

Alternate morphological pathways involved in climbing ability in ten Neotropical anuran species

IRENE CEBALLOS-CASTRO^{1,2,*}, JIMMY CABRA-GARCÍA^{2,*} and JHON JAIR OSPINA-SARRIA^{1*}

¹Calima, Fundación para la Investigación de la Biodiversidad y Conservación en el Trópico, Cali, Colombia

²Departamento de Biología, Universidad del Valle, A.A. 25360, Cali, Colombia

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Convergent evolution has been shown to be a prominent feature of anuran evolutionary history. Studying the morphological pathways involved in the evolution of a convergent character allows us to test whether deterministic or contingent forces drive the evolution of characters. Here, we have assessed the morphological pathways associated with arboreal habits in species of six families of anurans (Hylidae, Eleutherodactylidae, Strabomantidae, Centrolenidae, Bufonidae and Hemiphractidae) through a comparative analysis of 19 phenotypic characters related to climbing ability. All species showed differences in the assessed characters, exhibiting variations in the distribution of their states and different ranges in all limb lengths. These variations implied a wide distribution across the morphospace as defined by a non-metric multidimensional scaling analysis (NMDS), with *Rhinella paraguas* (Bufonidae) being the most distinctive species, presenting unique characters such as the absence of intercalary elements, adhesive pads, subarticular tubercles and interphalangealis muscles of the hands. Our findings demonstrate that the morphological mechanisms involved in climbing ability of the ten species are different, suggesting distinct morphological pathways. Consequently, we advocate that historical contingency has an essential role in the evolution of arboreal habits among the species studied.

ADDITIONAL KEYWORDS: convergent evolution – determinism – historical contingency.

INTRODUCTION

Convergent evolution is interpreted as the recurrent evolution of similar characters between phylogenetically distant taxa (Stayton, 2008, 2015; Wake *et al.*, 2011; Losos, 2017; Morinaga & Bergmann, 2017). Convergence is considered to be ubiquitous throughout the tree of life, being recognized as a central subject in evolutionary biology (Stayton, 2015; Sackton & Clark, 2019; Law, 2021). It has been proposed that convergent characters can be produced by deterministic means driven through similar selective pressures or constraints acting on similar aspects of organisms, resulting in the predictability of evolution (Morris, 2003; Stayton, 2008; Losos, 2017; Blount *et al.*, 2018). Conversely, historical contingency has been considered an essential hallmark of evolution (Gould, 1990), whereby evolutionary changes are

unique and unpredictable historical events and should not be interpreted as replicates for statistical analysis (Kluge, 2005). Accordingly, convergent phenotypes can emerge, but they would occur through different underlying mechanisms. In other words, the evolutionary histories of each lineage might have different solutions to shared selective issues (Moen *et al.*, 2016; Dolezal & Lipps, 2019). Several authors express that determinism and contingency provide opposite but not mutually exclusive explanations of how convergence occurs. This is a topic of constant debate among scientists who attempt to demonstrate the importance of each force in driving the evolution of characters (Gould & Lewontin, 1979; Losos, 2011; Bergmann & Morinaga, 2019).

Several studies of convergent evolution provide evidence of both determinism and historical contingency. A classic example is the evolutionary radiation of *Anolis* lizards on different islands in the Greater Antilles, where the same ecomorphs evolved

*Corresponding author. E-mail: jhon.sarria@fundacioncalima.org

on the islands, but different evolutionary events drove the order in which these ecomorphs appeared on each island (Losos, 1992; Losos *et al.*, 1998). Likewise, similarities have been found in the swimming mode of lamnid sharks and tunas, although they exhibit notable differences in their anatomy (Donley *et al.*, 2004). For example, in tunas the primary force-transmitting tendons are in the horizontal septum; however, in the lamnid sharks, as in other sharks, the horizontal septum is reduced in the posterior half of the body, hence the primary linkage to the tail appears to be the hypaxial lateral tendons (Donley *et al.*, 2004). Also, the convergence of elongated phenotypes in salamanders and carnivorous mammals has been associated with different morphological mechanisms of body elongation (Wake *et al.*, 2011; Law, 2021). The increase of body length in salamanders can be associated with the elongation of individual vertebrae, whereas in carnivorous mammals it can be attributable to the elongation of the thoracic region, thoracic vertebrae or the lumbar region (Wake *et al.*, 2011; Law, 2021). In the particular case of anurans, Moen *et al.* (2016) suggested repeated convergence at large spatial and temporal scales, based on phenotypic similarity in relationship to microhabitat use, revealing that specialists in similar microhabitats (e.g. fossorial, aquatic or arboreal) have evolved repeatedly across clades and regions. Dugo-Cota *et al.* (2019) reported similar results of morphological convergence in frogs of the genus *Eleutherodactylus* owing to a strong association between morphological characters and habitat types. Those authors suggested that their results are a consequence of adaptation, because each habitat presents distinctive challenges to the species that are solved in a similar manner.

One of the habitats in which strong and unique selective pressures have been postulated, including predator avoidance, osmoregulation, foraging strategies and locomotion, is the arboreal habitat (Cartmill, 1985; Baken & Adams, 2019). Currently, arboreal habits are considered a clear example of evolutionary convergence in amphibians, given that climbing ability has evolved multiple times independently among lineages (Sustaita *et al.*, 2013; Moen *et al.*, 2016; Dugo-Cota *et al.*, 2019). Hence, arboreal habits in anurans are an ideal model for studying evolutionary convergence across different levels of organization in terms of function and form (Speed & Arbuckle, 2017; Bergmann & Morinaga, 2019). A higher level of biological organization specifies functional capabilities (i.e. climbing ability), whereas a lower level specifies the morphological mechanisms involved in achieving this general function. It is worth noting that convergence at higher levels of biological organization does not necessarily indicate convergence at a lower level (Doolittle, 1994; Losos, 2011; Maeso

et al., 2012; Dolezal & Lipps, 2019). Consequently, only a detailed comparison of the evolutionary pathways involved in arboreal habits will elucidate whether convergence extends to lower mechanistic levels. Evolutionary pathways include the sequences of genes (i.e. genetic pathways), developmental programmes (i.e. developmental pathways) or morphological modifications (i.e. morphological pathways) underlying the phenotype evolution (Arendt & Reznick, 2008; Morinaga & Bergmann, 2017; Bergmann & Morinaga, 2019).

In the present study, we assessed the morphological pathways involved in the arboreal habits of six phylogenetically distant families of anurans. We performed a comparative analysis of morphological and anatomical characters directly involved in climbing ability. Our results provide new insights into the interplay of contingency and determinism in the evolution of arboreal habits in Neotropical frogs.

MATERIAL AND METHODS

SPECIES SELECTION

We studied ten arboreal species of six distantly related families of anurans: Hylidae (*Hyloscirtus larinopygion*, *Boana pugnax* and *Agalychnis spurrelli*), Eleutherodactylidae (*Eleutherodactylus johnstonei*), Strabomantidae (*Pristimantis palmeri* and *Pristimantis molybrignus*), Centrolenidae (*Nymphargus ignotus* and *Centrolene heloderma*), Bufonidae (*Rhinella paraguas*) and Hemiphractidae (*Gastrotheca dendronastes*) (Supporting Information, Appendix S1). A species is considered arboreal if it is usually active in vegetation (higher than 0 m and up to 6 m) during the night, with diurnal retreat sites in vegetation or on the ground (Dugo-Cota *et al.*, 2019). In total, we examined 120 adult specimens belonging to the Colección de Prácticas Zoológicas, Universidad del Valle (CPZ-UV) and the Colección de Anfibios y Reptiles, Universidad del Valle (UV-C), which are listed in the Supporting Information (Appendix S2). The sex and maturity of specimens were determined by the presence of secondary sexual characters (i.e. vocal slits and nuptial pads) and/or direct examination of gonads and oviducts by making a small posterolateral incision. Amphibian taxonomy follows Frost (2022).

CHARACTER SELECTION

Morphological and anatomical characters were selected based on the literature, including characters related to climbing ability on smooth, vertical surfaces. In total, we scored 12 qualitative and seven quantitative characters. We have provided a detailed description of the coded characters (Table 1). The sources of evidence

Table 1. Characters and states

Character	State
<i>Hand musculature</i>	
1. Interphalangealis of digits IV and V	Absent (0), present (1)
2. Degree of development of interphalangealis with respect to superficial tendon coverage	Poorly developed (0), highly developed (1) The poorly developed state is defined when more than half or all the superficial tendon is visualized in the phalanx
3. Position of flexor teres III with respect to the transversus metacarpi muscle	Dorsal (0), ventral (1)
4. Position of flexor teres IV respect to the transversus metacarpi muscle	Dorsal (0), ventral (1)
5. Extensor breves distal of digit III–V	Absent (0), present (1)
6. Elongation of the extensor breves profundi of digits III–V	Not extended (0), extended beyond the basal phalanx (1)
<i>Foot musculature</i>	
7. Lateral interphalangeal of digit V	Absent (0), present (1)
8. Position of flexor teres II with respect to the transversus metatarsi muscle	Dorsal (0), ventral (1)
9. Intercalary elements	Absent (0), present (1)
10. Adhesive pads	Absent (0), present (1)
11. Hyperdistal subarticular tubercle	Absent (0), present (1)
12. Distal and basal subarticular tubercles	Absent (0), present (1)

used to score these characters include examination of museum specimens, published descriptions and field observations (Supporting Information, [Appendix S3](#)).

QUALITATIVE CHARACTERS

Hand and foot musculature (Figs 1, 2)

Interphalangealis and flexor teres muscles allow flexion of the fingers by bending the phalanges where they are inserted ([Burton, 1998b, 2001](#); [Blotto *et al.*, 2020](#)). Therefore, we described the associated variation and degree of development of interphalangealis muscles of the hands in digits IV and V. We also reviewed the occurrence of the lateral slip of muscle interphalangei digit V in the foot ([Ospina-Sarria, 2012](#); [Pereyra *et al.*, 2021](#)). In the case of the muscle flexor teres, we reviewed the dorsal or ventral position concerning the transversus metacarpi/metatarsi muscles in digits III and IV of the hand and digit II of the foot ([Burton, 1998c](#)). The muscle extensores breves distales and profundi allow extension of the phalanges and assistance with detachment of the adhesive discs ([Blotto *et al.*, 2020](#)). The associated variation (presence, absence and extension) of the muscle extensores breves distales and profundi in digits III–V was described. Dissection protocols and terminology of the musculature followed [Burton \(1996, 1998a, b, c\)](#). We studied the muscles of the specimens with the aid of a stereomicroscope (Zeiss Stemi 2000-C), and digital images were obtained through the ocular lens using a Samsung camera.

Intercalary elements

The intercalary elements are additional skeletal structures between the terminal and penultimate phalanges. The presence of these elements has been related to other morphological specializations to facilitate locomotion between vertical and slippery surfaces ([Manzano *et al.*, 2007, 2017](#)). On this basis, we scored the distribution of the variation (presence or absence) of the intercalary elements in the digits.

Adhesive pads and subarticular tubercles (Figs 3, 4)

[Endlein *et al.* \(2017\)](#) demonstrated that adhesive pads and subarticular tubercles of the fingers in the Chinese gliding frog *Zhangixalus dennysi* are ideal for climbing smooth surfaces and narrow substrates, such as twigs or branches. We scored the presence or absence of distal and basal subarticular tubercles and adhesive pads of digits III and IV in the foot and hand, following the terminology of [Lynch \(1999\)](#) for the adhesive pads and subarticular tubercles. Likewise, we evaluated the occurrence of the hyperdistal subarticular tubercle in digits III and IV of the hand, which is located beneath the joint between distal phalanges ([Ospina-Sarria & Duellman, 2019](#)).

QUANTITATIVE CHARACTERS

We measured the lengths of the following characters: humerus, radio-ulna, hand, femur, tibiofibula, calcaneus and foot. We took all measurements using Ubermann digital callipers to the nearest 0.01 mm.

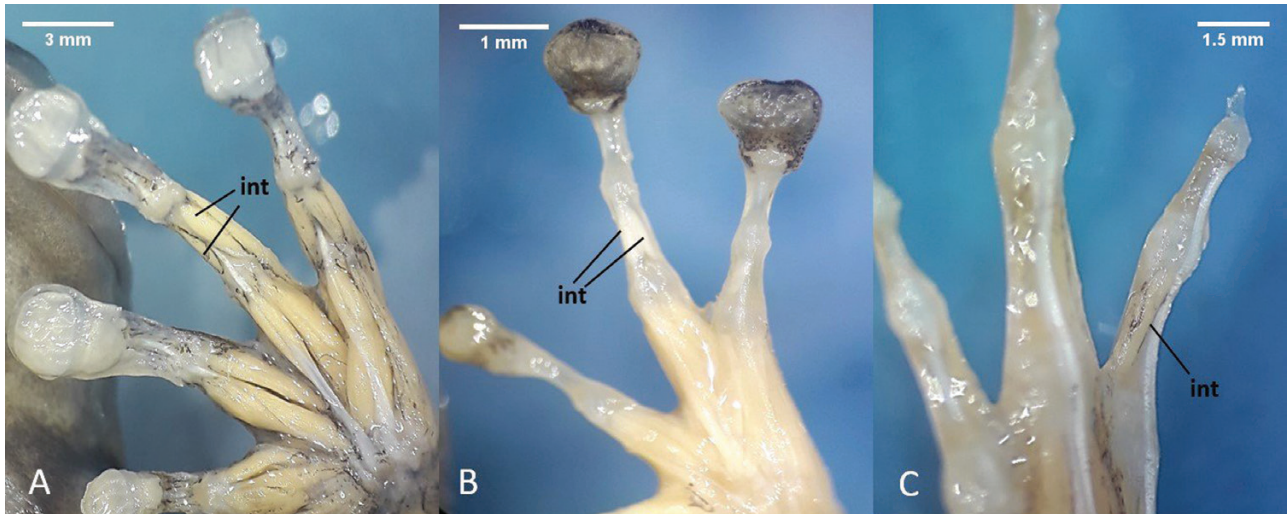


Figure 1. Interphalangeal muscles of hands and feet. **A**, highly developed interphalangeal muscles of digit IV in the hand of *Hyloscirtus larinopygion*. **B**, poorly developed interphalangeal muscles of digit III in the hand of *Pristimantis palmeri*. **C**, medial interphalangeal muscle of digit V in the foot of *Rhinella paraguas*. Abbreviation: int, interphalangeal muscles.

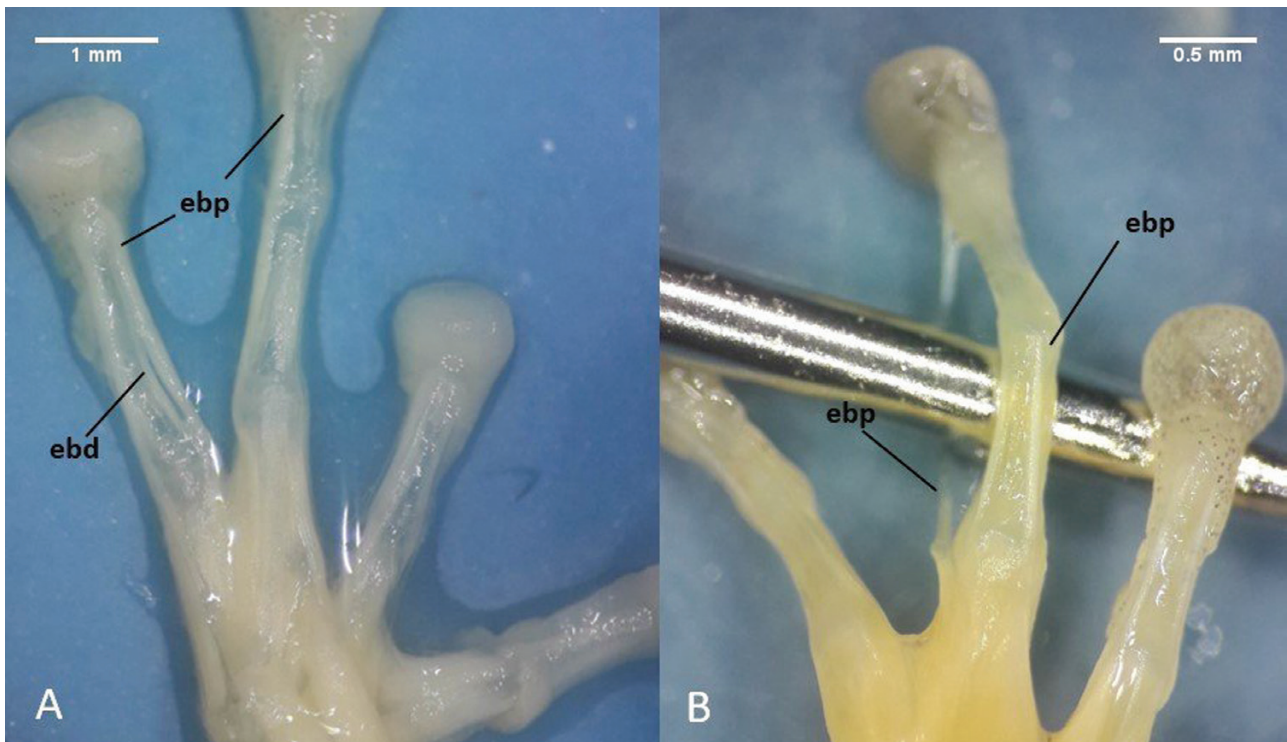


Figure 2. Extensores breves distales and profundus. **A**, extensor brevis distalis and elongation of extensor brevis profundus in the hand of *Nymphargus ignotus*. **B**, extensor brevis profundus fibres extending beyond the base of the basal phalanx in the hand of *Eleutherodactylus johnstonei*. Abbreviations: ebd, extensor brevis distalis; ebp, extensor brevis profundus.

Using the raw measurements of the limbs (see Supporting Information, [Appendix S4](#)), we calculated the relative value of the characters, correcting them for size by dividing them by snout–vent length (see

Supporting Information, [Appendix S5](#)). In addition, we performed an exploration of sexual dimorphism in size across the ten species. As a result of this exploration, we found that *E. johnstonei*, *P. palmeri*, *P. molybrignus*

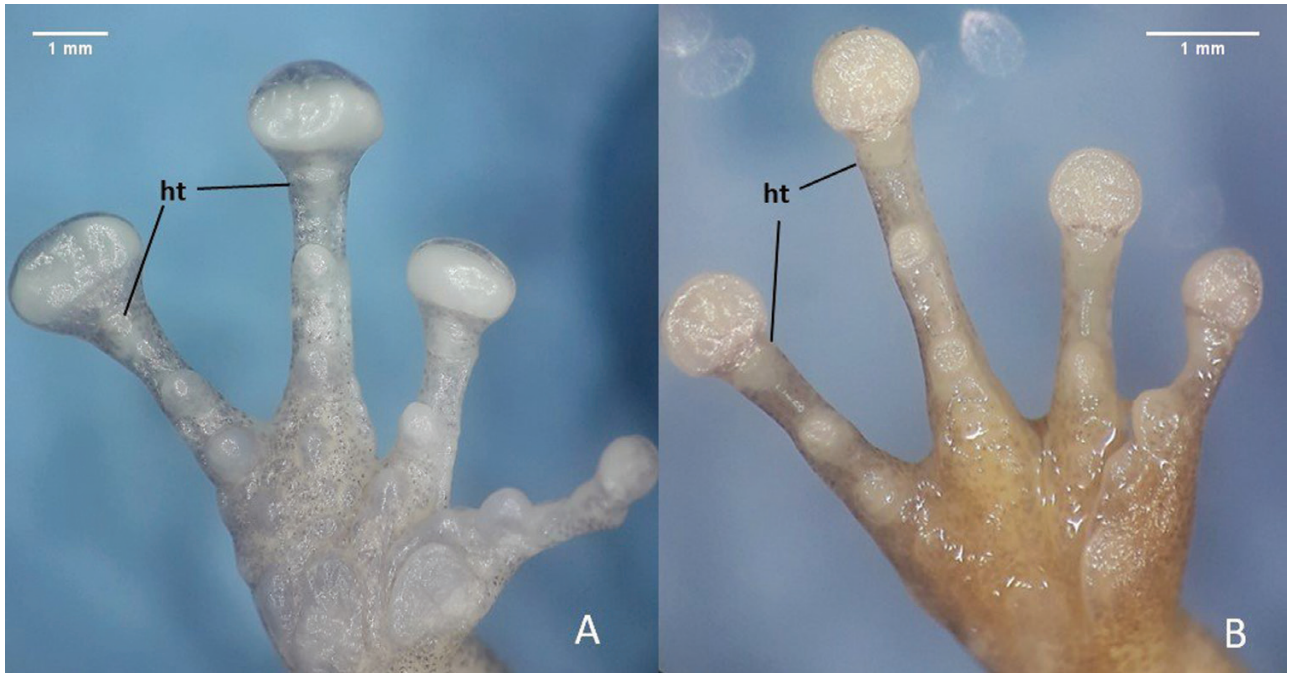


Figure 3. Hyperdistal subarticular tubercles in digits. Hand, ventral view. **A**, *Pristimantis palmeri*. **B**, *Eleutherodactylus johnstonei*. Abbreviation: ht, hyperdistal subarticular tubercle.



Figure 4. Subarticular tubercles and adhesive pads. Hand, ventral view. **A**, *Hyloscirtus larinopygion*. **B**, *Rhinella paraguas*. Abbreviations: ap, pads; st, subarticular tubercles.

and *R. paraguas* exhibit sexual dimorphism in size (Lynch, 1986, 1996; Ortega *et al.*, 2005; Grant & Bolívar-G, 2014), whereas *A. spurrelli*, *B. pugnax*, *H. larinopygion*, *G. dendronastes*, *C. heloderma* and *N. ignotus* do not (Duellman, 1981; Lynch, 1990; Krynak *et al.*, 2018; Ortiz *et al.*, 2018; Escalona *et al.*, 2019; Chasiluisa *et al.*, 2022; Ron *et al.*, 2022). Based on these results, females and males of *A. spurrelli*, *H. larinopygion*, *G. dendronastes*, *C. heloderma* and *N. ignotus* were analysed together; however, only males of *B. pugnax* were analysed because they were the only available specimens for this species. In the case of *E. johnstonei*, *P. palmeri* and *R. paraguas*, the analyses were performed exclusively on females, because the number of males in each case hampered the statistical analysis (see Supporting Information, Appendix S5). A similar situation occurred with *P. molybrignus*; however, in this case the sample size was adequate only for males.

CHARACTER ANALYSES

We implemented the following two analytical approaches.

Direct comparison

We built a phenotypic data matrix for character scoring using the software MESQUITE v.3.70 (Maddison & Maddison, 2019). We compared characters and their character states directly, in order to identify differences or similarities in their distribution across the ten species, following the approach of Liu *et al.* (2017).

Non-metric multidimensional scaling

We analysed all characters using a non-metric multidimensional scaling (NMDS) method. This method uses ranges of distances from a data matrix to represent the distribution of species according to the similarity or difference in their morphological characters (Palacio *et al.*, 2020). We performed NMDS analysis using the R package ‘vegan’ v.2.44 (Oksanen *et al.*, 2013) with the metaMDS function, k=2, autotransforms set to false, and Gower dissimilarity (Gower, 1971).

RESULTS

DIRECT COMPARISON OF MORPHOLOGICAL CHARACTERS

Nineteen phenotypic characters were analysed in ten species of anurans with arboreal habits, disclosing variations in the distribution of their states in addition to differences in each of the ranges of the limbs (see Supporting Information, Appendices S5 and S6).

All species except *R. paraguas* exhibit the presence of adhesive pads, distal and basal subarticular tubercles, lateral interphalangealis of digit V of the foot and interphalangealis of digits IV and V of the hand (see Supporting Information, Appendix S6). Regarding the occurrence of the last of these characters, there are different degrees of development. A well-developed interphalangealis covering the upper part of the superficial tendon appears in *H. larinopygion*, *A. spurrelli*, *B. pugnax*, *C. heloderma* and *N. ignotus*, whereas a poorly developed interphalangealis (i.e. more than half or all the superficial tendon is visualized in the phalanx) occurs in *G. dendronastes*, *E. johnstonei*, *P. molybrignus* and *P. palmeri* (Fig. 1; see Supporting Information, Appendix S6).

The flexor teres muscles of digit III of the hand are found in a position dorsal to the transversus metacarpi muscle in *A. spurrelli*, *B. pugnax*, *H. larinopygion*, *G. dendronastes*, *P. palmeri* and *P. molybrignus*, whereas in *R. paraguas* and *E. johnstonei* and centrolenids (*C. heloderma* and *N. ignotus*) they are observed ventral to the transversus metacarpi muscle. The position of the flexor teres of digit IV of the hand in *N. ignotus* and *C. heloderma* emerges ventral to the transversus metacarpi muscle. The muscles extensores breves distales of digits III–V of the hand, and intercalary elements, are present only in hylids (*A. spurrelli*, *B. pugnax* and *H. larinopygion*) and centrolenids (*C. heloderma* and *N. ignotus*). Elongation of the muscle extensor breves profundi is found in digits III–V of the hand in *E. johnstonei*, *C. heloderma* and *N. ignotus* (Fig. 2). The ventral position of flexor teres II with respect to the transversus metatarsi muscle is shared between *G. dendronastes* and *R. paraguas*. Additionally, hyperdistal tubercles are present exclusively in *E. johnstonei* and *P. palmeri* (Fig. 3).

As mentioned above, *R. paraguas* is the most distinctive species by lacking adhesive pads, hyperdistal tubercles, distal and basal subarticular tubercles (see Fig. 4), muscle extensores breves distales, elongation of the muscle extensores breves profundi and interphalangealis on the palmar surface. Moreover, although the interphalangealis muscles of the plantar surface are present in all the studied species, *R. paraguas* is the only one that lacks the lateral interphalangei in digit V of the foot (see Supporting Information, Appendix S6).

Regarding the quantitative characters, *B. pugnax* exhibits differences in the range of humerus length from most of the species, overlapping only with *G. dendronastes* and *R. paraguas* (see Supporting Information, Appendix S5). Moreover, *B. pugnax* has a different range in radio-ulnar length from *R. paraguas*, *H. larinopygion*, *N. ignotus* and *P. molybrignus*, being *R. paraguas* the one with the highest value (see Information, Appendix S5). Hand

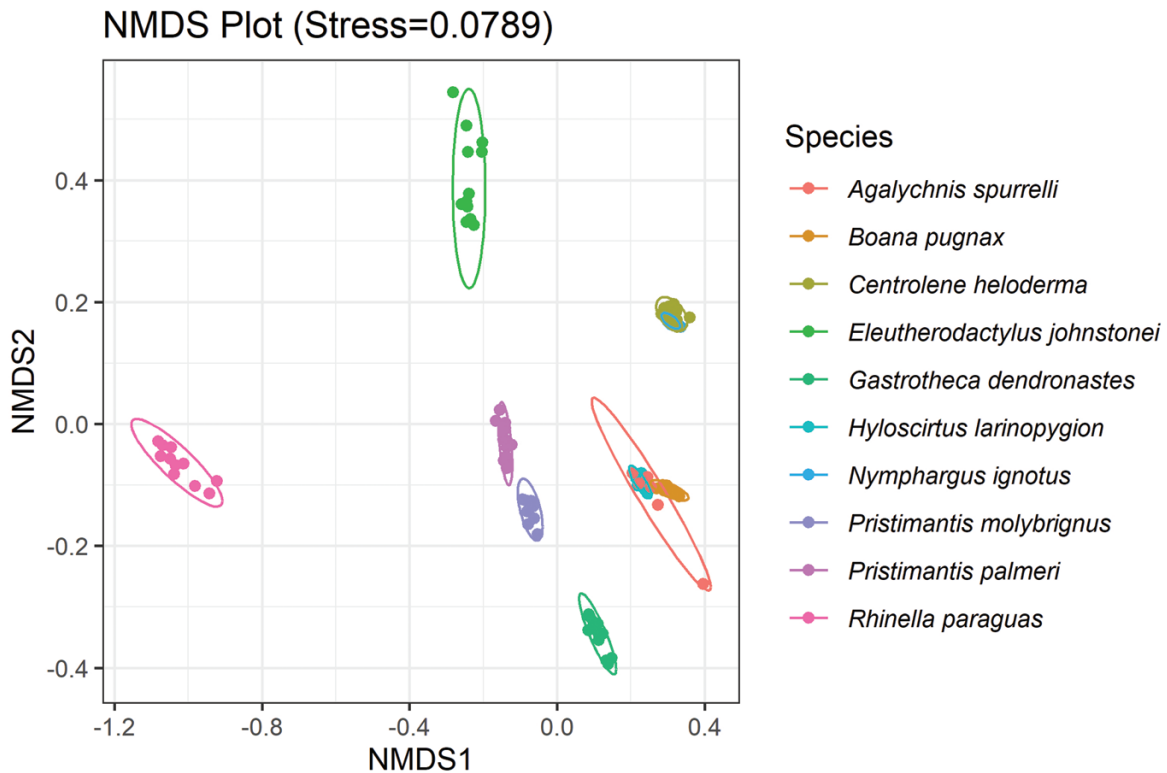


Figure 5. Non-metric multidimensional scaling (NMDS) analysis showing the distribution of the species within the morphospace.

length has the maximum ranges with respect to the length of the humerus and radio-ulna in *A. spurrelli*, *G. dendronastes*, *H. larinygion*, *N. ignotus* and *C. heloderma*.

In hindlimbs, femur length exhibits overlapping and lowest ranges among *E. johnstonei* and *R. paraguas*. *Gastrotheca dendronastes* differs from *H. larinygion*, *E. johnstonei* and *R. paraguas* in tibiofibular length. It is important to note that tibiofibular length is the most elongated character for all species, except for *R. paraguas* (see Supporting Information, [Appendix S5](#)). Finally, *R. paraguas* presents differences in calcaneus and foot lengths from most species, with the only exceptions being the overlapping in calcaneus length with *E. johnstonei* and *P. molybrignus* and overlapping in foot length with *B. pugnax* and *E. johnstonei* (see Supporting Information, [Appendix S5](#)).

NON-METRIC MULTIDIMENSIONAL SCALING ANALYSIS

The NMDS analysis revealed that the ten species are widely distributed throughout the two-dimensional space (see [Fig. 5](#)). *Rhinella paraguas* is the most distant species, followed by *E. johnstonei* and *G. dendronastes*. Also, the following three fairly distinct subgroups

emerge: (1) centrolenid species (*C. heloderma* and *N. ignotus*) and (2) hylid species (*A. spurrelli*, *B. pugnax* and *H. larinygion*), both with a high degree of overlap; and (3) the strabomantid species with no overlap (*P. molybrignus* and *P. palmeri*) (see [Fig. 5](#)).

DISCUSSION

In this study, we assessed a set of morphological characters involved in the climbing ability of ten phylogenetically distant anuran species. Overall, our results indicate that these species differ in the assessed characters and therefore present different morphological pathways involved in climbing ability.

DIFFERENT PHENOTYPIC MECHANISMS INVOLVED IN CLIMBING ABILITY

Taken together, the direct comparison of characters and the NMDS suggests that the climbing morphological mechanisms of the ten species are different ([Fig. 5](#); see Supporting Information, [Appendices S5 and S6](#)). Hylids (*A. spurrelli*, *B. pugnax* and *H. larinygion*) and centrolenids (*C. heloderma* and *N. ignotus*) present intercalary elements, which have been related to the



Figure 6. Field observations of *Rhinella paraguas* climbing vertical surfaces (photographs by Jhon Jairo Ospina-Sarria).

ability to climb vertically and/or to walk on slippery surfaces (Manzano *et al.*, 2017); however, as shown here, species lacking intercalary elements are also able to climb (i.e. *E. johnstonei*, *P. palmeri*, *P. molybrignus*, *G. dendronastes* and *R. paraguas*). Regarding this result, Endlein *et al.* (2017) demonstrated that the recruitment of tubercles increases the contact area and produces higher frictional stresses than adhesive pads, enhancing the grip for climbing on narrow substrates. Thus, it could be suggested that the presence of hyperdistal subarticular tubercles in *E. johnstonei* and *P. palmeri*, together with basal and distal subarticular tubercles, could enhance climbing ability on small-diameter structures that make up much of their arboreal habitat (Hill *et al.*, 2018). In order to achieve a better comprehension of the climbing ability in species lacking intercalary elements, it is necessary to perform biomechanical studies. In any case, a growing body of evidence demonstrates that species of anurans lacking intercalary elements can also climb (e.g. ceratobatrachids of the genus *Platymantis* and the microhylids of the genera *Cophyla* and *Platypelis*; Manzano *et al.*, 2007).

Interestingly, *R. paraguas* can climb vertical surfaces (Fig. 6) despite the lack of intercalary elements, adhesive pads, subarticular tubercles (Fig. 4) and elongation in muscle size (except in the medial interphalangeal muscle of digit V of the foot; see Fig. 1). Recently, Vassallo *et al.* (2021) demonstrated that *Rhinella arenarum* climbs by flexing the fingers and

toes, grasping the substrate displaying hookings (i.e. a form of subsidiary prehensility where the phalanx–metacarpal/metatarsal joints remain in a straight position, and only the two terminal phalanges flex) and partial grasping. Hence, both *R. arenarum* and *R. paraguas* are non-specialized species that achieve climbing by different climbing mechanisms in comparison to specialized tree frogs. In this regard, Vassallo *et al.* (2021) suggested that any species (as is the case in *Rhinella*) with the appropriate anatomy and morphology can climb and add such behaviour to their locomotion. It has been suggested that different behaviours in ecological contexts can drive alternative locomotor strategies related to morphological adaptations (Manzano *et al.*, 2019). Furthermore, performing a novel locomotor mode can be important for adapting to new niches involving sudden changes in habitats, including changes in the substrate on which animals must move (Gomes *et al.*, 2009).

EVIDENCE OF HISTORICAL CONTINGENCY IN ARBOREAL HABITS

The set of morphological and anatomical characters studied in ten Neotropical anuran species demonstrates distinct morphological mechanisms involved in climbing ability, suggesting that different morphological pathways underlie the evolution of arboreality among the species studied. These findings corroborate that convergence at the highest level

of biological organization (i.e. the ability to climb) is insufficient evidence of determinism at lower levels. Similar findings of so-called incomplete or imperfect convergence have been documented in several studies (Wray, 2002; Stayton, 2006; Young *et al.*, 2010; Collar *et al.*, 2014; Morinaga & Bergmann, 2017; Bergmann & Morinaga, 2019). This type of convergence has been interpreted as a hallmark of historical contingency, whereby several lineages are observed to achieve similar function through different evolutionary pathways owing to many-to-one mapping of phenotype to function, leading to the evolution of distinct phenotypes (Wainwright *et al.*, 2005; Morinaga & Bergmann, 2017; Bergmann & Morinaga, 2019; Blotto *et al.*, 2021). Furthermore, the multiple morphological pathways involved in many-to-one mapping are thought to expand the number of solutions at similar selective pressure, and therefore, this can increase morphological diversity (Wainwright *et al.*, 2005, 2007; Muñoz, 2019).

It is worth noting that arboreal habits within anurans have been coded historically as a single character. Consequently, it is considered that this condition emerged several times independently in the Anura tree of life, establishing a classic homoplasy (Moen *et al.*, 2013, 2016; Vidal-García *et al.*, 2014; Dugo-Cota *et al.*, 2019). Nevertheless, as suggested by our results, the morphological pathways involved in climbing ability in the studied species are different, and the mode of locomotion and climbing behaviour across the species are also very likely to be distinct. Hence, the so-called arboreal habits might conceal several distinct conditions. The origin of this problem is attributable to the difficulty of delimiting the arboreal habits, because climbing behaviour involving a grasping action with the hands and feet (Burton, 1998b) can be found in anurans that are terrestrial during the day but climb at night, in addition to those that rarely descend to the ground and are found at great heights in the forest. Menzies (1976) made a distinction between the two cases to solve this problem, categorizing them as scansorial and arboreal species, respectively. Therefore, the proper coding of the arboreal habits in future studies should include ecological and behavioural analyses that focus on the modes of locomotion adopted by arboreal species, because each might be associated with novel characteristics (Duellman & Trueb, 1994; Manzano *et al.*, 2008; Reynaga *et al.*, 2018; Abdala *et al.*, 2022).

Likewise, to elucidate the morphological pathways involved in arboreal habits in anurans further, future research should focus on increasing the number of species, specimens and studied characters. With an increase in species, additional analyses, such as evolutionary integration analysis, could be performed

to estimate the correlated change of multiple characters within an organism (Klingerberg, 2008; Morinaga & Bergmann, 2017). A preliminary exploration conducted with our data on limb measurements suggests a limited role of evolutionary integration as an explanation for convergent arboreal habits (Supporting Information, Appendix S7). In addition, it is worth noting that it is crucial to identify the possible presence of sexual dimorphism, which might affect character analyses and interpretations (Emerson, 1991; Lee, 2001; Hudson *et al.*, 2016). Finally, regarding the morphological characters, it is suggested to assess opposable fingers and the divergence of the digits by means of tendons, reduction or loss of sesamoids, in addition to the insertion, elongation and increases in muscle size (Manzano & Lavilla, 1995; Manzano *et al.*, 2007, 2008, 2019; Herrel *et al.*, 2013; Sustaita *et al.*, 2013; Dang *et al.*, 2018).

CONCLUSION

In the present study, we found that species of the six families of Neotropical anurans presented different morphological pathways involved in their climbing ability. This is a case of incomplete or imperfect convergence, whereby the species studied have evolved different morphological solutions to similar selective pressures, pointing to an essential role of historical contingency in the evolution of arboreal habits. Nevertheless, future research on morphological pathways involved in climbing ability should increase the number of species and morphological characters and include ecological and behavioural analyses that focus on the modes of locomotion adopted by arboreal anurans.

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DATA AVAILABILITY

The data underlying this work are available in the Supporting Information.

REFERENCES

- Abdala V, Ponssa ML, Fratani J, Manzano A. 2022.** The role of hand, feet, and digits during landing in anurans. *Zoologischer Anzeiger* **296**: 187–197.
- Arendt J, Reznick D. 2008.** Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? *Trends in Ecology & Evolution* **23**: 26–32.
- Baken EK, Adams DC. 2019.** Macroevolution of arboreality in salamanders. *Ecology and Evolution* **9**: 7005–7016.
- Bergmann PJ, Morinaga G. 2019.** The convergent evolution of snake-like forms by divergent evolutionary pathways in squamate reptiles. *Evolution* **7**: 481–496.
- Blotto BL, Biju SD, Pereyra MO, Araujo-Vieira K, Faivovich J, Grant T. 2021.** Hand and foot musculature of Sooglossioidea: synapomorphies, convergences and hind limb digging behaviour in anurans. *Biological Journal of the Linnean Society* **135**: 336–393.
- Blotto BL, Pereyra MO, Grant T, Faivovich J. 2020.** Hand and foot musculature of anura: structure, homology, terminology, and synapomorphies for major clades. *Bulletin of the American Museum of Natural History* **443**: 1–155.
- Blount ZD, Lenski RE, Losos JB. 2018.** Contingency and determinism in evolution: replaying life's tape. *Science* **362**: eaam5979.
- Burton TC. 1996.** Adaptation and phylogeny in the hand muscles of Australo-Papuan hylid frogs (Anura: Hylidae: Pelodyadinae). *Australian Journal of Zoology* **44**: 611–623.
- Burton TC. 1998a.** Variation in the hand and superficial throat musculature of neotropical leptodactylid frogs (Anura: Leptodactylidae). *Herpetologica* **54**: 53–72.
- Burton TC. 1998b.** Are the distal extensor muscles of the fingers of anurans an adaptation to arboreality? *Journal of Herpetology* **32**: 611–617.
- Burton TC. 1998c.** Pointing the way: the distribution and evolution of some characters of the finger muscles of frogs. *American Museum Novitates* **3229**: 1–13.
- Burton TC. 2001.** Variation in the foot muscles of frogs of the family Myobatrachidae. *Australian Journal of Zoology* **49**: 5391–5321.
- Cartmill M. 1985.** Climbing. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, eds. *Functional vertebrate morphology*. Cambridge and London: Harvard University Press, 73–88.
- Chasiluisa V, Coloma LA, Frenkel C, Félix-Novoa C, Pazmiño-Armijos G. 2022.** *Gastrotheca dendronastes*. In: Ron SR, Merino-Viteri A, Ortiz DA, eds. *Anfibios del Ecuador*. Museo de Zoología, Pontificia Universidad Católica del Ecuador. Available at: <https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Gastrotheca%20dendronastes>. Accessed 29 October 2022. Ecuador.
- Collar DC, Reece JS, Alfaro ME, Wainwright PC, Mehta RS. 2014.** Imperfect morphological convergence: variable changes in cranial structures underlie transitions to durophagy in moray eels. *The American Naturalist* **183**: E168–E184.
- Dang N-X, Wang J-S, Liang J, Jiang D-C, Liu J, Wang L, Li J-T. 2018.** The specialisation of the third metacarpal and hand in arboreal frogs: adaptation for arboreal habitat? *Acta Zoologica* **99**: 115–125.
- Dolezal M, Lipps J. 2019.** Digest: how many ways to make a snake? Evidence for historical contingency of the convergence of squamate reptiles. *Evolution* **73**: 626–627.
- Donley JM, Sepulveda CA, Konstantinidis P, Gemballa S, Shadwick RE. 2004.** Convergent evolution in mechanical design of lamnid sharks and tunas. *Nature* **429**: 61–65.
- Doolittle RF. 1994.** Convergent evolution: the need to be explicit. *Trends in Biochemical Sciences* **19**: 15–18.
- Duellman WE. 1981.** Three new species of centrolenid frogs from the Pacific versant of Ecuador and Colombia. *Occasional Papers of the Museum of Natural History, University of Kansas* **88**: 1–9.
- Duellman WE, Trueb L. 1994.** *Biology of Amphibians*. Baltimore: Johns Hopkins University Press.
- Dugo-Cota A, Vilà C, Rodríguez A, Gonzalez-Voyer A. 2019.** Ecomorphological convergence in *Eleutherodactylus* frogs: a case of replicate radiations in the Caribbean. *Ecology Letters* **22**: 884–893.
- Emerson SB. 1991.** A biomechanical perspective on the use of forelimb length as measure of sexual selection in frogs. *Journal of Evolutionary Biology* **4**: 671–678.
- Endlein T, Ji A, Yuan S, Hill I, Wang H, Barnes W, Dai Z, Sitti M. 2017.** The use of clamping grips and friction pads by tree frogs for climbing curved surfaces. *Proceedings of the Royal Society B: Biological Sciences* **284**: 20162867.
- Escalona MD, Juncá FA, Giaretta AA, Crawford AJ, Marca E. 2019.** Contrasting genetic, acoustic, and morphological differentiation in two closely related gladiator frogs (Hylidae: *Boana*) across a common Neotropical landscape. *Zootaxa* **4609**: 519–547.
- Frost DR. 2022.** *Amphibian species of the world: an online reference. Version 6.1*. New York: American Museum of Natural History. Available at: <https://amphibiansoftheworld.amnh.org/index.php>
- Gomes FR, Rezende EL, Grizante MB, Navas CA. 2009.** The evolution of jumping performance in anurans: morphological correlates and ecological implications. *Journal of Evolutionary Biology* **22**: 1088–1097.
- Gould SJ. 1990.** *Wonderful life: the Burgess shale and the nature of history*. New York: W. W. Norton & Company.
- Gould SJ, Lewontin RC. 1979.** The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proceedings of the Royal Society of London Series B, Biological Sciences* **205**: 581–598.
- Gower JC. 1971.** A general coefficient of similarity and some of its properties. *Biometrics* **27**: 857–874.
- Grant T, Bolívar-G W. 2014.** A new species of semiarboreal toad with a salamander-like ear (Anura: Bufonidae: *Rhinella*). *Herpetologica* **70**: 198–210.
- Herrel AP, Perrenoud M, Decamps T, Abdala V, Manzano A, Pouydebat E. 2013.** The effect of substrate diameter and incline on locomotion in an arboreal frog. *The Journal of Experimental Biology* **216**: 3599–3605.

- Hill IDC, Dong B, Barnes WJP, Ji A, Endlein T. 2018.** The biomechanics of tree frogs climbing curved surfaces: a gripping problem. *Journal of Experimental Biology* **221**: jeb168179.
- Hudson CM, Brown GP, Shine R. 2016.** Athletic anurans: the impact of morphology, ecology and evolution on climbing ability in invasive cane toads. *Biological Journal of the Linnean Society* **119**: 992–999.
- Jetz W, Pyron RA. 2018.** The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution* **2**: 850–858.
- Klingenberg CP. 2008.** Morphological integration and developmental modularity. *Annual Review of Ecology, Evolution, and Systematics* **39**: 115–132.
- Kluge AG. 2005.** Testing lineage and comparative methods for inferring adaptation. *Zoologica Scripta* **34**: 653–663.
- Krynak KL, Wessels DG, Imba SM, Lyons JA, Guayasamin JM. 2018.** Newly discovered population of Bumpy Glassfrog, *Centrolene heloderma* (Duellman, 1981), with discussion of threats to population persistence. *Check List* **14**: 261–265.
- Law CJ. 2021.** Different evolutionary pathways lead to incomplete convergence of elongate body shapes in carnivorous mammals. *Systematic Biology* **71**: 788–796.
- Lee JC. 2001.** Evolution of secondary sexual dimorphism in the toad *Bufo marinus*. *Copeia* **2001**: 928–935.
- Liu W, Golovatch S, Wesener T, Tian M. 2017.** Convergent evolution of unique morphological adaptations to a subterranean environment in cave millipedes (Diplopoda). *PLoS One* **12**: e0170717.
- Losos JB. 1992.** The evolution of convergent structure in Caribbean Anolis communities. *Systematic Biology* **41**: 403–420.
- Losos JB. 2011.** Convergence, adaptation, and constraint. *Evolution* **65**: 1827–1840.
- Losos JB. 2017.** *Improbable destinies: fate, chance, and the future of evolution*. New York: Riverhead Books.
- Losos JB, Jackman TR, Larson A, de Queiroz K, Rodriguez-Schettino L. 1998.** Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279**: 2115–2118.
- Lynch JD. 1986.** New species of *Eleutherodactylus* of Colombia (Amphibia: Leptodactylidae) II: four species from the cloud forests of the western Cordilleras. *Caldasia* **15**: 629–647.
- Lynch JD. 1990.** A new ocellated frog (Centrolenidae) from western Colombia. *Proceedings of the Biological Society of Washington* **103**: 35–38.
- Lynch JD. 1996.** New frogs of the genus *Eleutherodactylus* (family Leptodactylidae) from the San Antonio region of the Cordillera Occidental. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* **20**: 331–345.
- Lynch JD. 1999.** Lista anotada y clave para las ranas (Género *Eleutherodactylus*) chocoanas del Valle del Cauca, y apuntes sobre las especies de la cordillera Occidental adyacente. *Caldasia* **21**: 184–202.
- Maddison WP, Maddison DR. 2019.** *Mesquite: a modular system for evolutionary analysis. Version 3.61*. Available at: <http://www.mesquiteproject.org/>
- Maeso I, Roy SW, Irimia M. 2012.** Widespread recurrent evolution of genomic features. *Genome Biology and Evolution* **4**: 486–500.
- Manzano AS, Abdala V, Herrel A. 2008.** Morphology and function of the forelimb in arboreal frogs: specializations for grasping ability? *Journal of Anatomy* **213**: 296–307.
- Manzano AS, Fabrezi M, Vences M. 2007.** Intercalary elements, treefrogs, and the early differentiation of a complex system in the Neobatrachia. *The Anatomical Record* **290**: 1551–1567.
- Manzano AS, Fontanarrosa G, Abdala V. 2019.** Manual and pedal grasping among anurans: a review of relevant concepts with empirical approaches. *Biological Journal of the Linnean Society* **127**: 598–610.
- Manzano AS, Fontanarrosa G, Prieto YA, Abdala V. 2017.** La prensibilidad en anfibios y reptiles: perspectivas evolutivas basadas en la anatomía y la función. In: Abdala V, Manzano A, Vasallo A, eds. *Morfología de Vertebrados: Hacia una integración de conceptos, metodologías y grupos de investigación del país*. Mar del Plata: EUDEM Editorial Universitaria de Mar del Plata, 59–81.
- Manzano AS, Lavilla EO. 1995.** Notas sobre la miología apendicular de *Phyllomedusa hypocondrialis* (Anura, Hylidae). *Alytes* **12**: 169–174.
- Menzies JI. 1976.** *Handbook of Common New Guinea Frogs*. Papua New Guinea: Wau Ecology Institute.
- Moen DS, Irschick DJ, Wiens JJ. 2013.** Evolutionary conservatism and convergence both lead to striking similarity in ecology, morphology and performance across continents in frogs. *Proceedings of the Royal Society B: Biological Sciences* **280**: 20132156.
- Moen DS, Morlon H, Wiens JJ. 2016.** Testing convergence versus history: convergence dominates phenotypic evolution for over 150 million years in frogs. *Systematic Biology* **65**: 146–160.
- Morinaga G, Bergmann P. 2017.** Convergent body shapes have evolved via deterministic and historically contingent pathways in *Lerista* lizards. *Biological Journal of the Linnean Society* **121**: 858–875.
- Morris SC. 2003.** *Life's solution: inevitable humans in a lonely universe*. Cambridge: Cambridge University Press.
- Muñoz MM. 2019.** The evolutionary dynamics of mechanically complex systems. *Integrative and Comparative Biology* **59**: 705–715.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Oksanen MJ. 2013.** *Package 'vegan' community ecology package* **2**: 1–295.
- Ortega JE, Serrano VH, Ramírez-Pinilla MP. 2005.** Reproduction of an introduced population of *Eleutherodactylus johnstonei* at Bucaramanga, Colombia. *Copeia* **2005**: 642–648.
- Ortiz DA, Coloma LA, Frenkel C, Ron SR, Quiguango-Ubillús A, Páez-Rosales N. 2018.** *Hyloscirtus larinopygion*. In: Ron SR, Merino-Viteri A, Ortiz DA, eds. *Anfibios del Ecuador*. Museo de Zoología, Pontificia Universidad Católica del Ecuador. Available at: <https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Hyloscirtus%20larinopygion>. Accessed 15 July 2021.

- Ospina-Sarria JJ.** 2012. *Revisión morfológica de las ranas del género Strabomantis (Anura: brachycephalidae sensu Frost et al. 2006)*. Unpublished MSc. Thesis, Universidad Nacional de Colombia, Colombia.
- Ospina-Sarria JJ, Duellman WE.** 2019. Two new species of *Pristimantis* (Amphibia: Anura: Strabomantidae) from southwestern Colombia. *Herpetologica* **75**: 85–95.
- Palacio FX, Apodaca M, Crisci JV.** 2020. *Análisis multivariado para datos biológicos: teoría y su aplicación utilizando el lenguaje R, 1st edn*. Ciudad Autónoma de Buenos Aires: Fundación de Historia Natural Félix de Azara. Argentina.
- Pereyra MO, Blotto BL, Baldo D, Chaparro JC, Ron SR, Elias-Costa A, Faivovich J.** 2021. Evolution in the genus *Rhinella*: a total evidence phylogenetic analysis of neotropical true toads (Anura: Bufonidae). *Bulletin of the American Museum of Natural History* **447**: 1–156.
- Reynaga CM, Astley HC, Azizi E.** 2018. Morphological and kinematic specializations of walking frogs. *Journal of Experimental Zoology. Part A, Ecological and Integrative Physiology* **329**: 87–98.
- Ron SR, Read M, Pazmiño-Armijos G.** 2022. *Agalychnis spurrelli*. In: Ron SR, Merino-Viteri A, Ortiz DA, eds. *Anfibios del Ecuador*. Museo de Zoología, Pontificia Universidad Católica del Ecuador. Available at: <https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Agalychnis%20spurrelli>. Accessed 9 October 2022. Ecuador
- Sackton TB, Clark N.** 2019. Convergent evolution in the genomics era: new insights and directions. *Philosophical Transactions of the Royal Society B: Biological Sciences* **374**: 20190102.
- Speed MP, Arbuckle K.** 2017. Quantification provides a conceptual basis for convergent evolution. *Biological Reviews of the Cambridge Philosophical Society* **92**: 815–829.
- Stayton CT.** 2006. Testing hypotheses of convergence with multivariate data: morphological and functional convergence among herbivorous lizards. *Evolution* **60**: 824–841.
- Stayton CT.** 2008. Is convergence surprising? An examination of the frequency of convergence in simulated datasets. *Journal of Theoretical Biology* **252**: 1–14.
- Stayton CT.** 2015. What does convergent evolution mean? The interpretation of convergence and its implications in the search for limits to evolution. *Interface Focus* **5**: 20150039.
- Sustaita D, Pouydebat E, Manzano A, Abdala V, Hertel F, Herrel A.** 2013. Getting a grip on tetrapod grasping: form, function, and evolution. *Biological Reviews of the Cambridge Philosophical Society* **88**: 380–405.
- Vassallo AI, Manzano A, Abdala V, Muzio RN.** 2021. Can anyone climb? The skills of a non-specialized toad and its bearing on the evolution of new niches. *Evolutionary Biology* **48**: 293–311.
- Vidal-García M, Byrne PG, Roberts JD, Keogh JS.** 2014. The role of phylogeny and ecology in shaping morphology in 21 genera and 127 species of Australo-Papuan myobatrachid frogs. *Journal of Evolutionary Biology* **27**: 181–192.
- Wainwright PC, Alfaro ME, Bolnick DI, Hulsey CD.** 2005. Many-to-one mapping of form to function: a general principle in organismal design? *Integrative and Comparative Biology* **45**: 256–262.
- Wainwright PC, Carroll AM, Collar DC, Day SW, Higham TE, Holzman RA.** 2007. Suction feeding mechanics, performance, and diversity in fishes. *Integrative and Comparative Biology* **47**: 96–106.
- Wake DB, Wake MH, Specht CD.** 2011. Homoplasy: from detecting pattern to determining process and mechanism of evolution. *Science* **331**: 1032–1035.
- Wray GA.** 2002. Do convergent developmental mechanisms underlie convergent phenotypes? *Brain, Behavior and Evolution* **59**: 327–336.
- Young RL, Sweeney MJ, Badyaev AV.** 2010. Morphological diversity and ecological similarity: versatility of muscular and skeletal morphologies enables ecological convergence in shrews. *Functional Ecology* **24**: 556–565.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article on the publisher's website.

Appendix S1. Families of anurans studied. Anuran tree, showing the phylogenetic position of the taxa included in the present study (marked in green). Tree modified from [Jetz & Pyron \(2018\)](#).

Appendix S2. Specimens examined. Specimens used for the present study and their collection codes at the Colección de Prácticas Zoológicas, Universidad del Valle (CPZ-UV) and the Colección de Anfibios y Reptiles, Universidad del Valle (UV-C).

Appendix S3. Sources of evidence used for each qualitative character.

Appendix S4. Raw measurements of limb length and snout–vent length.

Appendix S5. Quantitative characters. Measurements of morphological characters in the ten examined species. The range of lengths corresponds to the smallest and largest value of specimens per species; values are given as the range (mean \pm SD). Length measurements are controlled by body size.

Appendix S6. Qualitative characters. Coding of morphological characters in the ten anuran species examined. Inapplicable: ---.

Appendix S7. Evolutionary integration.