



# Can Anyone Climb? The Skills of a Non-specialized Toad and its Bearing on the Evolution of New Niches

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## Abstract

Several studies of arboreal anuran species show morphological specializations for clinging onto narrow substrates. However, little is known about these capacities in non-specialized anurans, which is crucial to understand the initial phases of adaptation to a new niche. To assess the functional requirements related to the evolution of arboreality in anurans we analyzed climbing performance, and correlated anatomical traits, in the terrestrial toad *Rhinella arenarum*, a species chosen as a proxy for the ancestral condition regarding the evolution of this specialized niche. We studied the impact of a substrate of wooden rods with different diameters, arrangements, and slopes on locomotion, grasping, and climbing with a comparative framework. Animals were confronted with climbing tests, video recording their behaviors. Preserved specimens were dissected to assess limb myology, osteology, and tendons' characteristics. Our results show that how terrestrial toad *R. arenarum* climbs is different from those displayed by specialized tree frogs. Animals flexed their fingers and toes, grasping the substrate displaying hookings and partial graspings. The palm was scarcely involved in the grip, as in specialized anurans. These actions were performed although flexor and extensor muscles of the digits are highly conserved and generalized. Further, we formally assess the evolutionary history of ecological and anatomical traits related to climbing among *Rhinella* species to improve the comprehension of the relation between morphofunctional patterns and behavioral climbing skills. Our experiments revealed that this terrestrial toad possesses unexpected climbing capacities, suggesting a way in which evolution of new niches could have developed in the evolution of anurans.

**Keywords** Anurans · Grasping and climbing · Muscle–tendon morphology · Terrestrial toad *Rhinella arenarum* · Evolution of new niches

## Introduction

The evolution of new niches is a relevant phenomenon but one that can rarely be evaluated directly by researchers. This is because the direct scrutiny of the ancestral forms and the circumstances underlying the origin of a new niche are no longer accessible, or because the extant forms have marked specializations in which the initial phases of the phenomenon cannot be assessed. Data from extant species within focal lineages and the use of evolutionary comparative methods have proven to be useful to understand adaptation and to infer ancestral character states (Tulli et al., 2012). Another way to circumvent that difficulty is to investigate the performance of species that serve as a proxy for ancestral character states (Aubret et al., 2007), analyzing in detail their capacities and limitations to carry out new functions and behaviors (where learning could also play a role when a new niche

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is being colonized; Beltman et al., 2004). The ability to carry out new functions or behaviors ultimately relies on the adaptive plasticity of species, an attribute that in recent years has generated renewed interest as one of the causes of the origin of diversity (West-Eberhard, 2005). It has been argued that, in addition to the variability resulting from genetic variation, the ability to produce innovations, whether functional, behavioral or by modifying other phenotypic traits, might be an important source of variation on which selection can operate.

Although, in general, animals have a characteristic primary locomotor mode, which is performed during their current activities, it is known that several species may be able to move in more than one way through habitats or substrates that are not the usual ones (Karantanis et al., 2017). Whereas there are many anecdotal reports on this issue the ability to move through non-typical habitats has been scarcely assessed in the scientific literature (Dagg & Windsor, 1972; Hyams et al., 2012; Pizzatto et al., 2017). This ability may be critical for individuals in exceptional circumstances, such as during dispersal into new habitats, or to access new dietary items, facing stressful environmental situations, or during the generation of new ecological niches by actively contributing to drive their evolution and build their niches (Diogo, 2017; Odling-Smee et al., 2003). Therefore, the ability to perform a novel locomotor mode is of great importance for the evolution of new niches that involve sudden changes in the habitat and in the characteristics of the substrate on which the animals must move (Gomes et al., 2009).

Arboreality and climbing have independently evolved in various vertebrate lineages (Sustaita et al., 2013). Besides a diverse array of anatomical modifications shown by the different taxa, there are distinct substrates on which animals must adhere and move (Herrel et al., 2013; Tulli et al., 2012). Climbers have two basic requirements: (1) avoid falling, both during displacement and at rest, and (2) travel on a three-dimensional discontinuous substrate (Hildebrand & Goslow, 2001). In climbing vertebrates, grasping is a usual action observed in several taxa, being a function of an appendage (hand, tail, foot) that exerts normal forces concerning the support surface on which the animal is situated (Manzano et al., 2018). This action requires the opposition of parts in the grasper device. In tree climbing Anura, the ability to perform grasping has been associated with opposability and digits divergence, with the lengthening of the metacarpals, and other attributes of the autopodium (Manzano et al., 2008, 2018; Sustaita et al., 2013). It has been observed that highly specialized anuran species like *Phyllomedusa bicolor* close the hand (i.e., execute a power grip) to generate a balancing torque while walking on narrow branches. A general elongation and increase in the size of hand muscles and tendons seem to be related to the ability to perform power grip (Manzano et al., 2008).

To avoid falling at rest, some climbing taxa that do not use grasping, instead use a curved structure such as claws, digits, to cling to the substrate (Hildebrand & Goslow, 2001), an action called hooking. Hooking was defined by Napier (1993) as a form of subsidiary prehensility where the phalanx-metacarpal/metatarsal joints remain in a straight position, and only the two terminal phalanges flex. Although it is not an accurately grasping movement, it is enough to allow the animal to climb high substrates.

Although without reaching the degree of climbing morphological specialization shown by species of hylids such as *Phyllomedusa* and *Litoria* (Manzano et al., 2007, 2008), several species of *Rhinella* (Bufonidae)—a mostly terrestrial genus—have evolved semi-arboreal and arboreal habits (e.g., Chaparro et al., 2007; Grant & Bolívar, 2014), particularly those that occupy tropical moist forest of northern South America and Andean forests. For example, climbing ability and the occupation of the arboreal (*R. manu*) and semi-arboreal (*R. margaritifera*) niches are frequent (Chaparro et al., 2007; de Noronha et al., 2013).

From a functional-morphological perspective, it might be intriguing to evaluate the performance of organisms during the generation of a new ecological niche (Diogo, 2017); for example, the arboreal niche in anurans, since new, perhaps unexpected functions, could be performed to cope with new adaptive zones. It has been argued that the change of a function (Müller & Wagner, 1991), or the acquisition of a new function (Lauder, 1996), could be important factors allowing the evolution of new behaviors and niches. To tackle this issue, we propose to evaluate the climbing ability of a terrestrial anuran species without distinct anatomical adaptations concerning this specialized locomotor mode.

Due to its morpho-functional traits and phylogenetic relatedness with arboreal and semi-arboreal species belonging to the same genus, we chose the terrestrial toad *Rhinella arenarum* as a proxy for ancestral character states regarding the evolution of arboreality (Pramuk et al., 2008). The species is employed as an anuran model for different behavioral and learning situations (Daneri et al., 2007, 2011; Muzio et al., 1992, 2011; Puddington et al., 2018; Sotelo et al., 2015, 2019). Despite that, nothing is known yet about the effects of substrate characteristics on its locomotion performance in this terrestrial toad.

*R. arenarum* is found near small lagoons, ponds, or swamps, in temperate open habitats of southern South America. *Rhinella* comprises ~90 living species, which have diversified in South America since the Eocene. Phylogeny, biogeography, and adaptations of this genus are areas of active research (Jetz & Pyron, 2018; Pereyra et al., 2015; Pramuk et al., 2008).

It is known that the terrestrial toad *R. arenarum* has some slope encoding skills (Sotelo et al., 2017), but this ability has not yet been tested in the context of climbing. Based on

what is known about anatomical features related to climbing in vertebrates and amphibians in particular (reviewed in Manzano et al., 2018), it can be reasonably assumed that *R. arenarum* lacks conspicuous adaptations related to this habit, namely: (1) it has a long metatarsal II, which in grasper species is considerably shortened, (2) it shows no noticeable shortening of the preaxial digit, contrary to what is observed in climbing species, including primates, (3) lacks digits' adhesive discs, (4) its digits are short, which would not allow exerting adequate normal forces against the substrate. We focus first on the actions/behaviors that animals can perform, and if they enable climbing on different experimental substrates. Next, based on anatomical analysis, we try to interpret the feasibility or limitations to perform those actions. Besides, we reviewed the literature to gather information about the habitat, climbing capacity, and ecological niche of species belonging to the clade *Rhinella*, which includes the focus species of our study. These traits, along with anatomical ones (especially, characteristics of the distal phalanges) were analyzed within a phylogenetic context to explore the evolution of the arboreal niche within this clade of South American bufonids. We hope that with this study, we will help to better understand the issue of arboreality and the origin of new niches in anurans.

## Materials and Methods

### Subjects

13 sexually mature, experimentally naive terrestrial toads, *Rhinella arenarum* (Anura; Bufonidae), a species not listed as threatened (IUCN, ) were used. Eight adult females (mean body mass 209.4 g) and five adult males (mean body mass 141.6 g) were collected in natural environments of Buenos Aires province, in habitats constituted by natural grasslands dotted with small temporary ponds. Subjects were kept in the IBYME vivarium in group cages with accessible running water and were fed with *Tenebrio* larvae. After the end of the experiments, animals were released in their natural environment.

### Experimental Set-up

For the assessment of climbing abilities, animals were confronted with scaling test trials on different climbing substrates. The experimental device was a box of translucent Plexiglas (50 × 16 × 36 cm, L × W × H) consisted of a small 12 × 16 cm enclosure in which the animal was placed at the beginning of each test trial, adjacent to a climbing substrate that allows leaving the enclosure (Fig. 1). The climbing substrate was composed of wooden cylindrical rods. These were 6 or 8 mm in diameter and were parallel located 1.5 or

2 cm apart from each other (see Fig. 2A and B). The rods were perpendicular or parallel to the climbing direction and were inclined at 0°, 45°, 67.5° or 90° to the floor of the box. Increasing angles produce increases in the force component parallel to the support surface of the animal's weight, which must be counteracted to prevent sliding down. Rods with side supports of 3 mm in length were also used as a climbing substrate (see Fig. 2C). The enclosure was illuminated from the ceiling by an incandescent light bulb (75 w) hanging above the center of the experimental device.

### Behavioral Procedure

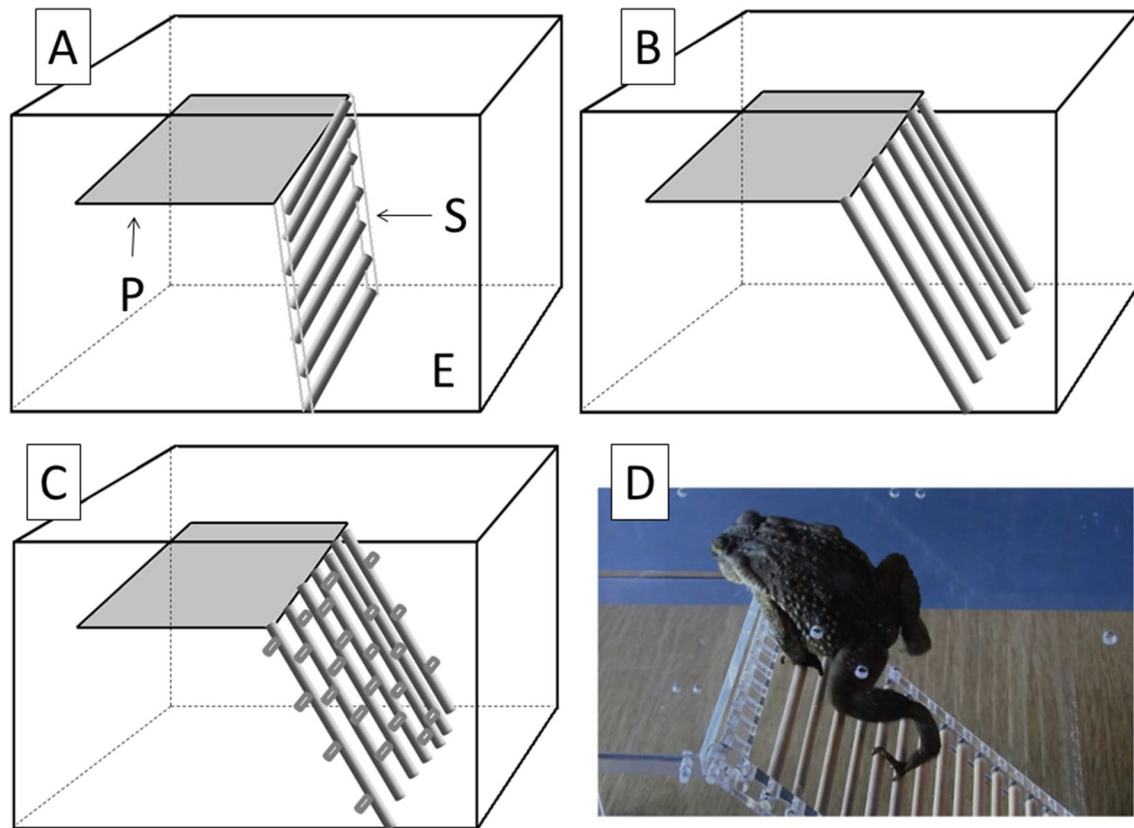
Each animal performed 1–4 climbing test trials on each of the different substrates and slopes. Toads were recorded on each climbing substrate type following a random order to prevent or minimize learning components (additionally, substrate slope and diameter rod selection were also randomized). Due to the small size of the enclosure, in general, the animals showed a tendency to climb immediately after the start of each test trial. In cases where this did not happen within three minutes, the test trials were repeated (this kind of trials were few and not systematic).

### Video Analysis

Animals were filmed and photographed during test trials using a Casio Exilim EX-F1 Digital video camera fixed on a tripod. The filming speed was set at 30 (1280 × 720 pixels) and 300 fps (512 × 384 pixels). The behavior used to climb each particular substrate was analyzed. During the video analyses, the actions and anatomical structures used to climb were recorded. The analysis aimed to recognize previously described climbing/grasping actions as well as new ones. Climbing success, as measured by the proportion of individuals who manage to reach the top arrival platform, and climbing time (time for a toad to reach the top arrival platform, measured from the moment the individual begins to move on the substrate until it places at least three limbs on the platform) were measured for different climbing substrates and slopes.

### Anatomical Dissections

12 specimens of *Rhinella arenarum* were dissected for this study. The specimens dissected belong to the herpetological collection of CONICET-Diamante (DIAM 177), Fundación Miguel Lillo (FML 29,859, 29,863), and a personal collection of M. L. Ponssa (MLP s/d, 9 specimens). Skeletal whole-mounts specimens were cleared and double-dyed with Alcian Blue and Alizarin Red S following Wassersug (1976) protocol. Some of the specimens were not cleared but used for anatomical descriptions of muscles and tendons. These



**Fig. 1** Experimental box of translucent Plexiglas (50×16×36 cm, L×W×H). A small enclosure E (12×16 cm) served as an animal starting place in each test trial, located adjacent to a climbing substrate S (composed of wooden cylindrical rods) that allowed leaving the enclosure to reach the arrival platform P. There were three types

of climbing substrate. **A** rods perpendicular to climbing direction. **B** rods parallel to climbing direction. **C** rods parallel to climbing direction with lateral supports. **D** example image of a male climbing a substrate of 6 mm diameter perpendicular rods, slope 45°

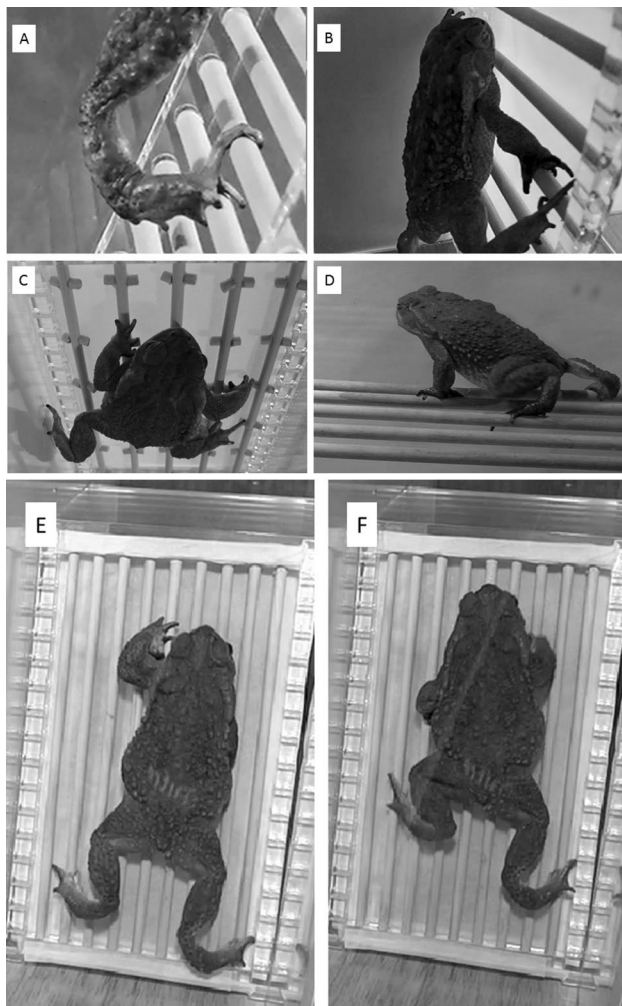
preparations were preserved in 70% ethanol and, at the time of observation, temporarily stained with an iodine solution to obtain a better contrast of the structures. Observations, illustrations, and photographs were made with Nikon SMZ1000 and with a Leica DM light microscope equipped with a digital camera. Muscles nomenclature follows Gaupp (1896), Dunlap (1960), and Blotto et al. (2020).

### Character Evolution Analysis

Information on habitat, climbing capacity, and ecological niche of species belonging to the genus *Rhinella* was gathered through a literature review (Table 1). This analysis reveals some degree of inconsistencies, given that some studies report the existence of climbing capacity in species whose ecological niche was assigned as terrestrial. The primary sources of information were taxonomic descriptions of new species, studies on species geographical distribution, and the IUCN database. As details about climbing abilities within *Rhinella* species are almost nonexistent, we also map the distal phalanges condition. According to unsystematic

preliminary own studies, distal phalanges curved are an excellent proxy of hooking and, then, of climbing abilities, which are also scarcely recorded. As we observed and found in the literature, part of the data refers to the skeleton of the distal phalanges. Part of the data was extracted from photographs showing the animals on the floor supported by their distal phalanges curved or straight (Table 1). Under the assumption of parsimony and by maximum likelihood of ancestral states, the evolutionary history of the characters in Table 1 was assessed through the Mesquite program (Maddison & Maddison, 2019). For the character habitat, the assigned states were “open”, “dry forest” and “moist forest”. Open habitat (not enclosed by trees) includes savannas, grasslands, Puna, desert areas, and margin of water bodies. Moist forest refers to tropical and subtropical premontane, montane and lowland forest, and cloud forest. Dry forest refers to Chaco, Cerrado, and Atlantic forest. For the character niche, the assigned states were “terrestrial”, “semi-arboreal”, “arboreal” and “aquatic.” For the distal phalanges character, we assigned “curved” or “straight”. We perform the character mapping using a combined phylogeny of the





**Fig. 2** Individuals of the terrestrial toad *Rhinella arenarum* climbing and moving on different substrates. **A** rods  $\varnothing$  6 mm, 67.5° slope, 1 cm rod separation, perpendicular to climbing direction: hooking by foot digit 4. **B** rods  $\varnothing$  8 mm, 90° slope, 2 cm separation, perpendicular: grasping by hand digits 2 and 3. **C** rods  $\varnothing$  6 mm, 90° slope, 2 cm separation, parallel, with side supports: hooking and weak intermediate grasping with hands and feet. **D** rods  $\varnothing$  6 mm, 0° slope, 2 cm separation, parallel. **E–F** Example of climbing sequence of a toad *R. arenarum*, substrate 6 mm diameter parallel to climbing direction. Note the hands and feet grasping the rods

genus *Rhinella* based on Chaparro et al. (2007), Pramuk et al. (2008), and Cusi et al. (2017).

## Results

### Behavioral Analysis

Animals were able to climb and move within the experimental box, facing different climbing conditions presented during the test trials (Figs. 2 and 3), and showing varying degrees of success to leave the enclosure (Table 2). We describe below the

most relevant actions and performance observed at the different climbing conditions.

### Rods $\varnothing$ 6 mm, 1 cm Separation, Perpendicular to the Climbing Direction

Animals were able to climb this substrate at the three angles presented: 45°, 67.5°, and 90°. Animals flexed the digits of the foot, hooking and clinging to the substrate. With this action they were able to support themselves, avoiding slipping (Fig. 3A). Animals flexed the digits of the hand and perform hooking on two or three consecutive rods (Fig. 3B). Animals supported themselves by simply settle the palm on the rod without grasping it, at the same time performing hooking with digit 4 of the foot (Fig. 2A), then extended the limbs and climbed. Animals avoided slipping by hooking the terminal phalanx of hand digits 4 and 5 to the wooden rods. By flexing the digits animals were able to grip on two adjacent rods at a time, whereas they failed to grip a simple rod. In the last situation, although the animals flexed the digits they did not cling probably due to the small diameter of a single rod. Animals performed intermediate grip (Manzano et al. 2018; also called “scissor grip” by Napier, 1993, and “scissor-like” grip by Anzeraey et al. 2017) in which the rod was grasped between lateral and medial sides of digits 3 and 4, respectively. Animals climbed the substrate at 90° with difficulty, which was evidenced by frequent slipping of the hands and feet and less secure attachment to the rods. Although animals showed greater difficulties in climbing the substrates with greater slopes, no significant differences were found in climbing success neither between slopes nor between sexes. Climbing time was shorter in the substrates with greater slopes (Table 2).

The hooking involved the last two phalanges, with a considerable ventroflexion performed by the tendon flexors action, and the consequent dorsioextension. All *Rhinella* species have the last phalanges of digits 3 to 5 of the hands, ventrally curved. At the feet, all digits have the last phalanx ventrally curved except that of digit 1, which is short and rounded (Fig. 6E).

### Rods $\varnothing$ 6 mm, 2 cm Separation; Perpendicular to the Climbing Direction

Animals had a climbing performance similar to the substrate described above. They flexed the fingers of the hand trying to surround a single rod producing only a partial grasping without palm intervention.

### Rods $\varnothing$ 8 mm, 2 cm Separation, Perpendicular to the Climbing Direction

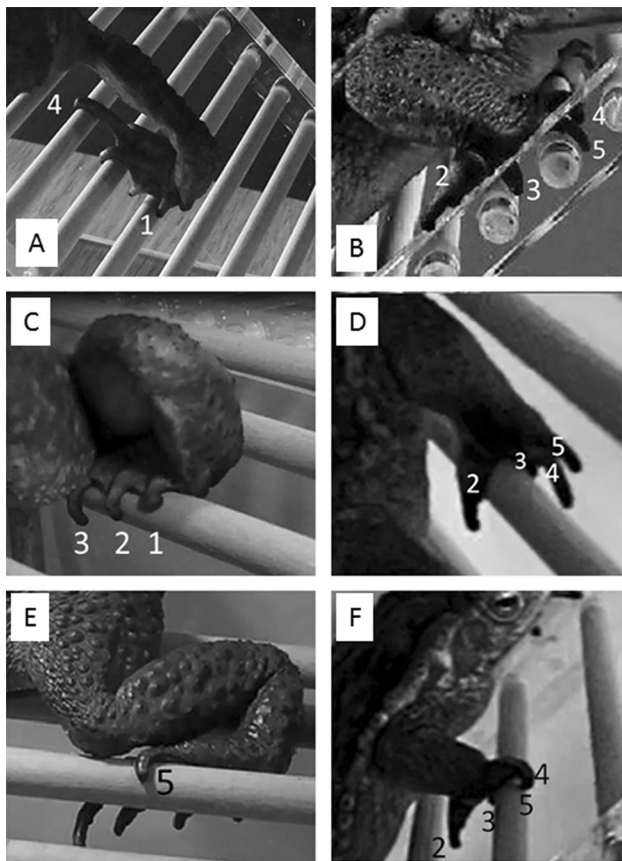
As with 6 mm rods, animals were able to climb this substrate at the three angles presented, showing a greater degree of

**Table 1** Type of distal phalanges, habitat, ecological niche and climbing ability of some species within the neotropical genus *Rhinella* (Bufonidae). Data still unknown are shown by the symbol “?”

| Species                   | Distal phalanges | Source   | Habitat   | Ecological niche | Climbing ability   | Source   |
|---------------------------|------------------|--|---|------------------|--|--|
| <i>R. dapsilis</i>        | Curved           | Ortiz & Coloma (2018)  | Lowland and premontane tropical moist forest  | Terrestrial      | ?  | Azevedo-Ramos et al. (2004)  |
| <i>R. castaneotica</i>    | Curved           | BNHM <a href="https://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+1009+1826">https://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+1009+1826</a>                         | Tropical moist old-growth lowland forests   | Terrestrial      | Climbs on leaves and low vegetation                                | De Noronha et al. (2013); IUCN SSC Amphibian Specialist Group (2015a, 2015b) |
| <i>R. margaritifera</i>   | Curved           | Our observations; Ortiz et al. (2018)  | Premontane and montane tropical moist forest, open areas  | Semi-arboreal    | Climbs vegetation  | de Noronha et al. (2013)   |
| <i>R. ocellata</i>        | Curved           | de Freitas et al. 2018; BNHM <a href="https://calphotos.berkeley.edu/cgi/img_query?enlarge=1111+1111+1111+6041">https://calphotos.berkeley.edu/cgi/img_query?enlarge=1111+1111+1111+6041</a> | Cerrado near waterbodies, moist savanna, subtropical or tropical moist shrubland, freshwater marshes            | Terrestrial      | ?  | Bastos et al. (2004)   |
| <i>R. nestotes</i>        | ?                | –  | Premontane and montane forest of the upper Amazon Basin   | Semi-arboreal    | Climbs vegetation; generally caught on leaves or inside bromeliads | IUCN SSC Amphibian Specialist Group (2018a, 2018b)                           |
| <i>R. manu</i>            | Straight         | BNHM <a href="https://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+1009+1823">https://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+1009+1823</a>                         | Cloud forest, montane, humid primary forest   | Arboreal         | Climbs on leaves and branches of arboreal ferns                    | Chaparro et al. (2007)   |
| <i>R. chavini</i>         | Curved           | Lehr et al (2001)  | Dense forest vegetation of small trees up to 10 m   | Terrestrial      | Climbs smooth, vertical surfaces                                   | Lehr et al. (2001)   |
| <i>R. festae</i>          | Straight         | Frenkel (2019)   | Tropical rainforest, premontane humid forest  | Semi-arboreal    | Climbs low vegetation  | Almendáriz et al. (2004); Cusi et al. (2017)                                 |
| <i>R. lilyrodriguezae</i> | Curved           | Cusi et al. (2017)   | Montane forest  | Semi-arboreal    | Climbs on leaves of bushes   | Cusi et al. (2017)   |
| <i>R. amboroensis</i>     | ?                | –  | Aquatic and terrestrial habitats within cloud forest  | Aquatic          | ?  | Chaparro et al. (2007)   |
| <i>R. veraguensis</i>     | Straight         | Padial et al. (2009); BNHM <a href="https://calphotos.berkeley.edu/cgi/img_query?enlarge=1111+1111+1111+1354">https://calphotos.berkeley.edu/cgi/img_query?enlarge=1111+1111+1111+1354</a>   | Montane tropical forests, cloud forests   | Semi-arboreal    | Climbs vegetation  | Lehr et al. (2001)   |
| <i>R. poeppigii</i>       | Curved           | Varela-Jaramillo and Ron (2018); Williams León de Castro and Rey Sánchez (2014)  | Tropical and cloud forest, tropical or subtropical montane dry forests, rivers, freshwater marshes, grasslands  | Terrestrial      | ?  | De la Riva (2002); IUCN SSC Amphibian Specialist Group (2020a, 2020b)        |
| <i>R. schneideri</i>      | Curved           | FLICKR <a href="https://www.flickr.com/photos/cdtirmm/8075491625">https://www.flickr.com/photos/cdtirmm/8075491625</a>   | From open, urban areas to the Chaco, Cerrado, and Atlantic Forest regions. It occasionally burrows in deep soil | Terrestrial      | Climbs on rocks and roots  | Wagley (2019)  |
| <i>R. marina</i>          | Curved           | Coloma et al. (2018); Thomas et al. (2016)   | Tropical and semi-arid open environments  | Terrestrial      | Climbs on rocks and tree trunks                                    | Hudson et al. (2016)   |
| <i>R. arenarium</i>       | Curved           | Our data   | Open and disturbed areas, variety of habitats with temporary freshwater bodies                                  | Terrestrial      | Climbs different substrates  | Lavilla et al (2000); Present study  |

Table 1 (continued)

| Species                | Distal phalanges | Source   | Habitat  | Ecological niche | Climbing ability | Source  |
|------------------------|------------------|--|--|------------------|------------------|---|
| <i>R. humboldti</i>    | Curved           | FLICKR <a href="https://www.flickr.com/photos/125903191@N07/15329580297">https://www.flickr.com/photos/125903191@N07/15329580297</a>                                 | Open areas, diverse habitats like low plains, savannas and dry forests                               | Terrestrial      | ?                | Torres-Suárez & Vargas-Salina (2014)  |
| <i>R. granulosa</i>    | Straight         | BNHM <a href="https://calphotos.berkeley.edu/cgi/img_query?enlarge=1111+1111+1111+2971">https://calphotos.berkeley.edu/cgi/img_query?enlarge=1111+1111+1111+2971</a> | Open areas, savannas, forests, river shorelines  | Terrestrial      | ?                | Silvano et al. (2010)   |
| <i>R. limensis</i>     | Curved           | GBIF <a href="https://www.gbif.org/es/species/5216960">https://www.gbif.org/es/species/5216960</a>   | Arid coastal valleys, agricultural areas close to rivers and streams, coastal sandy habitats in Perú | Terrestrial      | ?                | Angulo et al. (2004); IUCN SSC Amphibian Specialist Group (2014)                                  |
| <i>R. vellardi</i>     | ?                | –  | Montane dry forest, rivers, freshwater marshes   | Terrestrial      | ?                | Angulo et al. (2004); Rodríguez et al. (1993); IUCN SSC Amphibian Specialist Group (2018a, 2018b) |
| <i>R. spinulosa</i>    | Curved           | Urta (2013); SIB <a href="https://sib.gob.ar/especies/rhinella-spinulosa">https://sib.gob.ar/especies/rhinella-spinulosa</a>   | Valleys, mountains, highlands and arid steppes in central-western South America                      | Terrestrial      | ?                | Díaz-Páez and Ortiz (2003)  |
| <i>R. arequipensis</i> | Curved           | <a href="https://calphotos.berkeley.edu/cgi/img_query?seq_num=86671&amp;one=T">https://calphotos.berkeley.edu/cgi/img_query?seq_num=86671&amp;one=T</a>              | Montane scrubland and grassland habitats   | Terrestrial      | ?                | IUCN SSC Amphibian Specialist Group (2020a, 2020b)  |
| <i>R. atacamensis</i>  | Curved           | Urta (2013)  | Atacama Desert, shrubs, rocky slopes   | Terrestrial      | ?                | Correa et al. (2008)  |
| <i>R. arunco</i>       | Curved           | Urta (2013)  | Rivers, streams, adults in small caves or under rocks during the day                                 | Terrestrial      | ?                | IUCN SSC Amphibian Specialist Group (2015a, 2015b)  |



**Fig. 3** Actions performed by the terrestrial toad *Rhinella arenarum* at the different climbing conditions. **A** rods  $\varnothing$  6 mm,  $45^\circ$  slope, 1 cm rod separation, perpendicular to climbing direction: flexion of foot digits resulting in hooking. **B** rods  $\varnothing$  6 mm,  $45^\circ$  slope, 1 cm separation, perpendicular: hooking by flexion of hand digits 4 and 5. **C** rods  $\varnothing$  6 mm,  $0^\circ$  slope, 2 cm separation, parallel: hooking of foot digits 1, 2, and 3 resulting in a greater contact surface of the autopodium. **D** rods  $\varnothing$  8 mm,  $90^\circ$  slope, 2 cm separation, perpendicular: weak intermediate grasping (scissor grip) between lateral side of digit 2 and medial side of digit 3. **E** rods  $\varnothing$  6 mm,  $0^\circ$  slope, 2 cm separation, parallel: hooking by flexion of foot digit 5. **F** rods  $\varnothing$  8 mm,  $45^\circ$  slope, 2 cm separation, perpendicular: weak intermediate grasping by opposing hand digits 3 vs. 4, completed by a hooking of digits 4 and 5

difficulty at  $90^\circ$ . It was observed a weak intermediate grasping by opposing hand digits 3 vs. 4, completed by hooking of digits 4 and 5, without intervening digit 2 (Fig. 3F), and a weak intermediate grasping (scissor grip) between the lateral side of digit 2 and medial side of digit 3 (digits 4 and 5 did not participate; Fig. 3D). As observed, when grasping the rod the palm does not intervene, so in *Rhinella arenarum* the hand does not perform a power grip.

### Rods $\varnothing$ 6 mm, 1 cm Separation, Parallel to the Climbing Direction

At all substrate slopes, the animals performed with difficulty. Although the climbing success was lower on this climbing condition, no significant differences were found to perpendicular and parallel with side supports ( $\chi^2=4.89$ ,  $p=0.09$ ; Table 2). The hand performed weak intermediate grasping, between digits 4 and 5, to some extent effective in preventing slippage (see as example the Fig. 2E and F, showing a climbing sequence of a subject on this substrate with parallel vertical rods). Flexion of foot digits and weak intermediate grasping between digits 4 and 5, and between digits 4 and 3 were not effective to avoid slippage on this substrate.

### Rods $\varnothing$ 6 mm, 2 cm Separation, Parallel to the Climbing Direction, with Side Supports

Animals were able to climb this substrate at the three angles presented  $45^\circ$ ,  $67.5^\circ$ , and  $90^\circ$ . To secure the rods, they performed both hooking and weak intermediate grasping with their hands and feet (Fig. 2C). Animals climbed by extension of fore and hind limbs.

At higher angles, during climbing, animals brought the belly close to the rods (Fig. 2B and C), which lower the center of mass and decrease body weight moment.

### Horizontal Platform ( $0^\circ$ ) rods $\varnothing$ 6 mm, Parallel to the Climbing Direction

Animals flexed the digits of the hand and foot which resulted in a greater contact surface of the autopodium with the substrate. Intermediate grasping occurred between the lateral and medial surfaces of consecutive digits. Support on palmar pads as well as flexion of digits 4 and 5 resulting in hooking that prevents slippage were both observed (Figs. 2D, 3C, and E).

### Observations on Restricted Movements

Facing all the different climbing conditions, limited lateral rotation of the wrist joint was observed, so finger 2 remains directed towards the midline of the body, pointing backward (Fig. 3B and F). This could be a factor that has prevented a better grip of the digit 2 on the rods, with limited or no participation in intermediate grasping.

### Morphological Description

In the next section, we present the descriptions of those muscle-tendinous structures of the limbs that make the movements directly related to climbing and hooking. We also report information about the distal phalanges skeleton.



**Table 2** Climbing success (proportion of individuals who manage to reach the top arrival platform for a given climbing substrate), Climbing time (time for a toad to reach the top arrival platform), and Behaviors used (\*) to climb different substrates by individuals of *R. arenarum*

| Rods orientation                | Perpendicular |             |             | Parallel with side supports |             |            | Parallel      |             |             |
|---------------------------------|---------------|-------------|-------------|-----------------------------|-------------|------------|---------------|-------------|-------------|
|                                 | 45°           | 67.5°       | 90°         | 45°                         | 67°         | 90°        | 45°           | 67°         | 90°         |
| Climbing success                | 0.85          | 1           | 0.75        | 0.92                        | 1           | 0.77       | 0.83          | 0.75        | 0.58        |
| Chi-square                      | 3.21          |             |             | 3.90                        |             |            | 1.36          |             |             |
| <i>p</i>                        | 0.2           |             |             | 0.14                        |             |            | 0.5           |             |             |
| Mean climb time (s) (SD)        | 93.8 (78.5)   | 24.6 (25.8) | 29.9 (28.7) | 40.2 (29.2)                 | 19.5 (12.7) | 17.4 (9.1) | 145.9 (170.1) | 20.6 (18.1) | 20.2 (16.2) |
| <i>F</i>                        | 6.51          |             |             | 4.86                        |             |            | 4.21          |             |             |
| <i>p</i>                        | 0.004         |             |             | 0.014                       |             |            | 0.027         |             |             |
| Behaviors                       |               |             |             |                             |             |            |               |             |             |
| Hooking                         | *             | *           | *           | *                           | *           | *          |               |             |             |
| Intermediate (scissor grasping) | *             | *           | *           | *                           | *           | *          | *             | *           | *           |

### Forelimb Muscles (Fig. 4)

*Flexor carpi ulnaris* (Fig. 4A). It arises from the distal condyle of the humerus by a wide, short, and ribbon-like tendon and lies along the ulna covering it. It is a thick and wide muscle that inserts on the distal epicondyle of the ulna by a short tendon.

*Flexor carpi radialis* (Fig. 4A). It presents sexual dimorphism, being thicker and wider in males than in females. It is bulky and originates onto the distal 2/3 of the humerus, covering the tendon of the m. coracoradialis. It inserts on the base of the prepollex by a short, wide, and ribbon-like tendon.

*Flexor digitorum communis* (Fig. 4A). It is a strong muscle, fusiform that lies along the radio-ulna. It has two origins, one is long, wide tendinous from the distal condyle of the humerus; and the other is a short, narrow, and tubular tendon coming from the medial condyle of the humerus. Both origins are contiguous to each other. The muscle is inserted on the flexor plate by a wide, long, and rectangular tendon. The m. flexor digitorum communis presents fibers linked to the deeper face of the m. epitrochleoanconeus.

*Flexor plate* (Fig. 4B and C). It is a big and rounded tendon covering almost all the ventral face of the hand. It has an oval sesamoid embedded. The flexor tendons III, IV, and V, and the origin tendon of the m. lumbricalis longus IV and V originate from the flexor plate.

*Flexor accessorius* (*Palmaris profundus* sensu Gaupp, 1896) (Fig. 4B). Square and wide muscle that origins fleshy on the latero-distal condyle of the ulna. It inserts fleshy on the flexor plate. It extends obliquely on the lateral edge of the flexor plate.

*Flexor tendon + Flexor indicis superficialis propius digiti II* (Fig. 4B). Fusiform and thick muscle that covers almost all the surface of the metacarpal II. Origins wide and fleshy from the distal carpal III–V, at the level of digit 2. It

continues originating the Flexor tendon II inserting on the distal phalanx.

*Flexor tendon + Caput profundum III* (Fig. 4B and C). Fusiform and thick muscle that covers almost all the surface of the metacarpal III. Origins wide and fleshy from the distal carpal III–V, at the level of digit 3. It continues united to the tendon flexor III that inserts on the base of the last phalanx.

*Lumbricalis* (*Lumbricalis longus III, IV and V* sensu Gaupp, 1896) (Fig. 4C). Arise from the flexor plate beside the origin of the correspondent flexor tendons, joined to each tendon along each metacarpal IV and V surface.

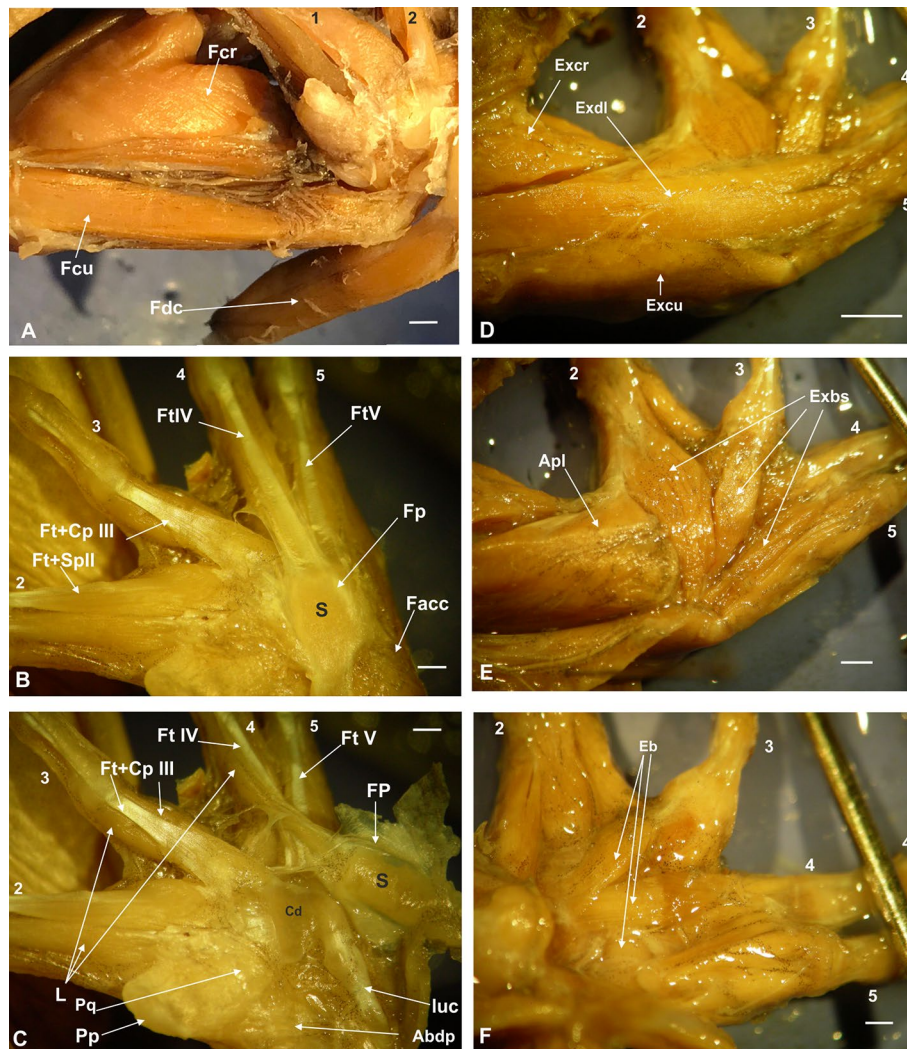
*Extensor carpi radialis* (Fig. 4D). It arises fleshy from the epicondyle medial of the humerus. A bulky muscle inserts on the radial by a long tendon. There is a sesamoid bone embedded into the tendon of the muscle that forms a saddle joint with the extreme of the radius.

*Extensor carpi ulnaris* (Fig. 4D). It arises from the lateral epicondyle of the humerus by a short and ribbon-like tendon. It is a flattened and fusiform muscle that inserts fleshy on distal epiphyses of the ulna. Some of their proximal fibers are interconnected with the m. extensor communis longus proximal fibers.

*Extensor digiti longus* (Fig. 4D). It originates from the epicondyle medialis of the humerus by a short tendon and extends covering the dorsal face of the forearm. It is a ribbon-like muscle split distally into two small ribbon-like branches. Both branches insert by short tendons, one on the base of metacarpal V, and the other, on the distal extreme of the metacarpal IV.

*Abductor pollicis longus* (Fig. 4E). It arises from the proximal 2/3 of the ulna. A bulky muscle extends obliquely through the radius and inserts by a strong and long tendon on the base of the metacarpal of digit II. It covers partially the m. extensor carpi radialis.

*Extensores digitorum breves* (superficial layer) (*Extensor breves superficialis* sensu Gaupp, 1896) (Fig. 4E). It is



**Fig. 4** Muscles of the *Rhinella arenarum* hand. A Ventral view of the antibrachium showing the superficial and deeper layer of muscles, flexor digiti communis muscle was removed. B Ventral view of the hand showing the flexor tendons-flexor plate complex, sesamoid, and muscles. C Ventral view of the hand with a close view of the dorsal sesamoid. The flexor tendons+flexor plate complex is raised to show the distal carpal 3–5 and the ulnocarpalis ligament. D Dorsal view of the hand, the superficial layer of extensor muscles is shown. E The extensor communis longus muscle was removed showing the deeper layer of extensor brevis superficialis muscles. F The extensor brevis superficialis muscles were removed showing the deeper layer of muscles extensores breves. *Abdp* abductor pollicis; *Apl* adductor

pollicis longus; *Cd* distal carpal 3–5; *Eb* extensores digitorum breves; *Ecr* Extensor carpi radialis; *Ecu* extensor carpi ulnaris; *Exbs* extensores digitorum breves (superficial layer); *Excr* Extensor carpi radialis; *Excu* extensor carpi ulnaris; *Exdl* extensor digiti longus; *Facc* Flexor accessorius; *Fcl* Flexor digiti comunis longus; *Fcr* Flexor carpi radialis; *Fcu* Flexor carpi ulnaris; *Fdc* Flexor digitorum communis; *Fp* flexor plate; *Ft + Cp III* Flexor tendon digit III + Caput profundum digiti III; *Ft + Sp II* Flexor tendon II + superficialis proprius digiti II; *Ft IV* flexor tendon digit IV; *Ft V* flexor tendon digit V; *L* lumbricalis; *luc* ulnocarpalis ligament; *Pp* prepollex; *Pq* pronator quadratus; *S* sesamoid. Digits are indicated in Arabic numbers. Scale: 1 mm

a complex of ribbon-like muscles that arise from the ulnar medial border. They extend superficially, like a fan, covering the carpals and inserts on the base of the distal extreme of each metacarpal by a short tendon.

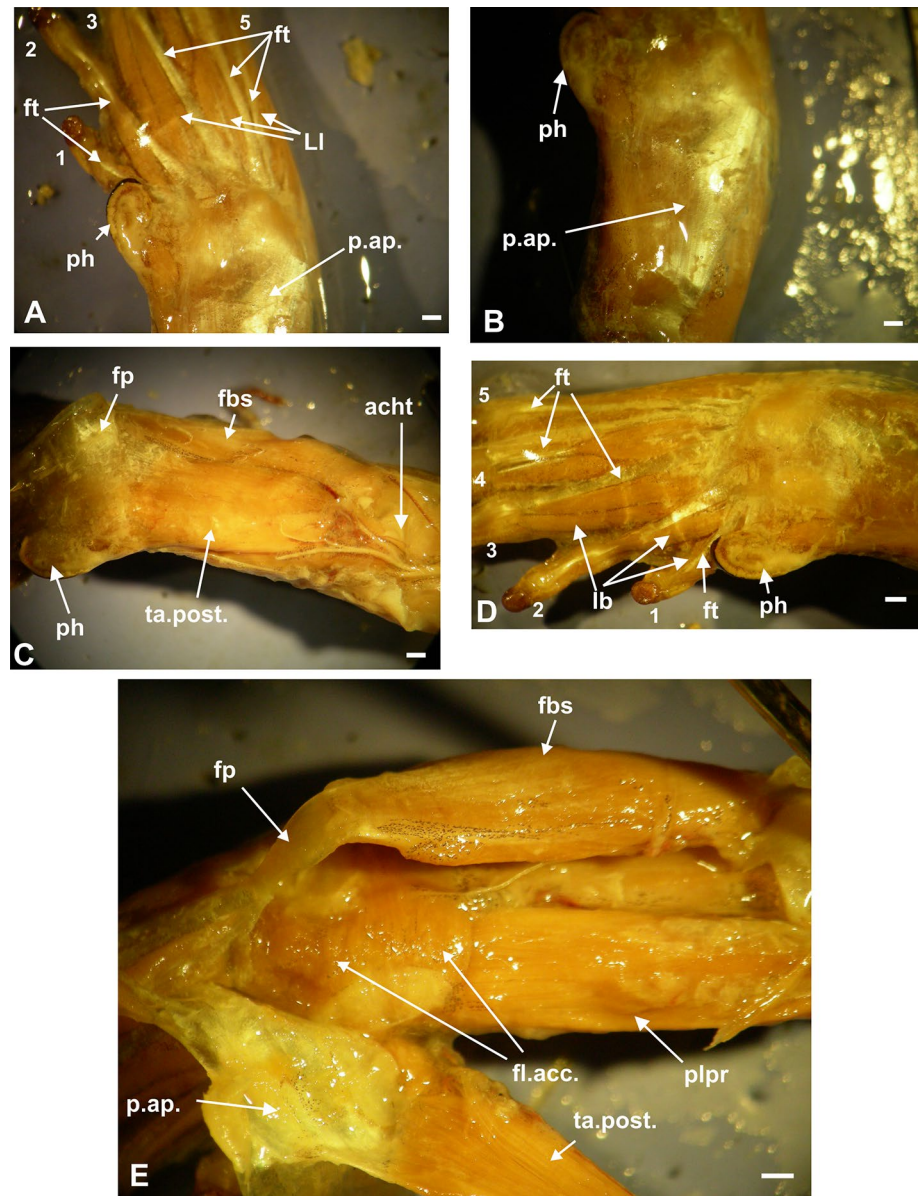
*Extensores digitorum breves* (*Extensor breves medii sensu Gaupp, 1896*) (Fig. 4F). It is a complex of short ribbon-like muscles that origin in part from radial bone and inserts on the base of metacarpals II, III, and IV. Those that insert on the digit V origins on the distal carpal III–V.

### Hindlimb Muscles (Figs. 5 and 6)

*Achilles tendon and plantar aponeurosis* (Fig. 5A, B and C). The Achilles tendon is a strong ribbon-like and thick sheath that covers all the plantar face from the ankle articulation and comes from the distal tendon of the m. flexor digitorum communis. It originates the plantar aponeurosis, which spread covering the superficial tarsal region. Give origin to the lumbricales muscles of each digit. Covering its



**Fig. 5** Ventral view of the muscles of *Rhinella arenarum* foot. **A** Superficial layer of the foot showing the plantar aponeurosis, the flexor tendons and the prehallux. **B** A detail of the plantar aponeurosis. **C** The plantar aponeurosis was removed showing the flexor plate, and the flexor digiti communis, and tarsalis posticus muscles. **D** A detail of the flexor tendons. **E** The tarsalis posticus muscle and the plantar aponeurosis were removed showing the deeper layer and a detail of the flexor plate formed by the distal tendon of the flexor digiti communis muscle. *acht* Achilles tendon; *fbs* flexor brevis superficialis; *fl.acc.* flexor accesorius; *fp* flexor plate; *ft* flexor tendons; *lb* lumbricalis brevis; *Ll* lumbricalis longi; *p.ap.* plantar aponeurosis; *ph* prehallux; *pl.pr.* plantaris profundus; *ta.post.* tarsalis posticus. Digits are indicated in Arabic numbers. Scale: 1 mm



distal portion, there is a subcutaneous fascia with tendons inserted on the skin and related to the flexor tendons. The plantar aponeurosis is continuous distally to the phalanges of each digit covering the flexor tendons as a thin layer. Flexor tendons I and II arise from a deeper layer of the plantar aponeurosis (Fig. 5A and D).

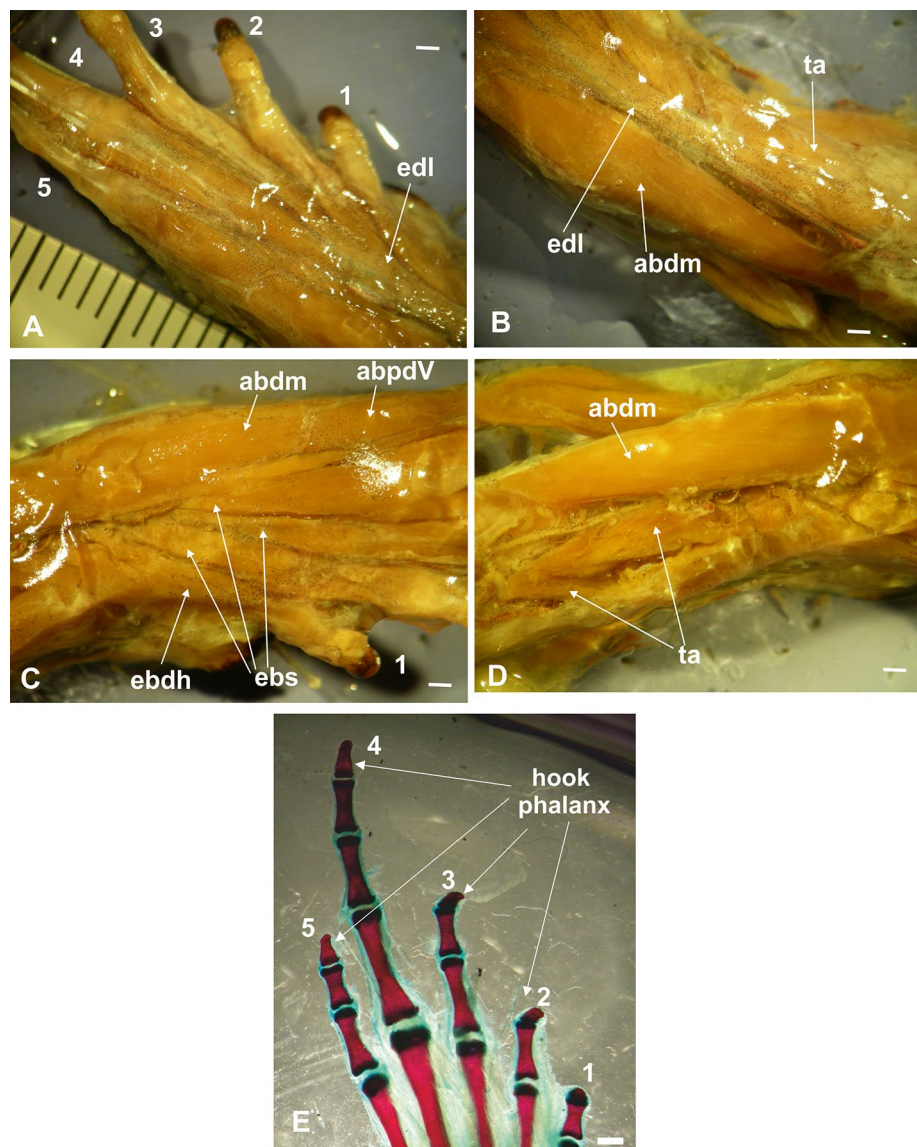
*Tarsalis posticus* (Dunlap, 1960) (Fig. 5C and E). It is a bulky, long, and fan-shaped muscle that arises from the Achilles tendon at the tibiale side. The superficial branch gives origin to two muscles: the abductor prehallucis and the abductor brevis plantae hallucis. The deeper branch surrounds the distal epiphysis of the tibiale and is attached fleshy to it. Also, the muscle is attached, tendinously, to plantar aponeurosis. It is closely related to other muscles, such as the plantaris profundus muscle (Dunlap, 1960) that

is joined proximally (Fig. 5E), and with several fibers that joint dorsolaterally to the tarsalis anticus (Fig. 6D).

*Flexor brevis superficialis* (*F. digitorum brevis superficialis*; Dunlap, 1960) (Fig. 5C and E). It is a bulky and fusiform muscle, originated tendinously from the ankle aponeurosis (Achilles tendon), continuous with it. Distally, it continues with a small tendinous flexor plate, which has a small, flat, and rounded sesamoid embedded. The flexor plate splits into three long flexor tendons that insert onto the distal phalanges of digits 5, 4, and 3 (Fig. 5A and D).

*Flexor accesorius* (*transversus plantae proximalis et distalis*; Dunlap, 1960) (Fig. 5E). A bulky, short, and fan-shaped muscle, which covers the tarsal region of the foot. Arises fleshy from the distal external border of the fibulare epiphysis and inserts by a wide tendon on the distal border

**Fig. 6** Dorsal view of the muscles of *Rhinella arenarum* foot. **A** Superficial layer of the foot showing the extensor digiti longus muscle and the digits. **B** Superficial layer of the foot at the tarsal level showing the extensor digiti longus, abductor medius, and the tarsalis anticus muscles. **C** The extensor digiti longus muscle was removed showing the deeper layer at the level of the digits. **D** The extensor digiti longus muscle was removed showing the deeper layer at the tarsal level. **E** Double stained cleared foot showing the hook-like ultimate phalanges of each digit. *abdm* abductor medii; *abpdV* abductor proprius digiti V; *abs* extensores breves superficialis; *edl* extensor digiti longus; *ta* tarsalis anticus. Digits are indicated in Arabic numbers. Scale: 1 mm



of the tibial epiphysis and Y element. There is a superficial layer that originates tendinously from the distal medial border of the fibulare epiphysis. It is bulky and short and covers the distal surface of the deeper layer. It has a small sesamoid embedded in the tendon of origin of the proximal portion of the muscles flexor accessorius, and of the muscles flexor brevis digiti V and abductor brevis V.

*Lumbricalis longi* (Fig. 5A). Arise from the medial flexor plate; they are fusiform, associated with the flexor tendons and inserts by long, thin tendons, to the distal phalanx of each digit. At digits 4 and 5, the lumbricalis split into two branches that insert fleshy at the lateral base of the proximal phalanx of digit 4, and at the medial base of the proximal phalanx of digit 5.

*Extensor digitorum longus* (Fig. 6A and B). It is a long, thin, and ribbon-like muscle that arises from the base of

the cruris, by a ribbon-like, long tendon. Distally, it split into three fusiform branches that insert on the dorsal fascia of metatarsals II to IV, at the level of the distal extreme of digit 4, or the first or second phalanx by thin tendons in the other digits.

*Extensor brevis superficialis* (Fig. 6C). It arises from the medial border of the fibulare. It splits into several branches that insert on the base of the first phalanx of digit 1 and 2 and the second phalanx of digit 3 by ribbon-like tendons.

### Distal Phalanges Skeleton (Fig. 6E)

*R. arenarum* presents all their distal phalanges curved at the tip, acquiring a hook shape.

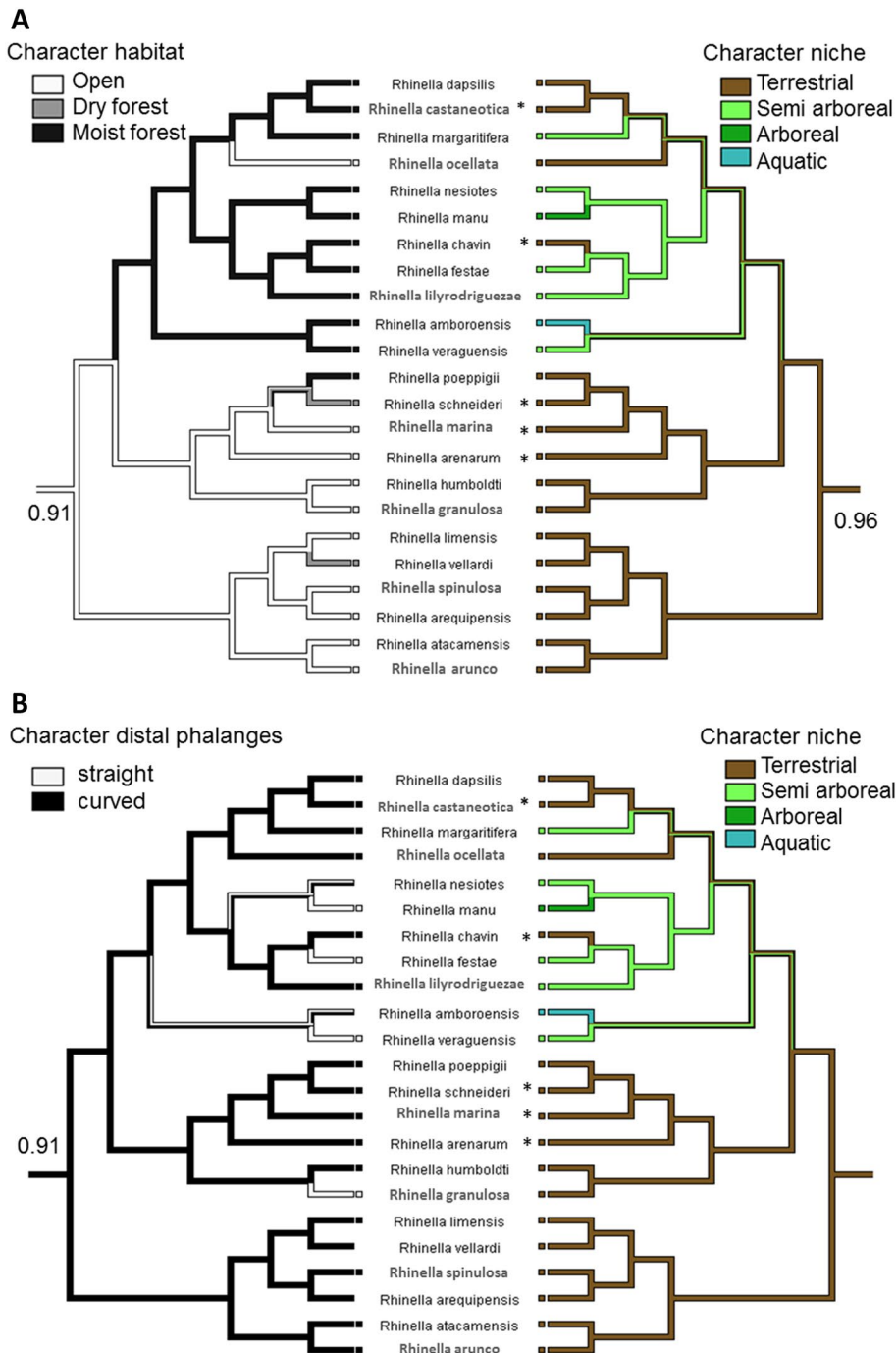


### Character Mapping

As previously mentioned, Table 1 summarizes gathered information regarding habitat, climbing capacity, ecological niche, and distal phalanges condition of species belonging to the genus *Rhinella*. Reconstruction of evolutionary history shows that the arboreal niche seems to be a derived condition in *Rhinella*, while the terrestrial niche

is traced at the base of the tree. It was clear that climbing ability arose frequently within the *Rhinella* clade, sometimes in species that occupy open habitats (Fig. 7). The curved distal phalanges are a basal character within the considered *Rhinella* species. However, this information should be taken with caution. There are terrestrial species of which climbing capacity is known that have curved distal phalanges, as well as arboreal species with straight phalanges.

**Fig. 7** Evolutionary history reconstruction for habitat (A), shape of the distal phalanges (B) and ecological niche in species belonging to the genus *Rhinella* (Bufonidae), under the assumption of parsimony. Numbers are proportional likelihoods of ancestral state reconstruction. Phylogeny based on Chaparro et al. (2007), Pramuk et al. (2008), and Cusi et al. (2017). Asterisks denote known climbing ability for terrestrial species (for *R. arenarum*, from the present study)



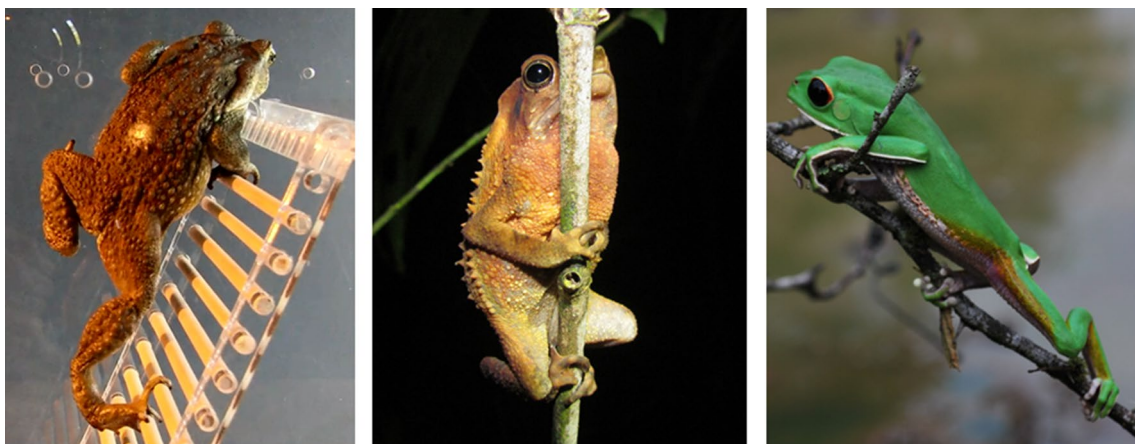
## Discussion

Usually, the common terrestrial toad *Rhinella arenarum* is not associated with any particular grasping skill or specialization, being hopping its primary locomotor mode (Gomes et al., 2009). Even the species of the genus exhibiting climbing abilities, such as *R. margaritifera* and *R. castaneotica*, lack any apparent morphological adaptations to climb (de Noronha et al., 2013), as was observed also in other species of Bufonidae (Gosá, 2008). Arboreal frogs, instead, show several specific traits related to their locomotion in the highly challenging arboreal habitat. For example, they present a bigger cerebellum than other anurans (Manzano, Herrel, et al., 2017); the main flexor tendons arise directly from the m. flexor digitorum communis, without the flexor plate and its embedded sesamoid (Manzano et al., 2008); and present distal extensor muscles of the fingers (muscles extensores breves distalis), strongly related to the arboreal mode of life (Burton, 1998). Figure 8 shows examples of these climbing abilities in anurans (from a generalized terrestrial form, *Rhinella arenarum*, passing through a semi-arboreal form, *R. margaritifera*, to a highly specialized arboreal form, *Phyllomedusa sauvagii*).

Our anatomical results suggest that the curved distal phalanges of *Rhinella arenarum* are held in tension due to the contraction of the m. flexor digitorum stretches the flexor tendons that attach to these phalanges. The superficial layer of each digit muscle, such as flexor indicis superficialis proprius II and caput profundum III and lumbricalis longus muscles also would contribute to sustaining this flexion in a hook position. The palmar sesamoid would help to maintain the flexor tendons in tension and, at the same time, it prevents total flexion of the hand around

the perch (see, e.g., *R. margaritifera*, and especially *Ph. Sauvagii*; Manzano et al., 2008). The lumbricalis muscles, which assist phalangeal metacarpal flexion, are also important in helping the proximal digit region to curve around the perch. The extensor muscles of the hands do not seem to intervene in the extension of the last phalanx, since there is no evidence of tendon insertion in the last phalanges. Thus, hooking in *Rhinella* is probably a contraction and relaxation action of the muscles of the ventral face of each autopodium, without including extension. In the hind limb, the plantar flexors exhibit a plesiomorphic state typical of nonarboreal clades (Blotto et al. 2020), attaching indirectly to the distal phalanges of the postaxial digits through the aponeurosis plantaris.

Despite its apparent lack of grasping skills, our data show that the specimens of terrestrial Argentinian toads are capable of not only walk through narrow substrates but also climbing substrates of different diameters and slopes. However, how terrestrial toad *R. arenarum* climbs is very different from that shown by specialized tree frogs. Our data show that the forelimbs of common toads are quite rigid. The hands exhibit a fixed position with the digit 2 directed to the midline of the body, with limited capacity for lateral movements. Only the distal phalanges have a limited range of flexion, enough to perform a hooking mode of the climb. Hooking is a movement involving only the terminal phalanges as a suspensory type of prehension (Napier, 1993). Hooking ability might be crucial for "clambering" over rocks or rough surfaces such as large diameter trunks. As mentioned above, contraction of the flexor digitorum communis flex the ventrally curved terminal phalanges through the flexor tendons, arising a hook that supports the body of the toad acting as claws. The foot digits present the same mechanism, although the movement is leading by digit 4. Even though we found sexual dimorphism in those muscles



**Fig. 8** Climbing in anurans. Left: generalized, terrestrial *Rhinella arenarum*. Center: semi-arboreal *R. margaritifera* (photo: Mariana Chuliver Pereyra, 2018). Right: highly specialized arboreal *Phyllomedusa sauvagii* (photo: Miriam C. Vera)

related to the amplexus such as the flexor carpi radialis, we do not observe apparent differences in hooking performance between males and females. The extensor layer of muscles, although strong, seem to be less involved in the dorsal extension of the hook phalanx, being probably more critical during amplexus, since they show sexual dimorphism in this species. Hooking grasping could also explain the abilities of the cane toad (*Rhinella marina*) to escalate through volcanic landscapes—probably during dispersal events to new habitats—and mesh cylinders under laboratory settings (Hudson et al. 2016). Interestingly, that study showed intraspecific differences in climbing abilities between individuals from four regions in Australia. These differences could be related to the fact that the local populations of *R. marina* could differ in the environmental conditions that they face, and how they influence the benefits of climbing, for example, to access prey items. Curiously, in some other anurans such as *Xenopus* sp., the last phalanx overpasses the skin forming a claw-like structure, which is used to tear the prey to pieces (Anzeraey et al., 2017).

A reduction or absence of a palmar sesamoid characterizes a grasper autopodium, a condition observed in specialized arboreal species such as *Phyllomedusa bicolor* (Manzano et al., 2008). The presence of a sizeable palmar sesamoid, which restricts the flexion of metacarpal-phalangeal joints (Abdala et al., 2009; Sustaita et al., 2013), seems to be a cause of *R. arenarum* not being able to perform a power grip, in which both the palm and digits intervene. It was clear that when climbing the narrow substrate constituted by the 6 and 8 mm diameter rods, *R. arenarum* has limitations to close all the fingers of the hand to grip the rod. The opposability of the hand could also be affected by the length of metacarpal 2, which is shortened in grasper species, contrary to the condition observed in *R. arenarum* and other non-graspers (Manzano et al., 2018). However, the weak independence of the digits and flexion of distal phalanges allow performing an intermediate grip that is used by this species to climb (Figs. 2C and 3F). This prevention of power grasping because the presence of the palmar sesamoid is also observed between squamates such as iguanid lizards (Abdala et al., 2009; Sustaita et al., 2013). However, it was clear that the flexing of the digits that *R. arenarum* can perform is sufficient to exert a hooking grip as well as achieve a greater contact surface of the autopodium with the substrate, to some extent effective in preventing slippage (Fig. 3C).

Our results demonstrate that the flexor and extensor muscles of the digits and toes, although highly conserved and generalized in *R. arenarum*, allow the hands and feet to perform hooking and, also, to make another type of grip sufficient to climb an experimental device that simulates an arboreal environment with thin branches. This fact suggests that the acquisition of a new function, here understood as the ability to climb, besides to perform terrestrial locomotion,

may have played a relevant role in the origin of the arboreal niche by amphibians. The arboreal niche exhibits considerable opportunities to survive either as a refuge, as resting sites to avoid predators, or as food availability alternatives (Granda-Rodríguez et al., 2008; Lindquist et al., 2007; Toledo et al., 2007). Our study supports the hypothesis of climbing as an alternative to escape, in our case, from a small enclosure, which has already been proposed by de Noronha et al. (2013). Table 1 and Fig. 7 show that, whereas semi-arboreal and arboreal niches, which necessarily require reliable climbing ability either through grasping or hooking on vegetal parts, have evolved in association with the occupation of closed forest habitats, some studies show that the ability to climb is also present in *Rhinella* species that have a terrestrial niche. Interestingly, the evolutionary history of an anatomical trait closely linked to the possibility of performing hooking such as the distal curved phalanges does not trace as essential to climbing. Thus, distal phalanges curved probably enhance climbing by hooking, not being essential to perform it.

But why would an animal like *Rhinella* choose a tree to live in? Many taxa have shown to choose these environments for the reasons explained above; in the case of *Rhinella*, the arboreality is obviously (as we can see in Table 1) associated with tropical environments, with a high canopy density and probably greater vulnerability at ground level and risk to be hunted or parasitized. Rezende Oliveira et al. (2017) have shown that different species of *Rhinella* are frequently preyed upon by snakes, which can increase the chances of using climbing tree branches or obstacles at height as an escape route.

The fact that *Rhinella* climb in a non-specialized form supports the argument that anyone, with the appropriate anatomy, can climb, having the possibility of adding it to its locomotor repertoire.

## Concluding Remarks: Implications to the Arboreal Niche Origin

The arboreal niche has triggered the evolution of morphological adaptations to climbing, in vertebrates in general and in amphibian species in particular, recurrently in different lineages. More generally, the idea that behavioral changes associated with the origin of new niches, through the generation of new selection pressures, have driven morphological evolution has been proposed as a working hypothesis by several researchers (Bateson, 2017; Losos et al., 2004; Wyles et al., 1983). It was argued elsewhere (Vassallo et al., 2019) that minimum requirements in terms of functional capacity should be met a priori for successful colonization of a new niche. In the case of the arboreal niche avoid falling, both during displacement and at rest,

and travel on a three-dimensional discontinuous substrate are two of those functional requirements. Our climbing experiments on the terrestrial species *R. arenarum* have not only shown that the species possess unexpected functional capacities which adequately fulfill the above-mentioned requirements but also suggest a way in which the origin of new niches could have been performed in anurans. Thus, the ability to perform terrestrial locomotion and climbing in *R. arenarum* could play a role in the case that the exploration of a new niche would be necessary. In that particular case, the hooked terminal phalanges combined with the action of the flexor tendons that allow performing hooking grasping, added to a weak intermediate (scissor) grasping shown during the experiments, would constitute an adaptation of the locomotion (Gould & Vrba, 1982; Manzano, Fontanarrosa, et al., 2017), that facilitate the exploration of heights and the possible establishment of arboreal niches. More generally, the results suggest the importance of analyzing in detail the functional skills (and limitations) of generalized species, as an approach to better understand the causes underpinning the origin of new niches.

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**Data Availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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