JOURNAL OF MORPHOLOGY 278:290–299 (2017)

Ecological Associations of Autopodial Osteology in Neotropical Geckos

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ABSTRACT Coevolution of form and function inspires investigation of associations between morphological variation and the exploitation of specific ecological settings. Such relationships, based mostly on traits of external morphology, have been extensively described for vertebrates, and especially so for squamates. External features are, however, composed by both soft tissues and bones, and these likely play different biomechanical roles during locomotion, such as in the autopodia. Therefore, ecological trends identified on the basis of external morphological measurements may not be directly correlated with equivalent variation in osteology. Here, we investigate how refined parameters of autopodial osteology relate to ecology, by contrasting climbing and nonclimbing geckos. Our first step consisted of inferring how external and osteological morphometric traits coevolved in the group. Our results corroborate the hypothesis of coevolution between external and osteological elements in the autopodia of geckos, and provides evidence for associations between specific osteological traits and preferred locomotor habit. Specifically, nonclimbers exhibit longer ultimate and penultimate phalanges of Digit V in the *manus* and *pes* and also a longer fifth metatarsal in comparison with climbers, a pattern discussed here in the context of the differential demands made upon locomotion in specific ecological contexts. Our study highlights the relevance of osteological information for discussing the evolution of ecological associations of the tetrapod autopodium. J. Morphol. 278:290–299, 2017. © 2017 Wiley Periodicals, Inc.

KEY WORDS: autopodium evolution; bone morphometrics; climbing; locomotion

INTRODUCTION

Identification of associations between morphological variation and specific ecological demands helps to clarify how phenotypes operate in a given ecological context, based on the biological premise that form and function coevolve (Lauder, 1995). Interest in investigating associations between morphology and ecology has consolidated with the development of an ecomorphological research program that fosters significant advances in understanding the evolution of the locomotor apparatus in a given environment (Williams, 1972; Arnold, 1983; Losos and Sinervo, 1989; Garland and Losos, 1994; Collar et al., 2010). For example, it has been stated that the evolution of remarkable morphological patterns in gymnophtalmid lizards is associated with the occupation of substrates that impose resistance to burrowing, with an elongated body, a flattened head, and reduced limbs likely favoring locomotor performance in fossorial species (Barros et al., 2011; Grizante et al., 2012).

Extensive attention has been paid to the coevolution of form and function in vertebrate limbs (Norberg and Rayner, 1987; Losos, 1990a,b; Bickel and Losos, 2002; Higham and Jayne, 2004; Young and Hallgrímsson, 2005), and the Squamata provides good examples of such associations. For instance, in tropidurid lizards the limb and foot proportions differ according to the preferred microhabitat of particular species (Kohlsdorf et al., 2001; Grizante et al., 2010), and within phrynosomatids, sand lizards run faster and have longer limbs than horned lizards, which mostly occupy herbaceous microhabitats (Bonine and Garland, 1999). Associations between limb lengths, locomotor performance and habitat use have also been described for *Anolis* lizards (e.g., Losos 1990a) and for the Scincidae (e.g., Melville and Swain, 2000). In fact, for several animal taxa ecological associations are most strongly expressed in the most distal region of the limb (i.e., the autopodium), which is the structure that directly interacts with different surfaces during locomotion (see Hertwig and Sinsch, 1995; Irschick et al., 1996, Zaaf and Van Damme, 2001; Bickel and Losos, 2002; Johnson et al., 2005; Collins et al., 2015).

Autopodial morphological diversity is often identified in association with colonization of structurally

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Additional Supporting Information may be found in the online version of this article.

Contract grant sponsors: FAPESP; Contract grant numbers: 2012/18269-3, 2014/26287-7, and 2016/03953-7 (to P.S.R.), 2013/14125-0 and 2016/01558-3 (to R.B.), 2011/18868-1 and 2015/07650-6 (to T.K.).

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Received 12 January 2016; Revised 29 September 2016;
Accepted 21 October 2016.

DOI 10.1002/jmor.20635

diverse environments by vertebrate lineages, a pattern particularly well described for squamates (e.g., Robinson, 1975; Rewcastle, 1983; Irschick et al., 1996; Aerts et al., 2000; Zani, 2000; Gamble et al., 2012). The vast majority of information available, however, concentrates on associations between a given ecological setting and parameters of external morphology, whereas osteological traits remain largely unexplored. For example, some ground-dwellers have elongated autopodial elements that are related to enhanced running speeds on horizontal surfaces (Miles, 1994; Irschick and Jayne, 1999; Melville and Swain, 2000; Higham and Russell, 2010), whereas climbers usually exhibit relatively short digits and tall claws that provide stability when grasping perches and vertical surfaces (Losos and Sinervo, 1989; Losos, 1990b; Zani, 2000). External autopodial morphology has a functional significance and is expected to be associated with variation in osteological elements. Some species of climbing lizards, for example, possess external adhesive toe pads that sometimes are associated with a very specific autopodial osteology, characterized by the presence of expanded skeletal structures at interphalangeal joints (named paraphalanges), and may also exhibit shortened fifth metatarsals (Russell, 1975, 2002; Russell and Bauer, 1988; Russell et al., 1997). Osteological variation in the fifth digit and metatarsal of the posterior autopodium (*pes*) is expected, given their biomechanical role, respectively, of grasping the surface and serving as the in-lever for the pedal flexors during locomotion (Robinson, 1975; Brinkman, 1980; Miles, 1994; Russell et al., 1997). However, the osteological specializations involving paraphalanges and a relative reduction of the fifth metatarsal have only been identified in certain species that are characterized by unique locomotor patterns. Hence, it remains poorly understood how external and osteological variation of homologous structures has coevolved during the colonization of different microhabitats. Identification of associations between external and osteological parameters of the autopodium is therefore the first step in understanding their integrated functional role when animals move along surfaces differing in orientation (such as vertical *versus* horizontal locomotion).

Despite arguments advocating a major incorporation of osteology into the study of functional ecomorphology in vertebrates (see Russell and Bels, 2001; Swartz et al., 2003; Dumont et al., 2016 for some examples), the major focus of the field remains based upon on traits of external morphology. For example, formal tests of whether autopodial osteological morphology of climbers and nonclimbers follows the same trends that have been identified from external morphology (see Grizante et al., 2010; Higham and Russell, 2010 for some examples) are underrepresented in the current literature (but see Johnson et al., 2005). Investigation of associations between external and osteological morphology is therefore essential for elucidating the ecological associations already established for the autopodia of climbers and nonclimbers, especially because rigid bones play a biomechanical role that differs from that of soft tissues, acting as links in a limb lever system (Lauder, 1995).

A direct association between external morphology and osteology in relation to ecological divergence is usually assumed (Vanhooydonck and Van Damme, 2001; Zaaf and Van Damme, 2001), but this has rarely been tested. As a result, the functional relationships among different autopodial elements during locomotion in specific environments may be oversimplified. The few studies that have investigated ecological associations of osteology in lizards have concluded that the long bones in the limb and some sets of small elements in the autopodium have coevolved in association with microhabitat usage (Bauer et al., 1996; Russell et al., 1997; Melville and Swain, 2000; Johnson et al., 2005), but how each of these bones evolved individually in specific ecological circumstances remains unclear. More precisely, ecological associations of metapodials and phalanges are expected, given the modified hooked shape of the fifth metatarsal in lizards that likely affects performance during horizontal locomotion (Robinson, 1975; Brinkman, 1980), and the associations of phalangeal morphology with the way climbing lizards grasp the substrate (Fontanarrosa and Abdala, 2016).

Here, we investigate how autopodial osteology relates to ecology by comparing geckos that differ in microhabitat usage. First, we inferred how external and osteological morphology coevolved in the lineage. Then, we compared species that use perches, leaves and trees (sometimes also moving along urban constructions) that are mostly found above 1.0 m from the ground (hereafter referred to as "climbers") with those moving exclusively along soil, rocks, roots of trees, and occasionally on low portions of trees (<1.0 m height), predominantly in horizontal or slightly inclined orientations (denoted "nonclimbers" in the present study). We use a broad taxonomic approach and phylogenetically informed analyses to test two main hypotheses for the Gekkota: 1) autopodial osteology and external morphology coevolved during the processes of ecological divergence in this lineage of lizards; 2) ecological associations of osteology in geckos have a functional basis, so that distal bones of the autopodium are more likely to be the ones associated with a given type of locomotion. A derived prediction from hypothesis 2 proposes that osteological patterns will differ between the *manus* and *pes* when comparing climbers with nonclimbers, because of the specific and differing biomechanical roles that the fore and hindlimbs play during locomotion on different surfaces and/or different inclines (see Russell and Bels, 2001).

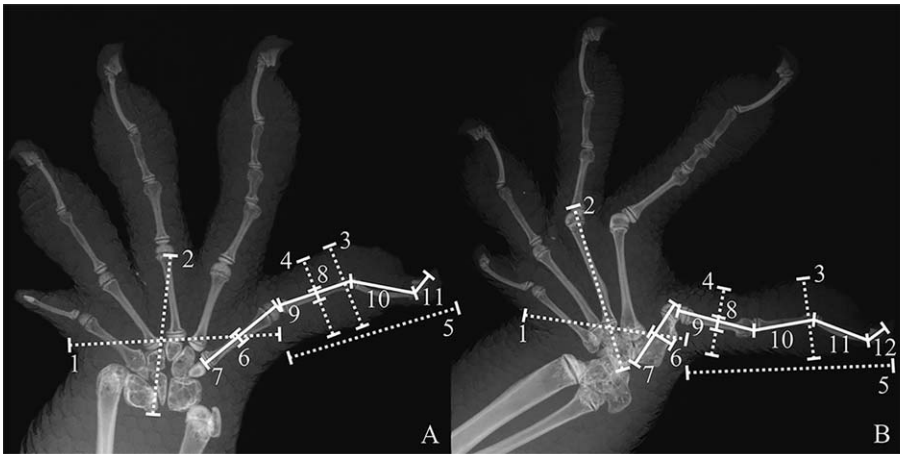

Fig. 1. Radiographs of the right *manus* (**A**) and *pes* (**B**) of the gecko *Phyllopezus pollicaris*, illustrating the external (dashed lines; 1–5) and osteological (solid lines; 6–12) measurements taken from Digit V and palm/sole. 1) width of palm of *manus* and sole of *pes*; 2) length of palm of *manus* and sole of *pes*; 3) maximum digit width; 4) proximal digit width; 5) digit length; 6) width of the fifth metapodial element at mid-shaft; 7) length of the fifth metapodial element; 8) phalanx I width; 9–12) length of each phalanx. Bars at the extremes of each line indicate the points that have been standardized for tracing linear distances.

The Gekkota is an appropriate biological lineage for conducting this kind of study, especially because it exhibits extensive morphological variation in the autopodium, which is often associated with the preferred locomotor habit (Irschick et al., 1996; Gamble et al., 2012). The Gekkota is also a very speciose group that is broadly distributed over several habitats that are structurally diverse (Bauer, 1999; Vitt and Caldwell, 2009; Losos, 2010; Gamble et al., 2011; Pyron et al., 2013). Here, we first tested for evolutionary associations between external morphological proportions (e.g., digit lengths and widths, plantar, and palmar lengths and widths) and osteology (e.g., phalangeal lengths and widths, metacarpal, and metatarsal lengths and widths) in the *manus* and *pes* of Neotropical geckos. Then, we tested for ecological associations of osteological elements in their autopodia, focusing on the contrast between climbers and nonclimbers. Based on the morphological and functional patterns described previously (Russell, 1975, 2002; Irschick and Jayne, 1999; Higham and Russell, 2010), we predict that most of the variation will be expressed in the distal part of the autopodium, where nonclimbers likely exhibit relatively longer elements than climbers. Our approach integrates morphological components representing both external and osteological traits of the autopodium and contributes to our understanding of how combined traits coevolve during the processes of ecological divergence involving shifts in locomotor habit.

MATERIAL AND METHODS

Dataset

Our dataset comprises 13 species of South-American geckos belonging to the Sphaerodactylidae, Phyllodactylidae, and Gekkonidae families. Species' choice was dependent of their availability in Brazilian museum collections at samples comprising more than 10 individuals, all presenting autopodia well preserved (i.e., we did not include specimens having deformed *manus*/*pes*, fragmented digits or curved autopodia). We measured 11 to 20 specimens of each species, borrowed from Brazilian herpetological collections (see Supporting Information Table S1 for details). Samples consisted of both males and females, but sex was often not determinable for the preserved specimens based on external traits. We disregarded possible sex effects on autopodial morphology in our study because only four species (30.8% of our sample) in our database exhibit clear sexual dimorphism that allows identification of males and females in all specimens based on external traits, and we were not allowed to dissect the specimens for inferring their sexes. We tested for sexual dimorphism in autopodial traits of *Hemidactylus mabouia*, one of the species where all specimens could be sex-determined, and none of the osteological traits were significantly different between males and females (ANCOVA using SVL as a covariate, all $P > 0.05$). We used a Faxitron LX 60 digital X-ray machine to radiograph the right anterior (*manus*) and posterior (*pes*) autopodium of each specimen, assuming a left-right symmetry. A copper bar radiographed together with the specimens provided the scale in all pictures. Both osteological elements and external dimensions were measured from the images (see Fig. 1). To avoid parallax errors in the images, we flattened the autopodia prior to imaging, so that they were parallel to the radiograph platform, holding them in this position with adhesive tape. Morphometric variables were obtained by directly measuring distances between standardized points (Fig. 1) on the digital images, using the software ImageJ version 1.45s (Schneider et al., 2012); measurements were converted from pixels to millimeters based on the copper scale bar of known length present in the digital images. We checked whether using tape for positioning the autopodia affected the data collected by comparing measurements of external traits using both a caliper and digital quantification from the X-ray images. We repeated eight measurements for each method on 14 specimens of one gecko species (*Hemidactylus mabouia*) and then performed a regression test for each trait using the caliper and digital data. For all the eight external traits, the regressions generated slopes that were not different from 1.0, with P-values < 0.05 and most r^2 values greater than 0.7, indicating that X-ray images of flattened and taped autopodia were appropriated for assessing external measurements.

Measurements were restricted to the fifth digit (Digit V) and the fifth metapodial (fifth metacarpal of the *manus* and fifth

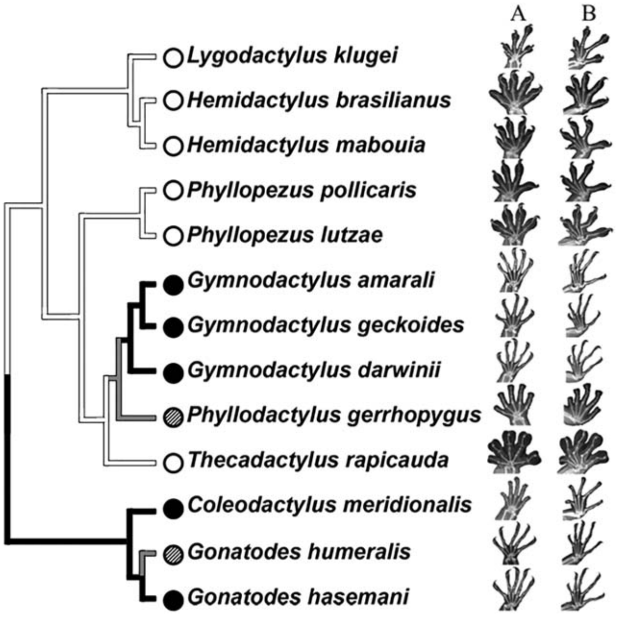

Fig. 2. Tree topology adopted including all species analyzed here. Families are indicated at the nodes: **A**) Gekkonidae; **B**) Phyllodactylidae; and **C**) Sphaerodactylidae. *Manus* and *pes* of each species are shown to the right of the species names. Locomotor habits are indicated by circles in black (nonclimbers) or white (climbers) at the tips of the tree. Ecological transitions of locomotor habit have been mapped along the topology's branches using parsimony, implemented in Mesquite; these do not reflect an ancestral reconstruction of locomotor habit for Gekkota due to the species' sample comprised here.

metatarsal of the *pes*). Elements associated with Digit V were selected to comprise our digital and metapodial database because of their particular and peculiar functional relevance to locomotor performance, especially in the hindlimb (Robinson, 1975; Brinkman, 1980). The external measurements taken were (see Fig. 1 and Supporting Information Table S2 for details): 1) width of the palm of the *manus* and of the sole of the *pes*; 2) length of the palm of the *manus* and the sole of the *pes*; 3) greatest digit width; 4) proximal digit width; 5) digit length. The osteological variables for the *manus* and *pes* were (see Fig. 1 and Supporting Information Table S2 for details): 6) width of the fifth metapodial element at mid-shaft; 7) length of the fifth metapodial element; 8) mid-shaft width of phalanx I; 9–12) length of each phalanx of Digit V (three phalanges in *manus* and four in *pes*). Using measurements 9–11 (*manus*) and 9–12 (*pes*), we also calculated the sum of all individual phalangeal lengths comprising Digit V. Snout-vent length (SVL) was measured using a digital caliper (Mitutoyo, ± 0.01 mm). Mean values of all morphological variables were calculated for each species (Supporting Information Table S3).

We also consulted literature sources to compile ecological information available for each species, enabling us to classify it as a "climber" (animals using perches, leaves, and trees, all over 1.0 m above the ground during most of their activity period) or "nonclimber" according to its preferred locomotor habit (Fig. 2 and Supporting Information Table S4). For two species studied, ecological information is ambiguous. In the case of *Phyllodactylus gerrhopygus*, some populations are described as climbers while others are classified as ground-dwellers (Dixon and Huey, 1970), and the specimens of *P. gerrhopygus* measured here are from a population that has no published ecological information associated with it. The other species, *Gonatodes humeralis*, seems generalist regarding habitat usage and locomotor habit: they mostly occupy the base of tree trunks and low height perches (approximately 1.5 m of mean height off the ground; Vitt et al., 1997), but frequently use the ground-level for oviposition (Avila-Pires, 1995; Vitt et al., 1997, 2000; Maciel et al., 2005); some authors also describe the species as a climber (Russell et al., 2015). Therefore, we performed our statistical analyses four times, classifying these species either as a climber or as a nonclimber, as further detailed.

Analyses

Our data were analyzed using phylogenetically informed statistics. We combined three available molecular phylogenetic hypotheses (Gamble et al., 2011; Pyron et al. 2013; Domingos et al., 2014) to build the topology used in our analyses (Fig. 2). The three hypotheses combined here were not inferred using the same molecular markers, so we lack equivalent data for the species comprising our dataset to calculate branch lengths corresponding to genetic distance or time. Therefore, we adopted the diagnostic method described by Garland et al. (1992) to identify which arbitrary branch lengths better standardized our data. Branch lengths employing the method of Grafen (1989) most successfully standardized our independent contrasts, so these were used in the subsequent analyses. We built the topology and transformed branch lengths using Mesquite version 2.75 (Maddison and Maddison, 2011).

All statistical analyses were implemented in R (version 3.2.4) using RStudio (099.896; R Core Team, 2016). Prior to analysis, the mean values of all variables were log10-transformed and then the autopodial traits were regressed against log10-SVL using phylogenetic generalized least-squares (PGLS; see Revell, 2009), to account for allometric effects. Size-free residuals were used in two phylogenetic Principal Component Analyses (PCAs) in *phytools* (Revell, 2012), with principal components (PC) having *eingenvalues* higher than 1 and explaining more than 20% of variance being retained (Jackson, 1993).

The first PCA performed comprised the entire size-corrected dataset, and was implemented to characterize morphological associations between the external and osteological traits measured. In this approach we performed separate PCAs for the *manus* ("*manus* morphological PCA") and the *pes* ("*pes* morphological PCA"), both including measurements 1–8 (see Fig. 1) and the sum of phalangeal lengths. We analyzed the *manus* and *pes* separately to identify specific correlations between external morphology and osteology in anatomically independent elements; if traits from both autopodia were analyzed together, it is very likely that correlated traits from the hand and foot would cluster together.

A second PCA approach was used to reduce the number of variables to further test for ecological associations of osteological traits. We performed a phylogenetic PCA using all size-corrected traits from the osteological dataset, this time evaluating the *manus* and *pes* together. This analysis is designated as the "osteological PCA," and includes variables 6–12 (illustrated in Fig. 1). The scores retained from the "osteological PCA" were then used as dependent variables in a PGLS with the ecological classification (climbers or nonclimbers). Estimation of PGLS included calculation of the degree of phylogenetic correlation (PGLS$_\lambda$ – see Revell, 2010 for details). As aforementioned, based upon literature records *P. gerrhopygus* and *Gonatodes humeralis* can be regarded either as ground-dwellers or climbers, so these analyses were performed four times to verify whether the ecological classification of these species influenced the ecomorphological associations identified in the autopodial osteology of Neotropical geckos. The four possible ecological combinations were: Ecological classification 1: *Phyllodactylus gerrhopygus* and *Gonatodes humeralis* assumed as being nonclimbers; Ecological classification 2: *P. gerrhopygus* classified as climber and *G. humeralis* as nonclimber*;* Ecological classification 3: *P. gerrhopygus* classified as nonclimber and *G. humeralis* as climber; Ecological classification 4: both *P. gerrhopygus* and *G. humeralis* assumed as being climbers.

TABLE 1. Loadings of morphological traits resulting from phylogenetic principal components analyses performed separately for hand (manus) and foot (pes) using external and osteological morphometrics of the hand palm/foot sole and the Digit V from geckos

	Manus		*Pes*	
	PC1	PC2	PC1	PC2
Hand palm/foot sole width	**0.704**	0.607	**0.701**	0.522
Hand palm/foot sole length	**0.935**	−0.073	**0.751**	−0.328
Digit width	−0.384	**0.714**	0.276	**0.684**
Digit proximal width	−0.408	**0.772**	0.094	**0.909**
Digit length	**0.600**	−0.381	**0.731**	−0.391
Metacarpus/metatarsus width	0.440	0.419	**0.766**	−0.038
Metacarpus/metatarsus length	**0.735**	0.386	**0.908**	0.052
Sum of phalanges lengths	**0.687**	−0.426	**0.779**	−0.350
Phalanx I width	**0.627**	0.450	0.493	0.298
Eigenvalue (% explained variance)	3.642 (40.47)	2.334 (25.94)	3.936 (43.74)	2.044 (22.70)

Variables contributing most to each principal component (PC) are indicated in bold.

RESULTS

Characterization of variation between external morphology and osteology were congruent between the *manūs* and *pedes*, and supported our first prediction that these traits are strongly correlated (Table 1). Both phylogenetic PCAs (for *manus* and for *pes*) retained two main principal components that explained more than 65% of the data variance. The first component has high positive loadings for the length measurements of Digit V, metacarpus/metatarsus, *manus* palm/*pes* sole, and for the sum of phalangeal lengths (Table 1). In the morphological PCA for the *pes*, the first component also grouped widths of the fifth metatarsal and the sole of the *pes*. These traits apparently coevolved in the same direction because the loadings of all variables grouped by the first principal component had the same signal (positive): animals having longer digits and a longer manual palm/pedal sole also exhibit higher sums of phalangeal lengths and longer metacarpals/metatarsals. The second principal component retained exhibited high positive loads for measurements related to external digit width (measurements 2 and 3 in Fig. 1; see Table 1).

The second analysis we performed tested for ecological associations of autopodial osteological traits. As mentioned before, we performed an osteological PCA combining the traits of the *manus* and *pes* together to further test for ecomorphological associations using scores from the retained principal components. The osteological PCA retained two principal components (Table 2): PC1 grouped the lengths of penultimate and ungual (ultimate) phalanges (phalanges 2 and 3 in *manus*, phalanges 3 and 4 in *pes*) and width and length of the fifth metatarsal positively; PC2 exhibited high loadings with positive values for the length of phalanx 1 (both *manus* and *pes*) and negative loadings for the width of phalanx I (both *manus* and *pes*) and for metacarpal width. Scores of PC1 were correlated with ecology in most combinations of ecological classifications, while results of ecological associations for PC2 were ambiguous and more dependent on the combination of ecological classification (Table 3). The phylomorphospace plot

TABLE 2. Loadings of morphological traits resulting from a phylogenetic principal components analysis performed exclusively for osteological variables, which combined data for the hand palm/foot sole and the Digit V of manus and pes of geckos

		PC1	PC2
Manus	Metacarpus width	−0.404	**−0.801**
	Metacarpus length	−0.492	−0.148
	Phalanx I width	−0.498	**−0.727**
	Phalanx I length	−0.570	**0.804**
	Phalanx II length	**−0.894**	0.350
	Phalanx III length	**−0.947**	0.133
Pes	Metatarsus width	**−0.715**	−0.551
	Metatarsus length	**−0.819**	−0.301
	Phalanx I width	−0.379	**−0.793**
	Phalanx I length	−0.646	**0.725**
	Phalanx II length	−0.385	0.179
	Phalanx III length	**−0.852**	0.229
	Phalanx IV length	**−0.968**	0.072
	Eigenvalue (% explained variance)	6.23 (47.92)	3.62 (27.82)

Variables contributing most to each principal component (PC) are indicated in bold.

TABLE 3. Results from PGLS models testing for associations between scores from the principal components (PC) retained in the osteological PCA and the locomotor habit

	Ecological classification	$t_{1,11}$	*P*
PC1	1	−3.076	0.0105*
	2	−4.631	0.0007*
	3	−4.153	0.0963
	4	−3.360	0.0064*
PC2	1	−1.498	0.1624
	2	−1.483	0.1662
	3	−2.648	0.0347*
	4	−2.374	0.0390*

Ecological classification 1: *Phyllodactylus gerrhopygus* and *Gonatodes humeralis* assigned as nonclimbers; Ecological classification 2: *P. gerrhopygus* classified as climber and *G. humeralis* as nonclimber; Ecological classification 3: *P. gerrhopygus* classified as nonclimber and *G. humeralis* as climber; Ecological classification 4: both *P. gerrhopygus* and *G. humeralis* assigned as climbers.
Significant values ($P < 0.05$) are indicated by *.

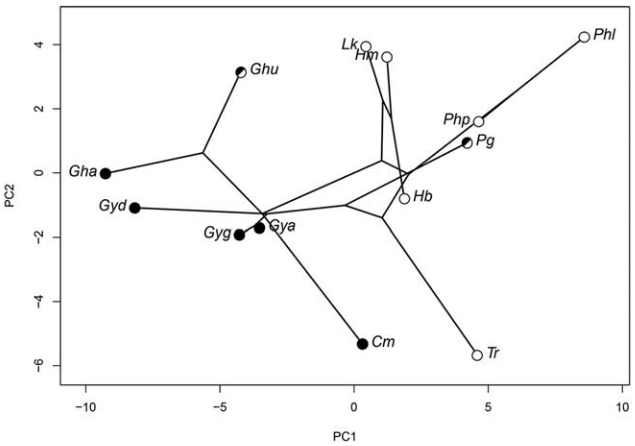

Fig. 3. Phylomorphospace graph of PC1 and PC2 scores for the osteological PCA. Locomotor habits are indicated by circles: black (nonclimbers) and white (climbers). The black lines represent the tree topology branches. Cm: *Coleodactylus meridionalis*; Gha: *Gonatodes hasemani*; Ghu: *Gonatodes humeralis*; Gya: *Gymnodactylus amarali*; Gyd: *Gymnodactylus darwinii*; Gyg: *Gymnodactylus geckoides*; Hb: *Hemidactylus brasilianus*; Hm: *Hemidactylus mabouia*; Lk: *Lygodactylus klugei*; Pg: *Phyllodactylus gerrhopygus*; Phl: *Phyllopezus lutzae*; Php: *Phyllopezus pollicaris*; Tr: *Thecadactylus rapicauda*.

of the two PC scores generated by the osteological PCA (Fig. 3) showed that climbers and nonclimbers occupy delimited morphospaces along the PC1 axis (clustering lengths of the two most distal phalanges and metatarsal dimensions), with the exception of *Coleodactylus meridionalis*, which is positioned near the positive region of the PC1 axis and closer to the climbing species. Data distribution along the PC2 axis (grouping length and width of phalanx I and metacarpus width) was much more dispersed, with climbers and nonclimbers having similar distributions along this axis and indicating osteological variation within the categories of locomotor habit. The direction of morphological variation can be visualized in this plot (Fig. 3): climbers exhibit shorter penultimate and ultimate phalanges and shorter metatarsals in comparison to nonclimbing species (PC1, Table 2).

DISCUSSION

This study provides evidence for evolutionary associations between ecology and autopodial osteology in Neotropical geckos, and reveals that in these lizards most of the examined components of osteological segments and external morphology coevolved in the same direction in morphospace, especially those related to the longitudinal parameters of digital elements (external traits [comprising several bones] and phalangeal size) and palmar dimensions (external relative proportions and metapodial sizes). Regarding the ecological associations of osteological morphology, we show that nonclimbing geckos exhibit longer distal phalanges in both the manual and pedal fifth digits, and longer fifth metatarsals in comparison to climbers. Although previous studies describe associations of ecology and morphological variation in external and osteological traits of the autopodium of geckos (Johnson et al., 2005; Russell et al., 2015), our study innovates by formally testing associations between these two morphological levels (external measurements and osteological segments) to explore autopodial evolution in the context of locomotor habit specialization. Our results contribute to a better understanding of how evolution of bone segments correlate with the locomotor habit performed in specific microhabitats.

A common expectation for limb morphological evolution is that hard and soft tissues should exhibit noticeable spatial associations due to both functional constraints and developmental restrictions (Manzano et al., 2012; Shwartz et al., 2012; Abdala et al., 2015; Diogo et al., 2015). Despite its extensive scope, this prediction has scarcely been tested using a phylogenetically informed comparative approach (Johnson et al., 2005). For Neotropical geckos, we showed that corresponding regions of the *manus* and *pes* exhibit equivalent associations between external morphology and osteology. These associations were identified for anatomical regions in which external morphology is mostly determined by the form of bones, rather than by other tissues, suggesting that these traits (external morphology and osteology) may have evolved under equivalent selective pressures. Associations between external morphology and osteology are not often perceptible. Digit width, for example, was not associated with bone width, being probably determined predominantly by fleshy tissues and epidermal organization—particularly in species that bear anatomical specializations associated with subdigital toe pads (Maderson, 1964; Russell, 1975, 2002; Irschick et al., 1996). Identification of associations between external morphology and osteology seems thus to be a relevant primary step in the understanding of the relevance of integrated morphology for locomotion in specific ecological settings.

Ecological divergence of locomotor habits in Neotropical geckos occurred in association with variation in osteological morphology in both the *manus* and *pes*. Besides descriptions of morphological changes in specialized structures of lizards associated with climbing or horizontal running (Russell 1975; Irschick et al., 1996; Zaaf et al., 1999; Zani, 2000; Russell et al., 2015; see also Johnson et al., 2005 for alternative approaches to address this type of question), variation in the relative proportions of autopodial structures has remained so far unexplored in the context of movement in different microhabitats (trees and perches vs. rocks, fallen trunks and soil), a question addressed here using climbing and nonclimbing geckos. Total limb proportions vary according to microhabitat use and locomotor habit in several squamate lineages—climbing lizards usually have shorter limbs than ground-dwellers (Losos and

Sinervo, 1989; Losos, 1990b; Sinervo and Losos, 1991; Miles, 1994; Irschick and Jayne, 1999). In geckos, however, this type of association has been barely detected within broad taxonomic samples (see Zaaf and Van Damme, 2001; Johnson et al. 2005), and generally specific adaptations, usually regarding the presence of adhesive toe pads, are those explored in this taxon (Irschick et al., 1996; Gamble et al., 2012; Higham et al., 2015). Using a combined dataset of external and osteological autopodial traits, we detected that variation of proportions among ecologically divergent gecko species actually exists, but is mostly confined to specific osteological elements of the autopodium. Together with published information about ecological associations of toe pad distribution among geckos (Johnson and Russell, 2009; Gamble et al., 2012, Collins et al., 2015, Higham et al., 2015), our results reinforce the hypothesis that the climbing specialization in Gekkota involved variation in relative proportions of osteological elements in the *manus* and *pes*.

Ecological associations of the autopodial osteology of geckos indicate that climbers and nonclimbers differ in the relative proportions of specific elements in the *manus* and the *pes*, and additional morphological variation very likely exists also within the same ecological category. Among climbers, the osteology of the fifth metacarpal and first phalanx of the *manus* and *pes* (PC2 of Table 2) is highly variable (illustrated in Fig. 3), and the different patterns identifiable within this ecological category likely relate to other autopodial peculiarities, associated, for example, with their adhesive systems. Adhesive toe pads evolved several times during the Gekkota radiation, and are often associated to most variable paraphalangeal structures (Russell and Bauer, 1988; Gamble et al., 2012). Most of the climbing species studied here actually have paraphalanges associated with toe pads (except *Phyllodactylus gerrhopygus* and *Gonatodes humeralis*, which are respectively leaf-toed and paddles, both ambiguously classified as climber and nonclimber in the literature), and remarkable morphometric and meristic diversity has been described in geckos presenting such structures (Russell and Bauer, 1988; Gamble et al., 2012). Paraphalanges support and control pad extremities (Russell and Bauer, 1988; Russell, 2002) and likely enhance pad attachment, but the biomechanical effects of morphological variation in paraphalanges remain obscure in the context of locomotion on different surfaces. For example, both *Thecadactylus rapicauda* and two species of *Phyllopezus* are climbers exhibiting similar patterns of paraphalangeal system (located at the penultimante phalangeal joint of digits II–V, the paraphalanges lay on adipose system and are tightly bound to lateral tendons, which tension provides a more efficient contact of the setae with the surface; Russell and Bauer, 1988), but they differ in relative sizes of the first phalanx and the metacarpus of the fifth digit (explained by the osteological PC2 at Table 2, see also Fig. 3). These species are classified as "climbers" in our analyses, but they differ in pad morphology and microhabitat use. Geckos from the Amazonian species *T. rapicauda* are often observed on tree trunks high in the canopy; they bear relatively large adhesive toe pads (see digits in Fig. 2) and phalanges that are short and wide (Russell and Bauer, 1988; Vitt and Zani, 1997; Bergmann and Russell, 2007). In contrast, *P. lutzae* climbs bromeliads, and we measured individuals of *P. pollicaris* from a Caatinga population that is found on rocky outcrops and buildings, mostly higher than 1.0 m above the ground (Vanzolini, 1974; Werneck and Colli, 2006; see also Supporting Information Table S1). The toe pads of *Phyllopezus* are proportionally smaller than those of *Thecadactylus*, and the proximal phalanx is longer but narrower. Interestingly, the species *Gonatodes humeralis* share traits that are common to both ecological classifications—their distal phalanges and metatarsals resemble that of nonclimbers, but dimensions of the first phalange and metacarpal define a climber's morphology. Such pattern is possibly related to the generalist ecology of this species (Avila-Pires, 1995; Vitt et al., 1997, 2000; Maciel et al., 2005), and the presence of incipient subdigital pads at the digits' medial region that enables clinging at low friction surfaces (Russell et al., 2015). Frictional structures likely represent a transitional stage between padless and padded autopodia, reflecting the transition from nonclimbing to climbing ecology in geckos (Russell et al., 2015). Despite clear autopodial differences among climbers, it remains undetermined whether such variation affects climbing biomechanics on different surfaces, encouraging further research to investigate the interactions of phalanges and paraphalanges relative to distinct surfaces and their implications the for evolution of different types of climbers among the Gekkota.

Evolution of toe pads in some species of geckos has led to distinctive changes in climbing biomechanics on vertical surfaces (Russell, 2002; Autumn et al., 2006; Russell and Higham, 2009; Higham et al., 2015). These specific locomotor patterns are also likely affected by the osteological variation detected here. During vertical locomotion, climbers possessing adhesive toe pads frequently employ hyperextension of the digits: the distal region of the digits (osteologically comprised of the ultimate and penultimate phalanges) curling dorsally and being the first part of the autopodium to detach from the surface (Russell, 1975, 2002). Nonclimbers, in contrast, are classically high-speed horizontal runners, and often exhibit longer limb segments and autopodia, which enhance the velocity propulsion (Irschick and Jayne, 1999). In this type of locomotion, digit release from the surface does not involve digital hyperextension from proximal to distal regions, and longer digit distal regions (comprising the ultimate and antepenultimate phalanges; here adopting Digit V as a proxy for all digits; see Melville and Swain, 2000; Grizante et al., 2010 for discussions about ecological associations using measurements from a single digit) possibly provide a longer lever arm to propulsion force, which likely enhances forward thrust during steady running (Kohlsdorf and Navas, 2012; Tulli et al., 2012). Therefore, effective substrate contact in nonclimbers may be endowed by autopodia that exhibit longer distal phalanges. The biomechanical roles of distal autopodial elements described above for climbers and nonclimbers support the hypothesis that size differences of Digit V between climbing and nonclimbing geckos may be correlated with the use of digital hyperextension during locomotion by one group but not the other.

In lizards, the fifth metatarsal has a modified hooked shape (see Fig. 1) associated with several muscle insertions that power pedal plantar flexion and raise Digit V during horizontal locomotion (Robinson, 1975; Brinkman, 1980). Our results confirm that nonclimbing geckos have a longer fifth metatarsal than do climbers, a pattern that may relate to the influence of metatarsal rotation on autopodial lifting particularly in geckos that lack adhesive toe pads (Zaaf et al., 1999; Russell and Bels, 2001). Moreover, we have also identified that climbers exhibit shorter distal phalanges. We recognize that *manus* and *pes* biomechanics differs during locomotion (see Russell and Bels, 2001), and in this study, we did not directly access the implications of the osteological elements measured in relation to the kinematics of locomotion in the species we studied. Our results, however, provide evidence that climbing performance in geckos likely evolved in association with autopodial osteological variation. This study provides a precursor for future studies that address the biomechanical implications of osteological variation in specific ecological settings, fostering the consideration of osteological segments to the morphological dimension of the Arnold's paradigm (Arnold, 1983), which has been biased in the literature by prevailing information derived from external traits composed by several bones.

ACKNOWLEDGMENTS

We acknowledge the curators and technicians of the following herpetological collections for specimen loans: Museum of Natural History of the Federal University of Alagoas (MUFAL), Museum of Zoology of the Federal University of Bahia (MZUFBA), Museum of Zoology of the University of São Paulo (MZUSP), National Museum of the Federal University of Rio de Janeiro (MNRJ), and the Herpetological Collection of the University of Brasília (CHUNB). We also thank to the members of the Laboratory of Tetrapod Evolution at Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, University of São Paulo, Brazil for helpful discussions in early drafts of the manuscript. Finally, we acknowledge two anonymous reviewers for significant contributions to this article.

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