Anatomical analysis of the lizard carpal bones in the terms of skilled manual abilities

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Abstract

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One of the most remarkable hand movements is grasping. This ability has been repetitively associated with the evolution of the human lineage towards the development of technology. Besides mammals, other tetrapods have also evolved significant, and in some cases surprising, forelimb prehensile capabilities. In this study, we present a qualitative analysis of the carpal bones in diverse lizard taxa. Our main goal is to make a survey of the carpal morphology of different squamatans and to interpret its variability in the context of both lizard skilled forelimb movements and the evolutionary history of Squamata.

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Introduction

The hand is a structure that arose during the Middle Devonian within a set of locomotive adaptations in the margins of aquatic ecosystems and terrestrial environments (Ahlberg and Clack 2006; Shubin *et al.* 2006). It is one of the main structures of Tetrapoda that enables physical interaction between the environment and the organism. The bone structure of the hand is composed of about 28 specialized bones. It is regionalized and includes, from the proximal to the distal region, the carpus, the metacarpus and the series of phalanges composing each finger, the carpus being one of the most complex regions of the skeleton because of their multiple joints (Romer 1956; Flower 1885; Renous-Lécuru 1973). In many tetrapod taxa, the ventral surface of the carpus is covered by a sesamoid bone, the palmar sesamoid (Jerez *et al.* 2009, Ponssa *et al.* 2010).

In many tetrapod taxa, the hand can perform skilled forelimb movements, which have been defined as 'the ability to reach objects, hold them in a hand or forepaw and manipulate them with the digits' (Iwaniuk and Whishaw 2000). Among them, one of the most remarkable movements is grasping behaviour, which helps the performance of numerous activities of manipulation and locomotion (Pouydebat *et al.* 2008). This ability has been repetitively linked to the evolution of the human lineage relative to the manufacture and use of tools allowing organisms to exploit the resources in their environment (Napier 1956; Susman 1998; Marzke and Marzke 2000; Pouydebat *et al.* 2008). Other tetrapods also present significant, and in some cases surprising, forelimb prehensile capabilities, whose recognition has led to a recent burgeoning interest in this phenomenon in no-mammalian taxa (Manzano *et al.* 2008; Abdala *et al.* 2009; Sustaita *et al.* 2013). Within Squamata, enhanced forelimb movements have been reported at least in five lizard lineages: Polychrotids, Dactyloids, Chamaleonids, Gekkota and Varanids (Abdala *et al.* 2009; Mendyk and Horn 2011; Sustaita *et al.* 2013). This issue was addressed from a few perspectives, such as comparative anatomy of muscle and tendon (Manzano *et al.* 2008; Abdala *et al.* 2009; Sustaita *et al.* 2013), biomechanics (Manzano *et al.* 2008; Abdala *et al.* 2009) and behaviour (Mendyk and Horn 2011). Although the skeletal structures are the basis of any movement, the bone configuration of the hand has received considerably less attention than other systems in studies of skilled forelimb movements (Sustaita *et al.* 2013).

In this study, we present a qualitative analysis of the carpal bones in diverse lizard taxa. Our main goal was to make a survey of the carpal morphology of different squamatans and to interpret it in the context of the lizard skilled manual movements, to find out whether the presence of this function requires particular morphologies for their performance. We interpret our morphological results in the context of the most

accepted phylogenetic hypothesis of the relationships of Squamata to assess whether some of the morphological traits of the hand skeleton of lizards can be proposed as an adaptation to the grasping function.

Materials and Methods

Morphology

Information of carpus anatomy was obtained from 72 specimens belonging to 43 species and 13 squamatan families (Appendix 1). We followed the criterion of maximizing (i) the morphological range sampled; (ii) the widest range of manual capabilities (Table 1); and (iii) the phylogenetic clades represented (i.e., species from different clades of the family).

Observations were made in clearing and double staining with Alcian Blue and Alizarin Red whole mounts obtained following the procedure described in Wassersurg (1976). Descriptions, illustrations and photographs were made with a stereo disection microscope (Nikon, SMZ-10, Nikon Corp., Tokyo, Japan). The available view of the carpus of each specimen was described (usually the ventral one). In addition, data on lizard wrist graphically reported on the literature were considered (e.g., Renous-Lécuru 1973).

Anatomical patterns were inferred based on the descriptions of the skeletal structures involved. We also present a qualitative approach to determining size. To do this, the size of each carpal element was also considered and was reported as an ordinal qualitative variable.

Table 1 Anatomical pattern of the carpus and manual capabilities of the species examined

Family	Species	Anatomical pattern of the carpus	Skilled forelimb movement?	Author
Gekkonidae	*Chondrodactylus angulifer*	3	Y	Juan Daza *
	Palmatogecko rangei	3	Y	Juan Daza *
Sphaerodactylidae	*Sphaerodactylus klauberi*	3	Y	Juan Daza *
	Pseudogonatodes sp	3	Y	Juan Daza *
	Lepidoblepharis xanthostigma	3	Y	Juan Daza *
Diplodactyilidae	*Hoplodactylus pacificus*	3	Y	Juan Daza *
	Lucasium damaeum	3	Y	Juan Daza *
Carphodactylidae	*Nephrurus deleani*	3	Y	Juan Daza *
Phyllodactylidae	*Phyllopezus lutzae*	3	Y	Juan Daza *
	Phyllopezus pollicaris	3	Y	Virginia Abdala+
	Homonota fasciata	3	Y	This work
	Thecadactylus rapicauda	3	Y	Juan Daza *
Scincidae	*Mabuya mabouya*	1	N	Virginia Abdala+
Gymnophthalmidae	*Cercosaura parkeri*	1	N	Virginia Abdala+
Teiidae	*Cnemidophorus longicaudus*	1	N	Virginia Abdala+
	Cnemidophorus ocellifer	1	N	This work
	Tupinambis merianae	1	N	This work
	Kentropix viridistriga	1	N	Virginia Abdala+
	Teius oculatus	1	N	Virginia Abdala+
	Ameiva ameiva	1	N	Virginia Abdala+
Varanidae	*Varanus griseus*	1	?	–
Helodermatidae	*Heloderma suspectum*	1	?	–
Anguidae	*Elgaria multicarinata*	1	?	–
Tropiduridae	*Tropidurus etheridgei*	1	N	Cruz, Tulli*
	Tropidurus melanopleurus	1	N	Cruz, Tulli*
Dactyloidea	*Anolis cuvieri*	2	Y	Abdala *et al.* 2009; #
	Anolis gundlachi	2	Y	Abdala *et al.* 2009#
	Anolis stratulus	?	Y	This work
Polychrotidae	*Polychrus acutirostris*	2	N	This work
Liolaemidae	*Liolaemus cuyanus*	1	N	This work
	Liolaemus bitaeniatus	1	N	This work
	Liolaemus ramirezae	1	N	This work
	Phymaturus ceii	1	N	Cruz, Tulli*
	Phymaturus panae	1	N	Cruz, Tulli*
Leiosauridae	*Leiosaurus catamarcensis*	1	N	This work
	Enyalius catenatus	1	N	W. Quatman*

Skilled forelimb movements (Y); Skilled forelimb absent (N). Manual capabilities were assigned based on empirical test own of this work, Literature data (#); Personal observations (+); Personal communication or photographs (*); Without data (?).

Skilled manual abilities

Live specimens of *Polychrus acutirostris* (Polychrotidae), *Liolaemus cuyanus* (Liolaemidae), *L. ramirezae* (Liolaemidae), *L. bitaeniatus* (Liolaemidae), *Leiosaurus catamarcensis* (Leiosauridae), *Anolis stratulus* (Dactyloidae), *Homonota fasciata* (Phyllodactylidae), *Cnemidophorus ocellifer* (Teiidae) and *Tupinambis merianae* (Teiidae) were observed to examine their manual capabilities [see Napier 1956 (hominids); Robinson 1975 (*Anolis equestris*); Gray 1997 (anurans); Iwaniuk and Whishaw 2000 (tetrapods); Mendyk and Horn 2011 (*Varanus beccarii*) for similar approaches]. In this context, skilled manual abilities refers to prehensile behaviour (Iwaniuk and Whishaw 2000), which is defined as the application of functionally effective forces by an appendage to an object for a task (Sustaita *et al.* 2013). Taking this definition into account, branches are considered objects grasped through the lizard hands. It should be noted that the authors are not referring to object manipulation because branches are not modified by grasping. The animals were observed at different points in the step cycle while moving across dowels of different diameters (3 and 6, and 10 cm), for the variable time they maintained their position, and photographs of the iconic postures were taken. Additionally, similar data on the remaining taxa surveyed were obtained from field observations. These data and the assignment of all lizard species examined to either the presence or absence of skilled manual abilities are summarized in Table 1.

Character mapping

Based on the comparative analysis, five characters that seem to be relevant in the study of the skilled manual movements of squamatans were selected (complete descriptions of the characters are given in Result section):

1. Skilled forelimb movements: (0) absent; (1) present.
2. Carpal Anatomical patterns: (0) Liolaemid-like; (1) Polychrotid-like; (2) Gecko-like.
3. Shape of the central bone: (0) subtriangular; (1) elongated; (2) saddle like.
4. Palmar sesamoid: (0) reduced or absent; (1) big.
5. Ulnare–radiale relationship. (0) united; (1) entirely separated by a free space in between; (2) partially united; (3) totally separated by the centrale, which occupies the space between them.

Character 1 has been selected following the statements in Abdala *et al.* (2009). Character 2 synthesizes the variability related to the carpal patterns. Characters 3 and 4 exhibit clear differences among the studied taxa and present an apparent association with grasping abilities (as seen in our exploratory analysis and in Abdala *et al.* 2009). Character 5 completes the information conveyed by the other characters. We also considered data on the tendinous patterns exhibited by the selected taxa, as described in Tulli *et al.* (2012), to make our discussion more comprehensive. The characters were optimized onto a cladogram of the squamatan relationships of Wiens *et al.* (2012) reduced to family level. For this purpose, we used TNT software (Goloboff *et al.* 2008).

Results

Common morphology of the carpus among the species studied

Most of the lizards analysed exhibit a carpus composed of nine bones: radiale, ulnare, centrale, five distal carpalia and pisiform (Fig. 1). All these bones are often ventrally covered by one or even two palmar sesamoids, which are embedded in the tendon of the flexor digitorum longus muscle. The radiale is a rectangular bone, articulating by its proximal portion with the radius and covering the distal surface of its epiphysis almost completely. The radiale exhibits a prominent structure located in the pre-axial region of the ventral surface. We named this structure 'radiale process', which takes different forms among the taxa (Fig 2). The radiale process is raised to reach spatial plane of the pisiform. These two structures – the pisiform and the radiale processes – delimit a concavity in the ventral surface of the carpus, which is restricted to the proximal region. The ulnare articulates with the ulna, covering its distal surface. The centrale articulates proximally with the radiale and the ulnare, and distally with the distal carpalia 1, 2, 3, and usually also with distal carpal 4, which possesses the biggest surface of all distal carpals. It is possible to trace three
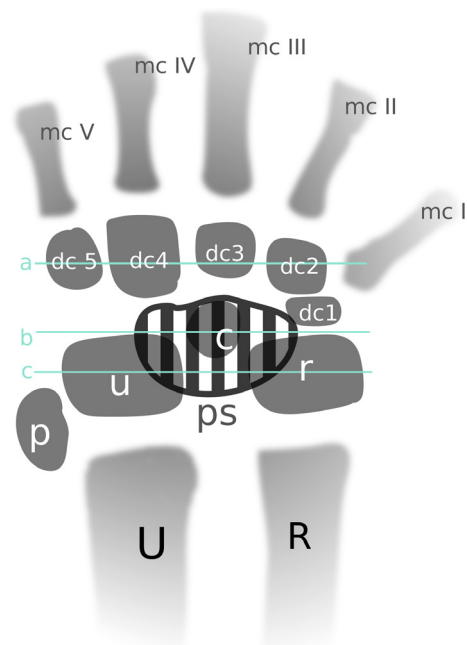

Figure 1—General scheme of the lizard carpus in ventral view. R, radius; U, ulna; r, radiale; u, ulnar; c, central; 1–5, distal carpalia 1–5; I–V, metacarpalia I-V; ps, palmar sesamoid. Reference lines: a: proximal line, b: central line, c: distal line.
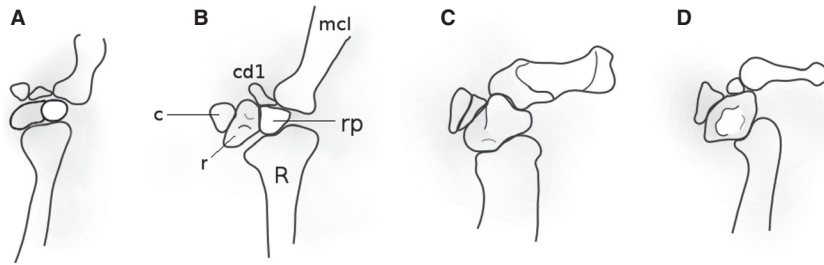
Figure 2—Radiale process. — **A.** *Liolaemus cuyanus*, — **B.** *Mabuya mabouya*, —**C.** *Polychrus acutirostris*; — **D.** *Phyllopezus lutzae*. The cross-section shape varies between quadrangular and triangular (A, B) to eliptical (C, D). The size ratio radiale process/radiale is bigger in *M. mabouya* than in the other species. The radiale process is projected ventrad (A, B, and D) or distad (C). Note the ball and socket joint between the radiale processes and the metacarpale 1 epyphisis in *P. acutirostris*. R, radius; r, radiale; rp, radiale process; dc 1 and dc 2, distal carpalia 1 and 2; mc I, metacarpal I.

imaginary axes parallel to one another and perpendicular to the medial axial of the hand as references crossing the carpus: a first line (a), proximally situated crossing ulnare and radiale; a second line (b), crossing the centrale; and a third line (c), distally situated crossing the distal carpalia (Fig. 1).

The carpalia connects with the metacarpalia through joints between each distal carpal and its respective metacarpal. Only the first metacarpal connects also with the radiale. The radiale and the distal carpal 1 form a cavity that hosts the first metacarpal epiphisis. The arch formed by the distal carpalia is asymmetric with respect to the median axis because its postaxial elements are more distally situated than the pre-axial ones.

All the lizards analysed exhibited the mesaxonic condition: the medial axis of the hand goes through the area between radiale and ulnare, the centrale, the distal carpal 3, the third metacarpal and the digit 3.

The bone structure of the lizard hand is asymmetric. The pisiform is exclusive of postaxial region; the radiale process is exclusive of the pre-axial region (Fig. 3). The radiale, the distal carpal 1 and the metacarpal 1 posses a topological arrangement that cannot be compared with the arrangement of the ulnare, distal carpal 5 and the fifth metacarpal. The distal carpal 1 (the most pre-axially situated) is totally surrounded by other bones in ventral view, whereas the distal carpal 5 (the most postaxially situated) exposes one of its sides towards the pre-axial region. The distal carpal 2 articulates with the metacarpal II and the metacarpal I. The distal carpal 4 articulates exclusively with its respective metacarpal. Of all the distal carpalia, the distal carpal 4, belonging to the postaxial half, is the biggest carpal of the series. Hence, it is bigger than the distal carpal 2, which is its specular element. The distal carpalia are roughly aligned in a transverse line with respect to the median axis of the hand, except for the distal carpal 1, which is more proximally located. The centrale articulates with the distal carpalia 1, 2, 3 and 4.

Relative sizes of the carpal elements

The palmar sesamoid is a variable structure in its relative size (Fig. 4), with the remaining structure being more stable.
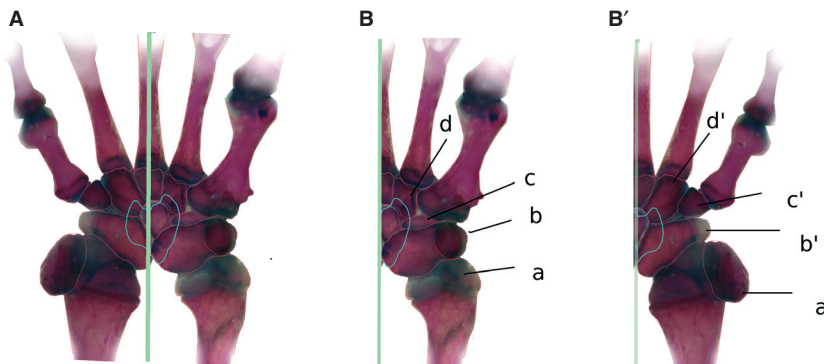

Figure 3—*Liolaemus cuyanus* showing asymmetric traits. Ventral view of the carpus of the right hand. — **A.** Whole hand. — **B.** and **B'.** comparison between the right and left halves. — **B.** Right half. a: absence of pisiform; b: radiale process; c: dc 1 more proximal located than dc2, smaller than dc5, and with its lateral face covered by mc I; d: dc2 smaller than dc4. — **B'.** Inverted left half of the hand. a': pisiform; b': absence of a process; c': dc5 aligned with dc4, showing a lateral face exposed, and bigger than dc1; d': cd4 bigger than dc2. The pisiform is exclusive of post-axial region, the radiale is exclusive of the preaxial region. R, radius; U, ulna; r, radiale; u, ulnare; c, central; 1–5, distal carpalia 1–5; I–V, metacarpalia I–V. The shape and position of the palmar sesamoid is shown in green.
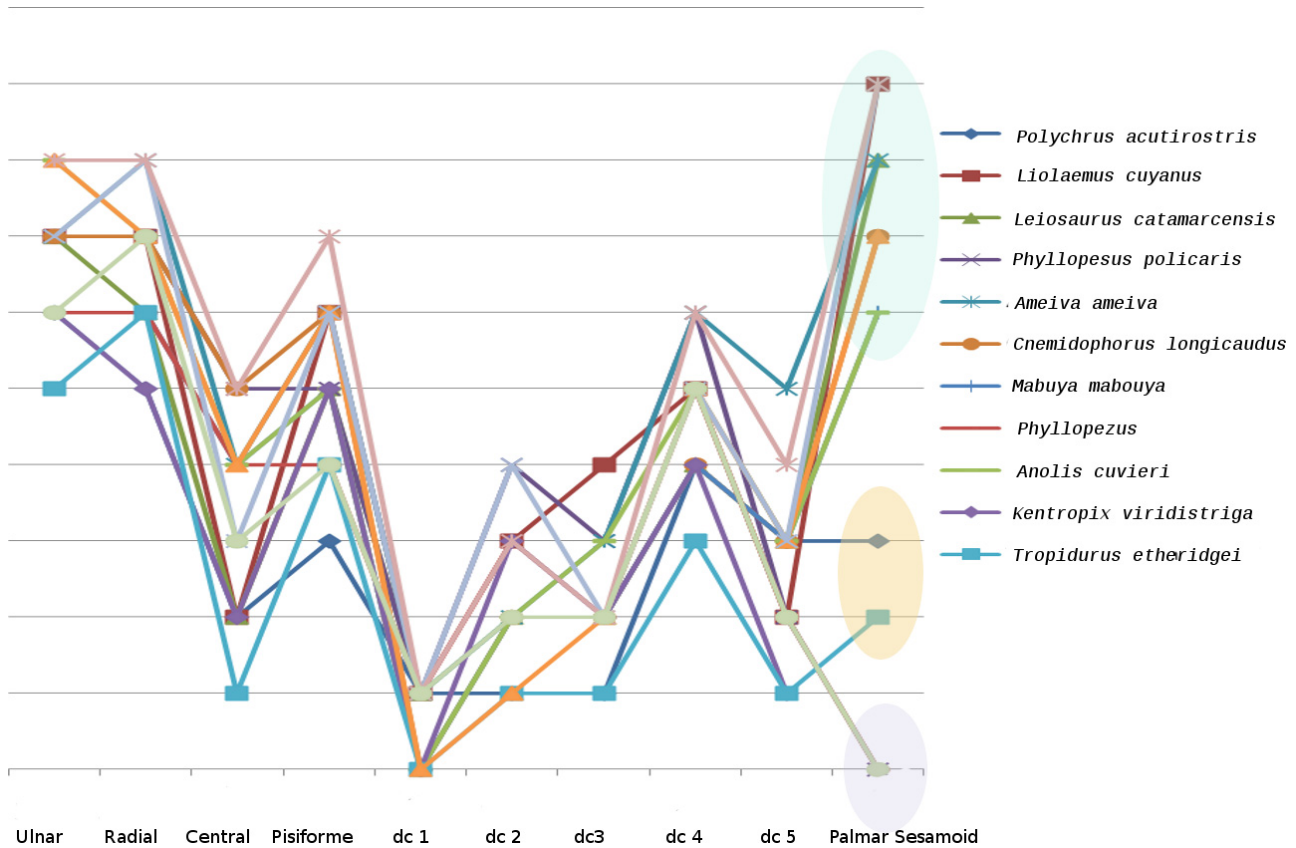
Figure 4—Line graphic comparing relative size of the carpal bones among different lizard species. Note that variations are detectable mostly in relation to the palmar sesamoid, which allows us to recognize three groups. a: big palmar sesamoid, b: small palmar sesamoid, c: palmar sesamoid absent

According to this variability, three groups of lizards are observed: lizards exhibiting a big palmar sesamoid (its size is similar to the ulnare size, which is one of the biggest bones of the carpus); those presenting a small one (smaller than the ulnare); and those without sesamoid.

Anatomical patterns

The anatomical characteristics of the carpal bones and their relationships with the metacarpal bones allowed us to identify three different skeletal patterns, which were named liolaemid-like, polychrotid-like and Gecko-like.

Liolaemid-like pattern. The ventral surface of the carpus is flat, because it does not present overlapping among pieces (Fig. 5A). The carpal bones are distributed in a circle, whose centre is occupied by the triangular centrale. Radiale and ulnare contact each other through their respective postaxial and pre-axial regions. Three imaginary axes can be recognized: a, b and c. Axis 'a' crosses the carpus through the radiale and ulnare, axis 'b' crosses through the centrale and axis 'c' crosses through the distal carpals (Fig. 1). Each metacarpal emerges parallel to the other and to the median axis of the hand, with all their epiphyses aligned.

Polychrotid-like pattern. The ventral surface of the carpus is slightly concave, with its pieces partially overlapped (Fig. 5B). The centrale is displaced from the central position, and for that reason only, axes 'a' and 'c' can be recognized. The centrale is elongated, with its major axis coinciding with the middle axis of the hand. Its proximal apex is introduced between radiale and ulnare, separating them. The metacarpalia emerge parallel to one another and with respect to the median axis of the hand. The first metacarpal is located in a more ventral plane in relation to the other metacarpalia. The epiphyses of the first metacarpal and the first distal carpal are displaced to a more proximal position than the remaining carpal and metacarpal elements.

Gecko-like pattern. The ventral surface of the carpus is plane, without overlapped pieces (Fig. 5C). It is similar to polychrotid-like pattern in relation to the centrale position and shape. It differs in the position of the metacarpals, which emerge radially from the central point of the carpus. This is an arrangement strongly defined by the perpendicular position of the third metacarpal with respect to metacarpalia I and V. The hand shows a perfect symmetry in relation to the middle axis, which crosses through the third finger (here, we refer to the symmetry of the hand specifically in terms of emergence
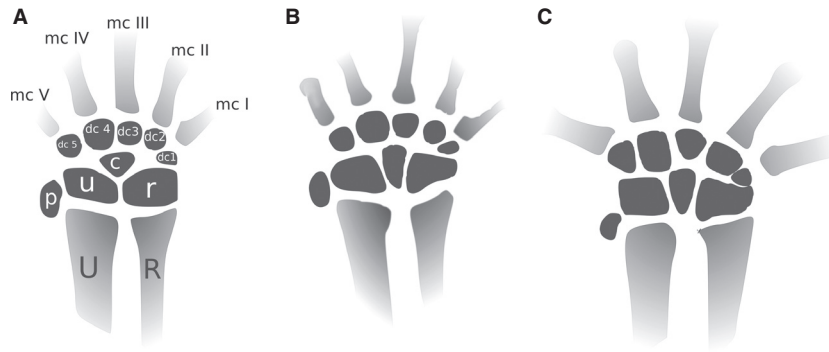
Figure 5—Carpal anatomical patterns: — **A.** Liolaemid-like. Note the carpal bones distributed in a circle, whose center is the triangular centrale; radiale and ulnare are in contact; the metacarpalia emerge parallel to each others. — **B.** Polychrotid-like. Note the centrale displaced from the centrale position with its proximal apex introduced between radiale and ulnare. The metacarpalia emerge parallel to the others. —**C.** Gecko-like the centrale is displaced from the central position with its proximal apex introduced between radiale and ulnare; the metacarpalia emerge radially from the central point of the carpus.U, ulna; u, ulnare; R, Radio; r, radiale; c, centrale; dc 1–5, distal carpalia 1–5; mc I–V, metacarpalia I-V.

angles of the digits). The carpalia and metacarpalia are disposed in a single plane, the radiale process and the pisiform being the only structures that advance to a more ventral plane.

Skilled manual abilities

Five of the lizard species examined perching on dowels were unable to close their manual digits around any of the perches: *Liolaemus cuyanus*, *L. catamarcensis* (Fig. 6A), *Liolaemus ramirezae*, *C. ocellifer and T. merianae*. By contrast, *P. acutirostris* (Fig. 6B), *A. stratulus* and *H. fasciata* (Fig. 6C) were able to curl their digits around the narrow perches (3 mm).

Mapping analysis indicated that only four characters showed convergent trends when their phylogenetic history was considered. The optimization of character 0 showed that the skilled forelimb movements have been independently acquired by Gekkota and the node of Dactyloidae + Polychrotidae (Fig. 7). In fact, there are two anatomical patterns, evolutionary isolated, that correspond with those lineages of enhanced abilities, as is observed by optimizing character 1 (Fig. 8). The elongated shape of the centrale (character 2) has been independently acquired three times by Gekkota, *Tropidurus melanopleurus* and Dactyloidae + Polychrotidae (Fig. 9). The character palmar sesamoid reduced or absent

appeared independently three times in the tree: Gekkota, Dactyloidae + Polychrotidae and Tropiduridae (Fig. 10). Finally, the optimization of character 3, separation between ulnare and radiale, shows that the ancestral state corresponds to the presence of a free space between ulnare and radiale (state 2), which is present in most of the taxa surveyed. The state character corresponding to both bones in full contact (state 0) appeared in some species of *Liolaemus*, *T. merianae*, *Cnemidophorus longicaudus*, *Elgaria multicarinata* and some sphaerodactylids. State 1 of this character, partial contact between both bones, appeared in *Kentropix viridistriga*, *Heloderma suspectum*, *L. catamarcensis* and *Phymaturus ceii*. Finally, state 3 appeared twice in the tree associated with a Gekkota and Dactyloidae (Fig. 11).

Discussion

According our data, the carpalia of squamatans is a skeletal complex highly conserved among different lineages. Most authors (Romer 1956; Fabrezi *et al.* 2007; Russell and Bauer 2008; Leal *et al.* 2010) consider that the lizard carpus is composed of nine bones. This number can increase to 10 because an additional small bone was reported in the hand of taxa such as *C. longicaudus*, *Heloderma suspectum*, *E. multicarinata*, *E-*
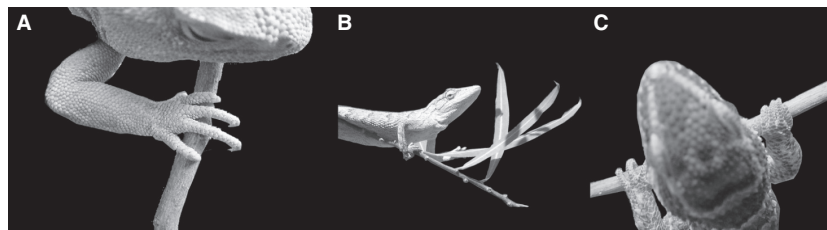

Figure 6—Selected images of: a: *Leiosaurus catamarcensis*; b: *Polychrus acutirostris*; c: *Homonota fasciata* contacting a narrow dowel. Note the rigidity of the hand in *L. catamarcensis* compared to the flexibility in *P. acutirostris* and *H. fasciata*.
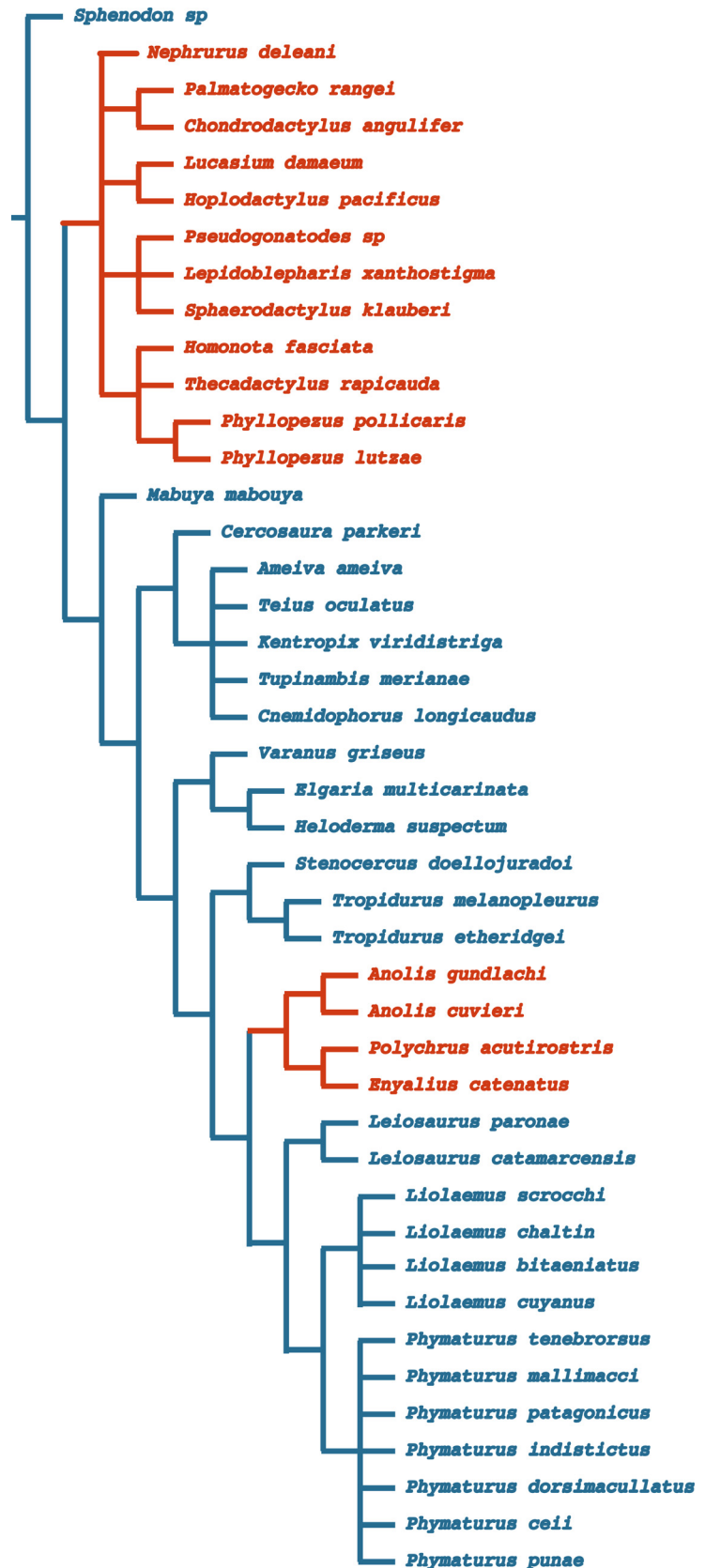
Figure 7—Ancestral character state reconstruction showing that the skilled forelimb movements have been acquired by Gekkota and the node Polychrotidae + Dactyloidae independently. Blue branches show the absence of skilled forelimb abilities as the ancestral state. Red branches show the presence of skilled forelimb movements as the derived state.
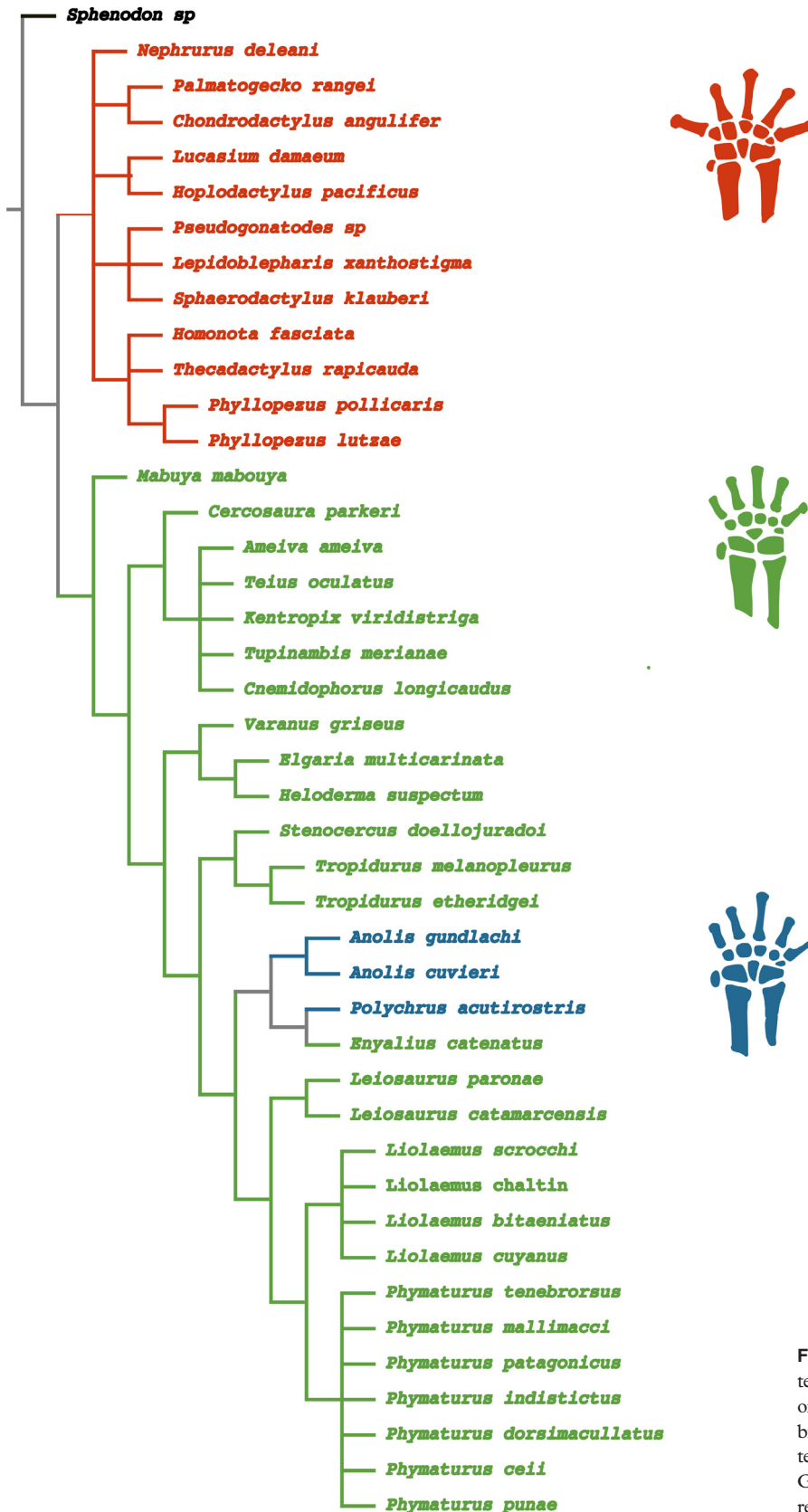
Figure 8—Reconstruction of ancestral character states, showing that each pattern appeared once in the tree. Polychrotid-like pattern (blue branches) nested within Liolaemid-like pattern (green branches). Red branches show Gecko-like pattern. The ancestral state is not resolved (gray branches)
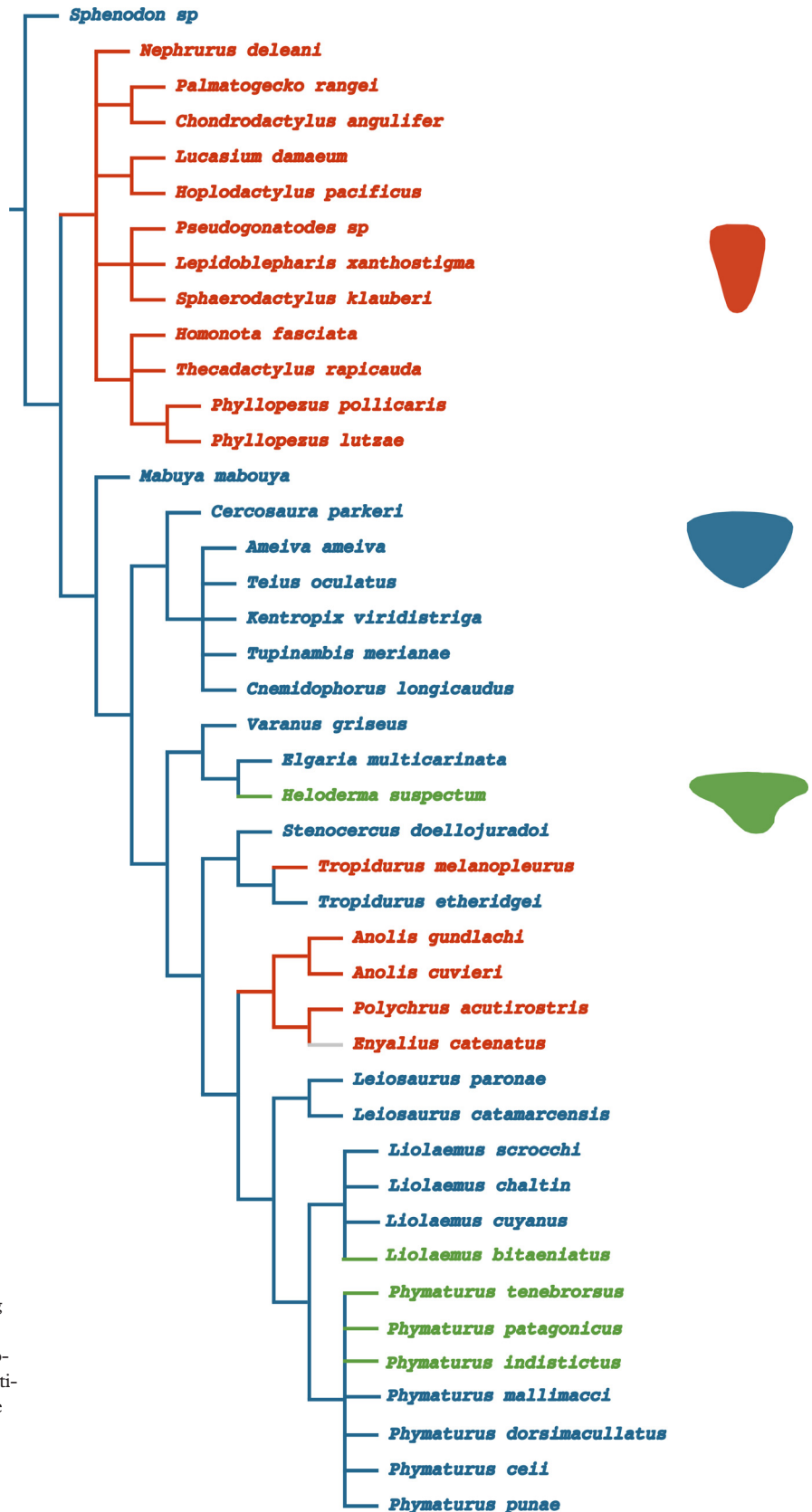
Figure 9—Ancestral reconstruction showing the elongated shape of the centrale (red branches) independently acquired by Gekkota, Tropidurus melanopleurus and Polychrotidae + Dactyloidae. Triangular centrale (blue branches) is the ancestral state. Saddle bike shaped centrale (green branches) appeared independently several times. Gray branches show ambiguous nodes.
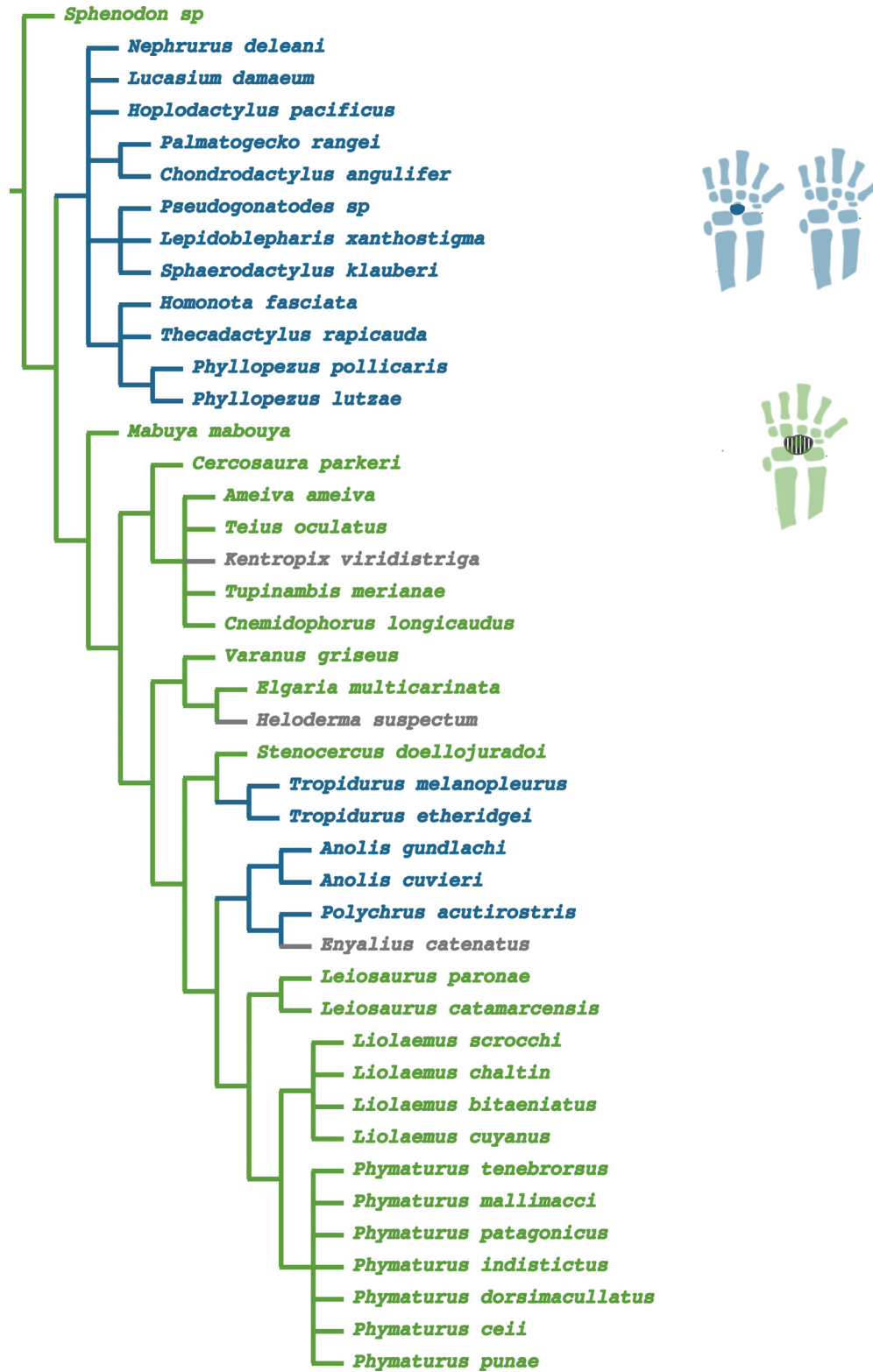
Figure 10—Ancestral character state reconstruction showing that a reduced or absent sesamoid character state appeared three times in the tree: Gekkota, Polychrotidae + Dactyloidea and Tropiduridae (blue branches). Green branches show the ancestral state (big sesamoid) widely distributed within the tree. Gray branches show ambiguous nodes.
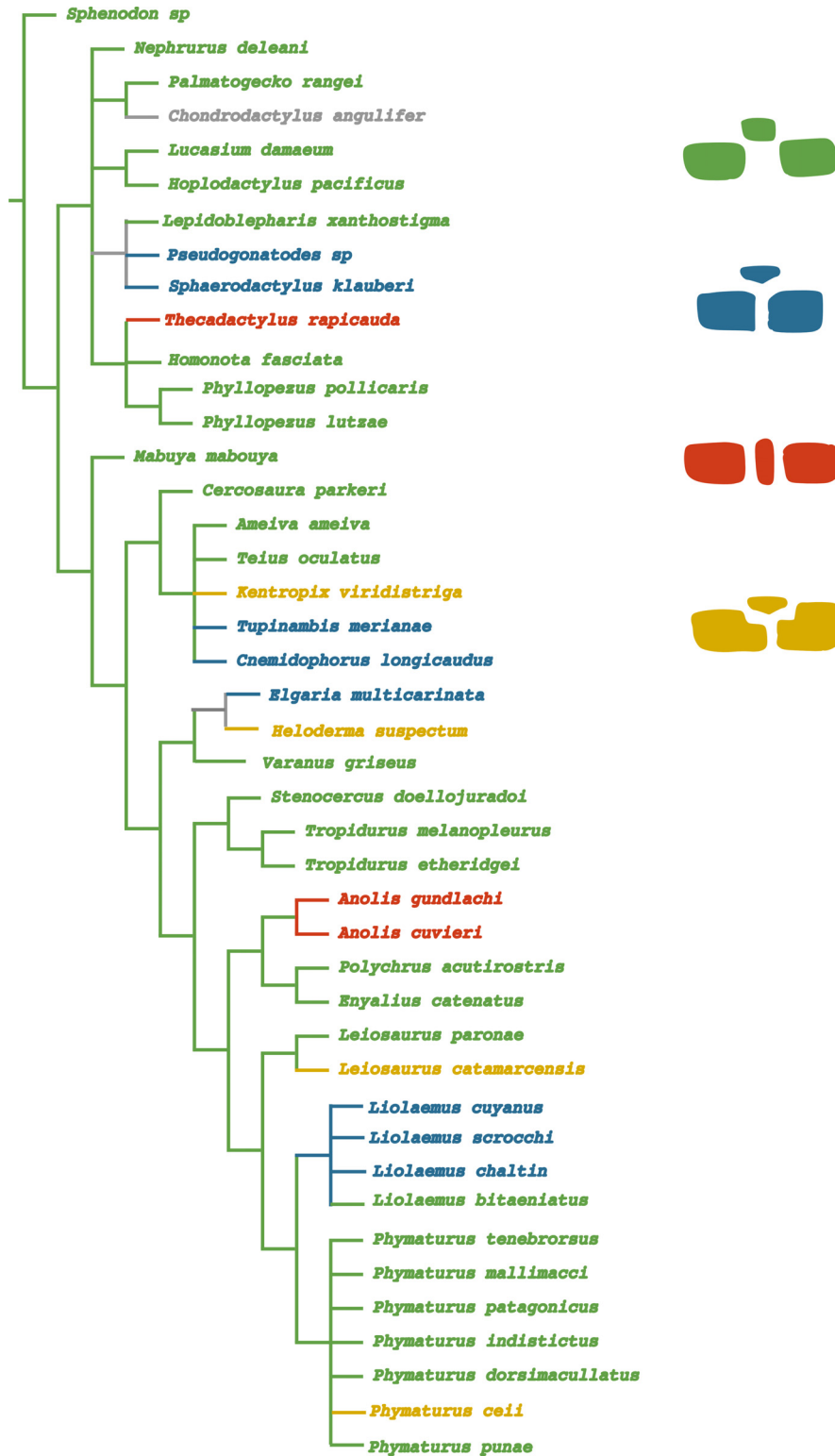
Figure 11—Reconstruction of ancestral character state showing that the ancestral state corresponds to the presence of a free space between radiale and ulnare (green branches). It is also the most widely distributed state. The 'ulnare-radiale whole united' state was acquired five times in the tree (blue branches). The 'ulnare-radiale partially united' state was acquired four times in the tree. The 'ulnare-radiale whole separated, with the centrale occupying the space between them' was acquired twice in the tree.

umeces, *Agama*, *Xenosaurus* and *Carsosaurus* (El-Toubi 1947; Stokely 1950; Romer 1956; Renous-Lécuru 1973; Caldwell *et al.* 1995; Fabrezi *et al.* 2007; this work). Renous-Lécuru (1973) identified that structure as a reduced intermedium. The intermedium would have been present in ancient tetrapods and it is still present in several groups, such as tuataras, turtles and mammals (Romer 1956; Flower 1885; Renous-Lécuru 1973). However, the evidence confirming the presence of an intermedium in lizards is rather weak (Fabrezi *et al.* 2007; Leal *et al.* 2010). Jerez *et al.* (2009) also reported the presence of a bone with a similar shape and topology in three species of Gekkonidae and one species of Gymnophthalmidae; however, those authors considered it as a sesamoid bone rather than an intermedium. Within the *C. longicaudus* specimens analysed (see also Otero and Hoyos 2013), the additional bone was occasionally present. Given that the eventual absence is characteristic of the sesamoid bones, the structure concerned is likely to be a sesamoid. Complete embryonic studies of the species possessing this bone are of fundamental importance for its identification. Renous-Lécuru (1973) reported the presence of one structure of the radiale, which clearly corresponds to the radiale process described in this study. According to that author, the radiale process and the pisiform delimit the carpal tunnel; hence, those structures are the insertion points of the flexor retinaculum (Haines 1950). The radiale process and the pisiform, located specularly symmetric with respect to each other, form the narrow channel at the base of the hand, through which tendons, ligaments and nerves run. In mammals, this tunnel exhibits a proximal region composed of the pisiform, the intermedium, ulnare and radiale, and a distal region composed of distal carpalia 1, 2, 3, and distal carpalia 4 and 5 fused (hamate). Even though in lizards the radiale process and the pisiform form also a channel that we homologate with the carpal tunnel, this structure is restricted to the proximal region of the carpus, the distal one being a plane region. Interestingly, *P. acutirostris* and *Anolis cuvieri*, two of the grasping species studied, show a subtle concavity in the region of the distal carpalia, suggesting an emerging differentiation that could function as a less complex carpal tunnel than in mammals. In both cases, the tunnel protects the pathways of communication between zeugopodium and autopodium and unites them in one package.

Some taxa present a morphological correspondence between the pre-axial edge of the palmar sesamoid and the postaxial edge of the radiale process, and both structures fit each other (e.g., *Teius oculatus*, *Ameiva ameiva* and *C. longicaudus*). In those taxa with a reduced or absent palmar sesamoid, the radiale process is a reduced flange that does not extend ventrally. The maximum reduction in the radiale process occurs in *P. acutirostris* and *Phyllopezus pollicaris*, both taxa with reduced or absent palmar sesamoid.

The skilled manual abilities are consistently associated with a pre-axial differentiation of the hand across all the tetrapod clade. *Phyllomedusa*, one of the most striking grasper among anurans, shows an opposable digit 2 (the most pre-

axial as the digit 1 is absent), which helps to execute grasping movements (Sheil and Alamillo 2005; Manzano *et al.* 2008). Among mammals, the arboreal shrew, *Tupaia minor*, the giant panda (*Ailuropoda melanoleuca*) and the red panda (*Ailurus fulgens*) can also flex the palm of the hand and grasp objects through a mechanism that involves a divergent digit 1 in the shrew (Sargis 2001) and a radiale sesamoid (false thumb) in the panda (Flower 1885; Gould 1980; Endo *et al.* 2001; Salesa *et al.* 2006). Specialization reaches its peak in primates, where many species also develop an opposable thumb and the ability to individualize the fingers, such as macaques, baboons, great apes and human (i.e., Pouydebat *et al.* 2008, 2009, 2011; Crast *et al.* 2009). Hence, specialized pre-axial structures do not always imply homologous structures, suggesting that they arose from independent and convergent evolutionary events. Accordingly, a common feature among tetrapods is the independent morphological variation of digit 1 in relation to the other digits, which also manifested in other evolutionary phenomena such as digit reduction. Indeed, digit 1 is the most frequently absent among tetrapods (Wagner and Vargas 2008). An analysis from a purely spatial perspective shows that the digits of the pre-axial zones of each hand are closer to each other and to the medial axis of the body than those of the postaxial regions. Moreover, the development of the carpal pieces also shows differences between both the pre-axial and postaxial regions. The temporal sequence of genesis of the skeletal elements of the carpus implies an early differentiation of the primary axis of the hand derived from condensation, bifurcation and a proximodistal branching of the ulna. The differentiation of the primary axis brings about the emergence of the fourth digit, from which the digital arch arises. Later, the radiale arises from the distal extreme of the radius (Shubin and Alberch 1986; Fabrezi *et al.* 2007; Wagner and Vargas 2008; Leal *et al.* 2010). This delay and independence of the pre-axial compared with the postaxial differentiation might imply a developmental lability of the pre-axial region that does not compromise the later development of the other pieces. Indeed, de Bakker *et al.* (2013) suggest that developmental constraints are weaker for digit 1 than for other digits, making it easier to modify its developmental pathways.

The relative sizes of the carpal elements are rather constant, except the palmar sesamoid, which can be big – of the same size as the ulnare, small – smaller than the ulnare, and absent. Interestingly, the classification of species according to the sesamoid size is consistent with the carpal anatomical patterns described above and the skilled forelimb movements. The skilled manual function is correlated with the absence (Gecko-like pattern) or reduction in the palmar sesamoid (Polychrotid-like pattern). A developed palmar sesamoid implies a kinetic impairment to the flexion of the palm of the hand, whereas its absence or reduction allows lizards to perform skilled hand movements (Abdala *et al.* 2009). The correlation between carpal anatomical pattern, palmar sesamoid and skilled manual abilities adds a new dimension to the results in Abdala *et al.* (2009), who reported a link between

the manual tendinous pattern and grasping in lizards. Hence, all these structures, carpal bones, sesamoids and tendons would be strongly interconnected parts composing a unit or module (Klingenberg *et al.*, 2004). These structural components of a module vary in a coordinated manner so that changes in one of them provoke changes in the others (Klingenberg *et al.*, 2001). This aspect of modular evolution could explain the consistent patterns obtained by the optimization of the different characters analysed.

Character mapping

Our data for squamatans show not only that the enhanced manual abilities occur in independent lineages, but also that the anatomical bases allowing these abilities are clearly different (e.g., Gecko-like and Polychrotid-like patterns). The fact that *Sphenodon* presents a big palmar sesamoid (pers. comm. Peter Johnston) suggests that it lacks skilled manual abilities; hence, skilled manual abilities would be a novelty that appeared twice in the context of the squamatan evolution. Our data show that even when the function could have been present very early in the evolutionary history of tetrapods, it may be achieved in different forms.

Character mapping of 'separation between ulnare-radiale' showed that the ancestral state corresponds to a free space between both bones (state 2). Interestingly, the ancestral state in squamatans resembles the condition of extant *Sphenodon*, which presents the intermedium filling the space between radiale and ulnare (Renous-Lécuru 1973). According to the transformation series, the intermedium loss in Squamata could have preceded the proximal displacement of the centrale (state 3) within some lineages and the contact between the radiale and ulnare within other lineages (Fig. 8). It is also interesting that patterns allowing skilled manual abilities, such as grasping (Gecko-like and Polychrotid-like patterns), are characterized by an elongated centrale that separates ulnare and radiale from each other, suggesting some possible mechanic relevance of the location and shape of this bone.

Conclusions

1. Three different anatomical patterns can be distinguished in the manual skeletal framework: Liolaemid-like, Polychrotid-like and Gekko-like patterns. Most of the lizard species present the first pattern. Skilled manual abilities are associated with Polychrotid-like and Gecko-like patterns.
2. The palmar sesamoid can be present, reduced or absent. Both reduced and absent conditions are associated with the skilled forelimb movements.
3. The lizard carpus presents a tunnel that resembles the carpal tunnel of humans, but of more simple formation.
4. arpal bones, sesamoids and tendons are strongly interconnected parts forming a unit or module that varies in a

coordinated manner and explains the consistent patterns obtained by optimizing the different characters analysed.

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Appendix 1

List of specimens included in the study:

Institutional Abbreviations

FBC, Félix Benjamín Cruz, field series; FML, Fundación Miguel Lillo (Tucumán, Argentina); GS, Gustavo Scrocchi; specimens previously donated to FML by the Museu de Zoologia from University of São Paulo (MZUSP) at São Paulo, Brazil; MCN, Museo de Ciencias Naturales-Universidad Nacional de Salta; PT: Universidad Puerto Rico; RT, Richard Thomas, field series; SDSU, San Diego State University (California, USA); UNNEC, Universidad Nacional del Nordeste (Corrientes, Argentina) and USNM, Smithsonian National Museum of Natural History, Washington, USA.

The list of all the specimens examined for this study is given below. For each species, we provide the Linnean binomial, the total number of specimens examined, and the institution where specimens were deposited (together with their unique identifier – voucher).

Gekkonidae: *Chondrodactylus angulifer*, N = 1, *Palmatogecko rangei*, N = 1 W/D; Sphaerodactylidae: *Sphaerodactylus klauberi*, N = 1, W/D; *Pseudogonatodes guianensis*, N = 1, USNM 32060; *Lepidoblepharis xanthostigma*, N = 1 W/D; Diplodactylidae: *Hoplodactylus pacificus*, N = 1 W/D; *Lucasium damaeum*, N = 1 W/D; Carphodactylidae: *Nephrurus deleani*, N = 1 W/D; Phyllodactylidae: *Homonota fasciata*, N = 1 W/D; *Phyllopezus lutzae*, N = 1, FML 23462; *Phyllopezus pollicaris*, N = 1, FML 02913; *Thecadactylus rapicauda*, N = 1, FML 23471; Scincidae: *Mabuya mabouya*, N = 2; Gymnophthalmidae: *Cercosaura parkeri*, N = 5, FML (00731, 02411, 01980, 01983, 1983-2); Teiidae: *Ameiva ameiva*, N = 2, FML 03637; *Cnemidophorus longicaudus*, N = 5, FML 02761, W/D; *Kentropix viridistriga*, N = 1, 1204; *Teius oculatus*, N = 1, FML 03625; *T. merianae*, N = 2; Tropiduridae:

T. etheridgei, N = 2, FML 01985, FBC 301; *T. melanopleurus*, N = 3, FML (02054, 02055, 02056); *Stenocercus doellojuradoi*, N = 2, FML (03521, 00503); Dactyloidea: *Anolis cuvieri*, N = 2, SDSU 2183, PT 005694; *Anolis gundlachi*, N = 2, RT (1-4478, 1-4479); Polychrotidae: *Polychrus acutirostris*, N = 4, UNNEC (08610-08611, W/D); *Enyalius catenatus*, N = 1, MZUSP 66153; Liolaemidae: *Phymaturus ceii*, N = 1, GS, 3136; *P. dorsimaculatus*, N = 4, MCN (921, 1488, 1487, 923); *P. indistictus*, N = 3, MCN (686, 1481, 1482); *P. mall-limacci*, N = 3, MCN (1484, 1483, 920); *P. patagonicus*, N = 2, MCN (908, 909); *P. punae*, N = 1, FBC 395; *P. tenebrosus*, N = 3, MCN (1490, 1491, 1492); *Liolaemus bitaeniatus*, N = 2 (FML: 1333, 2178); *L. chaltin*, N = 1, FML 1461; *Liolaemus cuyanus*, N = 3, FML 1803; *Liolaemus ramirezae*, N = 1; *L. scrocchi*, N = 1, FML 1757-1; Leiosauridae: *Leiosaurus catamarcensis*, N = 1, FML 00670-2; *L. paronae*, N = 1, FML 00035.