	**9**	**0.022**	**11**	**0.042**	**11**	**0.042**
Liolaemus curicensis	**4**	**<.001**	**16**	**0.017**	**10**	**0.003**
Liolaemus darwinii	21	0.299	**10**	**0.023**	30	0.918
Liolaemus elongatus	19	0.535	12	0.128	20	0.620
Liolaemus fitzgeraldi	**3**	**0.030**	6	0.126	6	0.126
Liolaemus fitzingerii	17	0.414	15	0.282	**7**	**0.029**
Liolaemus hellmichi	6	0.073	7	0.106	**0**	**0.003**
Liolaemus kingii	**3**	**0.002**	**6**	**0.007**	**9**	**0.022**
Liolaemus koslowskyi	36	0.331	26	0.080	24	0.056
Liolaemus lemniscatus	**6**	**<.001**	**4**	**<.001**	**4**	**<.001**
Liolaemus lorenzmuelleri	4	0.052	**2**	**0.017**	**2**	**0.017**
Liolaemus magellanicus	**4**	**0.004**	**0**	**<.001**	11	0.054
Liolaemus monticola	13	0.181	18	0.491	19	0.573
Liolaemus nigromaculatus	7	0.051	10	0.138	17	0.628
Liolaemus nitidus	14	0.145	**10**	**0.050**	11	0.066
Liolaemus ornatus	**0**	**<.001**	11	0.108	10	0.081
Liolaemus paulinae	25	0.321	28	0.481	24	0.277

Liolaemus pictus	19	0.068	27	0.274	**15**	**0.027**
Liolaemus platei	**13**	**0.033**	20	0.161	19	0.133
Liolaemus poconchilensis	**4**	**0.026**	**1**	**0.004**	11	0.310
Liolaemus pseudolemniscatus	**0**	**0.003**	**5**	**0.048**	**2**	**0.010**
Liolaemus ramonensis	6	0.126	12	0.662	**0**	**0.004**
Liolaemus schroederi	**1**	**0.009**	**3**	**0.03**	8	0.247
Liolaemus tenuis	17	0.142	**3**	**0.001**	**0**	**<.001**
Liolaemus valdesianus	14	0.639	15	0.755	12	0.432
Liolaemus wiegmannii	**5**	**0.030**	17	0.724	13	0.354
Liolaemus zapallarensis	**6**	**0.009**	11	0.054	11	0.054
Liolaemus lineomaculatus	4	0.073	4	0.073	9	0.412
Liolaemus multicolor	**0**	**0.036**	**0**	**0.036**	**0**	**0.036**
Liolaemus loboi	5	0.486	8	1.000	5	0.486
Liolaemus audituvelatus	3	0.167	**0**	**0.024**	**0**	**0.024**
Liolaemus moradoensis	0	0.057	2	0.229	4	0.629
Liolaemus buergeri	**0**	**0.024**	4	0.262	6	0.548
Liolaemus velosoi	10	0.762	10	0.762	5	0.171

Table 17. Average values for digit lenghts in *Liolaemus* sp.

Species	**Sex**	**DI**	**DII**	**DIII**	**DIV**	**DV**	**DI**	**DII**	**DIII**	**DIV**
Liolaemus alticolor	f	2.12	3.04	4.03	3.98	2.33	2.61	3.85	6.01	7.74
	m	2.12	3.30	4.61	4.79	2.86	3.09	4.71	6.74	9.15
Liolaemus andinus	f	3.21	4.55	6.04	6.11	3.71	3.42	5.26	7.28	9.75
	m	3.44	5.25	6.82	6.99	4.22	4.01	6.02	8.42	11.2
Liolaemus atacamensis	f	3.32	4.64	6.20	6.39	4.23	4.04	6.10	8.39	11.9
	m	3.75	5.58	7.14	7.37	4.91	4.87	7.23	10.3	14.0
Liolaemus audituvelatus	f	2.87	4.49	5.66	5.59	3.23	2.88	4.43	6.25	8.51
	m	3.14	4.52	5.99	6.06	3.65	2.98	5.01	6.99	8.74
Liolaemus bellii	f	2.87	4.45	5.98	6.14	3.83	3.92	5.90	8.29	11.4
	m	3.29	4.92	6.54	6.63	4.02	4.54	6.55	9.14	12.4
Liolaemus bibroni	f	2.08	3.10	4.07	4.01	2.49	2.40	3.83	5.81	7.53
	m	2.25	3.26	4.42	4.36	2.92	2.62	4.17	6.31	8.50
Liolaemus buergeri	f	4.01	5.86	7.85	8.20	5.65	4.96	6.78	9.64	12.5
	m	3.90	5.90	7.46	8.02	4.99	4.49	6.61	9.87	13.3
Liolaemus carlosgarini	f	3.89	5.69	7.44	7.64	4.70	4.44	6.77	9.50	12.9
	m	3.63	5.50	7.66	7.98	4.45	4.50	6.97	9.71	13.3
Liolaemus chiliensis	f	4.33	6.46	8.72	8.52	5.34	5.43	7.91	11.4	15.7
	m	3.86	6.40	8.76	8.63	5.21	5.28	8.16	11.6	15.9
Liolaemus constanzae	f	2.94	4.19	5.74	5.51	3.55	3.42	5.26	7.66	10.6

	m	3.35	5.16	6.75	6.87	4.17	4.35	6.65	9.16	12.3
Liolaemus curicensis	f	2.42	3.48	4.72	4.97	3.15	3.19	4.72	6.57	9.60
	m	2.64	3.96	5.52	5.62	3.68	3.49	5.37	7.52	11.0
Liolaemus darwinii	f	2.47	3.31	3.99	4.00	2.88	2.77	3.69	5.35	8.23
	m	2.35	3.20	4.19	4.08	2.92	2.75	4.05	5.64	8.69
Liolaemus elongatus	f	3.70	5.65	7.10	7.32	4.38	4.32	7.01	9.40	13.1
	m	3.87	5.80	7.73	8.00	5.24	4.57	7.52	10.1	13.5
Liolaemus fitzgeraldi	f	2.30	3.58	4.95	5.31	2.53	3.49	4.63	6.73	9.18
	m	2.42	3.36	4.99	5.10	2.68	3.29	4.94	6.87	9.52
Liolaemus fitzingerii	f	4.33	6.38	8.02	7.76	4.69	5.03	7.67	10.9	14.4
	m	4.03	6.02	7.89	7.52	3.92	5.04	7.40	11.0	14.7
Liolaemus fuscus	f	1.65	2.71	3.50	3.81	2.63	2.68	3.94	5.57	7.42
	m	2.12	3.19	4.39	4.47	2.85	2.95	4.49	6.25	9.06
Liolaemus gravenhorstii	f	2.56	4.23	5.93	6.33	3.70	3.51	5.47	8.19	11.6
	m	2.56	4.12	6.08	6.23	3.63	3.82	5.50	8.39	11.7
Liolaemus hellmichi	f	2.25	3.41	4.55	4.44	2.51	2.81	4.25	5.96	8.19
	m	2.48	3.82	5.29	5.14	2.97	3.31	4.97	7.05	9.47
Liolaemus kingii	f	4.09	5.48	6.68	6.81	3.97	4.13	6.26	8.75	12.1
	m	3.55	5.38	7.02	7.28	4.44	4.43	6.60	9.41	12.5
Liolaemus koslowskyi	f	2.66	3.51	4.71	4.86	3.00	3.07	4.26	5.90	8.88
	m	2.66	3.70	4.94	5.10	2.86	3.30	4.58	6.47	9.65
Liolaemus lemniscatus	f	1.81	2.88	4.22	4.40	2.42	2.48	3.93	5.73	7.96
	m	1.88	2.90	4.45	4.56	2.48	2.74	4.30	6.30	8.77
Liolaemus lineomaculatus	f	2.32	3.63	4.50	4.51	2.69	2.77	4.17	5.97	8.43
	m	2.61	3.90	4.74	4.95	2.92	3.17	4.87	7.14	10.3
Liolaemus loboi	f	3.22	4.56	5.88	5.47	3.33	3.79	5.60	8.02	10.3
	m	3.02	4.70	5.82	6.17	3.25	3.89	5.79	8.07	11.0
Liolaemus lorenzmuelleri	f	3.57	5.31	7.19	7.04	4.75	4.63	6.77	9.29	13.0
	m	4.63	6.79	8.51	8.76	5.78	6.01	8.53	11.9	15.8
Liolaemus lutzae	f	2.49	3.79	5.19	5.14	2.91	3.01	4.61	6.53	9.35
	m	2.68	4.40	5.97	6.07	3.08	3.53	5.52	8.22	11.1
Liolaemus magellanicus	f	2.31	3.57	4.56	4.34	3.09	2.72	4.21	6.00	7.79

	m	2.29	3.69	4.77	4.65	3.18	2.85	4.69	6.72	8.91	4.57
Liolaemus monticola	f	2.78	4.35	5.44	5.73	3.68	3.89	5.50	7.72	10.9	5.82
	m	2.80	4.54	6.11	6.24	4.13	3.84	6.04	8.78	12.1	7.64
Liolaemus moradoensis	f	2.69	3.99	5.84	5.93	3.36	3.43	5.38	7.72	11.0	6.36
	m	2.71	4.14	5.69	5.86	3.38	3.81	5.72	7.91	11.3	6.77
Liolaemus multicolor	f	3.03	4.58	6.01	5.99	3.57	3.69	5.31	7.61	10.4	5.33
	m	3.39	4.56	5.97	5.99	3.68	3.88	5.49	7.81	10.3	5.97
Liolaemus nigromaculatus	f	2.67	3.94	5.44	5.62	3.63	3.21	4.98	7.09	10.2	5.73
	m	3.13	4.55	6.25	6.63	4.13	3.86	5.99	8.39	12.1	6.44
Liolaemus nigroviridis	f	2.88	4.68	6.07	6.34	3.88	3.79	5.63	7.78	10.6	6.58
	m	3.89	5.66	7.25	7.64	4.71	4.74	7.09	9.70	13.1	8.14
Liolaemus nitidus	f	3.20	5.09	6.64	6.78	3.51	4.44	6.67	9.12	13.2	6.25
	m	4.18	6.28	8.54	8.50	4.82	5.69	8.39	11.9	16.3	9.40
Liolaemus occipitalis	f	2.58	3.70	4.92	4.88	2.85	3.06	5.01	6.29	9.05	4.76
	m	2.72	4.23	5.54	5.31	3.51	3.46	5.21	7.30	10.1	5.36
Liolaemus ornatus	f	2.59	3.47	4.83	4.93	2.96	2.86	4.08	5.92	8.59	4.56
	m	2.55	3.51	4.95	5.17	3.00	3.23	4.41	6.54	9.35	4.75
Liolaemus paulinae	f	2.25	3.34	4.44	4.59	2.64	2.77	4.67	6.33	8.81	4.32
	m	2.18	3.49	4.58	4.83	2.76	2.89	4.57	6.73	9.06	4.15
Liolaemus pictus	f	3.25	5.05	6.58	6.73	4.40	3.60	5.71	8.48	11.6	6.59
	m	3.46	5.21	6.76	7.23	4.26	4.01	6.59	9.22	12.0	7.31
Liolaemus platei	f	2.67	4.20	5.39	5.27	2.81	3.07	5.07	7.17	9.92	4.97
	m	2.55	4.22	5.62	5.62	2.58	3.37	5.60	7.82	11.1	5.57
Liolaemus poconchilensis	f	2.39	3.56	4.78	5.22	2.89	2.82	4.22	5.70	8.27	3.63
	m	3.06	4.22	5.37	5.89	3.22	3.17	4.51	6.42	9.40	4.09
Liolaemus pseudolemniscatus	f	1.61	2.30	3.46	3.57	2.13	2.09	3.28	5.04	7.29	2.97
	m	1.98	2.87	4.27	4.29	2.46	2.72	4.15	6.15	9.12	4.27
Liolaemus ramonensis	f	4.22	6.16	8.56	8.48	5.72	5.15	8.21	11.6	15.2	8.47
	m	4.64	6.77	9.14	9.51	5.77	5.91	8.69	12.2	16.3	9.16
Liolaemus schroederi	f	2.14	3.31	4.64	4.85	2.81	2.78	4.70	6.87	9.69	4.88
	m	2.30	3.68	5.02	5.44	2.87	3.56	4.97	7.27	9.77	5.21
Liolaemus tenuis	f	2.28	4.02	5.79	6.29	3.70	2.76	4.94	7.20	10.0	5.96
	m	2.45	4.04	5.90	6.17	3.54	3.16	5.57	7.78	10.6	5.56
Liolaemus valdesianus	f	4.18	6.23	8.15	7.78	4.89	5.09	7.72	10.6	14.0	7.97
	m	4.16	6.27	8.22	8.38	4.64	5.56	7.75	10.3	13.9	8.02
Liolaemus velosoi	f	1.73	3.05	4.13	4.05	2.31	2.71	3.91	5.78	8.11	4.35
	m	2.29	3.64	5.10	5.30	2.82	3.14	4.64	6.74	9.50	5.44
Liolaemus wiegmannii	f	2.00	3.11	3.99	3.57	2.31	2.30	3.44	4.80	7.08	3.35
	m	1.82	2.93	3.97	3.83	2.01	2.42	3.30	5.20	7.48	3.47
Liolaemus zapallarensis	f	2.87	4.46	5.99	5.77	3.66	3.55	5.69	7.84	10.8	5.41
	m	3.51	5.48	7.12	7.00	3.81	4.55	7.16	9.70	12.9	7.56

Species	Sex	SVL	TRL	SGL	SGH	PGL	PGH	HUL	RAL	FEL	TBL
Liolaemus alticolor	f	45.2	20.7	6.86	5.14	5.61	4.40	4.74	5.21	7.96	5.30
	m	47.6	20.6	7.51	5.58	5.62	5.02	5.09	5.55	9.06	5.73
Liolaemus andinus	f	58.8	28.9	11.0	7.46	7.52	5.86	7.30	6.98	11.4	6.69
	m	61.4	28.3	11.7	8.31	7.53	6.70	7.81	8.04	12.4	8.14
Liolaemus atacamensis	f	60.9	31.3	11.6	9.57	8.51	7.34	6.88	7.09	12.0	7.84
	m	70.7	34.5	13.4	11.3	9.65	8.70	8.36	8.59	14.4	9.46
Liolaemus audituvelatus	f	49.3	23.0	8.81	5.50	6.56	4.94	6.92	7.95	10.5	7.19
	m	49.4	20.8	8.60	6.44	5.29	5.50	7.58	8.20	11.1	8.72
Liolaemus bellii	f	63.1	31.3	11.2	9.82	8.69	8.00	6.67	7.53	11.7	7.01
	m	66.8	29.3	11.9	10.1	8.88	8.29	7.07	7.88	12.8	8.26
Liolaemus bibroni	f	42.8	20.4	6.14	5.86	4.50	4.97	3.98	5.04	7.18	4.68
	m	47.5	21.2	7.25	6.25	5.24	5.46	4.32	5.60	8.52	5.54
Liolaemus buergeri	f	80.0	38.9	15.0	12.0	11.1	9.77	7.03	9.49	14.2	8.56
	m	76.7	34.9	12.8	10.6	10.1	9.21	6.86	9.94	13.9	9.82
Liolaemus carlosgarini	f	71.3	34.6	12.6	9.21	9.65	7.95	8.02	9.03	13.6	8.31
	m	70.0	31.0	11.7	8.58	8.39	7.39	7.98	8.93	13.5	8.90
Liolaemus chiliensis	f	87.1	44.8	14.0	13.5	12.6	12.2	9.00	11.3	16.4	10.7
	m	81.3	37.9	12.3	11.6	11.1	11.0	9.12	10.6	15.6	10.8
Liolaemus constanzae	f	55.9	27.6	9.13	6.88	6.71	6.20	6.75	7.17	10.9	7.58
	m	66.1	30.6	10.7	8.25	7.59	7.11	7.48	8.09	12.6	9.02
Liolaemus curicensis	f	53.0	27.3	8.99	7.71	6.74	6.62	5.51	6.17	8.96	5.99
	m	58.2	29.3	10.6	8.58	7.61	7.42	6.09	6.97	10.6	7.28
Liolaemus darwinii	f	48.9	22.5	7.05	7.14	5.66	6.27	4.76	6.37	9.15	6.55
	m	50.8	22.8	7.15	6.58	5.42	6.03	5.09	6.37	9.75	6.96
Liolaemus elongatus	f	69.7	32.8	10.7	8.19	8.17	7.31	7.32	9.39	13.0	9.02
	m	74.5	34.5	10.7	8.55	8.55	7.66	7.90	9.91	14.2	10.1
Liolaemus fitzgeraldi	f	50.0	22.8	7.62	6.08	5.33	5.03	4.45	5.96	8.66	5.50
	m	46.0	20.6	8.44	5.01	5.80	4.89	4.38	5.51	8.03	5.50
Liolaemus fitzingerii	f	89.9	49.5	14.0	13.7	10.1	11.1	9.13	10.8	16.8	11.9
	m	90.0	45.0	14.0	13.6	10.3	11.0	9.16	11.0	16.4	11.6
Liolaemus fuscus	f	44.6	22.5	5.60	5.46	5.76	5.26	4.03	5.41	7.17	5.39

	m	49.4	21.8	7.27	6.08	6.32	5.69	5.12	6.22	8.51	6.15
Liolaemus gravenhorstii	f	59.9	31.9	8.44	7.39	7.84	7.09	6.24	7.57	10.6	7.60
	m	52.9	23.3	7.28	6.48	6.90	6.50	6.11	6.52	10.3	7.49
Liolaemus hellmichi	f	48.0	21.5	8.00	5.10	5.81	4.36	4.82	5.53	7.48	5.42
	m	55.4	22.2	8.47	6.59	7.12	5.30	5.59	6.61	10.0	6.59
Liolaemus kingii	f	81.1	43.5	13.1	11.5	10.2	8.80	7.71	9.66	13.6	8.72
	m	77.5	36.0	11.8	10.8	9.16	8.66	7.34	9.65	13.0	8.96
Liolaemus koslowskyi	f	52.6	26.7	8.38	7.54	6.88	6.44	6.04	7.23	9.99	7.53
	m	54.7	25.5	8.01	8.42	6.44	6.82	6.60	7.58	11.5	8.37
Liolaemus lemniscatus	f	47.4	23.8	7.02	6.00	5.37	5.38	3.82	5.01	7.55	4.93
	m	48.8	22.1	7.19	5.63	5.50	5.74	4.36	5.22	8.42	5.58
Liolaemus lineomaculatus	f	56.6	29.6	8.75	7.22	6.28	5.98	5.30	6.21	9.16	5.38
	m	58.5	28.0	8.96	7.69	6.68	6.68	6.14	6.89	9.95	6.16
Liolaemus loboi	f	61.1	30.5	10.1	8.55	7.05	6.64	6.69	7.39	11.4	7.97
	m	65.4	32.7	10.3	9.01	6.74	7.17	6.71	8.01	11.2	8.45
Liolaemus lorenzmuelleri	f	74.4	38.6	13.5	10.0	9.52	7.83	8.18	8.67	13.4	8.55
	m	92.0	46.3	17.2	13.4	11.9	10.9	9.40	10.7	17.9	10.5
Liolaemus lutzae	f	55.5	30.1	9.05	8.67	7.48	7.19	6.32	6.41	9.23	7.23
	m	67.2	34.4	11.2	10.5	8.75	8.98	7.10	8.19	11.5	8.96
Liolaemus magellanicus	f	55.1	27.7	8.64	7.21	6.08	6.39	5.30	6.03	8.80	5.78
	m	55.0	25.1	8.19	7.45	6.02	6.07	5.67	6.57	9.71	6.64
Liolaemus monticola	f	50.2	27.4	8.75	6.73	5.78	5.55	5.55	5.94	10.9	7.07
	m	55.1	28.5	9.39	6.97	6.27	6.29	6.02	7.67	12.2	7.57
Liolaemus moradoensis	f	54.8	25.0	8.94	8.51	7.10	7.41	5.56	6.78	10.5	7.12
	m	53.0	25.3	9.26	8.21	6.87	7.14	5.82	6.74	10.0	7.13
Liolaemus multicolor	f	63.8	31.3	10.4	8.06	7.69	7.38	6.83	8.37	11.5	8.10
	m	64.1	30.3	10.0	8.73	7.36	7.30	6.87	8.24	12.1	8.66
Liolaemus nigromaculatus	f	55.0	26.5	8.66	6.83	7.17	6.53	5.86	6.92	11.3	7.87
	m	66.1	31.3	10.5	8.94	8.90	7.57	7.50	8.52	13.0	9.26
Liolaemus nigroviridis	f	62.8	32.5	8.95	8.49	8.59	7.15	6.88	7.50	11.4	8.12
	m	80.8	38.4	11.2	11.1	11.4	9.44	9.03	9.97	14.9	10.5
Liolaemus nitidus	f	73.0	34.5	12.3	10.0	9.54	9.03	7.43	9.65	13.7	8.87

	m	87.2	39.1	15.2	12.5	11.0	11.6	8.71	11.2	17.0	11.1
Liolaemus occipitalis	f	55.9	28.3	8.02	7.37	7.13	7.12	5.96	6.40	7.52	5.97
	m	56.7	27.2	8.29	7.27	7.79	7.44	6.59	5.60	9.15	7.51
Liolaemus ornatus	f	55.1	25.8	8.41	7.21	7.04	6.56	5.93	6.71	9.96	7.38
	m	54.2	24.3	8.23	7.23	7.19	6.66	5.60	7.13	10.8	7.99
Liolaemus paulinae	f	46.5	21.6	6.71	5.83	5.14	5.08	4.70	5.44	8.03	5.43
	m	49.2	20.8	7.15	5.77	5.39	5.32	5.36	5.92	9.13	6.01
Liolaemus pictus	f	59.9	29.6	9.26	8.16	6.61	6.68	6.43	7.54	11.2	7.33
	m	60.9	27.6	9.90	8.43	6.32	6.71	6.64	7.65	11.5	7.68
Liolaemus platei	f	59.6	27.3	9.45	6.84	7.25	6.70	5.45	7.45	10.5	6.86
	m	58.3	25.0	8.44	7.13	7.19	6.56	5.89	7.75	10.8	7.10
Liolaemus poconchilensis	f	50.5	23.3	8.72	6.66	5.97	5.57	7.08	7.63	10.7	7.53
	m	51.6	22.0	9.17	7.34	5.98	5.13	7.28	8.00	12.1	8.48
Liolaemus pseudolemniscatus	f	42.7	20.6	5.64	5.22	4.65	4.92	3.46	4.55	6.75	4.71
	m	46.2	20.5	6.29	5.61	5.26	5.92	4.16	5.76	8.51	6.32
Liolaemus ramonensis	f	82.9	39.8	15.9	10.0	11.4	9.28	9.99	10.4	16.9	10.7
	m	89.5	40.1	16.9	11.3	11.9	9.77	9.92	11.2	18.0	10.6
Liolaemus schroederi	f	50.7	23.7	7.49	5.57	6.49	5.99	4.83	6.34	9.04	6.70
	m	49.0	22.4	7.75	5.42	6.30	5.64	5.57	6.21	8.53	6.36
Liolaemus tenuis	f	53.8	27.0	8.02	6.59	6.39	5.43	5.55	6.41	10.1	6.33
	m	51.1	23.3	8.34	6.46	5.69	5.59	5.88	6.65	10.1	6.76
Liolaemus valdesianus	f	80.2	38.1	15.9	11.9	11.2	10.8	8.55	10.2	16.9	10.6
	m	78.7	35.0	15.4	11.4	10.6	10.3	8.98	10.1	17.0	10.7
Liolaemus velosoi	f	44.1	19.1	7.00	4.17	4.78	3.94	4.64	5.29	8.34	5.60
	m	49.1	21.5	8.03	5.27	5.89	4.81	5.19	5.86	9.77	6.93
Liolaemus wiegmannii	f	49.8	24.5	7.81	6.95	6.18	6.39	5.10	5.82	8.24	5.91
	m	45.8	20.3	7.27	5.39	5.27	5.61	4.92	5.38	7.90	5.65
Liolaemus zapallarensis	f	62.6	31.4	10.5	9.01	8.45	7.36	7.34	8.01	11.7	7.78
	m	78.1	37.1	13.3	11.9	10.6	10.1	8.36	9.61	15.1	10.3

SUPPLEMENTARY MATERIAL – CHAPTER 2

Table S18. Descriptive statistics for the digits from the total of 329 adults of *T. catalanensis*. Abbreviations correspond to: female = f, male = m, digit = D.

Descriptive		*Manus*					*Pes*				
	Sex	**DI**	**DII**	**DIII**	**DIV**	**DV**	**DI**	**DII**	**DIII**	**DIV**	**DV**
Media	f	5.31	7.80	10.10	10.60	7.30	6.39	9.00	12.90	17.20	11.10
Media	m	6.36	9.35	12.10	12.80	8.69	7.86	11.1	15.70	20.60	13.70
Median	f	5.27	7.86	10.10	10.70	7.29	6.34	9.01	12.90	17.20	11.10
Median	m	6.40	9.38	12.10	12.70	8.77	7.75	11.0	15.80	20.70	13.80
Standard deviation	f	0.55	0.72	0.85	0.96	0.74	0.62	0.72	1.02	1.27	1.18
Standard deviation	m	0.67	0.86	1.05	1.04	0.77	0.84	0.84	1.12	1.44	1.34

Table S19. Descriptive statistics for body, limbs, and head traits from the total of 329 adults of *T. catalanensis*. Abbreviations correspond to: female = f, male = m, digit = D.

Descriptive	**Sex**	**SVL**	**TRL**	**SGL**	**SGH**	**PGL**	**PGH**	**HUL**	**RAL**	**FEL**	**TBL**	**HDL**	**HDH**	**HDW**
Media	f	83.6	39.8	16.4	12.3	12.5	10.4	11.9	12.9	20.6	14.4	22.4	10.0	14.4
Media	m	101.0	45.9	20.6	16.2	14.7	13.4	14.6	15.8	26.0	18.1	29.5	14.4	20.0
Median	f	86.6	39.9	16.2	12.2	12.3	10.4	11.8	12.8	20.6	14.3	22.3	9.9	14.4
Median	m	101.0	45.8	20.5	16.3	14.5	13.5	14.7	15.9	26.1	18.2	29.7	14.4	19.9
Standard deviation	f	7.3	4.3	1.7	1.6	1.5	1.3	1.5	1.3	2.0	1.5	2.2	1.1	1.5
Standard deviation	m	9.5	5.4	2.5	2.2	1.9	1.8	1.7	1.4	2.5	1.6	3.1	2.1	2.3

Table S20. Results of paired t-tests between left and right sizes from *T. catalanensis* from São Simão. Abbreviations correspond to: female = f, male = m, digit = D.

Autopodium	**Sex**	**DI**		**DII**		**DIII**		**DIV**		**DV**	
		t	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**
Manus	m	0.06	0.95	0.08	0.93	-0.92	0.37	-0.26	0.79	-0.93	0.36
	f	-0.90	0.92	-0.10	0.91	0.71	0.48	0.18	0.85	0.81	0.43

Pes	m	0.05	0.95	-0.71	0.49	-0.45	0.65	-0.58	0.56	-1.41	0.18
	f	-0.04	0.96	0.86	0.39	0.43	0.66	0.59	0.55	0.91	0.37

Table S21. Results of Mann-Whitney analyses testing for differences between sexes in body traits of *T. catalanensis* separated by locality; significant results (p <0.05) are highlighted in bold. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado (TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

Location	**SVL**		**TRL**		**SGL**		**SGH**		**PGL**		**PG**
	U	**p**	**U**	**p**	**U**	**p**	**U**	**p**	**U**	**p**	**U**
AC	**4**	**0.007**	20	0.620	17	0.383	18	0.456	24	1.000	21
AR	**24**	**0.002**	52	0.168	66	0.538	53	0.186	53	0.186	**30**
AU	**11**	**<.001**	86	0.051	**76**	**0.021**	**65**	**0.006**	93	0.089	128
BA	**0**	**<.001**	60	1.000	39	0.171	**17**	**0.003**	31	0.056	**17**
BR	**12**	**0.002**	**13**	**0.002**	52	0.917	44	0.508	34	0.169	44
CG	**28**	**0.008**	41	0.062	**33**	**0.019**	**11**	**<.001**	62	0.495	**28**
LA	**55**	**0.001**	**57**	**0.001**	141	0.708	**70**	**0.005**	111	0.173	133
LU	**0**	**<.001**	31	0.111	**24**	**0.034**	34	0.169	**23**	**0.028**	52
PI	**0**	**<.001**	78	0.581	**32**	**0.004**	72	0.399	50	0.053	57
RP	**11**	**0.011**	35	0.791	22	0.151	36	0.860	24	0.211	20
SS	**11**	**<.001**	**53**	**0.041**	94	0.892	92	0.821	57	0.065	95
TA	**11**	**<.001**	**125**	**0.043**	**124**	**0.040**	**123**	**0.038**	192	0.841	180
UN	**10**	**0.004**	24	0.109	25	0.129	32	0.351	**15**	**0.016**	38

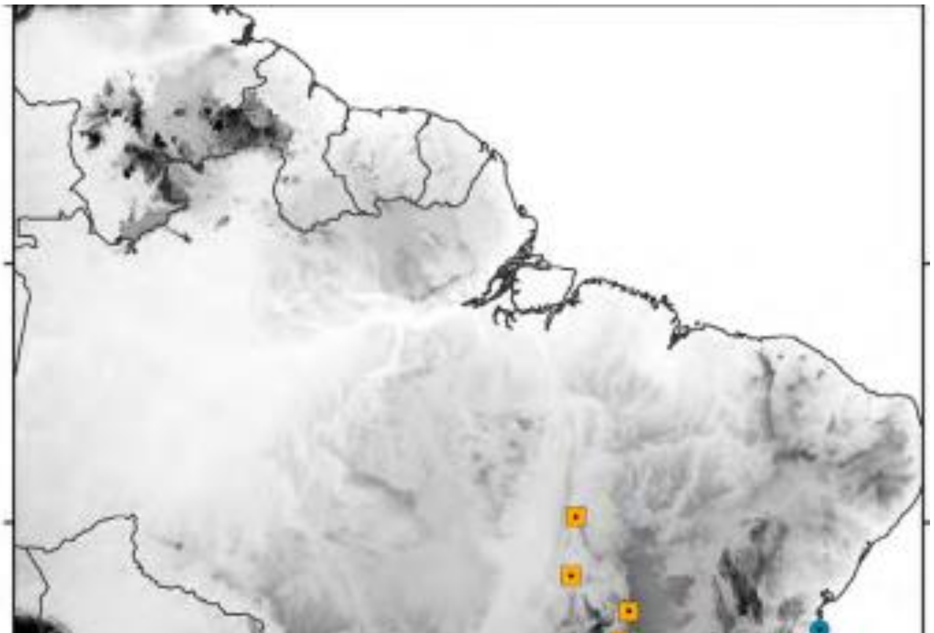
Table S22. Results of Mann-Whitney analyses testing for differences between sexes in limb and head traits of *T. catalanensis* separated by locality; significant results (p <0.05) are highlighted in bold. Abbreviations correspond to: AC = Alfredo de Castilho (SP), AR = Arinos (MG), AU = Aruanã (GO), BA = Batayporá (MS), BR = Brasilândia (MS), CG = Chapada dos Guimarães (MT), LA = Lajeado

(TO), LU = Luziânia (GO), PI = Piracicaba (SP), RP = Ribeirão Preto (SP), SS = São Simão (SP), TA = Tapirapé (MT) and UN = Unaí (MG).

Location	**HUL**		**RAL**		**FEL**		**TBL**		**HDL**		**HDH**		**HDW**	
	U	**p**	**U**	**p**	**U**	**p**	**U**	**p**	**U**	**p**	**U**	**p**	**U**	**p**
AC	18	0.456	23	0.902	19	0.535	23	0.902	20	0.620	21	0.710	20	0.620
AR	69	0.650	70	0.689	59	0.320	52	0.168	42	0.052	**23**	**0.002**	45	0.077
AU	103	0.179	114	0.336	**67**	**0.008**	**76**	**0.021**	**36**	**<.001**	**45**	**<.001**	**20**	**<.001**
BA	56	0.797	34	0.088	**22**	**0.010**	48	0.438	58	0.898	**27**	**0.028**	54	0.699
BR	29	0.082	53	0.972	50	0.808	33	0.148	**23**	**0.028**	31	0.111	33	0.148
CG	**10**	**<.001**	**23**	**0.003**	**15**	**<.001**	**45**	**0.103**	**4**	**<.001**	**4**	**<.001**	**3**	**<.001**
LA	**83**	**0.020**	100	0.083	**62**	**0.002**	103	0.103	**16**	**<.001**	**21**	**<.001**	**14**	**<.001**
LU	45	0.554	**4**	**<.001**	30	0.095	**16**	**0.006**	26	0.049	**11**	**0.001**	**17**	**0.007**
PI	90	1.000	88	0.943	81	0.683	79	0.614	65	0.236	88	0.943	77	0.548
RP	25	0.246	19	0.085	26	0.285	30	0.476	**7**	**0.003**	**4**	**<.001**	**10**	**0.008**
SS	83	0.525	79	0.413	59	0.080	91	0.786	75	0.316	83	0.525	**40**	**0.007**
TA	**111**	**0.015**	188	0.758	195	0.904	**125**	**0.043**	**44**	**<.001**	**66**	**<.001**	**79**	**<.001**
UN	39	0.717	40	0778	41	0.840	39	0.717	**16**	**0.020**	**16**	**0.020**	36	0.545

Table S23. Paired t-test in *T. catalanensis* neonates to verify the measurements between DF and APC. Two neonates were measured tree times.

Descriptive		**SVL**	**TRL**	**SGL**	**SGH**	**PGL**	**PGH**	**HUL**	**RAL**	**FEL**	**TBL**	**HDL**	**HDH**
1	**t**	-1.219	-0.205	3.569	-0.203	-2.548	-0.510	0.460	0.361	1.293	-5.159	-3.576	0.847
1	**p**	0.347	0.856	0.070	0.858	0.126	0.661	0.691	0.753	0.325	0.036	0.070	0.486
2	**t**	-1.167	1.076	-0.340	0.773	-2.087	-1.845	-0.241	0.504	0.685	-2.126	1.219	0.112
2	**p**	0.363	0.395	0.766	0.520	0.172	0.206	0.832	0.664	0.564	0.167	0.347	0.921



NEONATES DESCRIPTIVE

Legenda 24

Figure S3. Figure from De Sena (2015) to represent the *Tropidurus catalanensis* distribution.

Descriptive		*Manus*					*Pes*				
	Sex	**DI**	**DII**	**DIII**	**DIV**	**DV**	**DI**	**DII**	**DIII**	**DIV**	**DV**
Media	f										
Media	m										
Median	f										
Median	m										
Standard deviation	f										

Standard deviation	m

Legenda 24

Descriptive	Sex	SVL	TRL	SGL	SGH	PGL	PGH	HUL	RAL	FEL	TBL	HDL	HDH	HDW
Media	f	29.3	11.2	5.65	3.87	3.49	2.92	5.00	4.69	6.39	6.21	9.06	4.06	5.85
Media	m	29.4	11.4	5.61	3.45	3.51	2.84	4.78	4.56	6.54	6.09	8.86	3.92	5.62
Median	f	29.5	11.10	5.81	3.75	3.48	2.68	5.15	4.72	6.26	6.21	9.14	4.26	5.97
Median	m	29.6	11.10	5.71	3.70	3.67	2.66	4.68	4.54	6.51	5.84	9.05	4.05	5.73
Standard deviation	f	0.97	1.01	0.83	1.10	0.62	0.80	0.52	0.30	0.32	0.70	0.53	0.61	0.28
Standard deviation	m	0.92	0.91	0.69	0.48	0.81	0.766	0.72	0.67	0.28	0.78	1.00	0.58	0.45

Legenda 30

Descriptive		*Manus*					*Pes*				
	Sex	DI	DII	DIII	DIV	DV	DI	DII	DIII	DIV	DV
Media	f	1.26	2.33	3.58	3.85	2.15	1.45	2.83	4.78	6.57	4.25
Media	m	1.18	2.28	3.55	3.86	2.16	1.44	2.81	4.83	6.70	4.31
Median	f	1.21	2.30	3.53	3.81	2.14	1.42	2.82	4.79	6.64	4.28
Median	m	1.16	2.25	3.48	3.81	2.14	1.38	2.82	4.84	6.64	4.22
Standard deviation	f	0.17	0.24	0.38	0.39	0.34	0.14	0.30	0.48	0.74	0.39
Standard deviation	m	0.14	0.21	0.23	0.32	0.15	0.17	0.21	0.32	0.48	0.30

Legenda 30

Descriptive	Sex	SVL	TRL	SGL	SGH	PGL	PGH	HUL	RAL	FEL	TBL	HDL	HDH	HDW
Media	f	29.8	12.2	5.71	3.54	3.52	2.60	4.47	4.97	6.68	5.96	9.18	3.68	5.54
Media	m	29.7	12.5	5.80	3.51	3.35	2.80	4.82	4.94	7.19	5.86	9.42	3.64	5.54
Median	f	29.8	12.2	5.68	3.50	3.43	2.57	4.47	4.92	6.70	5.90	9.12	3.56	5.53
Median	m	29.8	12.5	5.68	3.42	3.35	2.62	4.85	4.96	7.16	5.91	9.33	3.63	5.52
Standard deviation	f	1.23	0.99	0.35	0.62	0.62	0.38	0.58	0.20	0.72	0.60	0.38	0.42	0.21

Standard deviation	m	1.07	1.12	0.37	0.56	0.54	0.56	0.46	0.26	0.59	0.44	0.34	0.40	0.29

Table S24. Results of Mann-Whitney tests implemented to identify differences between years (i.e 2021/2022) in *T. catalanensis* neonates digits*;* significant results (p <0.05) are highlighted in bold.

Morphological traits		**U**	**p**
Manus	I	308	0.074
	II	303	0.062
	III	361	0.333
	IV	399	0.698
	V	**296**	**0.048**
Pes	**I**	**138**	**< .001**
	II	318	0.103
	III	405	0.767
	IV	423	0.982
	V	344	0.219

Table S25. Results of Mann-Whitney tests implemented to identify differences between sexes *T. catalanensis* neonates from 2021 for 24ºC; significant results (p <0.05) are highlighted in bold.

2021, 24ºC			
Autopodium	**Digit**	**U**	**p**
Manus	DI	6	0.786
	DII	6	0.786
	DIII	4	0.393
	DIV	7	1.000
	DV	7	1.000
Pes	DI	5	0.571
	DII	7	1.000
	DIII	5	0.571
	DIV	6	0.786
	DV	4	0.393

Table S26. Results of Mann-Whitney tests implemented to identify differences between sexes *T. catalanensis* neonates from 2021 for 30ºC; significant results (p <0.05) are highlighted in bold.

2021, 30ºC			
Autopodium	**Digit**	**U**	**p**
Manus	DI	22	0.230
	DII	33	0.887
	DIII	31	0.740
	DIV	32	0.813
	DV	31	0.740
Pes	DI	23	0.270
	DII	23	0.270
	DIII	30	0.669
	DIV	26	0.417
	DV	20	0.161

Table S27. Results of Mann-Whitney tests implemented to identify differences between sexes *T. catalanensis* neonates from 2022 for 24ºC; significant results (p <0.05) are highlighted in bold.

2022, 24ºC			
Autopodium	**Digit**	**U**	**p**
Manus	DI	19	0.063
	DII	29	0.340
	DIII	22	0.113
	DIV	24	0.161
	DV	27	0.258
	DI	32	0.489

Pes	DII	31	0.436
	DIII	29	0.340
	DIV	35	0.666
	DV	22	0.113

Table S28. Results of Mann-Whitney tests implemented to identify differences between sexes *T. catalanensis* neonates from 2022 for 30ºC; significant results (p <0.05) are highlighted in bold.

2022, 30ºC			
Autopodium	**Digit**	**U**	**p**
Manus	DI	26	0.606
	DII	28	0.758
	DIII	31	1.000
	DIV	28	0.758
	DV	30	0.918
Pes	DI	17	0.142
	DII	25	0.536
	DIII	24	0.470
	DIV	20	0.252
	DV	20	0.252

Table S29. Results from the analyzes to verify the multicollinearity of variables using the VIF method from *T. catalanensis* and *Liolaemus*.

Climate variables	**Temperature**	**Precipitation**	**Altitude**	**Reproductio**
Tropidurus catalanensis	2.047	2.197	-	-
Liolaemus sp.	1.596	1.940	2.100	1.259