



Universidade de Brasília
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Zoologia

Variação fenotípica na lagartixa-doméstica

Hemidactylus mabouia (Moreau de Jonnès, 1818)

(Squamata, Gekkonidae)

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Brasília, Distrito Federal, Brasil

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Dissertação apresentada ao Programa de Pós-Graduação em Zoologia, Instituto de Ciências Biológicas, Universidade de Brasília, como requisito parcial para a obtenção do título de Mestre em Zoologia.

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Aos meus pais e ao meu irmão

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“Em algum lugar, algo incrível está
esperando ser descoberto.”

Carl Sagan

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Resumo

Um organismo é considerado invasor quando este consegue se estabelecer e dispersar para locais fora da sua área de distribuição nativa, o que frequentemente gera diversos impactos no ecossistema local. Apesar dos efeitos nocivos, sobretudo à biota local, espécies invasoras oferecem uma oportunidade única de acompanhar o processo de colonização de novas áreas por uma espécie. Como a seleção natural age sob a variação fenotípica presente em uma população, uma alta labilidade deve aumentar as chances de sucesso de estabelecimento de espécies exóticas pois possibilita a adaptação local. Assim, avaliamos a variação fenotípica de *Hemidactylus mabouia*, um lagarto invasor extremamente bem-sucedido, a fim de determinar o grau de labilidade e o potencial adaptativo da espécie. No primeiro capítulo, avaliamos a presença de variabilidade na fisiologia térmica de *H. mabouia* dentro de uma mesma população e entre populações habitando locais com condições climáticas distintas. No nível intrapopulacional, encontramos que juvenis possuem temperatura crítica mínima (CT_{min}) mais alta e temperatura crítica máxima (CT_{max}) mais baixa que adultos, bem como uma menor faixa de tolerância térmica. A diferença entre adultos e juvenis na CT_{min} e tolerância térmica pode ser explicada pelo efeito da massa. Comparando os dados obtidos para a população em região de Cerrado com dados publicados para indivíduos da região Amazônica, encontramos variação na temperatura preferencial, CT_{min} e CT_{max} , mas não na tolerância térmica. No segundo capítulo, descrevemos variação na morfologia externa entre populações de *H. mabouia* distribuídas ao longo de uma ampla faixa no Brasil, avaliamos os possíveis fatores por trás da diversificação morfológica, e descrevemos a ontogenia pós-natal do crânio e membros a fim de avaliar os períodos durante o desenvolvimento que devem ser mais lábeis. Nós observamos uma alta variação fenotípica entre populações de *H. mabouia*, sobretudo no comprimento do dígito IV do

pé e do antebraço. Em seguida, comparamos índices de diferenciação fenotípica, P_{ST} , com um índice de divergência genética em loci genéticos neutros, F_{ST} . *H. mabouia* possui valores de P_{ST} maiores que o F_{ST} , o que indica que o possível efeito da deriva genética não é suficiente para explicar a variação morfológica. O $P_{ST} > F_{ST}$ tradicionalmente é interpretado como efeito de seleção direcional sob os traços avaliados, no entanto, não conseguimos encontrar os possíveis fatores ambientais associados. Diferenças entre populações no tamanho relativo de partes do corpo são explicadas por mudanças no intercepto das curvas alométricas. Comparando a curva alométrica dos respectivos ossos entre juvenis e adultos de uma única população, encontramos que a trajetória de crescimento permanece a mesma durante o desenvolvimento pós-natal para a maioria dos traços. Esses resultados sugerem que mudanças entre populações tenham surgido durante o desenvolvimento embrionário. A análise de morfometria geométrica mostrou que maior parte da variação intrapopulacional na forma do crânio é explicada pela ontogenia, e a alometria sozinha explica mais da metade da variação. O estudo de espécies invasoras, além de crucial para a conservação da biodiversidade, também oferece uma oportunidade única de testar hipóteses evolutivas em populações naturais. Além disso, a compreensão dos processos que geram variação intraespecífica tem sido levantada como uma importante ferramenta para o entendimento da origem de diversidade em escalas macroevolutivas.

Palavras-chave

Alometria; Crânio; Esqueleto; Fisiologia térmica; Lagarto; Invasora; Morfologia; Ontogenia.

Abstract

An organism is considered invasive when it is able to establish and disperse outside its native range, frequently impacting the entire dynamic of the ecosystem. Despite the posed treat to the ecosystem, particularly to the native biota, invasive species still offer a unique opportunity to follow the colonization process of new habitats by a species. Once natural selection acts over the phenotypic variation present in a population, a high lability might increase the chances of establishment success of an exotic species as it enables local adaptation. Therefore, we evaluated the phenotypic variation in *Hemidactylus mabouia*, an extremely successful invader, in order to determine the degree of lability and the adaptive potential of the species. In the first chapter, we assessed the presence of variability in thermal physiological traits of *H. mabouia* within and between populations inhabiting areas with distinct climatic conditions. At the intrapopulation level, we found that juveniles exhibit higher critical thermal minimum (CT_{min}) and lower critical thermal maximum (CT_{max}) compared to adults, resulting in a narrower thermal tolerance range. The difference between adults and juveniles in the CT_{min} and thermal tolerance can be explained by the effects of mass on the thermal traits, but not in the CT_{max} . Comparing the data obtained for a population in the Cerrado region with data published for individuals from the Amazon region, we found a difference in preferred temperature, CT_{min} , and CT_{max} , but not in the thermal tolerance. In the second chapter, we described variation in external morphology among populations of *H. mabouia* distributed across a wide range in Brasil, then we evaluated the possible factors underlying the morphological diversification, further, we also described the post-natal ontogeny of the skull and limb bones to assess which developmental periods should be most labile. We observed a high morphological variation among populations, particularly in the foot digit IV and forearm. We compared an index of phenotypic differentiation,

P_{ST} , with an index of divergence at neutral genetic loci, F_{ST} . *H. mabouia* exhibits higher P_{ST} values than F_{ST} , indicating that drift alone cannot explain the phenotypic variation. A $P_{ST} > F_{ST}$ has been regarded traditionally as a signal of directional selection, however, we were unable to find the possible environmental factors associated. Differences between populations in the relative size of body parts are explained by shifts in the intercept of the allometric curves. Comparing the allometric curves for the respective bones among adults and juveniles of a single population, we found that the growth trajectory remains the same during post-natal development for most traits. These results suggest that morphological changes probably appeared during embryonic development. A geometric morphometric analysis showed that a high percentage of the within-population variation of the skull shape is explained by ontogeny, and allometry alone explains more than 50% of the total variation. The study of invasive species is not only crucial to biodiversity conservation, but also, offers a unique opportunity to test evolutionary hypothesis in natural populations. Besides, the comprehension of the processes that generate intraspecific variation have been proposed as an important tool to understand the origins of diversity at macroevolutionary levels.

Key Words

Allometry; Bone; Lizard; Invasive; Morphology; Ontogeny; Skull; Thermal physiology.

General Introduction

Invasive species are one of the many issues related to anthropic environmental change and are a prominent threat to the ecosystem. Although invasion biology has received much attention over the last decades, there is a great confusion regarding terminology in the literature as researchers from different disciplines have adopted different frameworks (Blackburn et al. 2011; Lockwood et al. 2013). Invasive species are defined by some authors as non-native organisms whose introduction generates an ecological or economic impact, while others define as non-native organisms that were able to establish and spread (Lockwood et al. 2013). Given the subjectivity and difficulty in determining impact (Jeschke et al. 2014), many authors advocated the use of the second definition (e.g., Richardson et al. 2000; Daehler 2001; Colautti and MacIsaac 2004). More recently, Blackburn et al. (2011) proposed a unified framework for invasion biology combining the frameworks proposed by Richardson et al. (2000), adopted by plant ecologists, and by Williamson and Fitter (1996), adopted by animal ecologists. This unified approach separates the invasion process into four stages: transport, introduction, establishment, and spread (Blackburn et al. 2011). In order to become invasive, the exotic organism must overcome, in each stage respectively, the barriers of geography, captivity, survival/reproduction, and dispersal/environment (Blackburn et al. 2011).

Exotic organisms can cause the extinction of native species by various mechanisms, such as competitive exclusion, predation, or introduction of parasites and diseases, and thereby can affect the dynamics of the entire ecosystem (Mooney and Cleland 2001; Lockwood et al. 2013). Biotic homogenization caused by the replacement of native, and frequently endemic species by widespread exotic species, is one of the main concerns related to environmental change (McKinney and Lockwood 1999). This

environmental impact can still be aggravated by the adaptation of the exotic organisms to the local environment (Mooney and Cleland 2001).

Biological invasions present interesting evolutionary problems regarding adaptation to novel environments and its study has enabled the understanding of various aspects of ecology and evolution (Baker and Stebbins 1965; Fridley and Sax 2014; Bock et al. 2015). For example, invasive species have been proposed as key model organisms to test hypotheses concerning evolutionary rate in nature, predictability of evolutionary patterns (Huey et al. 2005), and phenotypic plasticity (Pigliucci 2010). Because a spreading non-native population faces multiple establishment events, in which it needs to survive and reproduce over several generations under a wide range of environmental conditions (Blackburn et al. 2011), evolutionary shifts might extend the geographic limits of a colonizing population during range expansion (Lockwood et al. 2013). Studies show that the invasion success can be facilitated by the presence of variation upon which natural selection could act (reviewed in Lee 2002). Indeed, rapid evolution, especially in traits related to invasiveness, appears to be common during the colonization of novel habitats, with changes occurring at observational time scales (Reznick and Ghalambor 2001; Whitney and Gabler 2008). Therefore, the study of invasive species and a better comprehension of the invasion process is not only crucial for biodiversity conservation, but also offers unique opportunities to evaluate evolutionary theories once they provide almost a replicate experiment (Sax et al. 2007).

Among vertebrates, the lizard group Gekkota stands out for fostering a number of species that became successful invaders (Lever 2003; Bomford et al. 2009). Within Gekkota, the genus *Hemidactylus* is a particularly interesting group to study invasion biology. *Hemidactylus* is an extremely diverse genus with currently 173 recognized species (Uetz et al. 2021). Some species are very similar and easily confounded while

others show high intraspecific variation, rendering it a taxonomically difficult group (Carranza and Arnold 2006). The majority of *Hemidactylus* species have narrow distribution ranges confined in southern Asia and Africa (Carranza and Arnold 2006), but a few were able to establish outside their native range (e.g., Lever 2003; Bauer and Baker 2012). Among these, *H. mabouia*, *H. frenatus*, *H. turcicus*, *H. brookii*, and *H. garnotii* are the most preeminent and combined invaded almost the entire tropical, subtropical, and Mediterranean regions (Kluge 1969; Weterings and Vetter 2018).

Hemidactylus mabouia has the highest establishment success rate between invasive lizards, and when considering both invasive amphibians and reptiles, this gecko loses only to the thread snake *Indotyphlops braminus* (Bomford et al. 2009). *H. mabouia* is native from sub-Saharan Africa and was introduced to South, Central, and North America probably via slave ships (Kluge 1969; Powell et al. 1998; Gamble et al. 2011). This species occurs mainly in anthropic areas and its main dispersion vector is the accidental carriage via cargo transports (Kluge 1969; Short and Petren 2011). *H. mabouia* poses a risk to native geckos by their displacement through exploitative competition (Short and Petren 2012; Hughes et al. 2015) and predation (Lamb et al. 2020). In Brazil, *H. mabouia* colonized a diversity of natural environments (Rocha et al. 2011) where it competes with *Gymnodactylus darwinii* (Texeira 2002) and *Thecadactylus rapicauda* (Vitt and Zani 1997; Howard et al. 2001). Even though *H. mabouia* co-occurs and overlaps diet with *Phyllopezus pollicaris*, these two geckos seem to partition the temporal niche (Albuquerque et al. 2013). There are no records of the impact of *H. mabouia* on the native congeners *H. agrius*, *H. brasilianus*, and *H. palaichthus*, which are sympatric in some locations. Additionally, in the case of congeners, sexual interference could also be a possible displacement mechanism of natives by exotics in *Hemidactylus* (Dame and Petren 2006).

Considering the high invasiveness of *H. mabouia*, studying this species is crucial for biodiversity conservation and makes it a good model organism to evaluate evolutionary theories. Our main objective in this dissertation was to describe the phenotypic variation in *H. mabouia* in order to determine the degree of lability and adaptive potential of the species and infer possible causes of phenotypic change. Specifically, our objective in the first chapter was to describe variability in thermal physiological traits of *H. mabouia* within and between populations inhabiting areas with different climatic conditions. While in the second chapter we aimed to describe morphological variation within and between populations of *H. mabouia* distributed across a wide range in Brazil, suggest possible factors promoting this variation, and evaluate the possible underlying ontogenetic mechanisms.

Capítulo I

Labilidade da fisiologia térmica em *Hemidactylus mabouia*

(Moreau de Jonnés 1818)

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**Go with the flow: lability of thermal physiology in the invasive Tropical House
Gecko *Hemidactylus mabouia* (Moreau de Jonnès 1818) (Squamata, Gekkonidae)**

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Abstract

Thermal physiological traits are ecologically important to ectotherms and seem to be under natural selection in lizards. Physiological lability increases a species adaptive potential and is crucial to populations' persistence when facing climate change. It also appears to be advantageous during the colonization of novel habitats, therefore should benefit invasive species. The gecko *Hemidactylus mabouia* is an extremely successful invader that colonized a wide range of habitats with different climatic conditions. Understanding the thermal physiology of *H. mabouia* is crucial to a better comprehension of its invasion process. We evaluated if the ability of *H. mabouia* to colonize places with diverse climatic conditions could be associated with physiological lability. To analyze intraspecific variations in the thermal physiology of *H. mabouia*, we tested for differences in four traits between sexes, ontogenetic stages, and invasive populations in the Cerrado savanna and Amazon regions. Juvenile *H. mabouia* exhibit higher critical thermal minimum and narrower thermal tolerance than adults, which could be explained by effects of body size in the thermoregulation capacity. The Cerrado population shows higher preferred temperature, critical thermal minimum, and critical thermal maximum compared to the Amazon population, but not thermal tolerance. We suggest that labile thermal physiological traits should be an important factor in the colonization process of *H. mabouia*, as observed in other invasive lizards. Besides, this lability has also an

important role in buffering the effects of climate change in ectotherms and could reduce *H. mabouia*'s vulnerability to future temperature increases.

Key Words

Climate change; Ecophysiology; Exotic; Global warming; Lizard; Non-native; Reptile; Thermoregulation.

1. Introduction

Temperature is one of the major factors affecting the physiology of living organisms and is consequently related to fitness (Brown et al. 2004; Angilletta 2009). In the last decades, Earth's temperature has increased as a direct effect of global warming, which has already caused impacts in many systems (Deutsch et al. 2008; Huey et al. 2009). Studies revealed that climate change might alter species distributions, with some being benefited due to increases in the availability of thermally suitable habitats (Deutsch et al. 2008; Diele-Viegas and Rocha 2018), while others would suffer extinction (Diele-Viegas et al. 2019).

Ectotherms are especially susceptible to the effects of environmental temperature because they depend on external heat sources to regulate body temperature (Vitt and Caldwell 2013). Lizards, well-known vertebrate ectotherms, are active only in a narrow temperature range, in which their performance is optimized (Angilletta 2009; Huey et al. 2010). They coordinate behavioral (e.g., microhabitat choice, posture, and panting) and physiological mechanisms (e.g., evaporative water loss and cardiovascular alterations) to thermoregulate and maintain body temperatures inside their active range (Seebacher and Franklin 2005; Vitt and Caldwell 2013). Thermoregulation can be studied through thermal physiological traits such as preferred temperature and critical thermal limits (Angilletta 2009; Vitt and Caldwell 2013). These traits are ecologically important (Dawson 1975) and seem to be under natural selection (Gilbert and Miles 2017).

Physiological lability can buffer the negative impacts of temperature increases, being crucial to the survival and persistence of species affected by climate warming (Huey et al. 2012; Gilbert and Miles 2017; Diele-Viegas et al. 2019). Species with greater variability in thermal physiological traits are more likely to adapt to novel environmental conditions, which will probably be favored by selection under climate change scenarios (Pontes-da-Silva et al. 2018; Diele-Viegas et al. 2019). Similarly, high lability in thermal physiology should also facilitate the colonization of novel habitats and therefore can be especially important for invasive species. Physiological adaptation through phenotypic plasticity (i.e., acclimation), especially, should favor the establishment of exotic species since abiotic stressors serve as the first ecological filter to invasion (Olyarnik et al. 2009; Kelley 2014).

As invaders can drive native species to extinction or even disturb the whole ecosystem (Lockwood et al. 2013), the study of invasive species is indispensable to biodiversity conservation. As so, one of the main interests of invasion biology is to explain which factors allow colonizing species to be successful and established (Elton 1958; Kolar and Lodge 2001). Due to temperature's importance, understanding how exotic lizards respond to thermal variation may help elucidate invasion processes and patterns (Kelley 2014). Among invasive lizards, *Hemidactylus mabouia* has the highest establishment success rate (Bomford et al. 2009). *H. mabouia* is native from sub-Saharan Africa and invasive in South, Central, and North America, making it one of the most broadly distributed and prevailing species in the world (Kluge 1969; Powell et al. 1998). Usually this species invades anthropic environments, however, it has already been found colonizing several natural environments (Kluge 1969; Powell et al. 1998; Rocha et al. 2011). Studies have shown that *H. mabouia* can cause the complete displacement of resident species from urban habitats in less than a decade, posing a risk to native geckos

(Short and Petren 2012; Hughes et al. 2015). Despite its abundance in various countries and ecological importance, there is still a lack of knowledge about the biology of *H. mabouia*.

The objectives of this study were: (1) describe the preferred temperature, critical thermal minimum, critical thermal maximum, and thermal tolerance for an invasive population of *Hemidactylus mabouia* occupying urban areas in the Cerrado savanna region; (2) analyze if these traits differ between sexes or ontogenetic stages; (3) compare the data obtained with data already published for invasive populations in the Amazon region. Considering the high invasion capacity of *H. mabouia*, we expect that this species presents a high intraspecific variation in thermal physiology, indicating the lability of the traits analyzed. With this we aim to understand how *H. mabouia* responds to variations in environmental conditions, allowing better comprehension of its invasion process and insights into possible impacts of climate warming.

2. Material and Methods

2.1. Collection and animal care

We collected individuals of *Hemidactylus mabouia* by active search and captured them by hand in urban areas of Distrito Federal, Brazil (−15.7801, −47.9292), between March 2019 and February 2021. We took captured lizards to the laboratory in the Institute of Biological Sciences of the University of Brasilia, where we kept them in captivity. We kept the geckos in black plastic containers covered with thin mesh fabric, with sand and vermiculite as substrate, and refuges. We humidified terrariums daily and used heating plates suitable for reptile care to keep the ideal temperature for lizards—around 32° C (LLL Reptile and Supply Co., Inc. 2021. House Gecko. Available from <https://www.lllreptile.com/articles/25-house-gecko/> [Accessed 07 May 2021]). Room temperature ranged between 22–30° C, and was naturally illuminated, following a natural

photoperiod cycle. We fed geckos with live cockroaches (*Nauphoeta cinerea*), mealworms (*Tenebrio molitor*), or peanut beetle larvae (*Palembus dermestoides*)—to which we added calcium, phosphorus, and vitamin D supplements—and all individuals had water at their disposal. Animal collection and experiments were approved by Chico Mendes Institute for Biodiversity Conservation (ICMBio, n° 0632260220190624) and University of Brasilia Ethics Committee for Animal Use (CEUA–UNB, n° 116/2017), respectively.

2.2. Experimental procedure

We described the thermal physiology of 91 individuals of *Hemidactylus mabouia*, among juveniles and adults, using three measurements: preferred temperature; critical thermal minimum, critical thermal maximum, and thermal tolerance (sampling numbers for each measurement and each group in Table 1). Before experimental procedures, we weighed lizards in a precision balance and measured their body temperature at ambient conditions. We performed all experiments at night (i.e., after 1800) during the activity period of *H. mabouia* (Howard et al. 2001; Albuquerque et al. 2013). We used a thermocouple Tp-01 type K sensor connected to a digital thermometer to measure all body temperatures. We fixed the sensor to the lizard's abdomen with micropore surgical tape only when measuring the temperature. We tried to minimize handling time as much as possible so we would not influence the animal's body temperature. We did not use cloacal temperature because the sensor was too big for small juveniles and infrared thermometers showed ambient interference.

To measure the preferred temperature, we placed lizards individually in glass aquariums with a heating lamp on one extremity and ice on the other. Aquariums' dimensions varied between 21.5 × 44.5 cm and 34 × 80 cm long, creating a photothermal gradient ranging from 24–55° C in the smaller and from 16–55° C in the larger. Then,

we kept the lizard in the thermal gradient for 2 h and measured its body temperature every 20 min, resulting in six measurements. We used the mean of the six measurements to estimate the preferred temperature.

To obtain the critical thermal minimum (*CT_{min}*), we placed the lizards in a perforated plastic recipient, which we positioned inside a foam container cooled with ice. The temperature inside the cool chamber remained nearly constant at approximately 0.2° C during the time of the trials. Animals were turned backward and when they lost their righting response, we measured their body temperature. Loss of righting response indicates the loss of physiological response, being the most commonly used parameter to measure critical thermal limits in reptiles (Taylor et al. 2020). To obtain a critical thermal maximum (*CT_{max}*), we positioned the animal in the perforated plastic recipient inside a foam container heated with boiling water. The temperature in the heating chamber was kept around 50° C during the time of the trial, and we also kept lizards inside until the loss of righting response. *CT_{min}* and *CT_{max}* were not measured on the same day so that animals could recover, and we always chose to measure *CT_{min}* before *CT_{max}*, since the latter might get animals more impaired. Thermal tolerance is the difference between *CT_{max}* and *CT_{min}*.

After experimental procedures, animals were euthanized via intraperitoneal injection of lethal doses of Neocaine anesthetic, as suggested by the National Council for Animal Experimentation Control (CONCEA). Lizards were fixed in 10% formalin buffered with sodium chloride (NaCl) for 2–3 d and stored in 70% alcohol solution. Sex was determined by hemipenis eversion and gonad observation. Specimens with snout-vent length (SVL) over 46 mm were considered adults, following Anjos and Rocha (2008).

2.3. Comparison between methodologies

To analyze if the body's surface temperature measured with the thermometer placed in the lizard's abdomen corresponds to internal body temperature measured in the cloaca, we performed critical thermal minimum and critical thermal maximum experiments with 14 adults *Hemidactylus mabouia*. We performed two trials for each parameter: one with the thermocouple sensor fixed in the abdomen with micropore surgical tape, and another with the thermocouple sensor inserted in the cloaca. Each trial was separated by one week to allow the lizards' full recovery. All other experimental conditions were the same as described above.

2.4. Interpopulation variation

We compared thermal preference (T_{pref}), critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), and thermal tolerance (Tol) obtained in Distrito Federal—located in the Cerrado savanna region—with data obtained by Diele-Viegas et al. (2018) in the Amazon region—Amapa National Forest, Amapa (0.92, -51.60), and Adolpho Ducke Forest Reserve, Manaus (-2.95, -59.91). Briefly, to measure T_{pref} , Diele-Viegas et al. (2018) placed lizards for 2 h in a photothermal gradient of 16–40° C generated by placing ice in one end and a heating lamp on the other. They measured lizards' body temperature every 3–5 min and T_{pref} was the mean of all values. To obtain the CT_{min} and CT_{max} , Diele-Viegas et al. (2018) also placed lizards in containers cooled/heated with ice and boiling water, respectively, until the loss of righting response. In their study, body temperature in all experiments was measured with an infrared thermometer pointed to the lizards' abdomen.

Further, we compared climatic variables between Brasilia and Manaus to characterize climatic differences experienced by *H. mabouia* in both areas. We used values of diel mean, minimum and maximum air temperatures, and relative humidity

during the period when studies were carried out in each locality (2019–2021 in the Federal District and 2015 in the Amazon). We obtained data from the National Institute of Meteorology (INMET, <https://bdmep.inmet.gov.br/>) for the stations of Brasilia (Federal District) and Manaus. We used only data for Manaus because most of the animals were collected in this locality and there are no meteorological stations available close to where lizards were collected in Amapa, the closest one being more than 130 km away.

2.5. Data Analyses

We used the statistical software environment R 3.6.3 (R Core Team 2013) and RStudio 1.2.5019 (RStudio Team 2015) for all analyses. We log-transformed the trait values for all subsequent analyses to assure normality.

To assess the effects of mass and sex on the T_{pref} , CT_{min} , CT_{max} , and thermal tolerance, we performed ANCOVAs using sex as a factor and mass as a covariate for each trait separately. Because we did not have information about snout-vent length (SVL) for all individuals, we chose to use mass as a measure of size since both are highly correlated ($r = 0.97$, $P < 0.0001$). Besides, mass is directly related with the metabolic rate (Andrews and Pough 1985; Brown et al. 2004), and consequently might influence thermal physiology. Since sex did not affect the traits (Table 2), we performed ANOVAs to test for differences between adults and juveniles.

To compare CT_{min} and CT_{max} measured on the abdomen and in the cloaca, we used paired t -tests. We tested for differences in T_{pref} , CT_{min} , CT_{max} , and thermal tolerance between Cerrado and Amazon populations using an ANOVA. In addition, because of the differences between abdomen or cloacal temperature (see results), we compared the CT_{min} and CT_{max} between Amazon and Cerrado populations using body temperatures measured in the cloaca for the latter using an ANOVA.

Finally, we used Kruskal-Wallis test to compare the climatic variables (i.e., mean relative humidity, minimum, maximum, and mean air temperatures) between Brasilia and Manaus.

3. Results

Mean body temperature of *Hemidactylus mabouia* in ambient conditions was $28.92 \pm$ (SD) 2.88° C (range, 22.0 – 33.6° C) and descriptive statistics for thermal physiological measures can be observed in Table 1. The lowest value of the critical thermal minimum reached was 10.4° C and the highest critical thermal maximum was 44.8° C. Preferred temperatures and critical thermal limits had similar variation breadth (10 – 11° C), but thermal tolerance showed broader variation (Table 1). Only five individuals among 52, however, had preferred temperatures below 30° C.

Table 1. Thermal physiological trait values for *Hemidactylus mabouia*.

Trait	Cerrado				Amazon ¹		
	Mean \pm SD ($^\circ$ C)	Range ($^\circ$ C)	n		Mean \pm SD ($^\circ$ C)	Range ($^\circ$ C)	n (J/A)
			M/F	J/A			
T _{pref}	32.7 ± 2.3	26.8–36.8	21/20	24/28	27.4 ± 5.0	18.9–33.8	2/8
CT _{min}	15.4 ± 2.9	10.4–22.8	28/33	18/51	10.6 ± 3.5	5.6–16.8	2/7
CT _{max}	39.7 ± 2.0	34.1–44.8	27/27	12/49	36.1 ± 5.3	28.5–44.2	2/7
Tolerance	24.2 ± 3.7	14.9–29.9	24/24	12/43	25.5 ± 6.2	18.2–33.5	2/7

Variation range is described by minimum and maximum values. n, number of individuals analyzed; M, males; F, females; J, juveniles; A, adults. ¹Data for the Amazon population published by Diele-Viegas et al. (2018).

The ANCOVA analyses with temperature measured in the abdomen detected neither mass nor sex effects on preferred temperature and critical thermal maximum (Table 2). Preferred temperature did not differ between adults and juveniles ($F_{1,50} = 0.05$, $P = 0.81$) (Fig. 1A) but critical thermal maximum showed a significant difference between stages ($F_{1,59} = 3.89$, $P = 0.05$) (Fig. 1C). The critical thermal minimum and thermal tolerance were not affected by sex but was significantly affected by mass (Table

2) and both differed significantly between adults and juveniles ($CTmin$: $F_{1,67} = 17.56$, $P < 0.001$; Tol : $F_{1,53} = 17.47$, $P < 0.001$) (Fig. 1B,D).

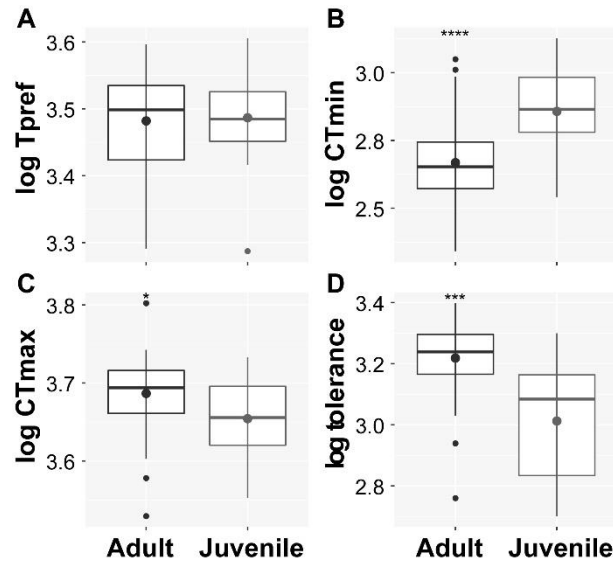


Figure 1. Boxplots showing the variation of the four thermal physiological traits analyzed between adult and juvenile *Hemidactylus mabouia*. (A) preferred temperature; (B) critical thermal minimum; (C) critical thermal maximum; and (D) thermal tolerance. Lower and upper box limits represent, respectively, first and third quartiles, internal bar the median, internal point the mean, and whiskers the minimum and maximal values inside the 99% interval. Ontogenetic stages differ in the critical thermal minimum, critical thermal maximum, and thermal tolerance (* $P = 0.05$, ** $P < 0.01$; **** $P < 0.0001$).

Table 2. ANCOVA testing the effects of mass and sex on the thermal physiology of *Hemidactylus mabouia*.

Trait	Mass		Sex		Mass:Sex		df
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
Tpref	2.71	0.11	3.16	0.08	0.20	0.66	37
CTmin	25.38	<0.001	0.18	0.67	0.009	0.92	56
CTmax	2.08	0.15	0.11	0.75	1.07	0.31	50
Tolerance	30.03	<0.001	0.0002	0.99	2.53	0.12	44

Both critical thermal minimum and critical thermal maximum measured on the abdomen were higher than cloacal temperatures ($CTmin$: $t = 3.20$, $df = 13$, $P < 0.01$; $CTmax$: $t = 2.19$, $df = 13$, $P = 0.05$) (Fig. 2).

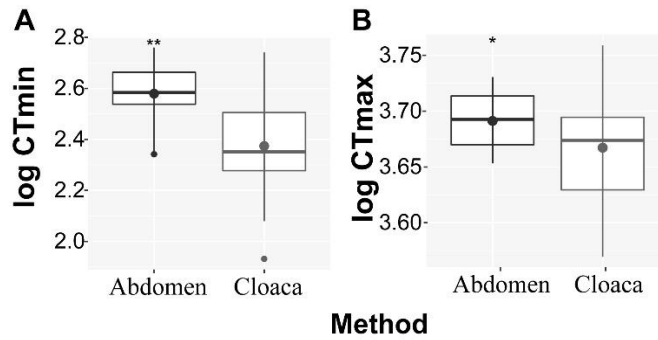


Figure 2. Boxplots showing variation between body temperature of *Hemidactylus mabouia* measured with the thermocouple sensor fixed on the abdomen and inserted in the cloaca. (A) Critical thermal minimum and (B) critical thermal maximum. Lower and upper box limits represent, respectively, first and third quartiles, internal bar the median, internal point the mean, and whiskers the minimum and maximal values inside the 99% interval. The two differ in the measures of critical thermal minimum (** $P < 0.01$) and critical thermal maximum (* $P < 0.05$).

Descriptive statistics for thermal physiological measures for Amazon populations provided by Diele-Viegas et al. (2018) can also be observed in Table 1. Cerrado and Amazon populations differed significantly in the preferred temperature ($F_{1,60} = 29.46$, $P < 0.001$), critical thermal minimum ($F_{1,76} = 30.27$, $P < 0.001$) and critical thermal maximum ($F_{1,68} = 16.92$, $P < 0.001$); but did not differ in the thermal tolerance ($F_{1,62} = 0.29$, $P = 0.59$) (Fig. 3). When using body temperatures measured in the cloaca in lizards from the Cerrado population, however, the two populations differ only in critical thermal maximum ($F_{1,23} = 14.54$, $P < 0.001$) and not in critical thermal minimum ($F_{1,19} = 0.61$, $P = 0.44$).

In 2015, Manaus had a diel mean \pm (SD) air temperature of $28.69 \pm 1.99^\circ\text{C}$ (range, $24.24\text{--}33.74^\circ\text{C}$), minimum air temperature of $25.66 \pm 1.49^\circ\text{C}$ (range, $21.50\text{--}29.10^\circ\text{C}$), maximum air temperature of $33.59 \pm 2.48^\circ\text{C}$ (range, $26.40\text{--}39.00^\circ\text{C}$), and mean relative humidity of $75.09 \pm 10.57\%$ (range, $45.25\text{--}95.00\%$). While between the years 2019 and 2021, Brasilia had a mean air temperature of $21.65 \pm 2.13^\circ\text{C}$ (range, $15.99\text{--}27.92^\circ\text{C}$), minimum air temperature of $16.81 \pm 2.41^\circ\text{C}$ (range, $8.5\text{--}22.8^\circ\text{C}$), maximum air temperature of $27.61 \pm 2.63^\circ\text{C}$ (range, $20.9\text{--}36.5^\circ\text{C}$), and relative humidity of $66.49 \pm$

16.67% (range, 20.91–93.67%). Both cities differed significantly in all four climatic variables: mean temperature ($\chi^2 = 520.73$, $df = 1$, $P < 0.001$), minimum temperature ($\chi^2 = 562.73$, $df = 1$, $P < 0.001$), maximum temperature ($\chi^2 = 433.86$, $df = 1$, $P < 0.001$), and relative humidity ($\chi^2 = 51.67$, $df = 1$, $P < 0.001$).

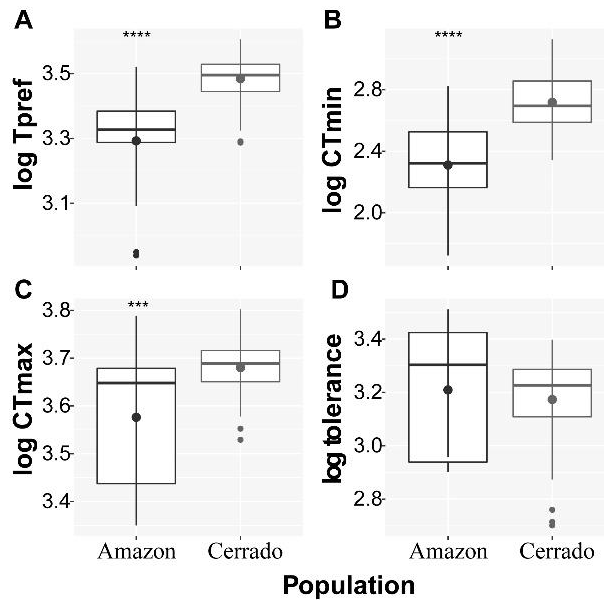


Figure 3. Boxplots showing variation of the four thermal physiological traits analyzed between Amazon and Cerrado populations of *Hemidactylus mabouia*. (A) preferred temperature; (B) critical thermal minimum; (C) critical thermal maximum; and (D) thermal tolerance. Lower and upper box limits represent, respectively, first and third quartiles, internal bar the median, internal point the mean, and whiskers the minimum and maximal values inside the 99% interval. Thermal tolerance is the only trait that does not differ between populations (***) $P < 0.001$, **** $P < 0.0001$).

4. Discussion

Hemidactylus mabouia is an invasive species with a high establishment success rate and is broadly distributed in the world (Kluge 1969; Powell et al. 1998; Bomford et al. 2009). Our aim in this study was to estimate if the ability of *H. mabouia* to colonize places with diverse climatic conditions is associated with labile thermal physiology. High variability in thermal physiological traits was demonstrated to increase the adaptive potential of a species (Pontes-da-Silva et al., 2018). Thus, we analyzed intraspecific variation in thermal physiology (i.e., preferred temperature, critical thermal minimum,

critical thermal maximum, and thermal tolerance) between individuals of the same population and between populations inhabiting areas with different climatic conditions.

To evaluate intrapopulation variation, we tested if sexes and ontogenetic stages differ in thermal physiology. Our results showed that there was no difference between males and females in any of the analyzed traits. Sexual differentiation in thermal physiology is usually related to temporal or spatial niche segregation between sexes (Paranjpe et al. 2013). Consistent with our results, males and females of *H. mabouia* show no difference in refuge choice or trophic niche, indicating that both use the same microhabitats (Rocha and Anjos 2007; Williams et al. 2016). Studies with other species of *Hemidactylus* also found no intersexual variation in preferred temperature (Hitchcock and McBrayer 2006; Romero-Báez et al. 2020). Accordingly, Huey and Pianka (2007), in a broad-scale analysis of the thermal biology of desert lizards, suggested that differentiation between males and females in thermal biology should be rare in lizards.

When comparing the thermal physiological traits between ontogenetic stages, we found that juveniles of *Hemidactylus mabouia* exhibit higher critical thermal minimum and lower critical thermal maximum, resulting in a narrower thermal tolerance breadth than adults. The ontogenetic variation in the critical thermal minimum and thermal tolerance is explained by the effect of mass, but not in the critical thermal maximum. Body size can affect the thermoregulation capacity of lizards by influencing thermal inertia (e.g., Zamora-Camacho et al. 2014; Claunch et al. 2020). Smaller animals have a higher surface area to volume ratio, which increases their cooling and heating rates (e.g., Martín and López 2003; Zamora-Camacho et al. 2014). In accordance, juveniles of *H. mabouia* have a higher water-loss rate compared to adults (Johnson et al. 2013), which could also protect them from overheating (Tattersall et al. 2006). Howard et al. (2001) observed that juveniles of *H. mabouia* preferred hotter substrates and were more active

earlier in the evening. The distinct microhabitat used by adults and juveniles could be related to differences in the critical thermal minimum and to the lower capacity to conserve heat.

In species exhibiting variation in the critical thermal limits between stages, juveniles usually show narrower thermal tolerance breadth (e.g., Xu and Ji 2006; Tang et al. 2013; Gilbert and Lattanzio 2016). Gilbert and Lattanzio (2016) proposed that ontogenetic variations could cause differential survival rates between adults and juveniles in the case of temperature changes, thus, influencing a population's ability to respond to climate change. And the higher sensitivity of juveniles to extreme temperatures can drive some populations to local extinction (Diele-Viegas et al. 2019). Similar to *H. mabouia*, the invasive species *H. turcicus*, *Tarentola mauritanica*, and *Podarcis siculus* show ontogenetic variation in thermal physiology also (Hitchcock and McBrayer 2006; Carretero 2008; Liwanag et al. 2018) and have colonized regions with climatic conditions highly different from their native habitat (Gomez-Zlata et al. 2006; Baldo et al. 2008; Kolbe et al. 2013). The presence of ontogenetic variation in invasive species suggests that this characteristic does not necessarily have a negative impact on populations' persistence facing stressful temperatures. Although, in such instances, the acclimation ability of juveniles to novel climatic conditions might be a critical factor in populations' persistence.

Body size has a great influence on lizards' thermal biology and important implications to experimental procedures (Camacho and Rusch 2017; Claunch et al. 2020). Some authors argue that differences between internal and surface temperature are negligible in small-size lizards because blood flow should equilibrate rapidly temperature throughout the body (e.g., Porter et al. 1973). However, our results indicate that this might not be true for all thermal physiological traits. When comparing critical thermal

limits measured in different body parts of *Hemidactylus mabouia*, we found that temperatures measured in the abdomen surface were higher than internal cloacal temperatures. In addition, the difference in the critical thermal minimum due to heterothermy was enough to alter the results of the comparison between Amazon and Cerrado populations.

When comparing abdomen temperatures from both populations, we observed that Amazon populations of *Hemidactylus mabouia* exhibit lower preferred temperature, critical thermal minimum, and critical thermal maximum compared to the Cerrado population. High physiological lability is advantageous during colonization and enables local adaptation to geographic gradients (Rodríguez-Serrano et al. 2009; Kelly 2019). For example, downward shifts in the thermal preference and critical thermal minimum are critical factors during the establishment and spread of *H. frenatus*, native from tropical Asia, in temperate regions of Australia (Lapwong et al. 2020, 2021). We suggest that physiological lability might be equally important during the invasion process of *H. mabouia*.

In natural environments, *Hemidactylus mabouia* occurs primarily on open vegetation areas with a high incidence of solar radiation (Rocha et al. 2011). There are no records of this species invasion in tropical rainforests (i.e., Amazon and Atlantic forests), where it occurs only in anthropogenically modified places (Vitt et al. 2008; Rocha and Bergallo 2011; Rocha et al. 2011; Telles et al. 2015). It has been suggested that the high humidity of forests imposes thermal restrictions on the nocturnal activity of *H. mabouia*, hampering the establishment in these areas (Rocha and Bergallo 2011; Rocha et al. 2011; Telles et al. 2015).

Another possible explanation for the lower establishment success rate of *Hemidactylus mabouia* in the Amazon rainforest could be its competitive ability against

native species. In the Amazon, *H. mabouia* and the native gecko *Thecadactylus rapicauda* exhibit similar habits and it appears to occur competitive exclusion between the two in urban areas (Vitt and Zani 1997). *H. mabouia* and *T. rapicauda* show similar preferred temperatures and critical thermal maximum, but the latter shows a much lower critical thermal minimum (Diele-Viegas et al. 2018). Less suitable climatic conditions and a limited capacity of physiological adaptation could cause fitness disadvantages to *H. mabouia*. Influences of climatic conditions on the competitive ability were described in *Lepidodactylus lugubris* and *H. frenatus* (Case et al. 1994). In Pacific islands, *L. lugubris* is displaced from urban areas by *H. frenatus*, but the former remains the most abundant gecko in forested environments, where *H. frenatus* is unable to invade (Case et al. 1994). The competitive superiority of *L. lugubris* in forests appears to be related to its thermal physiology better adapted to cooler and wetter environments, allowing *L. lugubris* to resist the competitive displacement by *H. frenatus* (Case et al. 1994).

Studies show that some species might be benefited from climate warming due to increases in the availability of thermally suitable habitats (Diele-Viegas and Rocha 2018). In the Americas, *Hemidactylus mabouia* occurs from southern Paraguay up to southern Texas (Powell et al. 1998; Fierro-Cabo and Rentfro 2014), and its potential distribution encompasses almost the entire tropical region (Rödder et al. 2008). Cold temperatures appear to be one of the main abiotic factors limiting the dispersal of *H. mabouia* both beyond and within the tropical region. Consequently, temperature increases caused by global warming could favor the invasion of *H. mabouia* in areas that are too cold for its survival, posing an additional threat to native geckos besides the temperature increase.

In conclusion, the high intraspecific variation of thermal physiological traits observed in *Hemidactylus mabouia* suggests a labile thermal physiology. Although more studies are necessary, especially analyzing more populations, our results indicate that

physiological lability should have an important role in the invasion process of *H. mabouia* and enables the colonization of areas with different climatic conditions. This physiological lability along with long-distance dispersal should render *H. mabouia* less vulnerable to climate change. *H. mabouia* could even be benefited from climate warming if it increases the availability of thermally suitable habitats. Future studies analyzing acclimation capacity will be essential to further understand the impacts of climate change in this species. Considering its broad geographical range and its threat to native geckos, a better comprehension of *H. mabouia*'s biology is important for biodiversity conservation in many areas.

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Capítulo II

Varição morfológica, ontogenia pós-natal e alometria em

***Hemidactylus mabouia* (Moreau de Jonnés 1818)**

Evaluating the drivers and engines of morphological diversification in the invasive gecko *Hemidactylus mabouia* (Moreau de Jonnès 1818)

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Abstract

Biological invasions provide the unique opportunity to observe evolutionary processes in natural populations, making invasive species good model organisms to study evolution. We described the morphological variation in the gecko *Hemidactylus mabouia*, an extremely successful invader, between populations distributed across a wide range in Brazil. We found high morphological variability between populations, particularly in forearm and foot digit IV length. Then, we compared indexes of morphological differentiation (P_{ST}) with divergence at neutral genetic loci (F_{ST}). Although a $P_{ST} > F_{ST}$ is regarded as a signal of directional selection, we were unable to find possible environmental factors that could be driving the morphological divergence. Further, we described skeletal post-natal ontogeny to assess which periods during development are most labile, thus more likely to generate morphological change. For most limb and skull traits, allometric trajectories remain the same throughout the post-natal development. As populations do not differ in their allometric coefficient, only in the intercept, differences in the relative size of head and limb traits likely appeared before hatching. Additionally, the geometric morphometric analysis showed that most of the within-population variation

in skull shape is explained by ontogeny. Heterochronic shifts have a prominent role in generating diversity in skull osteology in Gekkota through the extension or reduction of the terminal ossification process. Unexpectedly, we observed that adults of *H. mabouia* exhibit the supraoccipital, otooccipital, and prootic unfused, a condition considered a pedomorphic character in Squamata.

Key Words

Allometry, Intraspecific variation; Limb; Lizard; Microevolution; Morphometrics; Ontogeny; Skull.

1. Introduction

Invasive species are interesting to study because they enable the observation of evolutionary processes in natural populations, almost as replicate experiments (Sax et al. 2007). Non-native populations during the dispersal phase pass through multiple establishment events where they must survive and reproduce over many generations (Blackburn et al. 2011). The probability of local extinction of a colonizing population is determined by a conjunction of factors, such as initial population size, rate of population decline, and evolutionary potential (Reznick and Ghalambor 2001). Exotic populations often experience environmental conditions different from their native habitat, and rapid evolution (i.e., at observable time scales) appears to be common in biological invasions (Reznick and Ghalambor 2001; Whitney and Gabler 2008). Evolutionary change can arise as a response to natural selection, generating local adaptation (e.g., Clegg et al. 2002; Rasner et al. 2004; Kolbe et al. 2007), or due to plastic responses to environmental cues or stressful conditions (Ghalambor et al. 2007; Crispo 2008; Lande 2009). Additionally, the phenotypic divergence between populations also can be the result of random processes, as genetic drift (Lande 1976)—which could be particularly severe if population size is small over many generations (Sakai et al. 2001).

Morphological traits are closely related to ecology and performance, and they are frequently under divergent selection during colonization of novel habitats (Merilä and Crnokrak 2001; Leinonen et al. 2008). Thus, high morphological lability should increase the chances of invasion success. As morphological variation arises via changes in development, the comprehension of ontogenetic processes is crucial for understanding how biodiversity is originated (Raff 1996). The development acts as a channeling force that determines the variation upon which selection will act, consequently it has a major influence on the direction of the morphological change (Salazar-Ciudad 2006; Uller et al. 2018). Allometric relations represent the sum of all molecular and cellular processes involved during development and are a powerful tool to evaluate the ontogenetic bases of variation (Gould 1966; Alberch et al. 1979; Klingenberg 1998; Sanger et al. 2013). Evolutionary shifts can occur in the onset, offset, and/or rate of development, which are visualized in allometric curves as changes in the intercept and slope (Gould 1966; Alberch et al. 1979). Therefore, by comparing allometric curves, we are able to evaluate when variation arises during development and thereby the periods most labile (e.g., Sanger et al. 2012, Feiner et al. 2021).

Moreover, because macroevolutionary patterns can be difficult to interpret due to the accumulation of additional changes, a better comprehension of the processes generating intraspecific variation may help clarify the origins of diversity at deeper time scales (Sanger et al. 2011). Intraspecific variation was proposed to be the middle ground between development and macroevolution once it serves as the raw material for selection (Hanken and Hall 1993; Abouheif 2008; Sanger et al. 2011). Developmental dynamics and genetic cascades usually are conserved within species or between closely related species and may or not be remodeled after evolutionary changes in morphology (Klingenberg and Zimmermann 1992; Calsbeek et al. 2007; Piras et al. 2011; Sanger et

al. 2013; Urošević et al. 2013; Poe et al. 2021). Considering the utility of studying how the intraspecific variation arises, valuable insights may come from invasive species.

The gecko *Hemidactylus mabouia* is originally from the sub-Saharan Africa and was able to colonize a large portion of the Americas (Kluge 1969; Powell et al. 1998), being the most successful species among invasive lizards (Bomford et al. 2009). In Brazil, *H. mabouia* was able to establish in anthropic areas all over the territory as well as in a diversity of natural environments (Rocha et al. 2011). Within the genus *Hemidactylus*, some morphological characteristics exhibit high variability between species, such as body size, proportions of head, limbs, and tail, number and size of femoral pores in males, size and number of adhesive toe pads—particularly in foot digits I and IV—, presence or absence of the distal section of digit I, color, and pholidosis (for more details see Carranza and Arnold 2006). Despite the huge diversity and abundance of some species of the genus *Hemidactylus* (Carranza and Arnold 2006) there is still a lack of knowledge on their morphological evolution.

Our objectives in this work were: (1) evaluate the morphological lability of *Hemidactylus mabouia* by describing the variation in external morphometric traits among invasive populations distributed across a broad area in Brazil; (2) assess the possible factors driving diversification by comparing the extent of morphological differentiation with genetic divergence, climatic conditions, temperature variables, and geographical distance; (3) describe post-natal ontogeny of skull and limb bones and their allometric growth pattern; (4) infer which points of development are more liable and most likely to generate morphological variation. With this, we aim to better understand the factors and mechanisms driving morphological diversification in *H. mabouia*, and also demonstrate the potential use of invasive species and the study of intraspecific variation for a better understanding of evolutionary processes.

2. Materials and Methods

2.1. Animal collection

For individuals of *Hemidactylus mabouia* from the Distrito Federal, we collected lizards by active search and captured them by hand in urban areas of Brasilia, Brazil (-15.7801, -47.9292) between March 2019 and March 2021. Collections were approved by Chico Mendes Institute for Biodiversity Conservation (ICMBio, n° 0632260220190624). We euthanized captured lizards by Neocaine overdose via intraperitoneal injection, as suggested by the National Council for Animal Experimentation Control (CONCEA). Then, we fixed lizards in a solution of 10% formaldehyde buffered with sodium chloride (NaCl) for 2–3 days and conserved in 70% alcohol. Specimens were deposited in the scientific collection of the Laboratory of Comparative Vertebrate Anatomy, Department of Physiological Sciences, Institute of Biological Sciences, University of Brasilia.

We obtained specimens from other five populations of *H. mabouia* inhabiting urban areas in Brazil—Feira de Santana (-12.2635, -38.9299) and Salvador (-13.0002, -38.5100), Bahia; Recife, Pernambuco (-8.0509, -34.9485); Rio de Janeiro, Rio de Janeiro (-22.9099, -43.2361); and Palmas, Tocantins (-10.2034, -48.3488)—from the scientific collection of the Laboratory of Genetics and Biodiversity, Department of Genetics and Morphology, Institute of Biological Sciences, University of Brasilia.

We considered geckos with SVL higher than 46 mm as adults, following Anjos and Rocha (2008), and neonates were born in the laboratory from eggs found on the field. We sexed specimens by hemipenis eversion before fixing or by gonad observation. Voucher numbers of all specimens examined can be found in Appendix I.

2.2. Variation between populations

2.2.1. Morphological data

We measured a total of 134 specimens of *H. mabouia* from six invasive populations: Salvador (BA1, n = 17), Feira de Santana (BA2, n = 16), Brasilia (DF, n = 42), Recife (PE, n = 16), Rio de Janeiro (RJ, n = 16), and Palmas (TO, n = 27) (fig. 1A). To analyze morphological variation between populations of *H. mabouia*, we measured ten morphometric characters—SVL, thigh length, shank length, foot digit IV length, arm length, forearm length, hand digit IV length, head length, head width, snout length—using a digital caliper (fig. 1B). We measured the right side, however, in some specimens the right structure was damaged, in these cases we measured the left side. Measurements were made by the same person (J.L.) to avoid error between observers and we measured specimens twice to evaluate measurement repeatability.

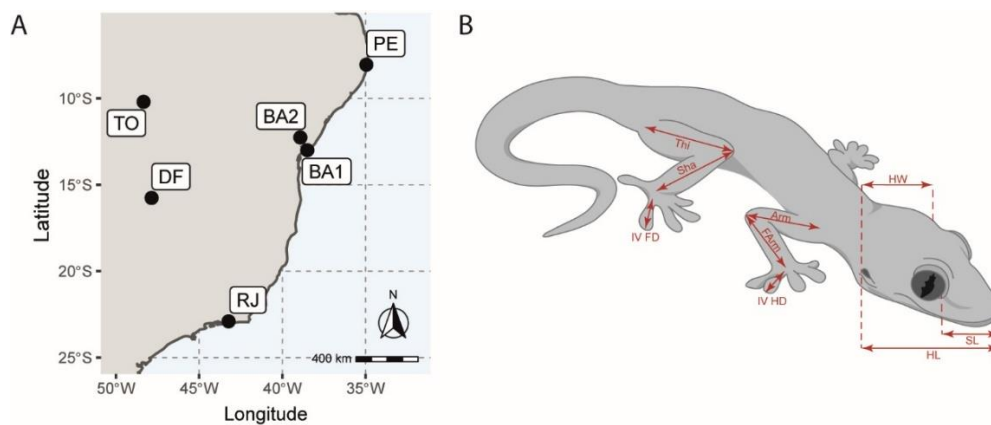


Figure 1. (A) Location of the sampled populations; BA1, Salvador, Bahia; BA2, Feira de Santana, Bahia; DF, Brasilia, Distrito Federal; PE, Recife, Pernambuco; RJ, Rio de Janeiro, Rio de Janeiro; TO, Palmas, Tocantins. (B) Morphometric characters measured; IV FD, foot digit IV length; IV HD, hand digit IV length; FArm, forearm length; HL, head length; HW, head width; Sha, shank length; SL, snout length; Thi, thigh length.

2.2.2. Genetic data

We used the F_{ST} as a measure of neutral genetic distance, which we obtained along with the geographical distances for the populations we analyzed morphological variation from Pontes (2017). Except for the population from Brasilia (DF), for all the other populations we used exactly the same set of individuals from the genetic analysis in the morphological analysis. F_{ST} was estimated by Pontes (2017) using 10 microsatellite loci,

which were isolated using the primers developed by Short and Petren (2008). From the 12 microsatellite markers characterized by Short and Petren (2008), Pontes (2017) excluded two loci from the analysis, Hm 136 and Hm 56, after tests of linkage disequilibrium, frequency of null alleles, and assessment of genotyping error. All pairwise F_{ST} estimates were statistically significant, indicating genetic differentiation among populations (Pontes, 2017).

2.2.3. *Environmental data*

To assess the influence of environmental conditions on the morphological variation, we obtained climatic data from the National Institute of Meteorology (INMET, <https://bdmep.inmet.gov.br/>) for the meteorological stations of Salvador, Feira de Santana, Brasilia, Recife, and Rio de Janeiro from 1965 until 2021, and of Palmas from 1993 until 2021. We used monthly mean values of mean temperature, maximum temperature, minimum temperature, temperature amplitude, insolation, relative humidity, total precipitation, and number of days with precipitation.

2.2.4. *Data analyses*

We used R 3.6.3 (R Core Team 2013) and RStudio 1.2.5019 (RStudio Team 2015) for the statistical analyses. To evaluate measurement repeatability, we used the intraclass correlation coefficient (ICC) (Lessells and Boag 1987) implemented in the “irr” package (Gamer et al. 2019). For all subsequent analyses, we used the natural log transformed mean between the two replicate measurements. First, we compared SVL using a two-way ANOVA with population and sex as factors, as sex had no effect (see results), we performed a post-hoc Tukey test on pooled males and females. To describe variation in the other traits, we used a MANCOVA with SVL as a covariate to analyze if there was overall difference between populations of *H. mabouia* followed by ANCOVAs to

evaluate which specific traits differed between populations. We used pooled sexes in the former analyses, but to assess the effect of sexual dimorphism, we performed two-way ANOVAs using the residuals of the linear regression between each trait and SVL as the response variable and sex and population as factors. As *H. mabouia* exhibited low levels of sexual dimorphism, which did not influence the results of the between population variation (see results), we used the pooled sexes sample for the rest of the analyses. To reduce the dimensionality and obtain independent variables, we used a principal component analysis (PCA) using size-corrected values of head and limb traits except for head width. We were unable to measure head width in some specimens once they were used to obtain brain tissue and the abductor muscle of the mandibula was damaged. To maintain statistical power, we opted for removing head width as the PCA including it showed that this trait was highly correlated with head length and removing it did not influence the results (results not shown). We used a MANOVA followed by ANOVAs to evaluate populational differences in each principal component (PC).

We used the P_{ST} - F_{ST} approach to assess possible evolutionary processes underlying morphological variation in *Hemidactylus mabouia*. The P_{ST} quantifies the proportion of among-population phenotypic variation and is used as a proxy for the Q_{ST} , which quantifies the among-population quantitative genetic variation (Spitze 1993). The F_{ST} informs the degree of differentiation at neutral marker loci and indicates the null expectation of differentiation attained by the combined effects of genetic drift and gene flow (Wright 1951; Lande 1992). If $P_{ST} = F_{ST}$, the pattern of phenotypic variation can be explained solely by random genetic drift; if $P_{ST} > F_{ST}$, phenotypic variation is higher than expected under random drift, and variation could be explained by directional selection; while if $P_{ST} < F_{ST}$, phenotypic variation is lower than expected under drift, suggesting stabilizing selection (Merilä and Crnokrak 2001; Sæther et al. 2007; Whitlock 2008). We

calculated separate pairwise P_{ST} values for the PCs which showed significant differences between populations. The P_{ST} is calculated by the formula:

$$P_{ST} = \sigma_{GB}^2 / (\sigma_{GB}^2 + 2\sigma_{GW}^2)$$

where σ_{GB}^2 is the among-population variance and σ_{GW}^2 is the within-population variance (Spitze 1993), which we obtained from an ANOVA for each PC and pairwise population combination (Raeymaekers et al. 2007; Wojcieszek and Simmons 2012). We tested for correlation between P_{ST} and F_{ST} using a Mantel test with 9999 permutations. Then, we calculated the bootstrap mean and 95% confidence interval for pairwise P_{ST} and F_{ST} values to compare both estimates (Wojcieszek and Simmons 2012).

We also examined if the morphological variation was correlated with environmental factors and if it was geographically structured. We performed a linear discriminant analysis (LDA) with the morphological data and calculated the Mahalanobis distance between populations. For the environmental data, we performed a PCA for all climatic variables combined and for temperature variables separately, then we calculated the Mahalanobis distance between localities considering climatic and temperature information. Finally, we tested for correlations between the morphological distance matrix and the climatic, temperature, and geographical distance matrices using Mantel tests with 9999 permutations.

2.3. Ontogeny

2.3.1. Specimen preparation

We diaphonized specimens from the Distrito Federal to analyze the post-natal ontogeny of skeletal traits. We cleared and stained for bones and cartilage using alizarin red and alcian blue, respectively, following the protocol of Wassersug (1976) with modifications: we performed the dehydration step in absolute alcohol in 24 h with three

solution changes, two within 4 h and one overnight; then, before clearing, we immersed specimens in 70% alcohol for 1 h and after in sodium tetraborate 0.5% also with three solution changes, two within 2 h and one overnight. Bones were identified following Daza et al. (2008).

2.3.2. *Skull geometric morphometrics*

We used geometric morphometrics to analyze sexual dimorphism and the morphological variation of the skull during ontogeny in 60 specimens (30 males and 30 females including adults and juveniles). We photographed diaphonized lizards with a digital camera fixed in a stand to assure a parallel position and at a constant distance in relation to the specimen to minimize the error due to distortion (Mullin and Taylor 2002). We used 30 landmarks in both sides of the skull in the dorsal view (Appendix II Table A1, fig. 2A). We chose landmarks based on a previous study in geckos (Daza et al. 2009), their presence in all specimens, and their ability to explain possible morphological variations. We used the program TpsUtil 1.4 (Rohlf 2008) to generate the file in the tps format and TpsDig 2.16 (Rohlf 2010) to digitize the landmarks. Digitization was performed by the same person (J.L.) to avoid error between observers. For the shape analyses, we used the program MorphoJ 2.0 (Klingenberg 2011). We performed a generalized Procrustes analysis (GPA) to remove the effects of position, scale, and rotation (Rohlf and Slice 1990) considering object symmetry (Klingenberg et al. 2002) and we generated the covariance matrix using only the symmetric component of the shape variation. To analyze the shape variation between groups, we performed a PCA of the Procrustes coordinates. We also performed a regression between the shape variable and centroid size to evaluate the effects of allometry in the variation. Finally, we used Procrustes ANOVA to test differences between adult males and females in the skull shape and centroid size in order to assess the presence of sexual dimorphism.

2.3.3. *Linear morphometrics*

For 63 individuals (32 males and 31 females including adults and juveniles), we made 10 measurements of limb bones length: femur, tibia, fibula, metatarsus IV, foot digit IV phalanges, humerus, radius, ulna, metacarpus IV, hand digit IV phalanges. We measured the right side, however, in a few specimens the right structure was damaged, then in these cases we measured the left side. We also used linear measurements of the skull of 73 individuals (35 males and 38 females including adults and juveniles) to describe the allometric patterns of different parts of the skull. For this, we made six measures on the dorsal view—skull length, skull width, snout length, snout width, interorbital distance, frontoparietal width—and two on the ventral view—mandible length and mandible width (Appendix II Table A2, fig. 2B). The only unilateral measurement was snout length, which we measured always on the same side. We measured the SVL using a caliper and the linear measurements of the skull and limbs from photographs using the software ImageJ 1.46 (Schneider et al. 2012). All measurements were made by the same person (J.L.) to avoid error between observers.

We used R 3.6.3 (R Core Team 2013) and RStudio 1.2.5019 (RStudio Team 2015) for statistical analyses. We randomly measured ten specimens twice and evaluated measurement repeatability using the intraclass correlation coefficient (ICC) (Lessells and Boag 1987) implemented in the “irr” package (Gamer et al. 2019). Measures were natural log transformed prior to all subsequent analyses. To compare ontogenetic trajectories between males and females we performed ANCOVAs for each trait using SVL as covariate. For the skull width, snout length, snout width, interorbital distance, and frontoparietal width we also performed ANCOVAs with skull length as a covariate. Additionally, we performed a PCA, for limb and skull measures separately, using the residuals from the regression between each trait and the SVL. After, we used a MANOVA

to test for differences between sexes and ontogenetic stages in the first two PC axes. To analyze the ontogenetic allometry, we used the slope of the linear regression of each trait in relation to the SVL as an estimate of the allometric coefficient, which we tested for difference from isometry (i.e., slope = 1) using the function “sma” implemented in the package “Smatr” (Warton et al. 2012). We also evaluated if ontogenetic stages differ in their allometric trajectory by performing ANCOVAs with stage as a variable and SVL as the covariate. For the traits which the slope differed between adults and juveniles, we tested for allometry in each curve separately.

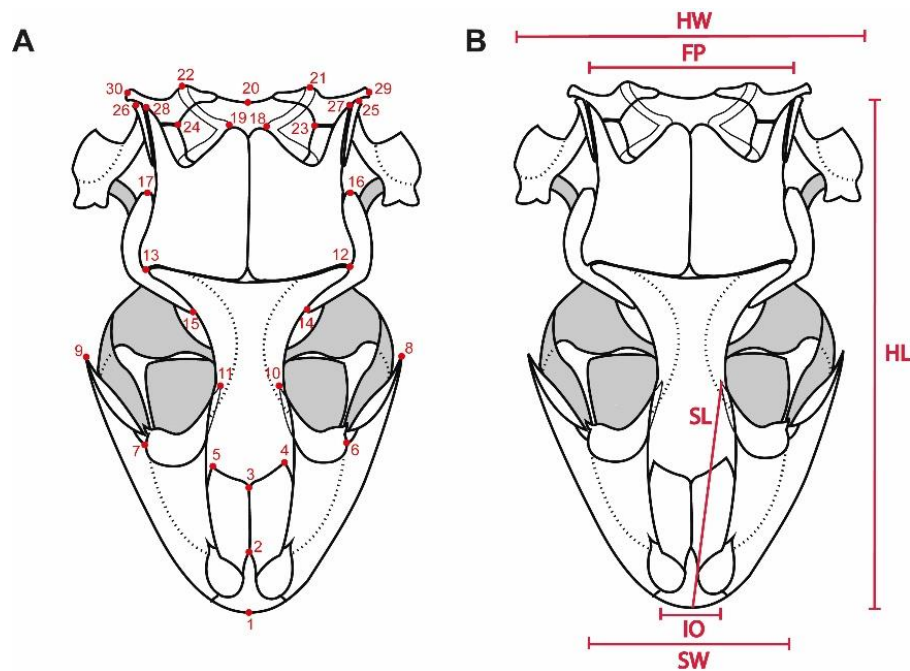


Figure 2. Illustrations of the skull of an adult of *Hemidactylus mabouia* with the landmarks used in the geometric morphometric analysis (A) and with the linear measures in the dorsal view (B). FP, frontoparietal width; HL, skull length; HW, skull width; IO, interorbital distance; SL, snout length; SW, snout width.

3. Results

3.1. Variation between populations

All measures were highly repeatable with ICC > 0.90 ($p < 0.001$) with the exception of the hand digit IV, which showed an ICC of 0.83. The two-way ANOVA found a significant difference in SVL between populations ($F_{5,122} = 2.80$, $p = 0.02$) but

not between sexes ($F_{1,122} = 0.63, p = 0.43$) nor an interaction between sex and population ($F_{5,122} = 0.58, p = 0.72$). However, when performing a Tukey test, DF and BA1 was the only pair that differed in SVL ($p < 0.01$). For the other traits, the MANCOVA with SVL as a covariate also found a significant difference between populations (Pillai's Trace = 1.55, $F_{40,580} = 6.51, p < 0.0001$). ANCOVAs showed that the traits that differ between populations are the foot digit IV, arm, forearm, head length, head width, and snout length—which differ only in the intercept but not in slope (i.e., there is no interaction effect between SVL and population) (Table 1, fig. 3). Two-way ANOVAs using size-corrected values and population and sex as factors showed that, although some traits present sexual dimorphism, there is no populational divergence in sexual dimorphism, and it does not influence the results of population comparisons (Table 2, Appendix III fig. A1). So, we continued all subsequent analysis with pooled sexes.

Table 1. ANCOVA results comparing morphometric traits between populations and ICC for each trait.

Trait	ICC	Population		Population:SVL		df
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	
Thigh	0.95	1.16	0.33	0.37	0.86	122
Shank	0.95	0.71	0.61	0.58	0.71	122
Foot digit IV	0.90	23.69	< 0.0001	0.30	0.91	118
Arm	0.94	7.43	< 0.0001	0.87	0.50	119
Forearm	0.94	16.66	< 0.0001	1.44	0.21	122
Hand digit IV	0.83	1.83	0.11	0.67	0.64	120
Head length	0.98	3.78	< 0.01	1.17	0.33	122
Head width	0.97	7.06	< 0.0001	0.38	0.86	122
Snout length	0.94	5.09	< 0.001	0.63	0.67	122

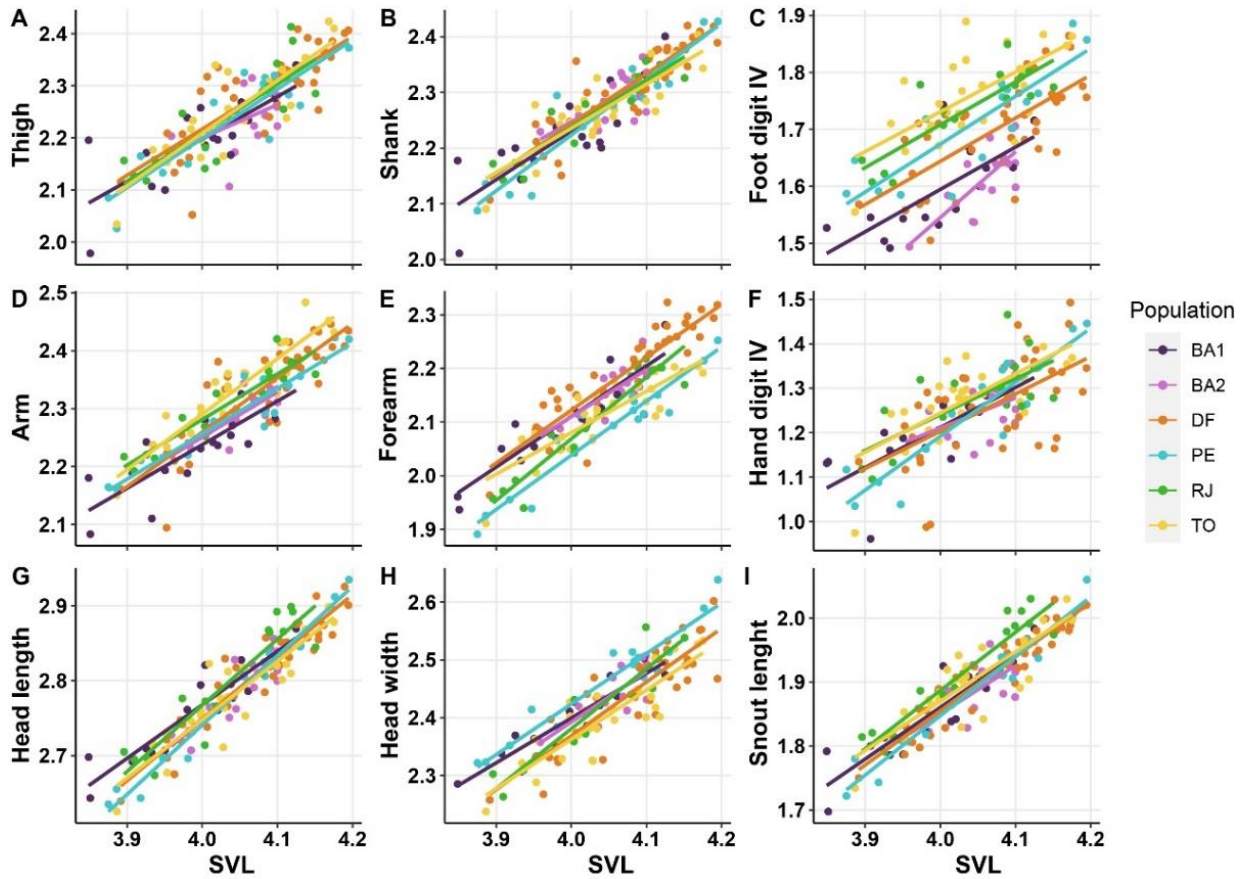


Figure 3. Regressions between traits and SVL for populations of *Hemidactylus mabouia*. BA1, Salvador; BA2, Feira de Santana; DF, Brasilia; PE, Recife; RJ, Rio de Janeiro; TO, Palmas.

Table 2. Two-way ANOVAs using size-corrected trait values testing the effects of population, sex, and their interaction.

Trait	Population		Sex		Population:Sex		df
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	
Thigh	1.16	0.33	7.68	< 0.01	0.99	0.43	114
Shank	1.24	0.29	18.08	< 0.001	0.78	0.56	114
Foot digit IV	24.31	< 0.0001	7.19	< 0.01	0.94	0.46	114
Arm	6.39	< 0.001	25.36	< 0.0001	0.52	0.76	114
Forearm	15.71	< 0.0001	0.94	0.33	0.42	0.83	114
Hand digit IV	1.57	0.17	5.58	0.02	2.20	0.06	114
Head length	3.83	< 0.01	1.08	0.30	0.76	0.58	114
Head width	9.05	< 0.0001	14.75	< 0.001	0.67	0.65	108
Snout length	4.73	< 0.001	1.37	0.24	0.51	0.77	114

The MANOVA using the eight PCs obtained from the PCA found a significant difference between populations (Pillai's Trace = 1.26, $F_{40,585} = 4.92$, $p < 0.0001$), and the ANOVA performed for each PC separately showed that populations differ in the PC1

($F_{5,120} = 3.07, p = 0.01$), PC2 ($F_{5,120} = 33.97, p < 0.0001$), PC3 ($F_{5,120} = 5.16, p < 0.001$), PC5 ($F_{5,120} = 2.72, p = 0.02$), and PC7 ($F_{5,120} = 4.36, p < 0.01$), but not in the PC4 ($F_{5,120} = 1.08, p = 37$), PC6 ($F_{5,120} = 1.96, p = 0.09$), nor PC8 ($F_{5,120} = 0.19, p = 0.97$) (fig. 4). The PC2 is mostly related to variation in the foot digit IV and forearm and separates populations in two clear groups: one formed by BA1, BA2, and DF, and other formed by PE, RJ, and TO (fig. 5, Appendix III fig. A2). Accordingly, the regressions in figure 3 also indicate that the foot digit IV and forearm are the traits with largest divergence between populations, the first being the most noteworthy.

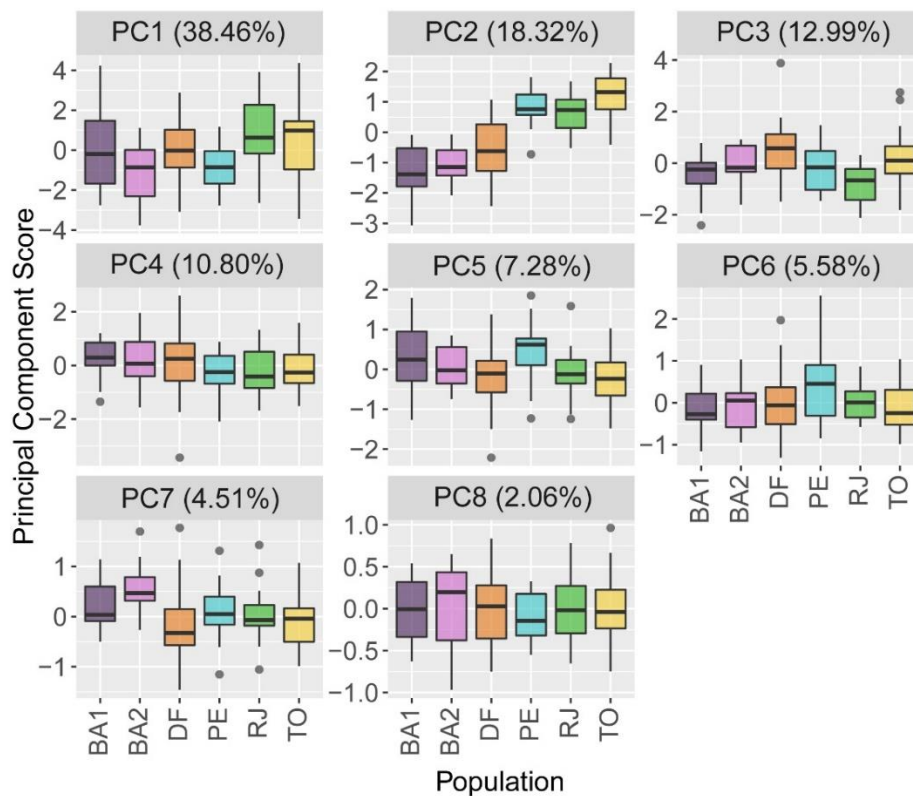


Figure 4. Boxplots showing the difference in PC score between populations of *Hemidactylus mabouia* and relative percentage of the total variation explained by each PC. BA1, Salvador; BA2, Feira de Santana; DF, Brasilia; PE, Recife; RJ, Rio de Janeiro; TO, Palmas. Lower and upper box limits represent, respectively, first and third quartiles, internal bar the median, and whiskers the minimum and maximal values inside the 99% interval.

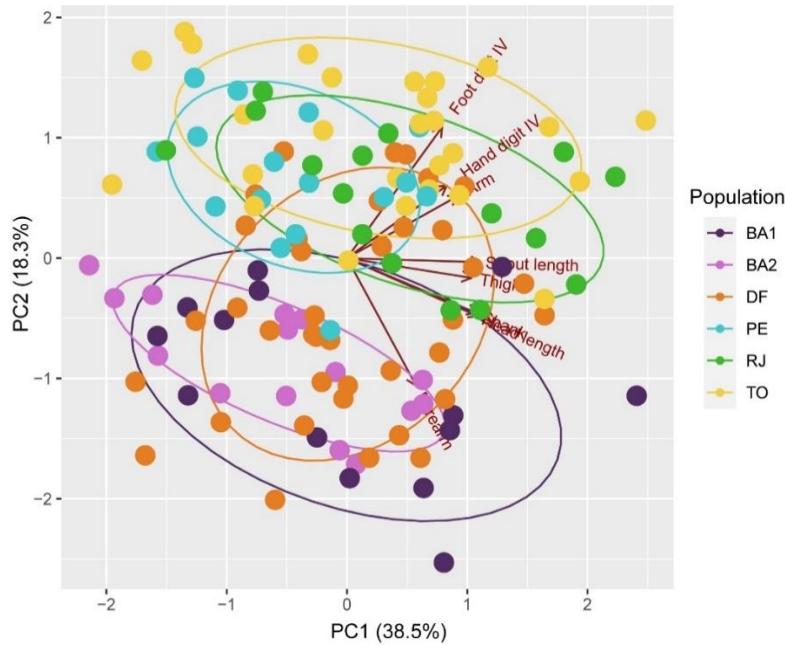


Figure 5. Plot between the first two axes from the principal component analysis with loading vectors for each trait analyzed. BA1, Salvador; BA2, Feira de Santana; DF, Brasilia; PE, Recife; RJ, Rio de Janeiro; TO, Palmas.

The Mantel test demonstrated that the pairwise P_{ST} estimates are not correlated with the F_{ST} for any of the PCs (PC1: $r = -0.29$, $p = 0.86$; PC2: $r = 0.01$, $p = 0.50$; PC3: $r = 0.11$, $p = 0.37$; PC5: $r = 0.17$, $p = 0.28$; PC7: $r = -0.55$, $p = 0.94$). In addition, pairwise P_{ST} values for all PCs did not fall upon the line of neutrality, as would be expected in a scenario of genetic drift (fig. 6). Besides, none of the P_{ST} values were within the range expected due to drift alone and the confidence limits calculated by bootstrap for all the P_{ST} estimates were higher than confidence limits of the F_{ST} (Table 3). Thereby suggesting that morphological differentiation among populations is higher than expected under random genetic drift.

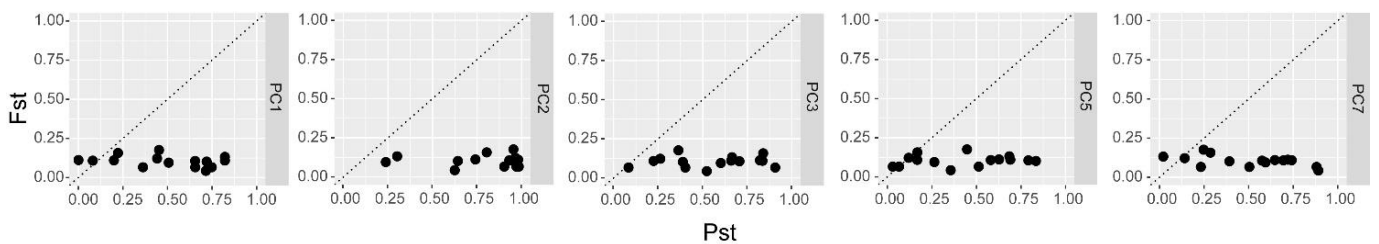


Figure 6. Population differentiation in morphology, P_{ST} , for each PC compared to neutral genetic differentiation, F_{ST} . Dashed lines represent the line of neutrality (i.e., $P_{ST} = F_{ST}$), when phenotypic variation could be explained solely by random genetic drift.

Table 3. Mean, standard error (SE), and 95% confidence interval limits calculated by bootstrap for pairwise F_{ST} and P_{ST} for each principal component (PC).

	Mean	SE	Lower 95% limit	Upper 95% limit
Pairwise F_{ST}	0.105	0.009	0.087	0.122
Pairwise P_{ST}				
PC1	0.493	0.068	0.361	0.627
PC2	0.799	0.060	0.679	0.916
PC3	0.549	0.063	0.432	0.678
PC5	0.422	0.070	0.288	0.562
PC7	0.505	0.070	0.367	0.641

The linear discriminant function also showed a significant difference between populations in morphology (Wilk's $\lambda = 0.14$, $F = 6.16$, $p < 0.0001$) and the first two axes explained, respectively, 70.15% and 18.42% of the total variation (fig. 7). The morphological distance calculated from the linear discriminants was not correlated with climatic distance (Mantel test, $r = -0.10$, $p = 0.54$), temperature distance (Mantel test, $r = -0.14$, $p = 0.69$), nor geographical distance (Mantel test, $r = -0.12$, $p = 0.63$).

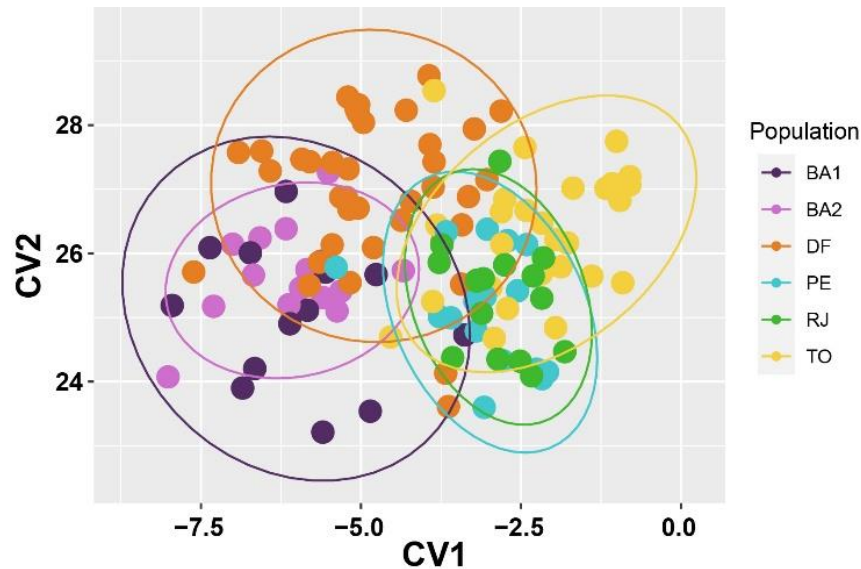


Figure 7. Plot between the first two axes from the linear discriminant function. BA1, Salvador; BA2, Feira de Santana; DF, Brasilia; PE, Recife; RJ, Rio de Janeiro; TO, Palmas.

3.2. Ontogeny

3.2.1. Post-natal skull ontogeny

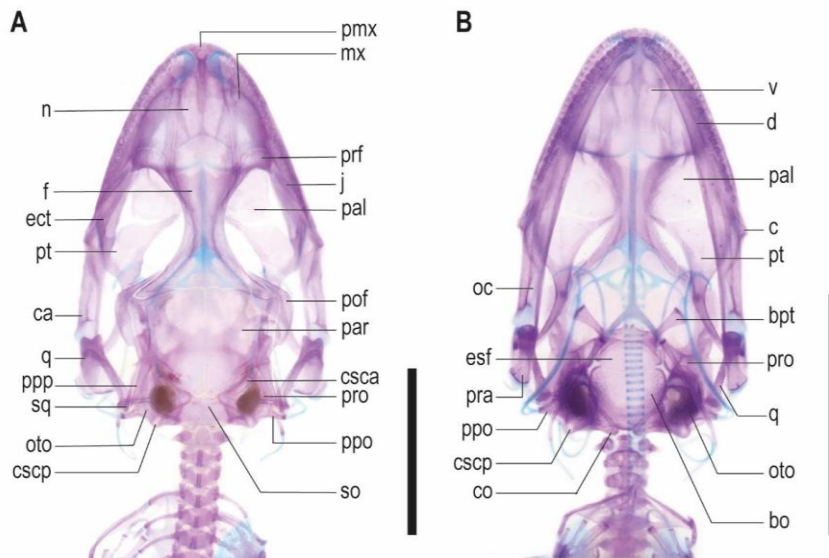


Figure 8. Identification of skull bones in two juveniles of *Hemidactylus mabouia* cleared and stained for bone and cartilage in dorsal (LACV_3508) (A) and ventral view (LACV_3512) (B). Scale bar = 5 mm. bo, basioccipital; bpt, basipterygoid process; c, coronoid; ca, articular condyle; co, occipital condyle; cscap, anterior semicircular canal; cscp, posterior semicircular canal; d, dentary; ect, ectopterygoid; esf, sphenoid; f, frontal; j, jugal; mx, maxilla; n, nasal; oc, compound bone; oto, otooccipital; pal, palatine; par, parietal; pmx, premaxilla; pof, postorbitofrontal; ppo, paroccipital process; ppp, postparietal process; pra, retroarticular process; prf, prefrontal; pro, prootic; pt, pterygoid; q, quadrate; so, supraoccipital; sq, squamosal; v, vomer.

Bone identification can be seen in figure 8. In neonates of *Hemidactylus mabouia*, the braincase bones are widely separated by cartilage, except the sphenoid and basioccipital, which are separated by a narrow fenestra (fig. 9A, fig. 10A). The postparietal process does not reach the anterior margin of the otooccipital, enabling the view of the prootic dorsally (fig. 9A). Individuals of *H. mabouia* are born with a poorly ossified dermatocranium: only the lateral and posterior margins of the parietal are ossified, and the posterior margin of the frontal is also unossified, delineating a large parietal fontanelle (fig. 9A). The anterior margin of the frontal is unfused medially and this bone does not contact the nasals (fig. 9B), and more rostrally, the nasals are also not in contact with the premaxilla in hatchlings (fig. 9B). The ossification of the rostral region of the skull seems to end shortly after hatching (fig. 9C). The posterior region of the

frontal, however, ossifies more slowly, advancing posteriorly until it reaches the parietals to form the frontoparietal suture (fig. 9C–F). During post-natal development, the anterior and posterior ossification fields of the parietal progress medially until both sides meet in the midline (fig. 9C–F). In most adults it is possible to observe the separation of bones in the otooccipital region (fig. 9H). Skull development appears to continue after sexual maturity. Near the maximum SVL found, we observed in some individuals the fusion between the supraoccipital and otooccipital, and the supraoccipital and prootic, while the suture between the otooccipital and prootic remains (fig. 9I).

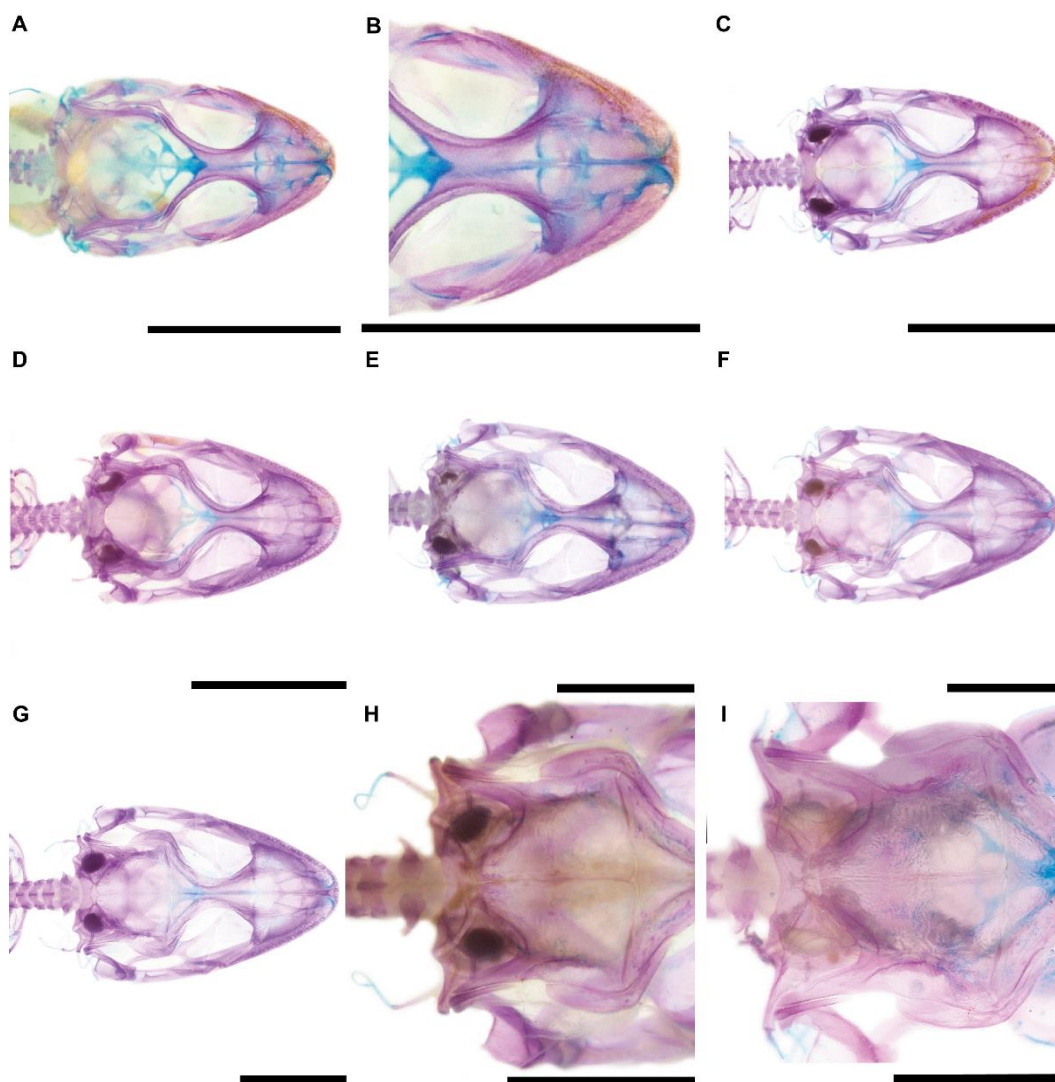


Figure 9. Post-natal ossification sequence of the skull of *Hemidactylus mabouia* in dorsal view. (A) SVL 21.7 mm (LACV_E_0125, neonate) and in (B) details of the skull showing incomplete ossification in the rostrum; (C) SVL 22 mm (LACV_3512, juvenile); (D) SVL 25 mm

(LACV_3509, juvenile); (E) SVL 29 mm (LACV_3544, juvenile); (F) SVL 30 mm (LACV_3508, juvenile); (G) SVL 35 mm (LACV_3507, juvenile); (H) SVL 52 mm (LACV_3334, adult) details of the skull showing unfused elements of the otooccipital region; (I) SVL 63 mm (LACV_JL_17, adult), details of the skull showing the fusion between the otooccipital and supraoccipital, and prootic and supraoccipital, while the suture between the otooccipital and prootic remains visible. Scale bar = 5 mm.

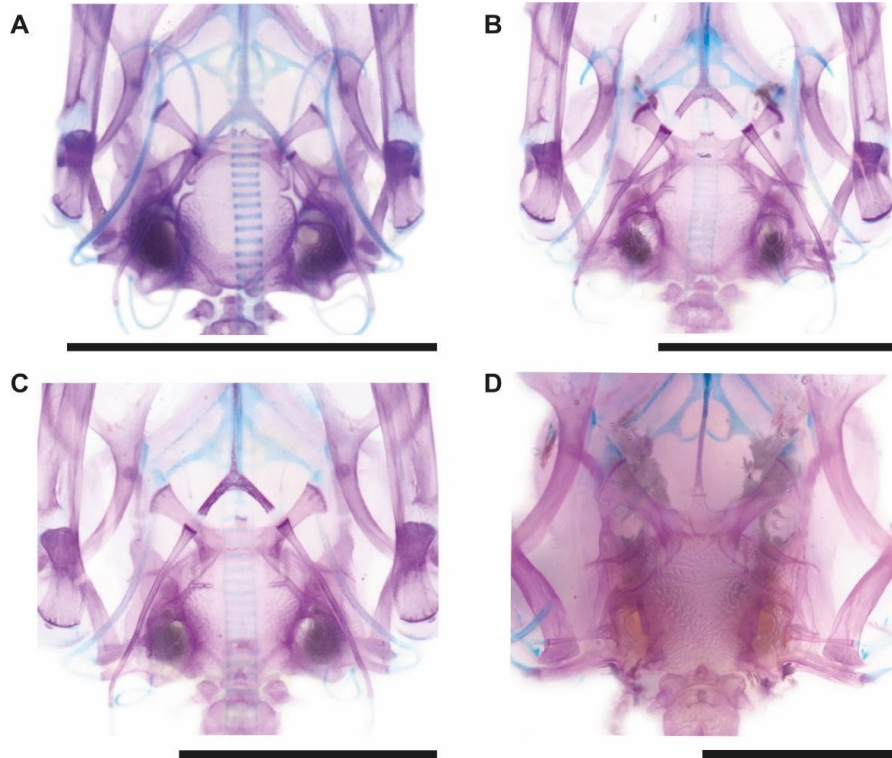


Figure 10. Post-natal ossification sequence of the basicranium of *Hemidactylus mabouia*. (A) SVL 22 mm (LACV_3512, juvenile); (B) SVL 35 mm (LACV_3508, juvenile); (C) SVL 41 mm (LACV_3507, juvenile); (D) SVL 63 mm (LACV_JL_17, adult). Scale bar = 5 mm.

3.2.2. Skull geometric morphometrics

In the PCA performed with the symmetrical component of the variation, the first two PCs explained together 72.46% of the total variation. Specifically, the PC1 explained 60.64 % of the variation and the PC2 11.82%. Plotting the PC1 versus PC2, there is no visual separation between males and females in the morphospace. This pattern was confirmed by the Procrustes ANOVA, which did not find significant shape differences between adult males and females ($F_{28,840} = 1.14, p = 0.28$), nor in centroid size ($F_{1,29} = 0.01; p = 0.93$). On the other side, it is possible to observe a clear separation of ontogenetic stages in the morphospace along the PC1, with negative values representing larger lizards

(fig. 11). Indeed, the regression between the shape variable and centroid size was significant ($p < 0.001$) and allometry explained 56.73% of shape variation (fig. 12).

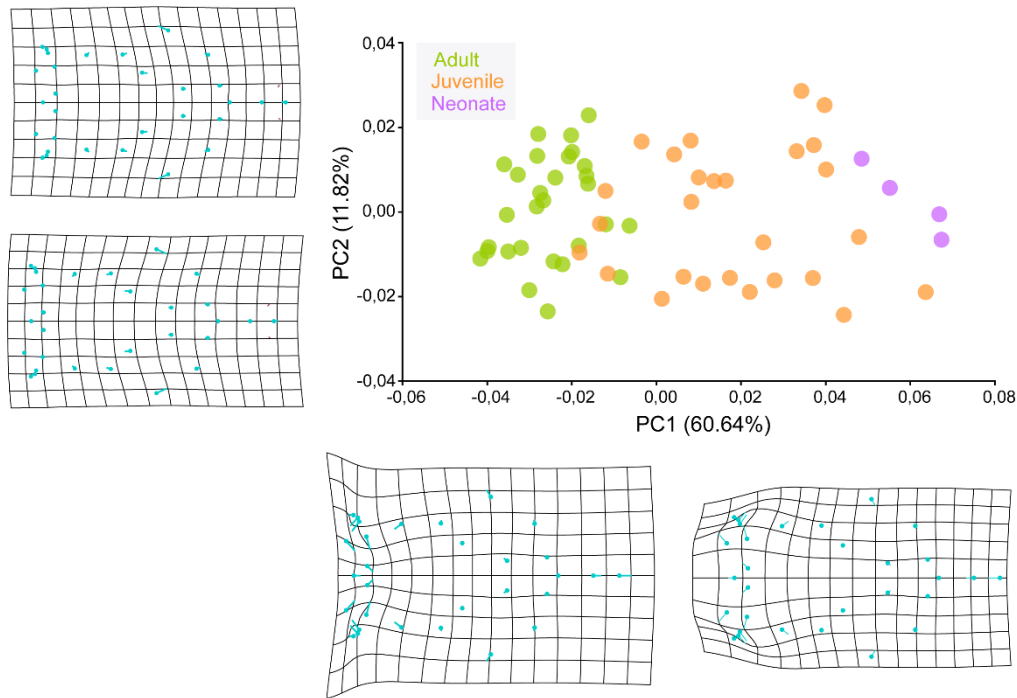


Figure 11. Principal component analysis (PCA) considering the symmetric component of shape variation in *Hemidactylus mabouia*. Deformation grids represent in clockwise order respectively: PC1 at 0.08, PC1 at -0.08, PC2 at -0.04, and PC2 at 0.04.

During post-natal ontogeny occurs the relative elongation of the snout due to the anterior displacement of the anterior edge of the premaxilla and the ascending nasal process of the premaxilla, as well as a subtle posterior displacement of the posterior edge of the jugal. Landmarks at the posterior part of the nasals (3–5), frontal (10–13), and at the anterior part of the postorbitofrontal (14 and 15) show almost no variation during ontogeny, indicating low variation in the middle face and postorbital region with growth (fig. 6). In contrast, the parietals and braincase show considerable changes during ontogeny, being responsible for most of the variation (fig. 11). The posterior edge of the postorbitofrontal (landmarks 16 and 17) displaces posteromedially, accompanying the straightening of the parietal—which passes from a bulbous to a more rectangular form in adults (fig. 11). Landmarks at the edge of the postparietal process (27 and 28), paroccipital

process (29 and 30), and squamosal (25 and 26) also displace posteromedially (fig. 11). Contrarily, the landmarks at the supraoccipital (20), semicircular canals (18–21) and prootic (23 and 24) displace anteromedially, indicating a relative shortening and straightening of the braincase (fig. 11). Changes along the PC2 are subtle and do not seem to have biological meaning (fig. 11).

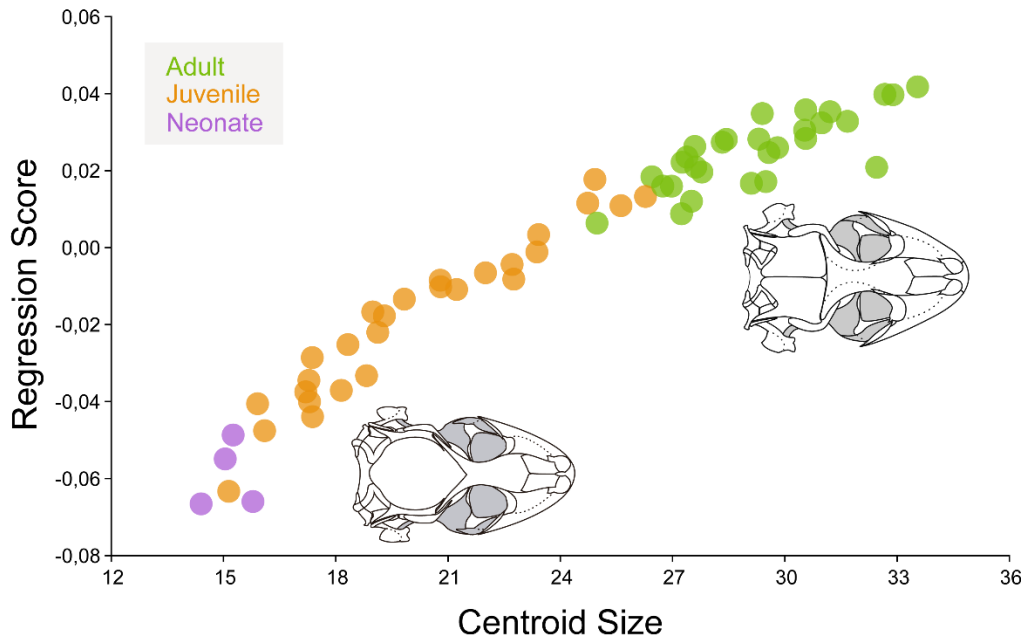


Figure 12. Regression between the shape variable and centroid size.

3.2.3. Linear morphometrics

All linear measurements showed high repeatability ($ICC > 0.90$, $p < 0.01$) (Table 4). Results from the skull linear morphometrics also revealed an overall absence of sexual dimorphism. ANCOVAs using sex as factor and SVL as a covariate showed that males and females do not differ in slope or intercept in all traits except frontoparietal width, which exhibited a marginally significant difference in slope between sexes (Table 4, Appendix III fig. A3). However, ontogenetic curves showed no sexual differentiation when performing ANCOVAs using skull length as the covariate for any trait, including frontoparietal width (Table 5). Results from the ANCOVAs comparing curves between adults and juveniles showed that the interorbital distance is the only measure exhibiting

a change in the ontogenetic trajectory after sexual maturity (Table 4, Appendix III fig. A4). In the PCA for size-corrected skull linear measures, the PC1 explained 74.5% of the variation and the PC2 12.6%. The PC1 is strongly correlated with variation in skull length, skull width, snout length, snout width, frontoparietal width, mandible length, and mandible width, but weakly with interorbital distance (fig. 13). While the PC2 is correlated only with variation in the interorbital distance. A MANOVA using the PC1 and PC2 as response variables showed no difference between males and females (Pillai's Trace = 0.03, $F_{2,69} = 1.00$, $p = 0.37$) nor adults and juveniles (Pillai's Trace = 0.03, $F_{2,69} = 0.93$, $p = 0.40$).

Table 4. Results for the ANCOVAs and intraclass correlation coefficient (ICC) for linear measurements of the skull and limbs of *Hemidactylus mabouia*.

	ICC*	Sex		Sex:SVL		df	Stage		Stage: SVL		df
		F	p	F	p		F	p	F	p	
Femur	1.00	0.40	0.53	0.33	0.56	59	0.02	0.88	4.77	0.03	59
Tibia	1.00	1.09	0.30	0.37	0.55	59	0.001	0.97	0.53	0.47	59
Fibula	1.00	0.06	0.81	2.05	0.16	59	0.24	0.62	0.21	0.65	59
Metatarsus IV	1.00	0.10	0.75	0.38	0.54	59	0.07	0.80	0.04	0.84	59
Foot digit IV phalanges	0.99	5.02	0.03	0.80	0.37	57	1.18	0.28	0.008	0.93	57
Humerus	1.00	0.22	0.64	0.15	0.70	58	0.004	0.95	0.58	0.45	58
Ulna	1.00	0.002	0.97	0.007	0.93	59	2.72	0.10	0.001	0.97	59
Radius	1.00	0.02	0.88	0.66	0.42	59	1.19	0.28	0.06	0.80	59
Metacarpus IV	0.99	0.04	0.84	0.57	0.45	58	2.58	0.11	0.06	0.80	58
Hand digit IV phalanges	0.99	4.48	0.04	2.30	0.13	56	2.75	0.10	1.67	0.20	56
Skull length	0.99	0.59	0.44	1.21	0.28	69	0.24	0.63	0.001	0.97	70
Skull width	0.99	1.98	0.16	0.68	0.41	69	1.98	0.16	0.04	0.85	70
Frontoparietal width	0.98	0.66	0.42	3.85	0.05	69	0.03	0.87	0.01	0.90	70
Interorbital distance	0.94	0.31	0.58	0.02	0.88	69	5.82	0.02	4.27	0.04	70
Snout length	0.98	0.007	0.93	0.14	0.70	69	0.41	0.52	0.14	0.70	70
Snout width	0.97	2.79	0.10	0.70	0.41	69	0.12	0.73	0.49	0.48	70
Mandible length	0.99	2.66	0.11	1.10	0.30	69	0.15	0.7	0.04	0.84	70
Mandible width	0.99	1.42	0.24	0.27	0.60	69	0.0002	0.99	0.0001	0.99	70

*All ICC values were significant with $p < 0.01$.

Tests for allometry revealed that skull length, skull width, snout length, snout width, frontoparietal width, mandible length, and mandible width follow an allometrically

negative growth trajectory in relation to SVL, while the interorbital distance exhibits isometric growth (Table 6). When testing the interorbital distance for allometry for adults and juveniles separately, we found that juveniles show a pattern of negative allometry (slope = 0.84, $R^2 = 0.69$, $p = 0.04$) and adults positive allometry (slope = 1.55, $R^2 = 0.55$, $p < 0.01$).

Table 5. Results of the ANCOVAs for skull linear measures with head length (HL) as covariate.

	Sex		Sex:HL		df
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	
Skull width	1.26	0.26	1.09	0.30	69
Frontoparietal width	0.02	0.90	1.45	0.23	69
Interorbital distance	1.16	0.28	0.46	0.50	69
Snout length	2.49	0.12	2.21	0.14	69
Snout width	2.64	0.11	2.30	0.13	69

In the linear morphometrics for limb bones, the ANCOVAs using sex as factor and SVL as covariate showed that the only structures exhibiting sexual differentiation are digit IV phalanges of both foot and hand (Table 4, Appendix III fig. A5). For these traits, males and females differ in the intercept but not slope, indicating that differences arise during embryonic development and sexes follow the same ontogenetic trajectory after birth. ANCOVAs with the ontogenetic stage as factor showed that only the growth curve of the femur differs between adults and juveniles, for all other traits, stages exhibit the same ontogenetic trajectory (Table 4, Appendix III fig. A6). In the PCA for the limb measures, the PC1 explained 53.5% of the total variation and the PC2 14.0%. Vectors for the foot and hand digit IV phalanges separate from the other hindlimb and forelimb bones, respectively, which show high correlation within each other (fig. 13). A MANOVA using the PC1 and PC2 as response variables showed no difference between males and females (Pillai's Trace = 0.002, $F_{1,56} = 0.05$, $p = 0.95$) nor adults and juveniles (Pillai's Trace = 0.01, $F_{1,56} = 0.33$, $p = 0.72$).

Tests for allometry showed that tibia, metatarsus IV, foot digit IV phalanges, metacarpus IV, and hand digit IV phalanges exhibit isometric growth, while femur, fibula, humerus, radius, and ulna exhibit positive allometry (Table 6). When testing the femur for allometry for adults and juveniles separately, we found that juveniles show a pattern of positive allometry (slope = 1.09, $R^2 = 0.94$, $p = 0.05$) and adults show isometric growth (slope = 0.95, $R^2 = 0.72$, $p = 0.63$).

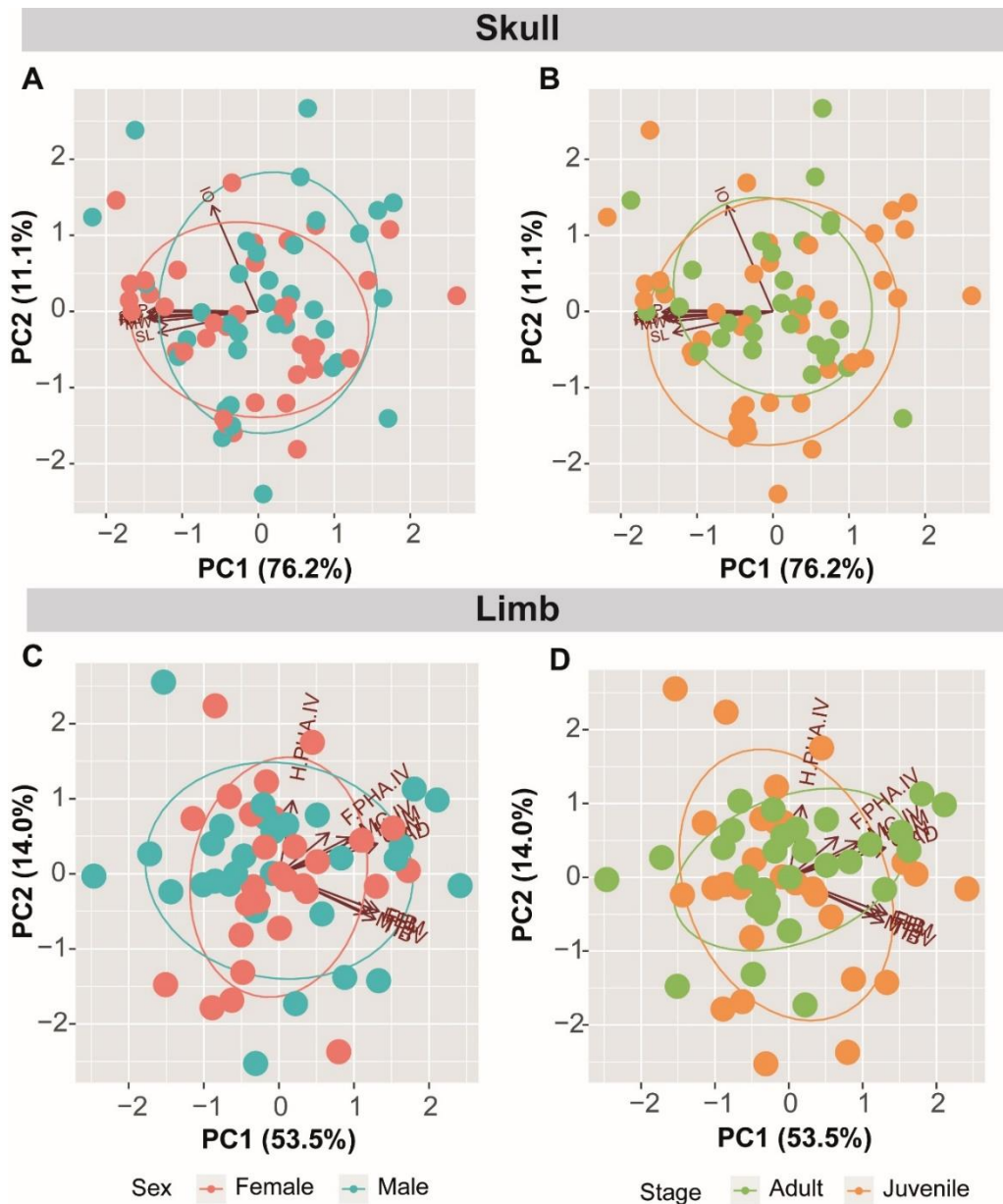


Figure 13. Principal component analysis (PCA) plot for size corrected linear measure of the skull (A, B) and limbs (C, D) showing overlap between sexes and ontogenetic stages. FEM, femur; FIB, fibula; FP, frontoparietal width; F.PHA.IV, foot digit IV phalanges; HUM, humerus; HL, skull length; H.PHA.IV, hand digit IV phalanges; HW, skull width; IO, interorbital distance;

MC.IV, metacarpus IV; MT.IV, metatarsus IV; RAD, radius; SL, snout length; SW, snout width; TIB; tibia; ULN, ulna.

Table 6. Results from the test of allometry.

	Slope	R^2	p
Femur	1.04	0.97	0.04
Tibia	1.03	0.97	0.13
Fibula	1.04	0.97	0.05
Metatarsus IV	1.00	0.97	0.82
Foot digit IV phalanges	1.00	0.93	0.94
Humerus	1.12	0.96	< 0.0001
Ulna	1.10	0.96	< 0.001
Radius	1.11	0.96	< 0.001
Metacarpus IV	1.01	0.93	0.75
Hand digit IV phalanges	1.01	0.92	0.73
Skull length	0.79	0.94	< 0.0001
Skull width	0.81	0.94	< 0.0001
Frontoparietal width	0.81	0.94	< 0.0001
Interorbital distance	0.94	0.89	0.12
Snout length	0.92	0.93	< 0.01
Snout width	0.83	0.95	< 0.0001
Mandible length	0.82	0.94	< 0.0001
Mandible width	0.83	0.94	< 0.0001

4. Discussion

Rapid evolution appears to be a common feature of invasive species when colonizing habitats with different environmental conditions (Reznick and Ghalambor 2001). Thus, high phenotypic lability could be related to the high invasion success by providing the raw material for selection to act (Lee 2002). Because *Hemidactylus mabouia* is an amazingly successful invader, we expected that it would be morphologically labile. As expected, our results demonstrated that *H. mabouia* exhibits high morphological variation among populations distributed across a wide range in Brazil.

To assess the possible evolutionary mechanisms that could be behind the morphological divergence in *Hemidactylus mabouia*, we compared estimates of

phenotypic and neutral genetic differentiation, P_{ST} and F_{ST} respectively. Under the null hypothesis of random accumulation of variation, populations with high neutral genetic divergence are predicted to be equally phenotypically divergent (Orgogozo et al. 2015). Our results showed that there is no correlation between P_{ST} and F_{ST} , indicating that random genetic drift alone cannot explain morphological variation among populations of *H. mabouia*. A P_{ST} value higher than the F_{ST} , as we observed in our results, has been regarded as a potential indicator of directional selection (Merilä and Crnokrak 2001; Whitlock 2008). We did not find, however, any associations between morphology and environment nor a geographic pattern, which, if significant, would suggest local adaptation (Stearns 1989; Ghalambor et al. 2007; Mullen et al. 2009). But once the P_{ST} is calculated from the phenotype of wild populations, other factors other than natural selection, such as environmental effects, can inflate estimates of the among-population variance, increasing the P_{ST} value and confounding the effects of plasticity with genetic evolutionary change (Merilä and Crnokrak 2001; Leinonen et al. 2008). An alternative hypothesis could be that non-adaptive plasticity is driving morphological variation in *H. mabouia*, either via the expression of cryptic genetic variation or disruption of the normal development (Ghalambor et al. 2007). As an invasive species, *H. mabouia* probably experiences stressful conditions during colonization of novel habitats, thereby non-adaptive plasticity is likely to contribute to at least part of the phenotypic variation among populations—especially if responses are not maladaptive.

Nevertheless, in the PC2 from the PCA using size-corrected limb and head measures, populations genetically distant converged morphologically and were separated in two clear groups, and the occurrence of a systematic pattern could be the result of selection by an unidentified factor (Whitlock 2008). Variation along the PC2 is most strongly related with variation in the foot digit IV and forearm length, the first being the

trait showing the highest variation among populations. Phalanx relative length seems to be related with climbing biomechanics in geckos and climbing species are morphologically diverse regarding proportions of autopodial bones (Zani 2000; Johnson et al. 2005; Rother et al. 2017). The relative length of distal limb structures is particularly evolvable in *Anolis* lizards (Toro et al. 2003; Mahler et al. 2010; Wakasa et al. 2015; Feiner et al. 2021) and differences in traits important to clinging ability also exhibit high variability within species, being associated with variations in habitat characteristics (e.g., Lee 1985; Calsbeek et al. 2007; Dill et al. 2013; Donihue et al. 2018; Donihue et al. 2020). More studies are necessary to determine the roles of plasticity and natural selection in the morphological divergence between populations of *Hemidactylus mabouia*, such as common-garden experiments. In addition, further studies evaluating if variation in digit length in *H. mabouia* has functional implications in performance would be interesting.

It is important to highlight that in tetrapods, several species exhibit sexual dimorphism of digit length, and this trait has been regarded traditionally as indicative of sex steroid concentration during development (Lofeu et al. 2020), being usually correlated with other sexually dimorphic characters (e.g., van Damme et al. 2015; Lofeu et al. 2017). Our results showed that sexual dimorphism in foot and hand digit IV phalanges arise during prenatal development in *Hemidactylus mabouia*, and after hatching males and females share the same ontogenetic trajectory. Despite the low level of sexual dimorphism, males of *H. mabouia* are territorial, aggressive towards other males, and show complex courtship behavior (Regalado 2003). Consequently, they must be under sexual selection (Kratochvíl and Frynta 2002, 2007), which could vary in strength between populations. Yet, we found that males and females vary similarly across populations, indicating no difference in the direction or magnitude of sexual dimorphism.

Thus, the high variation in the foot digit IV length is unlikely to be related to exposure to different hormonal environments during embryonic development.

Variation in morphology requires changes in development, which has a major influence on the course of evolution (Raff 1996; Salazar-Ciudad 2006; Uller et al. 2018); and allometry is a powerful tool to evaluate evolutionary and developmental changes in relative size (Klingenberg 1998). Covariation between traits within species are likely attributed to genetic cascades and developmental dynamics (Klingenberg and Zimmermann 1992). Voje et al. (2013) reported that, for non-genital traits in various animal species, variation of ontogenetic and static slopes between populations are rare and the intercept shows higher evolvability at the microevolutionary scale. Accordingly, our results showed that populations of *Hemidactylus mabouia* differ only in the intercept of the regression curves, also indicating that differences arise earlier in development. Except for femur length and interorbital distance, allometric relationships between skeletal traits and body size are constant over post-natal life stages of *H. mabouia*, which means that static allometric curves represent relative growth (Cheverud 1982). Therefore, if bone length measures are correlated with external measures (Rothier et al. 2017; but see Johnson et al. 2005), for the traits in which the ontogenetic and static allometric coefficient do not differ (i.e., all except femur), differences between populations likely appeared before hatching.

Moreover, we found that populations do not differ in the traits related to long bones (i.e. thigh and shank) but differ in digit length in the hindlimbs, while the inverse was observed in the forelimb. In the ontogenetic analysis, the PCA loading vectors showed that long bones and metapodials vary jointly in the same direction in both forelimb and hindlimb during post-natal ontogeny, while the phalanges' vector is separated from the other bone elements within the same limb. These observations suggest

a certain dissociation between limb elements. Long bones, metapodials, and phalanges are generated independently during morphogenesis, and appear to constitute different modules during pre- and post-natal development (Hall 2003; Rolian 2008; Kavanagh et al. 2013; Gilbert and Barresi 2016). Numerous studies in different tetrapod lineages, including lizards, suggest that the slope of ontogenetic curves of limb bones are more evolutionarily stable and point limb morphogenesis as a critical period for the emergence of different bone proportions both between and within species (e.g., Hall 2003; Rolian 2008; Sanger et al. 2012; Kavanagh et al. 2013; Wakasa et al. 2015; Andrews and Skewes 2017). By analyzing variation in limb length in two rodents, Rolian (2008) proposed that changes in morphogenesis should be the main mechanism underlying variation in relative length of long bones and metapodials while changes in bone growth should be related with variation in absolute length linked to differences in body size. Further, it was demonstrated that mechanical stress caused by changes in embryo motility affects the growth rate of limb bones and generates differences in their relative length, being a potential source of intraspecific variation in limb morphology (Pollard et al. 2017).

Geometric morphometric analysis of the skull of *Hemidactylus mabouia* revealed that most of the shape variation is related with ontogeny and allometry explains a great proportion the total variation within a population. The shape variation described during ontogeny in *H. mabouia* follows the general pattern seen in tetrapods of a relative reduction of the braincase and elongation of the snout (Emerson and Bramble 1993). Still, these changes are subtle and in the linear morphometric analysis adults and juveniles of *H. mabouia* do not differ in the first two axes of the PCA using size-corrected linear measures. The PCA also showed a strong association between all skull linear measures except the interorbital distance. The interorbital distance also exhibited a distinct allometric pattern from the other traits and differed between juveniles and adults—

growing relatively larger in the latter. This difference in the allometric curve of the interorbital distance between stages follows the variation described in the allometric curve of eye growth relative to body size between juveniles and adults of *H. turcicus* (Werner and Seifan 2006). Because the function of sensorial organs depend on their absolute size, there is a constraint to their reduction such that smaller geckos exhibit proportionally larger organs (Werner and Igic 2002; Werner et al. 2002; Werner et al. 2005; Werner and Seifan 2006). Our results corroborates the hypothesis that the presence of well-developed sensorial organs is an important factor shaping skull design in Gekkota (Herrel et al. 2007).

Interspecific variation of skull shape in geckos is also highly correlated with size (Daza et al. 2009). Differences between gecko species mirror changes observed during ontogeny and frequently generates paedomorphic-peramorphic patterns (Daza et al. 2009; Daza et al. 2015). Heterochronic shifts are commonly described in osteological characters in geckos, especially in miniaturized species (Kluge 1967; Daza et al. 2008). Interestingly, we observed that in most adults of *Hemidactylus mabouia* the supraoccipital, otooccipital, and prootic are unfused. In lizards, elements of the otooccipital region usually fuse during post-natal development before sexual maturity, and this is the ancestral state in Squamata (Maisano 2001; Tarazona et al. 2008)—a pattern also observed in *H. turcicus* (Villa et al. 2018). The unfused state of the elements of the otooccipital region appears to occur in adults of a few small-sized and miniaturized geckos (e.g., *Saurodactylus mauritanicus* in Daza et al. 2008; *Rhyncoedura ornata* and *Microgecko persicus* in Sullivan 2018) and is regarded as a paedomorphic character in squamates (Scanferla and Bhullar 2014). Therefore, this finding in *H. mabouia* was surprising considering it is a medium-sized gecko. Further studies on cranial evolution in

Hemidactylus are necessary to determine the significance of this characteristic in *H. mabouia*.

In conclusion, our results showed that *Hemidactylus mabouia* exhibits high morphological variation among populations, corroborating the hypothesis that invasive species are phenotypically labile. Although we were unable to determine if this divergence is the result of directional selection or plasticity, the presence of variability is the first step to evolution and suggests that *H. mabouia* should be able to adapt locally in the presence of selective pressures. Further studies evaluating the contribution of plasticity and natural selection to the morphological diversification of *H. mabouia* will provide interesting insights into processes of rapid evolution and its importance in biological invasions. Additionally, we reinforce the utility of invasive species as model organisms for testing evolutionary hypothesis in natural populations and of studying intraspecific variation to understand evolution at macroevolutionary scales.

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Appendices

Appendix I

List of specimens examined

Variation between populations: LGG_186, LGG_199, LGG_165, LGG_002, LGG_187, LGG_161, LGG_160, LGG_201, LGG_185, LGG_183, LGG_180, LGG_190, LGG_159, LGG_202, LGG_164, LGG_200, LGG_214, LGG_158, LGG_197, LGG_209, LGG_163, LGG_179, LGG_162, LGG_194, LGG_207, LGG_205, LGG_195, LGG_181, LGG_166, LGG_213, LGG_193, LGG_215, LGG_S/ID02, LGG_S/ID04, LGG_S/ID03, LACV_JL_33, LACV_JL_38, LACV_JL_37, LACV_JL_34, LACV_JL_32, LACV_JL_47, LACV_JL_36, LACV_JL_41, LACV_JL_40, LACV_JL_39, LACV_JL_30, LACV_JL_35, LACV_JL_F2, LACV_JL_42, LACV_JL_31, LACV_JL_49, LACV_JL_50, LACV_JL_51, LACV_JL_28, LACV_JL_29, LACV_JL_04, LACV_JL_02, LACV_JL_12, LACV_JL_11, LACV_JL_03, LACV_3579, LACV_JL_05, LACV_JL_44, LACV_JL_52, LACV_JL_53, LACV_JL_56, LACV_JL_55, LACV_JL_57, LACV_JL_58, LACV_JL_59, LACV_JL_60, LACV_JL_61, LACV_JL_62, LACV_JL_63, LACV_JL_64, LACV_JL_65, LGG_262, LGG_276, LGG_271, LGG_274, LGG_154, LGG_222, LGG_263, LGG_247, LGG_224, LGG_236, LGG_277, LGG_269, LGG_226, LGG_261, LGG_240, LGG_275, LGG_249, LGG_243, LGG_297, LGG_257, LGG_258, LGG_278, LGG_234, LGG_248, LGG_246, LGG_235, LGG_266, LGG_255, LGG_256, LGG_273, LGG_259, LGG_254, LGG_253, LGG_223, LGG_264, LGG_221, LGG_225, LGG_157, LGG_272, LGG_270, LGG_265, LGG_267, LGG_244, LGG_107, LGG_090, LGG_087, LGG_091, LGG_094, LGG_088, LGG_108, LGG_100, LGG_101, LGG_113, LGG_086, LGG_096, LGG_095, LGG_111, LGG_102, LGG_104.

Ontogeny: LACV_3514, LACV_3515, LACV_3516, LACV_3544, LACV_3545, LACV_3546, LACV_3575, LACV_3334, LACV_3335, LACV_3337, LACV_3509, LACV_3510, LACV_3511, LACV_3512, LACV_3513, LACV_3504, LACV_3505, LACV_3506, LACV_3507, LACV_3508, LACV_E_0113, LACV_3576, LACV_3577, LACV_3578, LACV_3584, LACV_3540, LACV_3541, LACV_3580, LACV_3582, LACV_3586, LACV_3534, LACV_3535, LACV_3539, LACV_3542, LACV_3588, LACV_3333, LACV_3338, LACV_3339, LACV_3341, LACV_3342, LACV_3336, LACV_3533, LACV_JL_00, LACV_3538, LACV_3543, LACV_3581, LACV_3589, LACV_3607, LACV_3612, LACV_3613, LACV_3608, LACV_3614, LACV_JL_06, LACV_JL_09, LACV_JL_25, LACV_3583, LACV_3609, LACV_3611, LACV_E_0125, LACV_E_0131, LACV_JL_07, LACV_JL_14, LACV_3536, LACV_3616, LACV_JL_01, LACV_E_0132, LACV_JL_10, LACV_JL_13, LACV_JL_15, LACV_JL_16, LACV_JL_17, LACV_JL_22, LACV_JL_23, LACV_JL_26, LACV_JL_27.

Appendix II

Description of skull morphometric traits

Table A1. Description of the landmarks used in the geometric morphometrics analysis of the skull of *Hemidactylus mabouia*.

Landmark	Description
1	Anterior-most part of the premaxilla
2	Posterior most part of the ascending nasal process of the premaxilla
3	Posteromedial contact point between nasals
4, 5	Posterolateral tip of the nasal
6, 7	Lateral contact point between the prefrontal and the maxilla
8, 9	Edge of the posterior process of the jugal
10, 11	Edge of the dorsal process of the prefrontal
12, 13	Lateral edge of the frontoparietal suture
14, 15	Edge of the anterior process of the postorbitofrontal
16, 17	Edge of the posterior process of the postorbitofrontal
18	Middle of the curvature of the supraoccipital
19, 20	Confluence between the anterior and posterior semicircular canals (i.e., <i>common crus</i>)
21, 22	Posterior most edge of the posterior semicircular canal
23, 24	Contact point between the prootic, otooccipital, and supraoccipital
25, 26	Edge of the posterior process of the squamosal
27, 28	Edge of the postparietal process
29, 30	Edge of the paroccipital process

Table A2. Linear measures of the skull of *Hemidactylus mabouia*.

Measure	Description
Skull length	From the anterior-most edge of the premaxilla to the middle of the curvature of the supraoccipital along the anteroposterior axis
Skull width	Between the two points of maximum curvature of the quadrate
Snout length	From the anterior-most edge of the premaxilla to the contact point of the dorsal process of the prefrontal with the frontal
Snout width	Between the two contact points of the dorsal processes of the prefrontal with the frontal
Interorbital distance	Between the edges of the dorsal processes of the prefrontal
Frontoparietal width	Between the lateral edges of the frontoparietal suture
Mandible length	From the posterior most edge of the retroarticular process to the anterior-most edge of the dentary
Mandible width	Between the posterior most edges of the retroarticular processes

Appendix III

Complementary figures

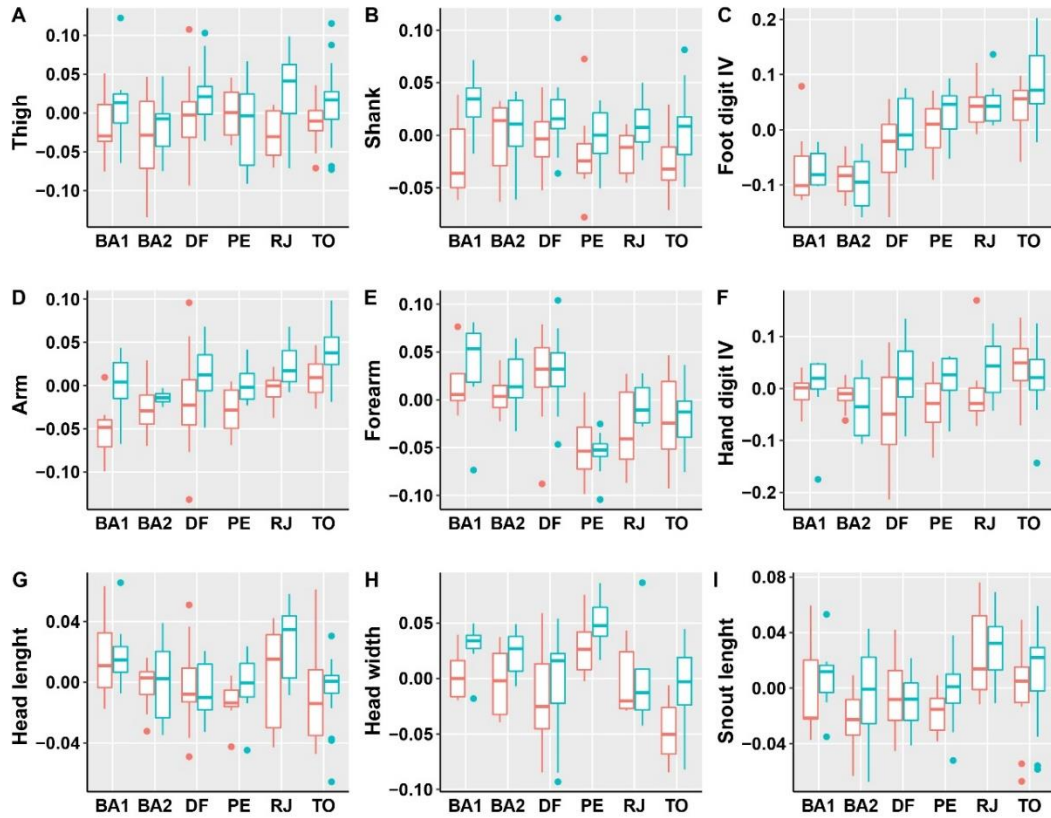


Figure A1. Boxplots showing population and sex differences in morphological traits. BA1, Salvador; BA2, Feira de Santana; DF, Brasilia; PE, Recife; RJ, Rio de Janeiro; TO, Palmas. Lower and upper box limits represent, respectively, first and third quartiles, internal bar the median, and whiskers the minimum and maximal values inside the 99% interval.

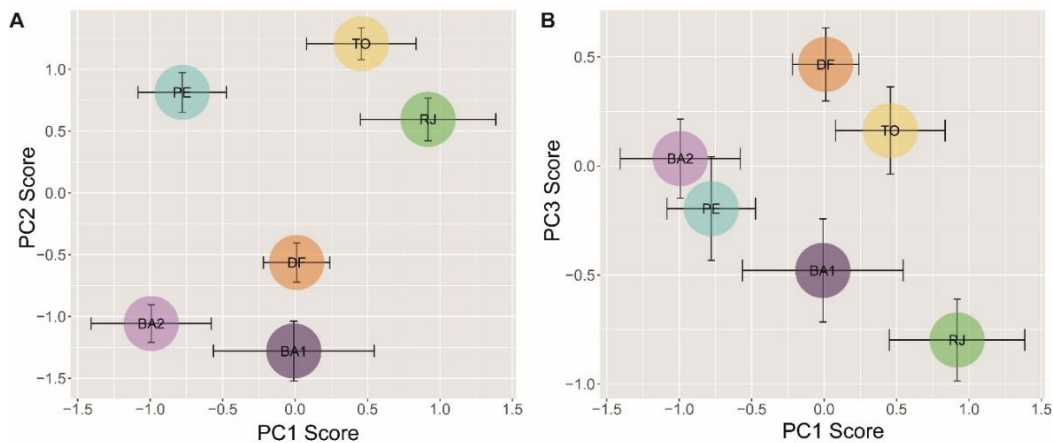


Figure A2. Clustering of principal component (PC) scores for PC1 versus PC2 (A) and PC1 versus PC3 (B). Circles represent the means between all individuals of that population and bars the standard errors.

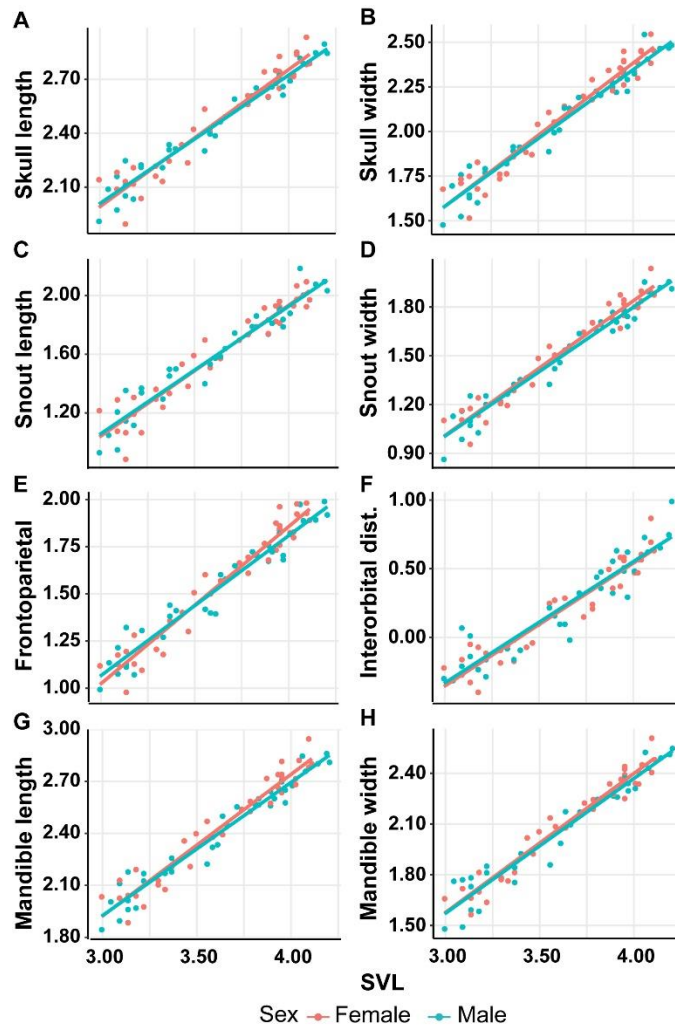


Figure A3. Regression curves between linear skull measures and SVL showing ontogenetic trajectories of males and females of *Hemidactylus mabouia*.

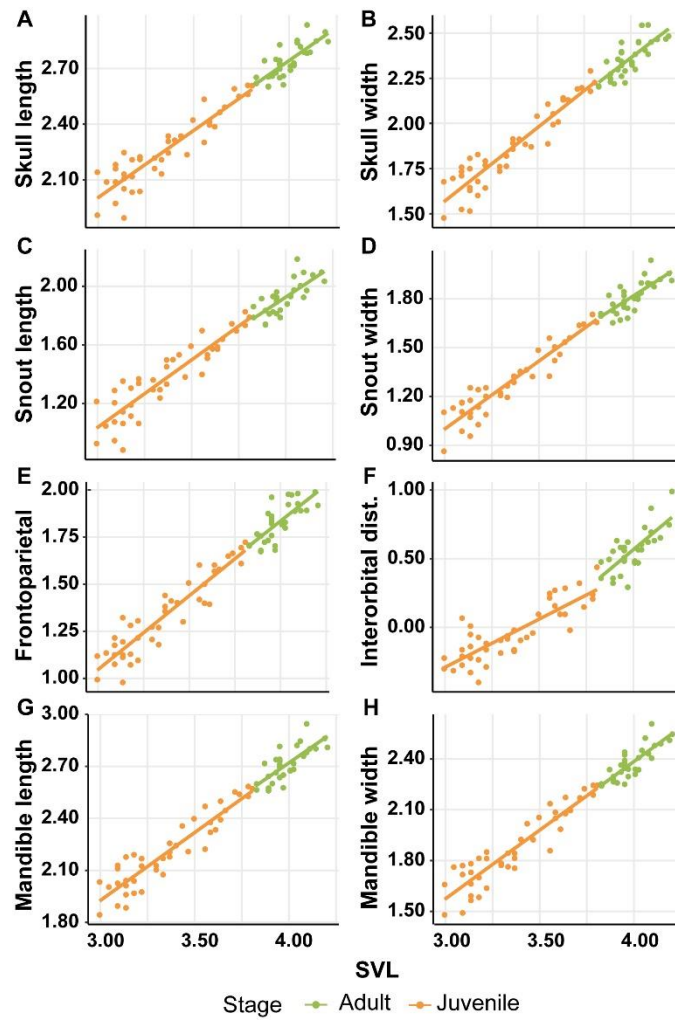


Figure A4. Regression curves between linear skull measures and SVL showing ontogenetic trajectories of adults and juveniles of *Hemidactylus mabouia*.

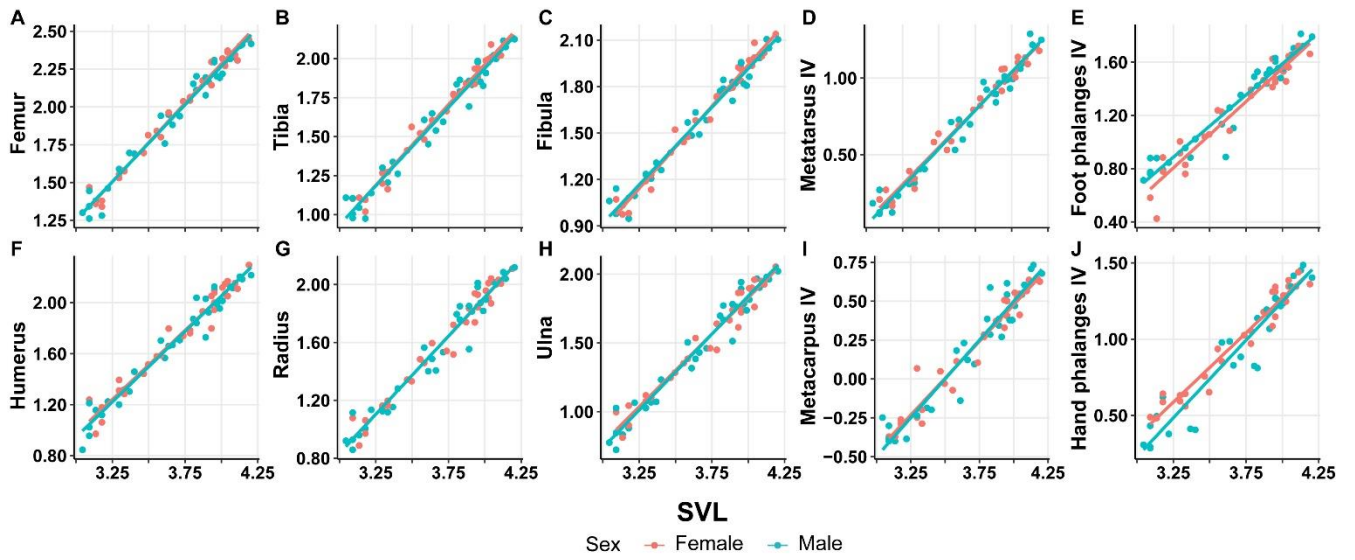


Figure A4. Regression curves between limb measures and SVL showing ontogenetic trajectories of males and females of *Hemidactylus mabouia*.

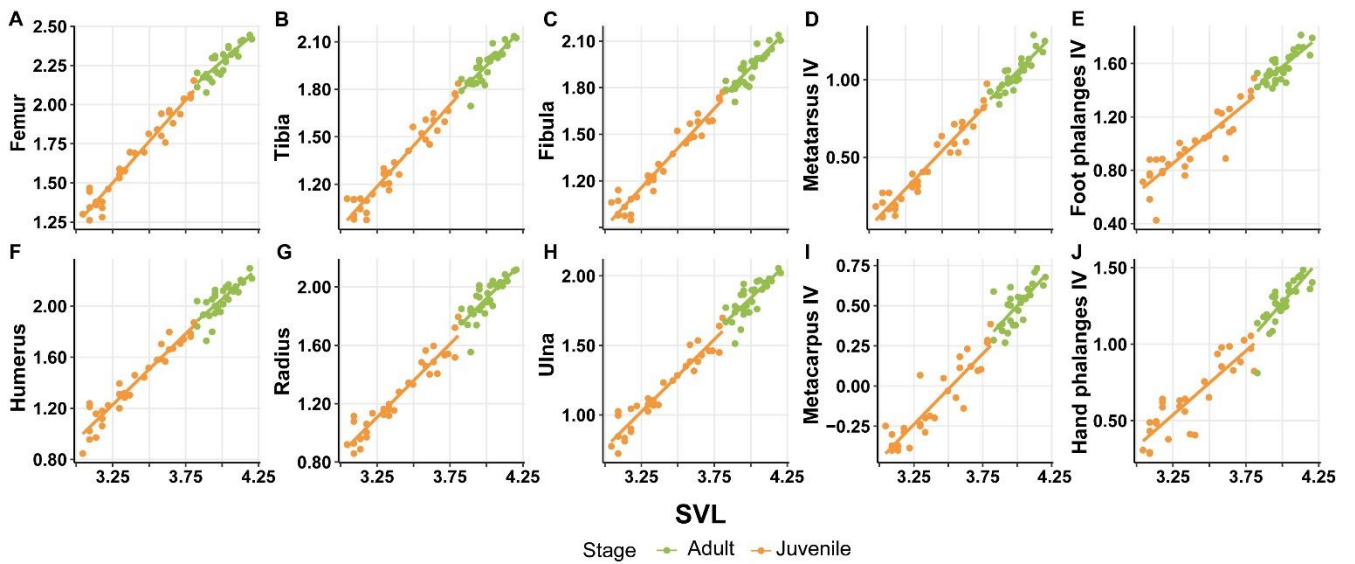


Figure A5. Regression curves between limb measures and SVL showing ontogenetic trajectories of adult and juveniles of *Hemidactylus mabouia*.