

Musculoskeletal Anatomical Changes That Accompany Limb Reduction in Lizards

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ABSTRACT Muscles, bones, and tendons in the adult tetrapod limb are intimately integrated, both spatially and functionally. However, muscle and bone evolution do not always occur hand in hand. We asked, how does the loss of limb bones affect limb muscle anatomy, and do these effects vary among different lineages? To answer these questions, we compared limb muscular and skeletal anatomy among gymnophthalmid lizards, which exhibit a remarkable variation in limb morphology and different grades of digit and limb reduction. We mapped the characters onto a phylogeny of the group to assess the likelihood that they were acquired independently. Our results reveal patterns of reduction of muscle and bone elements that did not always coincide and examples of both, convergent and lineage-specific non-pentadactyl musculoskeletal morphologies. Among lineages in which non-pentadactyl evolved independently, the degree of convergence seems to depend on the number of digits still present. Most tetradactyl and tridactyl limbs exhibited profound differences in pattern and degree of muscle loss/reduction, and recognizable morphological convergence occurred only in extremely reduced morphologies (e.g., spike-like appendix). We also found examples of muscles that persisted although the bones to which they plesiomorphically attach had been lost, and examples of muscles that had been lost although their normal bony attachments persisted. Our results demonstrate that muscle anatomy in reduced limbs cannot be predicted from bone anatomy alone, meaning that filling the gap between osteological and myological data is an important step toward understanding this recurrent phenomenon in the evolution of tetrapods. *J. Morphol.* 000:000–000, 2015. © 2015 Wiley Periodicals, Inc.

KEY WORDS: autopodium; digit loss; muscles; bones; Gymnophthalmidae; Tetrapoda

INTRODUCTION

Limb reduction in tetrapods is a common mechanism of limb evolution and has been extensively studied (Greer, 1987, 1990; Greer and Wadsworth, 2003; Shapiro et al., 2003, 2007; Sears et al., 2011; Lee et al., 2013), especially in relation to bone structures and body form (Wiens and Slingluff, 2001; Shapiro et al., 2007; Brandley et al., 2008;

Lee et al., 2013). However, with the exception of a few classical studies published more than a century ago (e.g., Fürbringer, 1870), changes to soft tissues such as muscles have never been addressed in the context of limb reduction. Considering the many examples of muscles that have changed their attachments over evolutionary time (Diogo and Abdala, 2010), we asked how the loss of metacarpals/metatarsals and phalanges affects limb muscle anatomy and whether these effects vary among lineages. Do muscles persist although the bones with which they are usually associated have been lost? Might similar but independently acquired osteological morphologies exhibit different muscle morphologies?

The main goal of this article is to describe the morphological changes in muscles that accompany various degrees of limb reduction in different gymnophthalmid clades. Gymnophthalmid lizards represent an ideal system for studies of limb evolution because they display remarkable diversity in limb and foot morphology and different grades of limb reduction involving the loss of

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several different autopodial elements (Presch, 1975; Pellegrino et al., 2001; Kohlsdorf and Wagner, 2006; Kohlsdorf et al., 2010). Previous studies focusing on osteology or external morphology suggest that limb reduction, which is usually associated with body elongation (see Skinner and Lee, 2009; Grizante et al., 2012) and involves digit loss (see Kohlsdorf et al., 2010), has evolved independently at least six times within Gymnophthalmidae (Rodrigues, 1991; Kizirian and McDiarmid, 1998; Pellegrino et al., 2001). These processes appear to follow two major patterns (Pellegrino et al., 2001): reduction predominantly of forelimbs (Gymnophthalmini), or reduction predominantly of hindlimbs (Cercosaurini). We compared muscle and bone anatomy between lizard species with reduced, non-pentadactyl limbs and optimized the patterns obtained in the cladogram of Pellegrino et al. (2001). We selected this phylogeny because it is based on molecular characters, thus preventing any possibility of non-independence of the characters in the phylogenetic mapping performed.

Limb reduction is a very frequent process recorded not only in Gymnophthalmidae but also in skinks (Greer, 1987, 1990; Shapiro et al., 2003; Lee et al., 2013), pygopodids (Stephenson, 1961), anguids (Greer, 1991; Wiens and Slingluff, 2001), and many other tetrapod taxa such as mammals (Lande, 1978). Thus, the results of this study will contribute to our understanding of the evolutionary processes that led to the origin of non-pentadactyly in gymnophthalmids and other tetrapods, as well as of the macroevolutionary patterns of interactions between muscles and bones.

MATERIALS AND METHODS

Information about limb anatomy was obtained from specimens belonging to 13 gymnophthalmid species from the three major clades (Pellegrino et al., 2001), and two teiid species that served as an outgroup (Fig. 1). While clade 1 (here mainly represented by tribe Gymnophthalmini) exhibits remarkable reduction of forelimbs, limb reduction in clade 3 (Cercosaurini) is more pronounced in the hindlimbs (Rodrigues, 1991; Kizirian and McDiarmid, 1998; Pellegrino et al., 2001; Kohlsdorf and Wagner, 2006). Two pentadactyl teiid species served as outgroups to reconstruct the plesiomorphic gymnophthalmid muscle pattern, and one monodactyl anguid species (*Ophiodes intermedius*) provided a comparative model of extreme limb reduction (spike-like appendages) from a different lizard family. Two scincid species, one with five digits (*Riopa* sp.) and one with four digits in the forelimbs and hindlimbs (*Leptosiaphos* sp.) were also included for comparison. A total of 67 gymnophthalmid specimens were included (see Appendix A for a complete list of specimens), following two main criteria: 1) maximization of the morphological range sampled (i.e., lineages presenting from five to zero digits), and 2) broad phylogenetic assemblage (i.e., species from different clades of the family). In our sample, clade 1 is represented by *Acratosaura mentalis* + Gymnophthalmini, clade 2 (Ecleopini) is represented by the pentadactyl species *Anotosaura vanzolinia*, and clade 3 (Cercosaurini) is represented by *Cercosaura parkeri* and three *Bachia* species.

We compared two datasets: 1) an osteological dataset obtained from X-rays and 2) a myological dataset assembled

from dissections of fixed specimens. Images of ossified long bones in the forelimb and hindlimb autopodia of all gymnophthalmid species sampled were obtained with a Faxitron Radiography System (LX-60), Lincolnshire, IL. Specimens were X-rayed with a 6× magnification, and the digital images were visually inspected to identify bones and infer the phalangeal formulae (Fig. 1). Exemplary X-ray images are accessible in the Supporting Information (SI1–SI2; URL:XXX).

Information on muscle anatomy was obtained from dissections of the specimens. Images of the dissected muscles were acquired using a Leica M16 stereomicroscope. Digital images at different strata (i.e., superficial and deep muscle layers) were acquired using a connected Leica DC 500 camera, Cambridge, London, and were assembled using the Auto-Montage Pro v5.02.0096 software. The images are accessible in the Supporting Information (SI13–SI30; URL:XXX). The nomenclature for forelimb muscles follows Abdala and Diogo (2010), Diogo and Abdala (2010), and Diogo and Tanaka (2012), while that for the hindlimb muscles follows Russell (1975, 1993), Hoyos (1990), Herrel et al. (2008) and Russell and Bauer (2008). Muscle anatomy data are placed in two major categories: a comparison of the reduction patterns among taxa including summary tables (Tables 1–5), and more detailed anatomical descriptions of each limb studied (Appendix B). The descriptions were placed in the appendix for the sake of fluency. Appendix B includes more specific anatomical descriptions of each type of limb morphology analyzed (pentadactyl, tetradactyl, tridactyl, didactyl, and monodactyl limbs as well as limbs lacking an autopodium). Following Shapiro et al. (2007), we assumed that digit reduction (e.g., loss of phalanges in a digit) is different from digit loss (loss of all phalanges and their supporting metacarpal or metatarsal).

To allow comparison between forelimbs and hindlimb, we defined patterns of muscle and bone reduction in terms of the numbers of muscles or bones in each limb segment (arm/thigh—stylopodium, forearm/crus—zeugopodium, manus/pes—autopodium) rather than the anatomy of particular muscles or bones. The anatomical data obtained were included in a 16 taxa × 20 characters dataset (Supporting Information Appendix C; URL:XXX). We then mapped these characters onto the selected phylogeny (Pellegrino et al., 2001) using the criterion of maximum parsimony in the computer program “Tree analysis using New Technology” (TNT), which is a program for phylogenetic analysis under parsimony with very fast tree-searching algorithms (Goloboff, 1999; Nixon, 1999; Goloboff et al., 2003). We chose this phylogeny because it was the first hypothesis using molecular data that included all the species in our dataset. The relationships among these species are congruent among all the phylogenetic studies available for this group (Casteo et al., 2004). With our morphological characters as the input data file, TNT produced lists of character changes and their fit to the selected phylogeny, and displayed those results in tree diagrams (with the menu options Optimize Characters, or with the command map; Goloboff et al., 2008). This character mapping allowed formal evaluations that the patterns detected using comparative anatomy represent independent acquisitions at the nodes in which they appear.

RESULTS

Comparisons Between Plesiomorphic Pentadactyl Autopodia of Teiidae and Reduced Autopodia of Gymnophthalmidae

Vanzosaura rubricauda and *Psilophthalmus pae-minosus* (Gymnophthalmini) exhibit a disparity between muscle and bone loss in the tetradactyl forelimbs. Both species lack digit I, yet some of the associated muscles such as the contrahentes digitorum, which usually insert on other digits, are distinguishable in *V. rubricauda* but not in *P.*

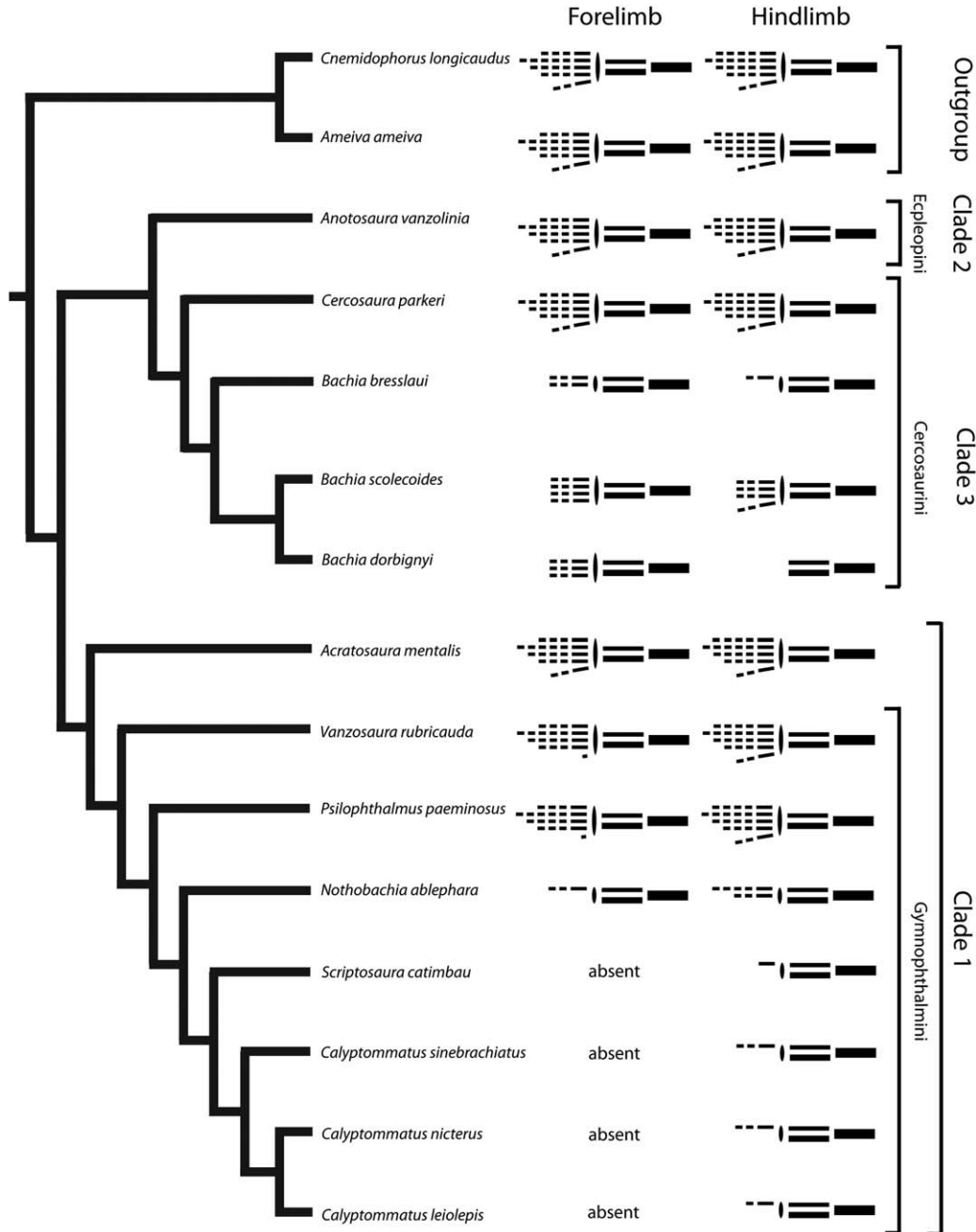


Fig. 1. Topology for Gymnophthalmidae with phalangeal formula of forelimb and hindlimb autopodia. In the interpretation of digital homology we follow Pellegrino et al. (2001).

paeminosus. Therefore, *P. paeminosus* retains for example, metacarpals of digit I to which the contrahentes attach in Teiidae, but the muscles themselves are not present. Hence, the bones have fewer associated muscles, and muscle loss seems more pronounced than bone loss. For comparison, data from the tridactyl forelimbs of *Bachia dorbignyi* (Cercosaurini) provide another example of different patterns of muscle versus bone reduction (Supporting Information S19–S22; URL: XXX). In this species, most of the intrinsic muscles

of the lost digits I and V are absent, but the mm. abductors, the most external ones, which in the plesiomorphic pattern insert on digits I and V, remain present and insert instead on digits II and IV (Figs. 2c and 4). Similarly, the mm. dorsometacarpales, which plesiomorphically attach to distal phalanges 2 (digit I), 3 (digit II), 4 (digit III), 5 (digit IV), and 3 (digit V), in the referred species attach to the persistent second phalanx (see Fig. 1 for phalangeal formulae of Cercosaurinae). Conversely, the intermetacarpales, which attach to all

TABLE 1. *Forelimb stylopodial and zeugopodial muscles*

	A. Teiidae	B. Gymnophthalmidae with five digits (clades 1 and 2)	C. Gymnophthalmidae with four digits (clades 1 and 3)	D. Gymnophthalmidae with three digits (clade 3)	E. Gymnophthalmidae with two digits (clade 3)	F. Gymnophthalmidae with one digit (clade 1)
Coracobrachialis	O I	Coracoid Humerus				3 dorsal and 2 ventral muscles
Biceps brachii	O I	2 branches Coracoid Humerus, ulna via brachialis fascia	2 branches	2 branches, flattened	Humerus	2 branches
Triceps	O I	Humerus Proximal ulna				
Brachialis anticus	O I	Very bulky Less bulky Deltopectoral crest	Not bulky	Flattened		
Extensor digitorum	O I	Biceps fascia Humerus Metacarpals II, III, IV Subtriangular, flattened				Persistent digits
Extensor antebrachii et carpi radialis	O I	Humerus Entire shaft of radius and base of metacarpal II Very robust, 2 branches				Shaft of radius 2 branches
Extensor antebrachii et carpi ulnaris	O I	Proximal humerus Distal ulna via aponeurosis, pisiform Subtriangular, flattened				Distal ulna Sub-triangular, flattened ?
Abductor pollicis longus	O I	Distal ulna Base of metacarpal I Not flattened	Radiales Reduced	Flattened		Absent ?
Extensores digitorum breves	O I	Ulnare Distal ends of metacarpals				
Palmaris longus	O I	Humerus Distal phalanges via connection with flexores breves superficiales				
Flexor carpi radialis	O I	Strong and narrow Distal humerus		Flattened		
Flexor carpi ulnaris	O I	Distal radius Long and slender Distal humerus		Flattened		
Pronator teres	O I	Blended with the extensor digitorum and distal ulna Bulky, 2 branches Distal humerus				3 dorsal and 2 ventral muscles
Flexor digitorum longus	O I	Distal radius Long and slender Humerus Distal phalanges via flexor plate			Flattened	

Table 1. (continued).

	A. Teiidae	B. Gymnophthalmidae with five digits (clades 1 and 2)	C. Gymnophthalmidae with four digits (clades 1 and 3)	D. Gymnophthalmidae with three digits (clade 3)	E. Gymnophthalmidae with two digits (clade 3)	F. Gymnophthalmidae with one digit (clade 1)
Epitrochleoconceus		3 branches to flexor plate, which gives rise to 5 tendons O Distal humerus I Proximal ulna	Same as A, but flattened	Same as A, but flattened	Same as A	?
Pronator accessorius		Small O Ulna, distal humerus I Radius Flattened; oblique fibers	Small, flattened	Small, flattened		
Pronator quadratus		O Radius I Ulna Broad	Flattened	Flattened		

Species in each category: A. *Cnemidophorus longicaudus*; B. *Anotosaura vanzolinia*, *Acratosaura mentalis*; C. *Psilophthalmus paeminosus*, *Vanzosaura rubricauda* (both species presenting digits II–V and metacarpal of digit I), *Bachia scolecoides* (digits II–V present); D. *Bachia dorbignyi* (digits II–IV present); E. *Bachia bresslaui* (digits II and III present); F. *Nothobachia ablephara*. A question mark (?) represents missing data.

digits in the plesiomorphic pattern, were entirely absent (Fig. 4).

The extreme cases of limb reduction represented by monodactyl limbs suggest that severe losses of bones were accompanied by similarly severe reduction of muscle elements (Tables (1–4); Figs. 2–4). Such osteological losses did not lead, however, to complete lack of positional identity in the remaining bones: metatarsals can be distinguished from phalanges, and proximal, middle, and distal phalanges can also be easily identified (Figs. 1 and 4). Although the osteological elements retain their identity, the remaining muscles are so reduced that in most cases it is not possible to assign them a functional or positional identity (Figs. 2e, 3d–f; Supporting Information SI27, SI30; URL: XXX).

The anatomical patterns identified in the musculature of non-pentadactyl limbs of gymnophthalmids (Tables (1–5)) show that some limbs possessing the same number of digits differ in muscle anatomy. Myological differences are especially apparent between didactyl species (i.e., between the forelimb in Cercosaurini, Tables 1 and 3, and the hindlimb in Gymnophthalmi, Tables 2 and 4, although some of these differences may arise from inherent differences between the forelimb and hindlimb rather than differences between clades). There are, however, general trends observable in the limbs of both species: in didactyl forelimbs (e.g., *Bachia bresslaui*), the stylopodium and zeugopodium retain much of their plesiomorphic pattern (Table 1), but muscles of the autopodium are often absent (Supporting Information SI17, SI18; URL: XXX). In this two-fingered manus, the flexor digitorum longus and the extensor digitorum insert on the digits, while neither extensores digitorum breves nor intrinsic manus muscles are identifiable (Fig. 2d). The few muscle fibers present on the radial and ulnar surfaces of the two digits probably correspond, because of their topology, to the abductor pollicis brevis and abductor digiti minimi, which normally insert on digits I and V, respectively. In the hindlimb autopodia, however, myological reduction can already be detected in the stylopodium when three digits are absent (didactyl hindlimb, e.g., *Notobachia ablephara*; Fig. 3c; Supporting Information SI28, SI29; URL: XXX). The zeugopodial muscles are also reduced (Table 2). The two remaining digits are connected mainly to the muscles of the crus (hindlimb zeugopod) and the intrinsic muscles of the hindlimb autopodia are absent (except for a few fibers whose topology indicates that they are probably remnants of the flexores breves complex; Table 4).

The autopodium exhibits the most profound changes during digit loss: in extremely reduced morphologies, it loses its intrinsic muscles and retains only attachments for the muscles of the zeugopodium (Tables (1–4)). Because tetradactyl

TABLE 2. Hindlimb stylopodial and zeugopodial muscles

	A. Teiidae	B. Gymnophthalmidae with five digits (clades 1 and 2)	C. Gymnophthalmidae with four digits (clade 3)	D. Gymnophthalmidae with three digits	E. Gymnophthalmidae with two digits (clade 1)	F. Gymnophthalmidae with one digit or no digits (clades 1 and 3)
Iliofibularis	O	Ilium		N/A	3 dorsal and 2 ventral muscles	3 dorsal and 2 ventral muscles that originate from the pelvic girdle and insert tendinously onto the distal phalanx
	I	Proximal fibula				
		Parallel-fibered, slender				
Iliotibialis	O	Lateral surface of ilium				
	I	Cnemial crest of tibia				
		Very bulky and strong; part of quadriceps femoris				
Femorotibialis	O	Shaft of femur				
	I	Cnemial crest via tendon of iliobtibialis				
		Part of quadriceps femoris				
Ambiens	O	Pubis and proximal femur via 2 tendons				
	I	Cnemial crest of tibia				
		Part of quadriceps femoris				
Iliofemoralis	O	Ilium				
	I	Proximal femur				
		Nearly triangular				
Tibialis anticus	O	Tibia				
	I	Proximal end of metatarsal I				
		Parallel fibered, slight torsion around tibia				
Peroneus longus	O	Lateral epicondyle of femur				
	I	Tubercle of metatarsal V				
		Gracile and parallel-fibered				
Peroneus brevis	O	Entire shaft of fibula				
	I	Head of metatarsal V				
Extensor digitorum longus	O	Femur			Femur	
	I	Metacarpals II and III			Digits III and IV	
		Fusiform			Very flat	
		Joint capsule of knee			Joint capsule of knee	
Femorotibial gastrocnemius	O	On digit V, associated with flexor tibialis externus			Digits III and IV	
	I					
		Broad belly				
Femoral gastrocnemius	O	Femur				
	I	Plantar aponeurosis				
Flexor digitorum	O	Femur primarily; also fibula and astragalocalcaneum				
	I	Digits via aponeurosis				
		Not flat				
Pronator profundus	O	Shaft of fibula				
	I	Proximal end of metatarsal I via aponeurosis				
		Parallel-fibered				
Popliteus	O	Proximal tibia				
	I	Proximal fibula				
		Triangular				
Interosseus cruris	O	Distal tibia				
	I	Distal fibula				

Species in each category: A. *Cnemidophorus longicaudus*; B. *Anotosaura vanzolinia*, *Acratosaura mentalis*, *Psilophthalmus paeminosus*, *Vanzosaura rubricauda*; C. *Bachia scoloides* (digits II–V present); E. *Nothobachia ablephara* (digits III and IV present), *Autopodial hindlimb muscles were absent in analyzed Gymnophthalmidae species that present only a single digit (Calyptommatus leiolepisand, Scriptorosaura catimbau)* and those lacking digits in the hindlimb (*Bachia dorbignyi*). N/A no Gymnophthalmidae species with three digits in the hindlimb was dissected in this study.

TABLE 3. Forelimb autopodial muscles

	A. Teiidae	B. Gymnophthalmidae with five digits (clades 1 and 2)	C. Gymnophthalmidae with four digits (clades 1 and 3)	D. Gymnophthalmidae with three digits (clade 3)	E. Gymnophthalmidae with two digits (clade 3)	F. Gymnophthalmidae with one digit (clade 1)
Flexores breves superficiales	O I	Flexor retinaculum Lateral surface of distal phalanges			absent	absent
Lumbricales	O I	5 muscles Flexor plate Proximal phalanges of digits II, III, IV 3 muscles	4	3	?	
Abductor digiti minimi	O I	Pisiform Lateral surface of metacarpal V	Proximal phalanges	?	?	
Abductor pollicis brevis	O I	Radiale Distal end of metacarpal I	Distal end of most medial metacarpal present	?		
Contrahentes digitorum	O I	Metacarpal I Metacarpal V	Radiale Metacarpal V Flexor digitorum V transversus I and II sensu Abdala and Moro (2006)	?	absent	
Flexores profundi	O I	Carpals Each side of the five digits			Greatly reduced	
Intermetacarpales	O I	Lateral aspects of metacarpals Distal ends of adjacent metacarpal on the lateral side		absent	absent	
Dorsometacarpales	O I	Metacarpals Distal phalanges 5 bicipital muscles		3 bicipital muscles		

See Table 1 for species and abbreviations.

TABLE 4. Hindlimb autopodial muscles

	A. Teiidae	B. Gymnophthalmidae with five digits (clade 1)	C. Gymnophthalmidae with four digits (clade 3)	D. Gymnophthalmidae with three digits (clade 3)	E. Gymnophthalmidae with two digits (clade 1)	F. Gymnophthalmidae with one digit or no digits (clades 1 and 3)
Flexores digitores breves	O	Aponeurosis of the femoral gastrocnemius Metatarsophalangeal joints		N/A	absent	Absent/vestigial in <i>Scriptosaura catimbau</i>
Lumbricales	I	3 muscles Tendinous plate Proximal ends of proximal phalanges	4 muscles			
Extensores digitores breves	O	3 muscles	Number unknown			
Extensor hallucis	I	Metatarsals, Distal phalanges	Tibiofibulare		Tibofibulare Distal phalanges?	
Flexor hallucis	O	5 muscles	Number unknown		absent	
Abductor digiti quinti	O	Proximal fibula				
Contrahentes digitorum	I	Metatarsal I				
Interossei plantares	O	Fourth distal tarsal Proximal end of the first metatarsal				
	O	Astragalocalcaneum	absent			
	I	Proximal end of metatarsal V				
	O	Metatarsals	?			
	I	Proximal phalanges				
	O	Metatarsals I, II, III, IV				
	I	Metatarsals II, III, IV	3 muscles			

See Table 2 for species and abbreviations.

TABLE 5. General trends inferred from our data

FORELIMB	Limb morphology	Stylopodium	Zeugopodium	Autopodium	Clade(s) and species	Bone/muscle differences
	Tetradactyl	Plesiomorphic	Plesiomorphic	Bones of digit I completely absent Vestigial metacarpal of digit I. Muscles show a plesiomorphic pattern	Cercosaurini (<i>B. scolecooides</i>) Gymnophthalmini (<i>P. paeminosus</i> , <i>V. rubricauda</i>) Cercosaurini (<i>B. dorbignyi</i>)	Unknown
	Tridactyl	Plesiomorphic	Plesiomorphic	Bones and muscles of digits I and V absent. Intermetacarpals (muscles) reduced, but metacarpals (bones) present	Cercosaurini (<i>B. bresslaui</i>)	Muscle loss greater than bone loss (intermetacarpales)
	Didactyl	Plesiomorphic	Insertions more distally located than in the plesiomorphic pattern.	Only bones of digits IV and V are present, they are served mainly by the extrinsic muscles of the hand. Intrinsic muscles of the hand are absent	Cercosaurini (<i>B. bresslaui</i>)	Muscle loss greater than bone loss (intrinsic hand muscles)
	Monodactyl	Reduced to adductor and abductor bundles	Reduced to adductor and abductor bundles	Reduced to adductor and abductor bundles	Gymnophthalmini (<i>N. ablephara</i>)	?
HINDLIMB	Tetradactyl	Plesiomorphic	Plesiomorphic	Bones and muscles of digit 5 absent	Cercosaurini (<i>B. scolecooides</i>)	None
	Didactyl	Reduced to three dorsal and two ventral bundles.	Few recognizable muscles	Intrinsic muscles of the digits almost completely absent	Gymnophthalmini (<i>N. ablephara</i>)	Muscle loss greater than bone loss (intrinsic foot muscles)
	Monodactyl	Reduced to adductor and abductor bundles	Reduced to adductor and abductor bundles	Intrinsic muscles absent	Cercosaurini (<i>C. leirolepis</i> , <i>S. catimbau</i>)	Muscle loss greater than bone loss (intrinsic foot muscles)
	No digits	Reduced to adductor and abductor bundles	Reduced to adductor and abductor bundles	Autopodium absent	Gymnophthalmini (<i>B. dorbignyi</i>)	?

B. Results

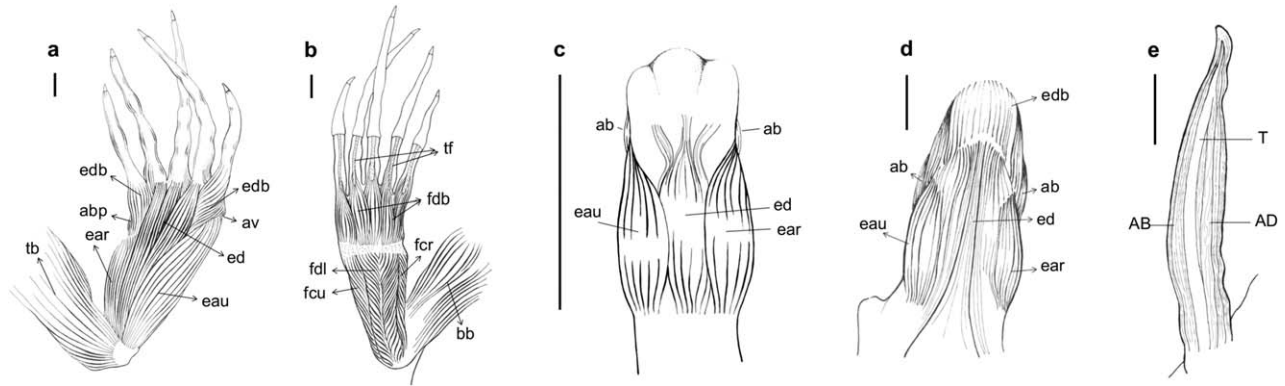


Fig. 2. Comparative myology of the forelimbs with different number of digits. (a) *Cnemidophorus longicaudus*, dorsal view of the stylopodium and ventral view of the zeugo- and autopodium of the plesiomorphic muscle pattern. (b) *Cnemidophorus longicaudus*, ventral view of the stylopodium and dorsal view of the zeugo- and autopodium of the plesiomorphic muscle pattern. (c) *Bachia dorbignyi*, dorsal view anatomical pattern of the tridactyl autopodium. (d) *Bachia bresslaui*, dorsal view note the zeugopodium with the extensor digitorum breves almost indistinguishable. (e) *Nothobachia ablephara*, lateral view of the complete spike-like appendix ab = abductors of the most preaxial and postaxial digits; AB = abductor complex; AD = adductor complex; aV = abductor digiti V; b = biceps brachii; ecr = extensor antebrachii et carpi radialis; ecu = extensor antebrachii et carpi ulnaris; edb = extensores digitorum breves complex; edl = extensor digitorum; fdbc = flexores digitorum breves complex; fdl = flexor digitorum longus; fcr = flexor carpi radialis; fcu = flexor carpi ulnaris; sec = stylopedial extensor complex; T = tendon; t = triceps brachii; tf = flexor tendons. Scale bar 6 mm.

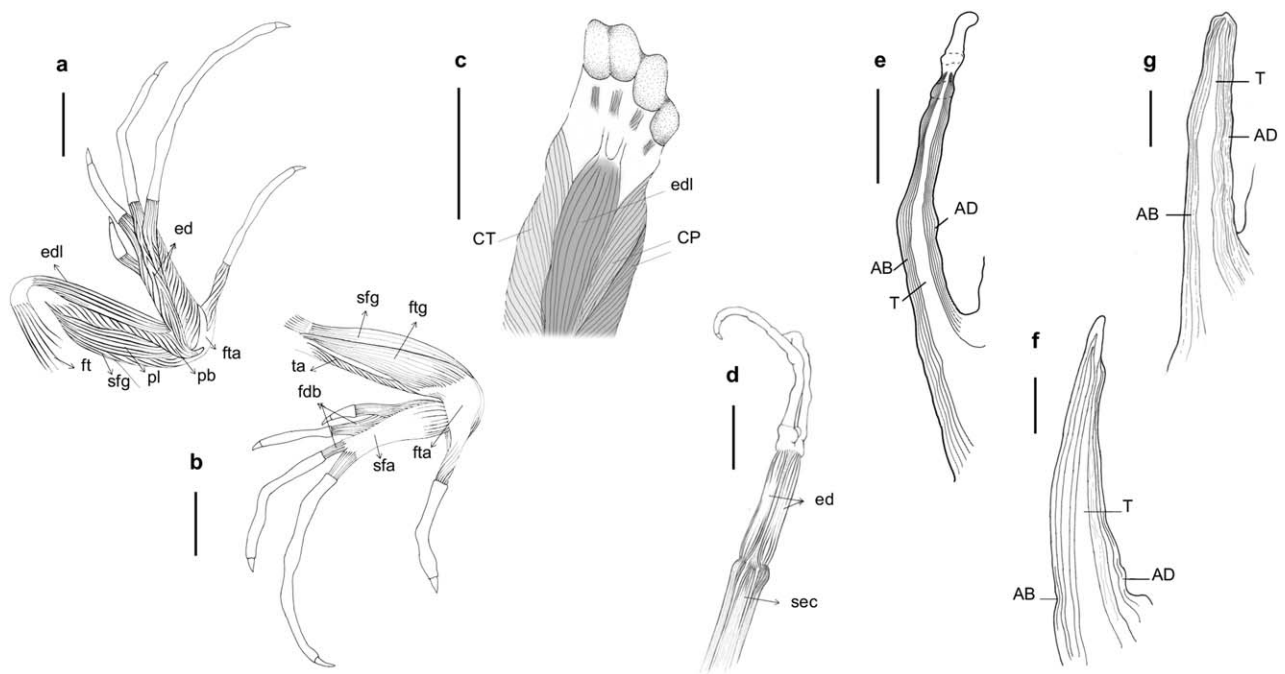


Fig. 3. Comparative myology of the hindlimbs with different number of digits. (a) *Cnemidophorus longicaudus*, lateral view of the stylo- and zeugopodium, and dorsal view of the autopodium of the plesiomorphic muscle pattern. (b) *Cnemidophorus longicaudus*, lateral view of the stylo- and zeugopodium and ventral view of the autopodium of the plesiomorphic muscle pattern. (c) *Bachia scolecoides*, dorsal view tetradactyl limb of. Because of the lack of the hooked fifth metatarsal, the hindlimb of *B. scolecoides* is more similar to the forelimb of *B. dorbignyi* (Fig. 2c) than to the hindlimb of the plesiomorphic *C. longicaudus* (a, b); (d) *Nothobachia ablephara*, dorsal view the stylopodial muscles are highly reduced; the two digits are connected to the extensor digitorum; (e) *Calyptommatius nicterus*, lateral view of the complete spike-like appendix; (f) *Scriptosaura catimbau*, lateral view of the complete spike-like appendix; (g) *Bachia bresslaui*, lateral view of the spike-like appendix in spite of the fact that these last species belong to different clades, their monodactylus forelimbs are characterized by a significant reduction of the limb muscles. The muscles in the stylopodium and zeugopodium are fused and lack identity, in the autopodium the intrinsic muscles are lost, the remaining digit being connected to muscular and tendinous fibers connected to the more proximal segment. AB = abductor complex; AD = adductor complex; ad 5 = adductor digiti quinti; CT = tibialis complex; CP = peroneus complex; edl = extensor digitorum longus; ed = extensores digitorum breves complex; fdb = flexores digitorum brevis complex; ft = femorotibialis; fta = femorotibialis aponeurosis; ftg = femorotibialis gastrocnemius; pb = peroneus brevis; pl = peroneus longus; sfa = superficial femoral aponeurosis; sfg = superficial femoral gastrocnemius; T = tendon; ta = tibialis anticus. Scale bar 6 mm.

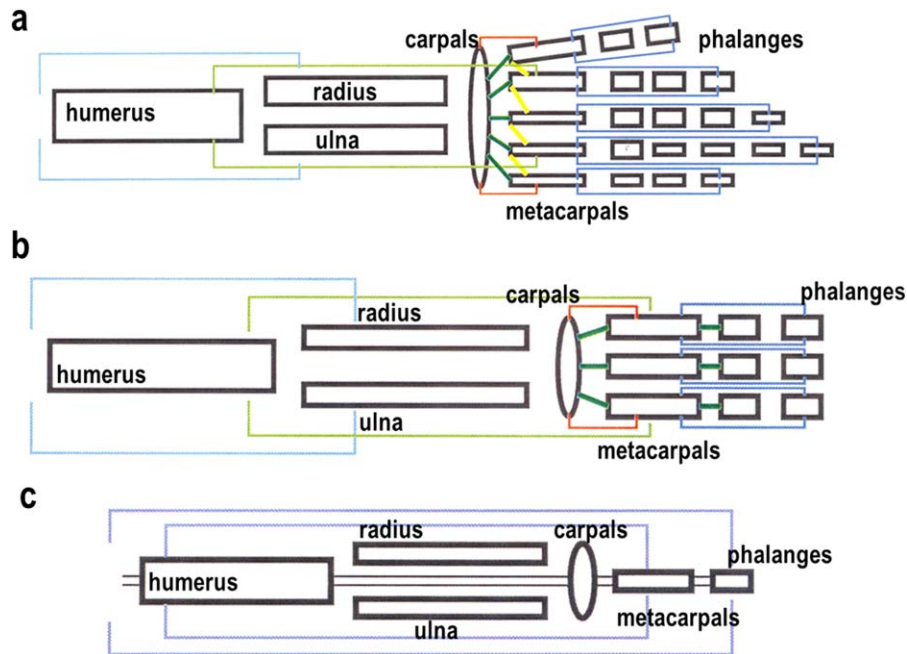


Fig. 4. General scheme of the major reduction events involving changes in extensor layers of the forearm muscles. (a) plesiomorphic pattern. (b) Tridactyl pattern. (c) Spike-like pattern. Color code: blue = dorsometacarpals; light blue = biceps; dark-green = contrahentes; light green = extensor digitorum; red = abductor pollicis and abductor digiti V; violet = reduced complex; yellow = intermetacarpals. Note the absence of the intermetacarpals (yellow) in the tridactyl pattern (b) and the insertion of the dorsometacarpals (blue) in the distal phalanges (2nd) of the autopodium.

forelimbs in the two lineages apparently evolved through different processes (primary reduction in e.g., *V. rubricauda* versus reversal of digit loss in *Bachia scolecoides*; see Kohlsdorf and Wagner, 2006), we could not determine whether the differences were caused by lineage-specific characteristics. Dissection of the species with a tridactyl manus (*B. dorbignyi*) revealed plesiomorphic patterns in the stylopodium and the zeugopodium (Table 1; Fig. 2c; Supporting Information SI06, SI19, SI20, SI21, SI22; URL: XXX): the extensores digitorum breves and flexores breves superficiales were present and identifiable, but the intermetacarpals could not be found (Table 3). Muscles that seemed to correspond to abductor pollicis brevis and abductor digiti minimi—which normally attach to digits I and V, respectively—inserted instead on digits II and IV (Table 3; Fig. 4).

Comparison of Muscle Anatomy Between Reduced Limbs of Gymnophthalmini and Cercosaurinae

The myological data obtained for species of Gymnophthalmini and Cercosaurinae suggest that the evolution of non-pentadactyl morphologies in these two lineages involved different patterns of muscle reduction. The dorsoventral flattening characteristic of all *Bachia* species (regardless of the number of digits that are present) is only observed in Gymnophthalmini in extremely reduced morphologies, such as the monodactyl

limbs of *Calyptommatus* species (Fig. 3e). Over the evolutionary course of progressive digit loss from pentadactyl to monodactyl limbs, major differences between Cercosaurinae and Gymnophthalmini become apparent at the tridactyl stage. Tridactyl limbs are only observed in Cercosaurinae, and didactyl species of the two lineages exhibit remarkable differences in muscle anatomy (see details above). The comparison between didactyl morphologies from different lineages is based on only one didactyl forelimb (*B. bresslaui*; Supporting Information SI01, SI02, SI03; URL: XXX) and one didactyl hindlimb (*N. ablephara*; Supporting Information SI28, SI29; URL: XXX), and thus involves the confounding effect of comparing forelimbs and hindlimbs (see Diogo et al., 2013).

The Particular Case of Spike-Like Reduced Limbs

The discrepancy in muscle anatomy between forelimbs and hindlimbs and different clades of gymnophthalmid lineages in which digit loss has occurred cannot be detected in extremely reduced morphologies. Monodactyl forelimbs (e.g., *N. ablephara*) are characterized by a significant reduction of the limb muscles: the muscles in the stylopodium and zeugopodium were fused and we could not identify individual muscles by visual inspection (Table 1); in the autopodium the intrinsic muscles are lost, and the remaining digit is connected only to muscular and tendinous fibers

originating from more proximal segments (Table 3). A similar pattern is observed in hindlimbs: when the entire hindlimb autopodium is absent (e.g., *B. dorbignyi*), the muscles of the stylopodium and zeugopodium are extensively modified and exhibit the same general configuration as those of limbs presenting only one digit (Table 2; Supporting Information SI23; URL: XXX).

The Particular Case of *Bachia scolecoides*

The comparison between tetradactyl species in our dataset must be considered separately because the evolutionary processes leading to the origin of tetradactyl limbs may have differed between Cercosaurinae and Gymnophthalmi. As mentioned previously, although both tetradactyl forelimbs lack digit 1, the tetradactyl forelimb of species such as *P. paeminosus* (which retains the first metacarpal) evolved from a pentadactyl ancestor, while the tetradactyl limb of *B. scolecoides* (Supporting Information SI24; URL: XXX) likely reflects a reversal of digit loss. Nevertheless, the persistent digits in both *B. scolecoides* and *P. paeminosus* consist of multiple phalanges (2 and 3, 4, 5, and 3 in each digit, respectively) and share similar muscle anatomy. Our data for the forelimbs indicate that, even when the number of bones present in the autopodia was established through different evolutionary processes, their associated muscles can exhibit similar anatomical patterns (Table 1 and 3; Appendix B).

In contrast to loss of the first digit in the forelimb, loss of the fifth digit in the hindlimb of *B. scolecoides* produced a major change in muscle anatomy in the autopodium: the muscles attaching to digit V are lost, and the remaining muscles lack individual identity. The tetradactyl hindlimb autopodium of this species grossly resembles a manus because the absence of digit V, with its hooked metatarsal that normally elevates the fifth digit above the others, produces a plate-like, flattened, autopodium (Fig. 3C; Supporting Information SI12, SI09, SI12, SI26; URL: XXX). While tetradactyl cercosaurines (e.g., *B. scolecoides*) exhibit a striking similarity in muscle anatomy between forelimbs and hindlimbs, characterized by lack of identity in digit muscles, the tetradactyl manus of Gymnophthalmi retains the plesiomorphic pattern of muscle anatomy (Fig. 5).

CHARACTER MAPPING

The phylogenetic character mapping allowed us to detect evolutionary patterns, such as the independent acquisition of similar patterns of muscle anatomy in didactyl limbs in both Gymnophthalmi and Cercosaurini. Character mapping of forelimb reduction showed independent reductions in Anguidae (*Ophiodes*) and Gymnophthalmidae (Table 6; Fig. 5 character 0: in red). Character

mapping of muscle patterns of the autopodium suggests that the configuration in which vestigial muscles are attached to the first metacarpal was independently acquired in scincid (*Leptosiaphos* and *Riopa*) and gymnophthalmid taxa (Table 6; Fig. 5 character 7: in green), and that the patterns present in the three *Bachia* species are autapomorphies of each taxon (Table 6; Fig. 5 character 7: in yellow, violet, and blue). The configuration in which muscles in the stylopodium of the hindlimb are reduced to adductor and abductor bundles seems to have been independently acquired in the evolutionary history of *Ophiodes* and in the common ancestor of *Scriptosaura* and *Calyptommatus* (Table 6; Fig. 5 character 11: in green). The configuration in which muscles are reduced to three dorsal and two ventral bundles also seems to have been independently acquired in *B. bresslaui* and *N. ablephara* (Table 6; Fig. 5 character 11: in blue). The pattern in which intrinsic muscles of the autopodium are highly reduced was likely acquired independently in *B. scolecoides* and *N. ablephara*, while the configuration in which muscles are reduced to abductor and adductor bundles probably evolved independently in *Ophiodes*, *B. bresslaui* + *B. dorbignyi*, and *Scriptosaura catimbau* + *Calyptommatus leiolepis* (Table 6; Fig. 5 character 17: in blue and green, respectively). Bones of digits seem to have been lost mostly through independent processes (Table 6; Fig. 5 character 18: in yellow). Differing patterns of muscle and bone reduction were evident in several lineages, including *Ophiodes*, *Nothobachia*, *Scriptosaura*, and *Calyptommatus*.

DISCUSSION

Muscles and bones exhibit clear spatial associations and form intimate connections within tetrapod limbs. Here, we asked how closely muscle reduction parallels bone reduction in non-pentadactyl limbs, and whether musculoskeletal morphology of reduced limbs differs between lineages in which it has evolved independently. Our approach to this question was a novel comparison of adult morphologies that deviate from the pentadactyl norm and phylogenetic optimization of the resultant patterns. In our sample, we observed differences between the pattern and degree of muscle versus bone reduction and examples of both convergent and lineage-specific non-pentadactyl musculoskeletal morphologies. In several of the non-pentadactyl gymnophthalmid specimens examined, loss or reduction of limb bones was not accompanied by corresponding loss or reduction of the muscles that plesiomorphically attach to them.

Extremely reduced (spike-like) autopodia are very similar between Cercosaurini and Gymnophthalmi, while the morphologies of didactyl forms tend to differ among clades. Thus, the degree of

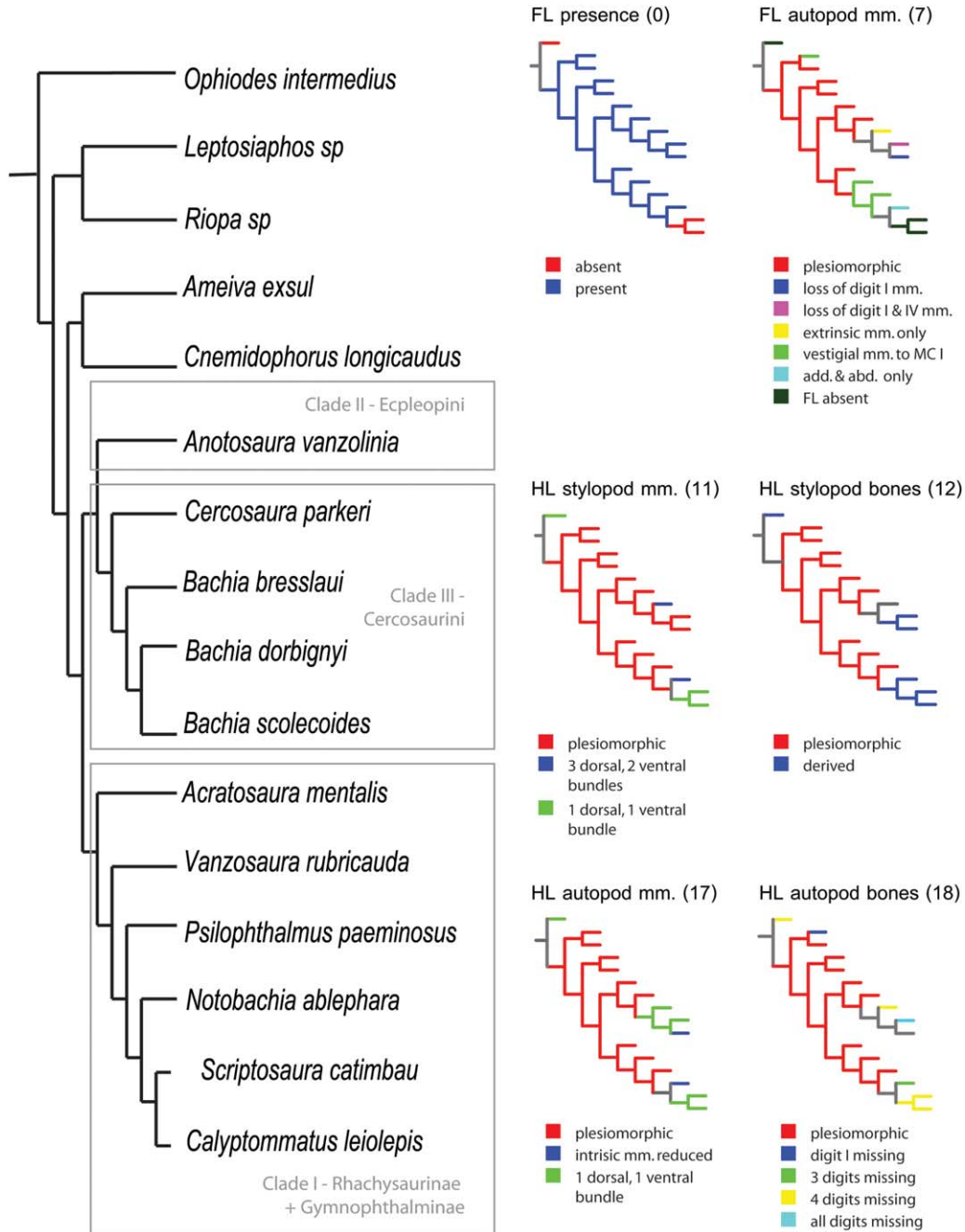


Fig. 5. Optimization of six selected characters onto the phylogeny of Pellegrino et al. (2001). State characters are represented in colors. Number in parentheses corresponds to character list. *FL* (*forelimb*) *presence* (0), red = forelimb absent; blue = forelimb present. *FL autopod mm. (muscles)*(7), red = plesiomorphic autopodium muscle pattern; blue = muscles inserting on digit I absent; lilac = muscles inserting on digits I and V absent; yellow = digits served by extrinsic muscles; pink = forelimb absent; light green = vestigial muscles to the first metacarpal; light blue = muscles reduced to abductor and adductor masses. *HL* (*hindlimb*) *stylopod mm. (11)*, red = plesiomorphic muscle pattern of the hindlimb stylopodium; blue = muscles reduced to three dorsal and two ventral bundles; green = muscles reduced to one dorsal and one ventral bundle. *HL stylopod bones* (12), red = hindlimb stylopod plesiomorphic bony pattern; blue = derived pattern. *HL autopod mm.*(17), red = plesiomorphic muscle pattern of the hindlimb autopodium; blue = intrinsic hindlimb autopodium muscles highly reduced; green = hindlimb autopodium muscles reduced to one dorsal and one ventral bundle. *HL autopod bones* (18), red = hindlimb autopodium plesiomorphic bony pattern; blue = bones of digit I absent; green = bones of three digits absent; yellow = bones of four digits absent; light blue = bones of all digits absent. Results: Muscle anatomy of adults of analyzed taxa

TABLE 6. Character list

0. Forelimb	16. Bone and muscle coupling
0) Absent	0) Present
1) Present	1) Absent
Stylopodium	Autopodium
1. Muscle pattern	17. Muscular pattern
0) Plesiomorphic	0) Plesiomorphic
1) Muscles extremely reduced to adductor and abductor bundles	1) Intrinsic muscles highly reduced
2. Bone pattern	2) Muscles reduced to abductor and adductor bundles
0) Plesiomorphic	18. Bone pattern
1) Derived	0) Plesiomorphic
3. Bone and muscle coupling	1) Bones of digit V absent
0) Present	2) Bones of three digits absent
1) Absent	3) Bones of four digits absent
Zeugopodium	4) Without digits
4. Muscle pattern	19. Bone and muscle coupling
0) Plesiomorphic	0) Present
1) Muscles extremely reduced to adductor and abductor bundles	1) Absent
5. Bone pattern	
0) Plesiomorphic	
1) Derived	
6. Bone and muscle coupling	
0) Present	
1) Absent	
Autopodium	
7. Muscular pattern	
0) Plesiomorphic	
1) Muscles of digit I totally absent	
2) Vestigial muscles to metacarpal I	
3) Digits served by extrinsic muscles	
4) Muscles reduced to abductor and adductor bundles	
8. Bone pattern	
0) Plesiomorphic	
1) Bones of digit I totally absent	
2) Persistent vestigial metacarpal of digit I	
3) Bones of digits I and V absent	
4) Bones of digits I, II, and III absent	
5) Bones of all digits except digit 4 absent	
9. Bone and muscle coupling	
0) Present	
1) Absent	
10. Hindlimb	
0) Present	
1) Absent	
Stylopodium	
11. Muscle pattern	
0) Plesiomorphic	
1) Muscles reduced to three dorsal and two ventral bundles	
2) Muscles extremely reduced to adductor and abductor bundles	
12. Bone pattern	
0) Plesiomorphic	
1) Derived	
13. Bone and muscle coupling	
0) Present	
1) Absent	
Zeugopodium	
14. Muscle pattern	
0) Plesiomorphic	
1) Muscles reduced to three dorsal and two ventral bundles	
2) Muscles extremely reduced to adductor and abductor bundles	
15. Bone pattern	
0) Plesiomorphic	
1) Derived	

convergence among gymnophthalmids during evolution of non-pentadactyl autopodia depends on the number of digits still present. The threshold for typical spike-like autopodial anatomy seems to be slightly different between clades: in *Gymnophthalmini* the shift to an extremely reduced limb seems to occur in the transition from four to two digits, while in *Cercosaurini* the spike-like morphology is apparently established in the transition from two digits to one. It is interesting that Shapiro (2002) already noted that when digits are reduced in the skink *Hemiergis*, the number of phalanges of the digits tend to match the plesiomorphic formula, except when three or more digits have been lost. Then, the phalangeal number is reduced, for example, in the didactyl *H. quadrilineata*. Future studies may elucidate whether this apparent threshold for convergence reflects peculiarities of the evolutionary history of each clade (e.g., processes involving only digit reduction versus possible reversals of digit loss), developmental and functional constraints applicable to any organism presenting spike-like appendages, or a combination of the two.

Disparities Between Muscle and Bone Reduction/Loss

Our results show that loss or reduction of limb bones is not necessarily accompanied by corresponding loss or reduction of the muscles that plesiomorphically attach to them. We found examples of muscles that persisted after the bones to which they normally attach were lost, and muscles that were lost although their bony attachments persisted. Skeletal and connective tissue patterning can be experimentally uncoupled, demonstrating a degree of autonomy in the development of the musculoskeletal system (Li et al., 2010). Empirical data also show that bone patterning is established before individual muscles can be recognized (e.g., Dunlap, 1966; Muntz, 1975; Kardon, 1998; Ponssa et al., 2010; Manzano et al., 2013). Remarkably, a

pattern of proximal wing muscles similar to that in plesiomorphic pentadactyl limbs can appear in chickens in the absence of wing skeletal elements (Lanser and Fallon, 1987), and, conversely, the limb skeleton develops with a normal pattern in the absence of muscles (Brent et al., 2005). Furthermore, many limb muscle attachments are more closely related to bone topology than bone identity (Diogo et al., 2015); in *B. bresslaui* and *N. ablephara* a few muscle fibers persist on the radial and ulnar surfaces of their two digits that probably correspond to the abductor pollicis brevis and abductor digiti minimi which normally insert on digits I and V; likewise, the abductor pollicis brevis in chickens usually inserts on the most radial pedal digit (which develops from the anlage of digit II), while in pentadactyl taxa this muscle always inserts on the digit that derives from the first, and not the second, anlage (e.g., Abdala and Diogo, 2010; Diogo and Abdala, 2010).

Phylogenetic Constraints

We report that reduced autopodia are morphologically different between Cercosaurini and Gymnophthalmi, suggesting that processes of digit loss differ among clades. A similar finding has been previously reported: major reduction in the forelimbs of Gymnophthalmi (see Pellegrino et al., 2001), *versus* more pronounced reduction of hindlimbs in Cercosaurini (see Kizirian and McDiarmid, 1998; Kohlsdorf and Wagner, 2006). Some lineage-specific trends toward limb reduction are noticeable even in pentadactyl species. For example, *Anotosaura* (Eupleopini, sister species of Cercosaurini, which contains the miniature-limbed *Bachia*) exhibits short fingers, a dorsoventrally flattened forelimb, and significant decrease in muscle size, although its muscular limb configuration resembles that of pentadactyl teiids. This pattern contrasts with the slender autopodia observed in Gymnophthalmi, evident not only in the tetradactyl *Vanzosaura* and *Psilophthalmus* but also in the pentadactyl *Acratosaura*. Presumably, the processes leading to the evolution of non-pentadactyl autopodia in these two clades were influenced by phylogenetic constraints imposed by the ancestral autopodial morphology of each lineage.

The influence of the phylogenetic histories of the lineages Cercosaurini and Gymnophthalmi is particularly relevant to comparisons among species that have acquired tetradactyl limbs through different evolutionary processes, as shown by the optimizations of characters 7 (Fig. 5; muscle pattern of the forelimb autopodium) and 8 (not shown: bony pattern of the forelimb autopodium). Tetradactyl autopodia in both *Vanzosaura* and *Psilophthalmus* likely result from loss of digit I (Pellegrino et al., 2001), and in these taxa the limbs

tend to conserve the plesiomorphic myological pattern. Similarly, rodents with only four digits exhibit manus and pes muscles with the same arrangement as that found in autopodia with all five digits (Rocha-Barbosa et al., 2007). In contrast, the muscular arrangement of the tetradactyl autopodia of *B. scolecoides*, thought to be an example of evolutionary reversal of digit loss (see Kohlsdorf and Wagner, 2006; Kohlsdorf et al., 2010), appears to have been independently acquired based on our optimization analysis (character 8). The unique myological organization observed in the four-toed *B. scolecoides* is characterized by a lack of identity in both manus and pes muscles associated with the four remaining digits, a pattern previously documented for the osteological autopodial elements of this species (Kohlsdorf and Wagner, 2006). Interestingly, this autopodial morphology lacking digit identity resembles that of polydactyl mice produced through the disruption of *Gli3* expression in knockouts of *Sonic Hedgehog*, in which all digits exhibit the identity of digit I and muscles also lack individual identity (te Welscher et al. 2002; see also Lopez-Rios et al. (2012) for additional information on phenotypic effects of disruption in *Gli-3* expression). The lack of digit identity in *B. scolecoides*, now identified in both, muscles and bones, supports the hypothesis that tetradactyly evolved through reversal of digit loss in this lineage, arguing against Galis et al. (2010) (see Kohlsdorf et al., 2010 for a recent discussion), because digit identity is retained in other groups in which tetradactyl limbs evolved through digit reduction. These apparently “re-evolved” digits of *B. scolecoides* would, therefore, be homoplastic, not homologous (see Diogo et al., 2013) with those of the tetradactyl Gymnophthalmi.

Differences in the trends of muscle reduction between clades were indeed expected. Shapiro (2002) already noted that skeletal limb reduction in *Bachia* contrasts with the pattern of digit loss observed in *Hemiergus* and *Lerista*, which is thus not generalized for all cases of limb reduction in lizards.

Convergence in Extremely Reduced Limb Morphologies

The muscular anatomy of spike-like appendages (e.g., those observed in the forelimb of *N. ablephara*) resembles a fin-like configuration in that most muscles of the stylo- and zeugopodium are lost/undifferentiated. Spike-like autopodia are morphologically similar between Cercosaurinae and Gymnophthalmi from a myological perspective. However, this myological similarity does not necessarily imply osteological similarity; for example, the monodactyl limbs of *N. ablephara* (forelimb) and *S. catimbau*, and *C. sinebrachiatus* (hindlimb) are myologically similar but differ in

their phalangeal formulae (yet all three belong to clade 1). Spike-like morphologies have also been independently acquired in members of other squamate families (e.g., *O. intermedius*, also included in this study), and the general trends are similar. Dorsoventral flattening is very pronounced, the limbs show a paddle-like aspect, the distal limb muscles are reduced to mainly abductor and adductor complexes, and the zeugopodium and autopodium contain almost no muscle (see also Fürbringer, 1870). Thus, our data corroborate previous reports of a seemingly widespread trend in limb reduction among tetrapods: as digit loss progresses, the intrinsic autopodial muscle groups are usually more severely reduced than those of the other limb regions (Fürbringer, 1870). Our data show that muscle loss is often more advanced than bone loss. Accordingly, in the spike-like appendages observed in Gymnophthalmidae the autopodial muscles tend to disappear.

A comparison between our data and the results of Fürbringer (1870) reveals further examples of morphological convergence in digit reduction of lizard limbs. That author described more profound modifications in all three segments (autopod, zeugopod, and stylopod) of the hindlimb muscles in *Pygopus lepidopus*, a lizard with four digits in the pes, than those observed in the gymnophthalmids we dissected. However, similar general trends can be recognized between descriptions, including those of lizards showing reduction of two and three digits: muscle fusions that lead to new functions (e.g., adduction and abduction of the limbs) and homologies to plesiomorphic muscles are no longer recognizable (see Fürbringer, 1870, p 55 and his figures XV and XVIII).

GENERAL COMMENTS AND FUTURE DIRECTIONS

Anatomical and developmental studies usually focus on pentadactyl autopodia, and most of those dealing with deviations from the norm focus on reduction or gain of cartilages and/or bones (especially of the phalanges). Much less attention has been paid to changes in soft tissues that occurred during the evolution of non-pentadactyl autopodia. This study mainly focuses on muscle anatomy and contributes toward filling the gap between osteological and myological data on limb reduction and digit loss in lizards and tetrapods in general. Further studies of limb reduction in other tetrapod taxa, such as amphibians and mammals, might corroborate the generality of the trends reported here. These might be complemented by data on development and biomechanics in species exhibiting limb reduction and digit loss, which together will clarify the causes and consequences of a phenomenon so recurrent in the evolution of tetrapods. This line of inquiry will reciprocally inform

the study of normal and abnormal development in human and other tetrapod limbs.

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APPENDIX A: LIST OF SPECIMENS INCLUDED IN THE STUDY

The specimens dissected in this study were obtained from the following institutions: Coleção Herpetológica de Ribeirão Preto (CHRP) from University of São Paulo at Ribeirão Preto, Brazil; Coleção Herpetológica from University of Brasília (CHUNB) at Brasília, Brazil; Coleção Herpetológica from Federal University of Mato Grosso (UFMT) at Cuiabá, Brazil; Colección Herpetológica de la Fundación Miguel Lillo at Tucumán, Argentina (FML); and specimens previously donated to FML by the Museu de Zoologia from University of São Paulo (MZUSP) at São Paulo, Brazil; Colección Herpetológica del Museo de Ciencias Naturales de Salta, Argentina (MCN). The list of all alcohol-preserved specimens examined for this study is given below. For each species, we provide the Linnean binomial, the institution where specimens were deposited (together with their unique identifier—voucher), and the total number of specimens examined. All dissections and muscle

identifications were made by the same person (VA).

- Anguidae—*Ophiodes intermedius*: FML 014548, 1 specimen.
- Scincidae—*Riopa* sp.: MCN 4692, 1 specimen; *Leptosiaphos* sp.: MCN 4725, 1 specimen.
- Teiidae—*Cnemidophorus longicaudus*: FML, un-catalogued, 7 specimens; *Ameiva exsul*, FML, un-catalogued, 1 specimen.
- Gymnophthalmidae—*Acratosaura mentalis*: CHR 555, 558, 560, 3 specimens; *Anotosaura vanzolinia*: CHR 547, 548, 2 specimens; *Bachia dorbignyi*: CHUNB 22331, 22332, 2 specimens; *Bachia bresslaui*: CHUNB 50258, 51322, 2 specimens; *Bachia scolecoides*: UFMT 6652, 8835, 2 specimens; *Calyptommatus leiolepis*: CHR 238, 239, 241, 244, and MZUSP 71339 (donated to FML), total of 5 specimens; *Calyptommatus nicterus*: CHR 551–554, 4 specimens; *Calyptommatus sinebrachiatus*: CHR 171, 173, 176, 177, 182, 184–186, 188, 222–228, 405, 17 specimens; *Cercosaura parkeri*: FML 00803 (without data), 6758 (w/d), 2 specimens; *Nothobachia ablephara*: CHR 549, 550, 2 specimens; *Psilophthalmus paeminosus*: CHR 245–253, 9 specimens; *Sriptosaura catimbau*: CHR 523, 526–528, 4 specimens; *Vanzosaura rubricauda*: CHR 059, 060–068, 10 specimens.

APPENDIX B: RESULTS: MUSCLE ANATOMY OF DIFFERENT LIMB MORPHOLOGIES

Plesiomorphic Pentadactyl Limb (Teiidae): *Cnemidophorus Longicaudus* and *Ameiva Exsul*

The plesiomorphic stylopodium in the forelimb (Figs. 2a and 3a,b) is covered by three muscles on the ventral aspect: the coracobrachialis with two branches, the biceps brachii with two branches, and the brachialis. These muscles are rather slender and about the same thickness. Dorsally, the arm is covered by the three heads of the triceps, which are very bulky and insert through a short, strong tendon onto the proximal ulna. In the hindlimb, the plesiomorphic stylopodium (Fig. 3a,b) is dorsally covered by the iliofibularis, a slender, parallel-fibered muscle that inserts onto the proximal fibular shaft. Adjacent to the iliofibularis, the iliobtibialis muscle, which is very bulky and strong and forms a functional complex (the quadriceps femoris, see e.g., Russell and Bauer, 2008) with the ambiens and femorotibialis muscles. The iliobtibialis arises from the lateral surface of the ilium and inserts onto the cnemial crest of the tibia. The femorotibialis arises from the femoral shaft and converges with the insertion tendon of the iliobtibialis. The ambiens, which arises through two ten-

dons from the pubis and the proximal femur, shares an insertion with the other three components of the quadriceps femoris. The iliofemoralis is a nearly triangular muscle that arises from the ilium and inserts onto the proximal femur.

The dorsal aspect of the plesiomorphic forelimb zeugopodium (Fig. 2a,b) is covered by the extensor digitorum, which originates from the humerus and inserts tendinously onto the bases of the metacarpals II, III, and IV. It is a subtriangular, flattened muscle, and the portion above the dorsum of the manus consists of a tendinous sheet with some adherent muscle fibers. The extensor antebrachii et carpi radialis is a very robust muscle that originates from the humerus and inserts fleshily along the entire shaft of the radius. Two branches can be recognized: a lateral branch (which probably corresponds to the brachioradialis of mammals) composed of fibers running obliquely to the distal radius, and a medial branch (which probably corresponds to the extensor carpi radialis of mammals) whose fibers parallel the shaft of the radius. A cord-like portion of the fibers of the medial branch surpasses the distal end of the radius and inserts onto the base of the second metacarpal. The extensor antebrachii et carpi ulnaris is a subtriangular, flattened muscle which originates from the proximal humerus and inserts onto the strong aponeurosis that covers the distal ulna, with some fibers inserting onto the pisiform. The abductor pollicis longus originates fleshily from the distal ulna and inserts tendinously onto the base of the first metacarpal. The extensores digitorum breves originate from the ulnare and insert onto the distal end of each metacarpal. The palmaris longus is a strong but narrow muscle that blends with the flexores breves superficiales through an aponeurosis. The flexor carpi ulnaris is bulky with two branches: one blends with the distal portion of the flexor digitorum longus, while the other inserts onto the distal ulna. Both branches originate from the distal humerus. The flexor carpi radialis and the pronator teres are two long, slender muscles which originate from the distal humerus and insert onto the distal radius. Some fibers of the pronator teres insert fleshily onto the medial portion of the radius. The flexor digitorum longus is a very bulky muscle with three branches that give rise to the broad flexor plate. The flexor plate in turn gives rise to the five flexor tendons, which insert onto the distal phalanges of each digit. The epitrochleanconeus is a small muscle that originates from the distal humerus and inserts fleshily onto the most proximal portion of the ulna. The pronator accesorius, a flattened muscle composed of fibers running obliquely between the ulna and radius, originates from the distal humerus and inserts onto the distal radius. The pronator quadratus is a broad muscle that originates from the

radius and inserts onto the ulna, filling the gap between the two bones.

In the plesiomorphic zeugopodium of the hindlimb (Fig. 3a,b), the tibialis anticus originates from the tibia and inserts onto the proximal end of the first metatarsal. This parallel-fibered muscle exhibits slight torsion around the tibia. The peroneus longus and brevis are two gracile, parallel-fibered muscles that occupy the lateral border of the crus. The peroneus longus originates from the lateral epicondyle of the femur and inserts onto the tubercle of the fifth metatarsal. The peroneus brevis arises fleshily from the entire length of the fibular shaft and inserts onto the head of the fifth metatarsal. The extensor digitorum longus is a fusiform muscle that originates from the femur and inserts onto the metacarpals of digits II and III. Occupying the ventral portion of the crus, the broad-bellied femorotibial gastrocnemius originates through a tendon complex associated with the knee joint capsule. The muscle inserts onto digit V in association with the crural tendon of the flexor tibialis externus. The femoral gastrocnemius originates from the femur and inserts on the plantar aponeurosis with the femorotibial gastrocnemius. The flexor digitorum muscle is a complex whose main origin is from the femur with some fibers coming from the fibula, with a smaller distal head that arises from the astragalocalcaneum. The muscle communicates with an aponeurosis that gives rise to four thick tendons serving the digits, the tendon of digit V being separate from the main aponeurosis structure. The parallel-fibered pronator profundus arises from the fibular shaft and inserts through an aponeurotic complex onto the proximal end of the first metatarsal. The popliteus is a triangular muscle that originates from the proximal tibia and inserts onto the proximal fibula. The interosseus cruris originates from the distal tibia and inserts onto the distal fibula.

Regarding the plesiomorphic pentadactyl autopodium, in the forelimb (Fig. 2a,b), five dorsometacarpals originate from the metacarpals and insert tendinously onto the distal phalanges of digits I, II, III, IV, and V. The abductor pollicis brevis originates from the lateral surface of the radiale and inserts onto the distal end of the first metacarpal. The abductor digiti minimi originates from the pisiform and inserts onto the fifth metacarpal. The five flexores breves superficiales originate from the flexor retinaculum and insert tendinously onto the penultimate phalanges. Dorsal to this complex lies the flexor plate with its palmar sesamoid. The 10 flexores breves profundi lie dorsal to the contra-hentes digitorum and insert on the medial and lateral side of the five digits. The intermetacarpales originate from the lateral aspects of the first four metacarpals and bridge the gap between the metacarpal of origin and the adjacent metacarpal on the lateral side. The lumbricales lie deep to the

flexor plate and are associated with it, serving each digit and forming a complex network. In the hindlimb, the plesiomorphic autopodium (Fig. 3a,b) has five ribbon-like extensores digitores breves for digits I–V, which originate from and are located on the dorsal aspects of the metatarsals; they insert through ribbon-like tendons onto the terminal phalanges. Although these muscles do form a clear complex, the elevation of digit V above the plane of the other digits means that its extensor is partially separated and could be considered an extensor digiti minimi. The extensor of digit IV is the broadest of the extensors. The superficial femoral aponeurosis (Russell, 1993) runs between digits I–V. Dorsally to this aponeurosis lies another aponeurosis coming from the flexor digitorum longus that guides tendons to every digit. Dorsal to this aponeurotic system are the flexores digitores breves that originate from the aponeurosis of the gastrocnemius femoralis and insert fleshily at the level of the metatarsophalangeal joints. The short, parallel-fibered flexor hallucis is clearly distinguishable, originating from the fourth distal tarsal and inserting onto the proximal margin of the first metatarsal. The abductor digiti quinti is also clearly recognizable, running from the astragalocalcaneum to the base of the fifth metatarsal. The lumbricales are associated with the tendons of the flexor digitorum longus and insert onto the phalanges of the corresponding digits. The interossei plantares cross the space between the metatarsals, originating from the metatarsophalangeal joints and inserting fleshily onto the entire length of the adjacent metatarsal on the medial side. Notably, digit IV also seems to have an interosseous plantaris, although this muscle does not insert onto the fifth metatarsal because this bone is displaced and occupies another spatial plane. The same muscular pattern is also recognizable in the interosseus plantaris those gymnophthalmid and scincid species that possess pentadactyl hindlimbs (i.e., *Psilophthalmus paeminus*, *Vanzosaura rubricauda*, *Cercosaura parkeri*, and *Riopa*).

Tetradactyl Limbs: *Bachia Scolecoides* (Forelimbs and Hindlimbs); *Vanzosaura Rubricauda* and *Psilophthalmus Paeminus* (Forelimb)

The gymnophthalmid tetradactyl species can be separated into two groups according to the osteological morphology of the autopodium: *Bachia scolecoides* lacks metacarpals and phalanges of digit I and also exhibits reduction of the phalanges of the other digits (all presenting only two phalanges, Supporting Information SI07, SI10, SI11; URL: XXX), whereas in *Vanzosaura rubricauda* and *Psilophthalmus paeminus* digit I is reduced to a vestigial metacarpal while the phalanges of the other

digits follow the plesiomorphic formulae (Fig. 1). The scincid species *Leptosiaphos* present a reduced digit I. Besides the short digits (with fewer phalanges), limbs of *B. scolecooides* are also characterized by dorsoventral flattening (Supporting Information SI12, SI25, SI26; URL: XXX). The stylopodium and the zeugopodium in the tetradactyl limbs of all these species exhibit the plesiomorphic pattern of bones and muscles described above. In the tetradactyl forelimb autopodia, the extensor digitorum inserts onto digits II, III, and IV, and the abductor pollicis longus tends to be reduced and inserts onto the radiale. Digit II is served by the typical muscle complexes of the manus, and muscle fibers of a short abductor of digit II are recognizable, which seemingly corresponds to the abductor pollicis brevis muscle that inserts on digit I in the pentadactyl manus. In *Vanzosaura rubricauda*, part of the contrahentes digitorum (i.e., the “flexor digitorum V transversus I” sensu Abdala and Moro, 2006) is distinguishable on the deep surface of the manus; it originates from the radiale (instead of digit I as in most other lizards) and inserts onto metacarpal V. The contrahentes digitorum muscles are not as clearly visible in *B. scolecooides* and *Psilophthamus paeminosus*. The other intrinsic muscles of the manus follow the plesiomorphic pattern described above in the three tetradactyl species examined. The tetradactyl pes of *B. scolecooides* exhibits a myological configuration very similar to that of a typical lizard forelimb: although the plesiomorphic pattern described above can be recognized, the lack of digit V lends the pes a remarkable similarity to the manus (e.g., the m. tibialis anticus exhibits no torsion and the peroneus brevis and longus are rather flat) (Fig. 3c; Supporting Information SI26; URL: XXX). The extensores and flexores digitorum breves form homogeneous layers without differentiation of the muscles associated with digit V (e.g., the abductor digiti minimi); however, the extensor hallucis can be distinguished.

Tridactyl Limbs: *Bachia Dorbignyi* (Forelimb)

Only one gymnophthalmid species exhibiting a tridactyl morphology was examined, as autopodia presenting only three digits are considerably rare in the group (e.g., to our knowledge there are no tridactyl species in clades 1 and 2 of Gymnophthalmidae). The tridactyl species *B. dorbignyi* lacks metacarpals and phalanges of digits I and V, and the remaining digits have only two phalanges (Fig. 1; Supporting Information SI06; URL: XXX). The stylopodium and zeugopodium of this species possess all the bones and muscles of the plesiomorphic pentadactyl limb and are characterized by pronounced dorsoventral flattening (Fig. 2c; Supporting Information SI19–SI22; URL: XXX). In the

forearm, the extensor digitorum inserts onto digits II, III, and IV, and the abductor pollicis longus inserts onto the base of metacarpal I. Although reduced in number, the serial intrinsic muscles of the manus (lumbricales, flexores breves profundi, flexores breves superficiales, and contrahentes digitorum) are all recognizable and similar to the plesiomorphic pattern described above, except for the intermetacarpales, which are absent or indistinguishable (Fig. 4). A few fibers can be distinguished lateral to digits II and IV, which seemingly correspond to the abductor pollicis brevis and abductor digiti minimi of the pentadactyl manus.

Didactyl Limbs: *Bachia Bresslaui* (Forelimb) and *Nothobachia Ablephara* (Hindlimb)

The extremely reduced didactyl autopodia of Gymnophthalmidae are represented here by one species exhibiting two digits in the forelimb autopodia (*B. bresslaui*) and one with two digits in the hindlimb autopodia (*N. ablephara*; Fig. 1; Supporting Information SI29; URL: XXX). The didactyl forelimb of *B. bresslaui* is composed of humerus, radius, ulna, the proximal carpals, two metacarpals, and two phalanges in each of the two digits that are present (identified as III and IV, see Kohlsdorf and Wagner, 2006). All of the skeletal structures exhibit the dorsoventral flattening characteristic of *Bachia* species (Supporting Information SI01–SI03; URL: XXX). The joint between the humerus and the radius/ulna is mobile, and the muscles of the forearm are not reduced. The manus is very short and robust, with the shape and rigidity of a paddle (Fig. 2d; Supporting Information SI17, SI18; URL: XXX). The two digits are connected to the extensor digitorum and the flexor digitorum longus, but the extensores digitorum breves and the flexores breves superficiales and flexores breves profundi seem to be absent. The intermetacarpales seem to be absent or indistinguishable, and the rest of the intrinsic muscular complexes of the manus are also indistinguishable. A few fibers can be distinguished lateral to the two existing digits, which would correspond to the abductor pollicis brevis and abductor digiti minimi of the pentadactyl manus. Regarding the didactyl hindlimb of *N. ablephara*, the bones of the stylopodium and zeugopodium match the plesiomorphic pattern, digit III comprises a metatarsal and two phalanges, and digit IV comprises a metatarsal and four phalanges (Fig. 1). The joint between femur and tibia/fibula is mobile. The thigh and crus muscles are reduced: in the thigh there are three dorsal and two ventral muscles, while in the crus a superficial layer probably corresponding to the gastrocnemius femorotibialis is recognizable, its tendons apparently serving both digits. The flexor digitorum longus seems to be highly reduced

and the flexores breves profundi are also reduced to a very thin layer serving the two digits (Supporting Information SI28, SI29; URL: XXX). In the dorsum of the crus, an extensor digitorum is recognizable, with its tendons serving both digits (Fig. 3c). Two lateral tendons originating from the thigh and adhering to both the lateral and medial borders of the bones of the fin-like appendage are noticeable. The extensores digitorum breves are not recognizable.

Monodactyl Limbs: *Nothobachia ablephara* (Forelimb); *Calyptommatus* [*C. leiolepis*, *C. nicterus*, *C. sinebrachiatus*], *Scriptosaura catimbau*, and *Bachia bresslaui* (Hindlimbs)

The extremely reduced monodactyl limbs of Gymnophthalmidae are composed of a single digit (assumed to be digit IV), and the loss of osteological elements is accompanied by profound reduction of limb muscles (Figs. 2e, 3d–f). The only representative included here with a monodactyl forelimb is *N. ablephara*; in this species, the forelimb is dorsoventrally flattened and composed of humerus, radius, ulna, the fourth metacarpal, and one phalanx. The joint between the humerus and radius/ulna is mobile. In the forearm, three dorsal and two ventral muscle masses can be recognized whose tendons attach onto the digits, but the complex of the extensores digitorum breves and the intrinsic muscles of the manus are absent (Fig. 2e).

The three *Calyptommatus* species included here are characterized by monodactylus hindlimbs (Fig. 3d; Supporting Information SI27; URL: XXX) composed of femur, tibia, fibula, reduced tibiofibulare, metatarsal IV, and associated phalanges, which differ in number among the species (one phalanx observed in *C. sinebrachiatus* and *C. leiolepis*; two phalanges observed in *C. nicterus*). In these species, the articulations between femur and tibia/fibula, and between tibia/fibula tibia/fibulare, are rigid. All bones are dorsoventrally flattened, giving the limb the shape of a flattened spike, and the intrinsic muscles of the pes are absent. The tibiofibulare and metatarsal IV are dorsally and ventrally covered by muscular fibers coming from the zeugopodial muscles. In the stylopodium, there are three dorsal and two ventral muscles, all of which originate from the pelvic girdle and insert tendinously onto the distal phalanx. Thus, the distal phalanx receives the terminal tendons of all muscles of the appendage. The two most lateral muscles have ribbon-like tendons that adhere to the border of all bony structures along the length of the spike-like appendage. In the zeugopod, the lateral muscles are reduced to a few fibers that accompany the lateral tendons. The vestigial limbs are moved like fins, and, because of their position, the usual flexor and extensor muscles of the thigh and crus appear to be abductors and adductors of

the appendage (Fig. 3d). Another monodactylus species from this lineage, *Scriptosaura catimbau* (Supporting Information SI30; URL: XXX), also exhibits a vestigial hindlimb composed of femur, tibia, fibula, tibialefibulare, and one metatarsal corresponding to digit IV (Fig. 1). The articulations between femur and tibia/fibula and between tibia/fibula and tibialefibulare seem to be rigid, and the general pattern of muscle reduction follows that of *Calyptommatus*, the only difference being persistent muscular fibers in *S. catimbau* that cover the entire fin-like appendage to its distal end, which probably correspond to some of the intrinsic muscles of the pes.

The monodactylus hindlimbs examined in the Cercosaurinae lineage (*Bachia* species) are composed of femur, tibia, fibula, tibialefibulare, metatarsal, and one phalanx corresponding to digit IV. The hindlimb of *B. bresslaui* (Fig. 1) is truncated (Supporting Information SI04, SI05; URL: XXX), and the muscular arrangement is similar to that of *Calyptommatus* and *Scriptosaura*: in the thigh, five muscles insert tendinously on the bones of the zeugopodium, and the joint between femur and tibia/fibula seems to be rigid.

Limbs That Lack Autopodia: *Bachia dorbignyi* (Hindlimb)

The species *B. dorbignyi* presents a vestigial limb composed of bones of the stylopodium and zeugopodium; the bones of the autopodium are entirely absent. The limb is flattened, following the pattern described above for other members of the genus *Bachia* (Supporting Information SI23; URL: XXX), and the muscular complexes are reduced to three dorsal and two ventral muscles, probably functioning as adductor and abductor complexes.

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