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RESEARCH ARTICLE

Comparative musculoskeletal anatomy of chameleon limbs, with implications for the evolution of arboreal locomotion in lizards and for teratology

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Abstract

Chameleon species have recently been adopted as models for evo-devo and macroevolutionary processes. However, most anatomical and developmental studies of chameleons focus on the skeleton, and information about their soft tissues is scarce. Here, we provide a detailed morphological description based on contrast enhanced micro-CT scans and dissections of the adult phenotype of all the forelimb and hindlimb muscles of the Veiled Chameleon (*Chamaeleo calyptratus*) and compare these muscles with those of other chameleons and lizards. We found the appendicular muscle anatomy of chameleons to be surprisingly conservative considering the remarkable structural and functional modifications of the limb skeleton, particularly the distal limb regions. For instance, the zygodactyl autopodia of chameleons are unique among tetrapods, and the carpals and tarsals are highly modified in shape and number. However, most of the muscles usually present in the manus and pes of other lizards are present in the same configuration in chameleons. The most obvious muscular features related to the peculiar opposable autopodia of chameleons are: (1) presence of broad, V-shaped plantar and palmar aponeuroses, and absence of *intermetacarpales* and *intermetatarsales*, between the digits separated by the cleft in each autopod; (2) oblique orientation of the superficial short flexors originating from these aponeuroses, which may allow these muscles to act as powerful adductors of the “super-digits”; and (3) well-developed *abductor digiti minimi* muscles and *abductor pollicis/hallucis brevis* muscles, which may act as powerful abductors of the “super-digits.”

KEYWORDS

arboreal locomotion, contrast staining, lizard, MicroCT, myology, PTA, syndactyly, zygodactyly

1 | INTRODUCTION

The lineage leading to modern Chamaeleonidae diverged from its sister group, the Agamidae, (forming the clade Acrodonta) ~126–120 MYA during the early Cretaceous (Okajima & Kumazawa, 2010; Zheng & Wiens, 2016). The family Chamaeleonidae is hypothesized to have evolved ~60–65 MYA during the Late Cretaceous/Early Tertiary (Okajima & Kumazawa, 2010; Tolley, Townsend, & Vences, 2013). Chameleons are thus a relatively young clade of lizards that present a highly specialized suite of phenotypic traits associated with true arboreal locomotion, in contrast to the sprawling gait typical of lizards (Fischer, Krause, & Lilje, 2010; Higham & Jayne, 2004; Peterson, 1984; Webb & Gans, 1982) and most tetrapods.

Adaptations for life in the trees include an increased dependence on a projectile tongue for feeding, large eyes and increased dependence on processing visual sensory information, a laterally compressed body, a prehensile tail, and zygodactyly of the autopodia (manus and pes).

In chameleons, the ulnar and radial sides of each autopodium are widely separated from each other by a cleft. The syndactylous complexes formed by manual digits 1–3 and 4–5 and pedal digits 1–2 and 3–5 are often designated “super-digits” because each constitutes a single functional unit. The cleft, associated with other changes in the wrist and ankle, allows the two “super-digits” of each autopod to face in opposite directions, resulting in opposable autopodia that are related to the characteristic chameleon mode of autopodial grasping (Diaz &

TABLE 1 Specimens dissected for this study

Species	Specimen #	Snout-vent length	Preservation	Sex	Origin	Limbs dissected
<i>Chamaeleo calytratus</i> (Veiled Chameleon)	HUCC1-2	13 cm	Frozen	Adult Female	Captive	Left
	HUCC1-3	14 cm	Frozen	Adult Female	Captive	Left
	HUCC1-4	13 cm	Frozen	Adult Female	Captive	Left
<i>Trioceros melleri</i> (Meller's Chameleon)	HUTM1-3	27 cm	Alcohol	Subadult unsexed	Wild caught	Left and right
<i>Aspidoscelis uniparens</i> (Desert Grassland Whiptail)	HUAU1-1	7 cm	Frozen	Adult Female	Captive	Right
	HUAU1-2	6.5 cm	Frozen	Adult Female	Captive	Right
	HUAU1-3	7 cm	Frozen	Adult Female	Captive	Left FL, right HL

Trainor, 2015; for recent reviews, see Diaz et al., 2015a). These features represent an important morphological trade-off that has led to reduced speed of locomotion and a decreased dependence on olfaction, audition, the use of the snout for catching prey, and potentially a decrease in gustation (taste) (reviewed in Tolley & Herrel, 2013).

Because of these unusual traits, chameleons have recently been adopted as model organisms for squamate development and the study of macroevolution. These lizards represent a good model system to study embryonic development because of their well-known husbandry require-

ments and slow development (Diaz & Trainor, 2015; Diaz et al., 2015a, 2015b; Stower et al., 2015). Thus, chameleons allow us to study not only the skeletal development of the autopodia, but developmental integration of soft and hard tissues of the limb more broadly. These developmental studies may help us better understand how changes in locomotor mode and life history lead to anatomical changes (Abdala, Manzano, Tulli, & Herrel, 2009). Chameleons have also recently been adopted as models for linking evolution, development and pathology; for instance, development of the autopodium in chameleons has been studied as a possible

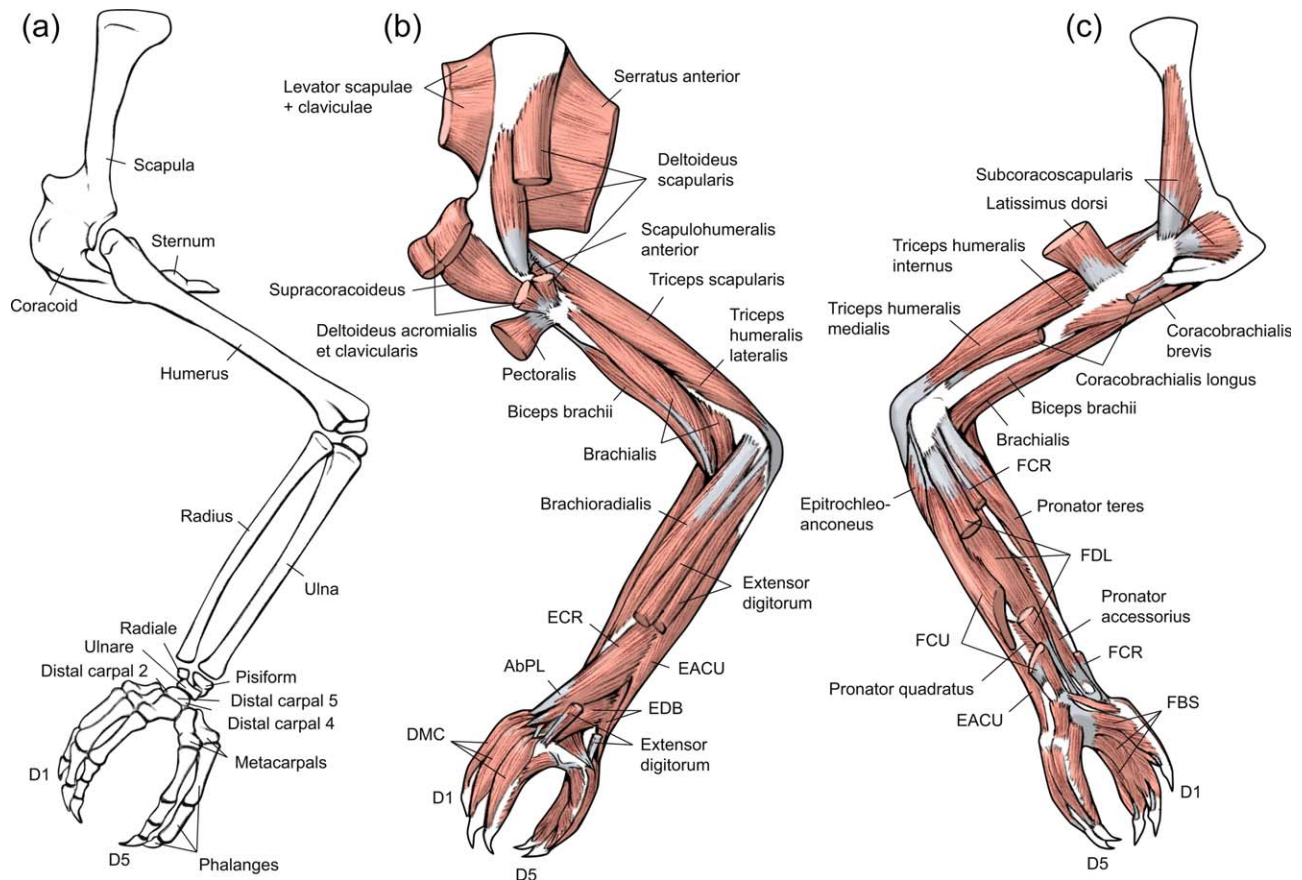


FIGURE 1 *Chamaeleo calytratus*, left pectoral limb. (a) skeleton in lateral view; (b) muscles in lateral view; (c) muscles in medial view. Abbreviations: digit (d)

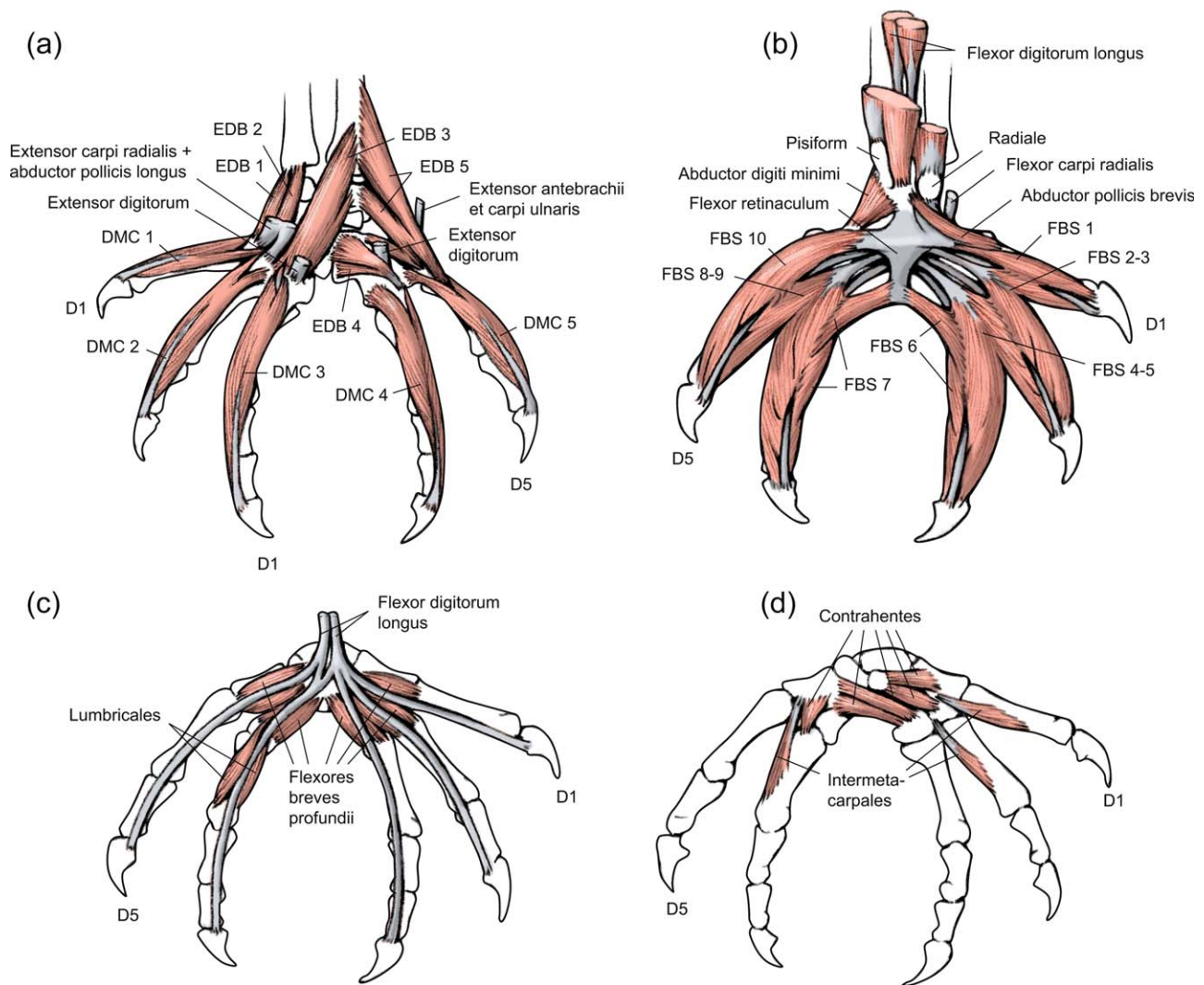


FIGURE 2 *Chamaeleo calytratus*, left manus. (a) Dorsal muscles; (b) superficial ventral muscles; (c) intermediate ventral muscles; (d) deep ventral muscles. Abbreviations: digit (d) dorsometacarpal (dmc), extensor digitorum brevis (edb), flexor brevis superficialis (fbs).

model for the genetic and developmental processes that lead to split hand/foot (SHFM) and syndactyly in humans (Diogo, Guinard, & Diaz, 2016). If the distinctive autopodia of chameleons arise via deviation of “normal” development, they could also be seen as an example of an evolutionary teratology *sensu* Guinard (2015), or even as “hopeful monsters” *sensu* Goldschmidt (1940; Diogo, Guinard, et al., 2016).

However, these recent studies were mainly based on the skeleton, and the soft-tissue anatomy of chameleons, particularly that of the autopodia, is poorly known. Here, we describe the adult musculature of the forelimbs and hindlimbs of *Chamaeleo calytratus* and compare its muscles to those of other chameleons and other lizards. We also discuss the locomotor implications of the distinctive features of chameleon limb muscle anatomy, particularly for grasping, and the striking similarities between the muscles of the distal regions of the forelimb and hindlimb. This descriptive work will serve as a basis for better understanding chameleon anatomy and evolution and to pave the way for linking anatomical studies with developmental and experimental studies on these new model organisms, including the mechanisms that

lead to phenotypes such as syndactyly and zygodactyly, which resemble human congenital malformations.

2 | MATERIALS & METHODS

All comparative analyses of muscles were conducted through laboratory dissection of soft tissues, while micro-CT imaging was only conducted in *Chamaeleo calytratus* for visualization of the intact musculoskeletal complex. All euthanasia protocols were approved by the Institutional Animal Care and Use Committee (IACUC) through La Sierra University.

2.1 | Dissections

We dissected forelimbs and hindlimbs of two adult chameleon species: three specimens of the Veiled Chameleon, *Chamaeleo calytratus*; Duméril and Duméril, 1851, and one specimen of Meller's Chameleon, *Trioceros melleri*; (Gray, 1865) (Table 1). *C. calytratus* and *A. uniparens*

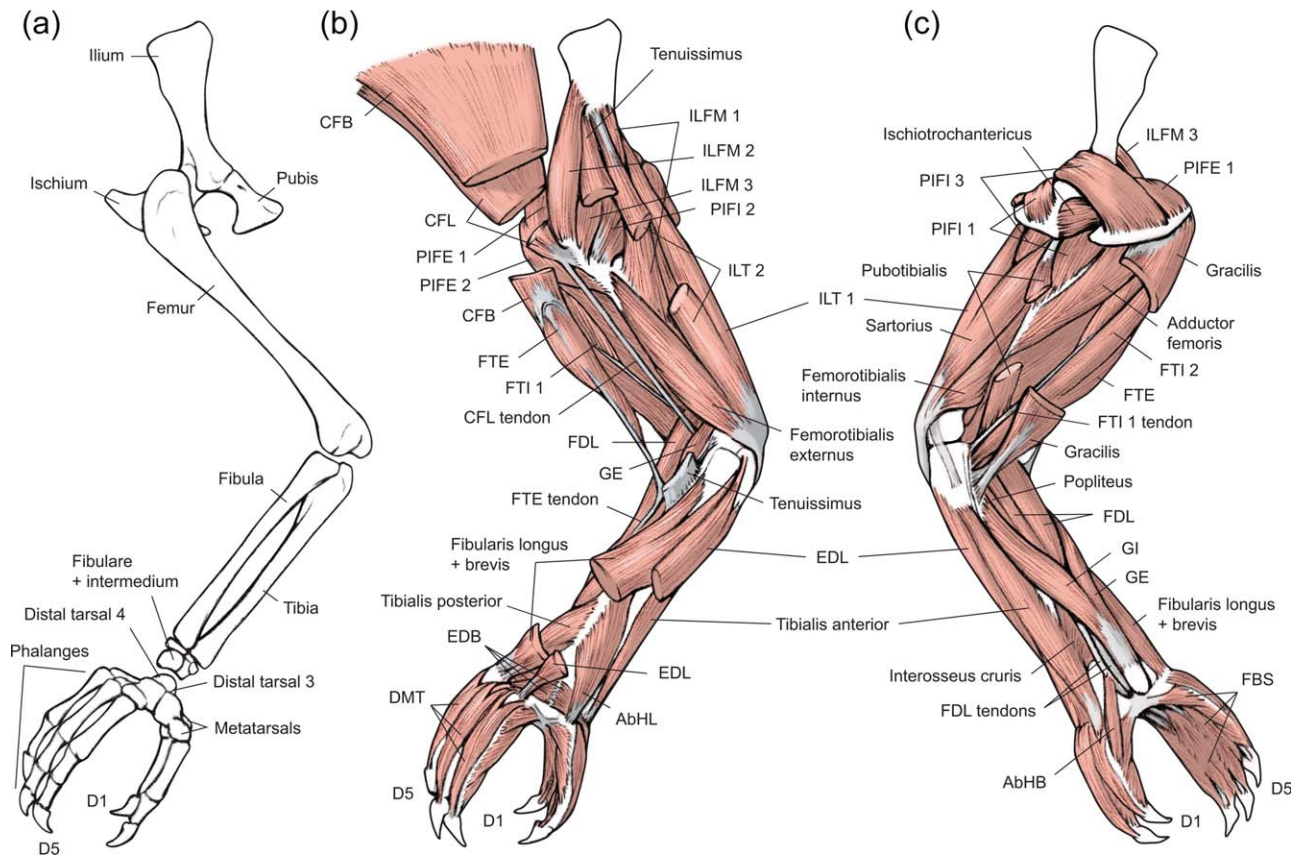


FIGURE 3 *Chamaeleo calytratus*, right pelvic limb. (a) skeleton in lateral view; (b) muscles in lateral view; (c) muscles in medial view. Abbreviations: abductor hallucis brevis (abhb), abductor hallucis longus (abhl), caudifemorales brevis (cfb), caudifemorales longus (cfl), digit (d), dorsometatarsals (dmt), extensor digitorum brevis (edb), extensor digitorum longus (edl), flexor digitorum longus (fdl), flexor tibialis externus (fte), flexor tibialis internus (fti), gastrocnemius externus (ge), gastrocnemius internus (gi), iliofemorales (ilfm), iliotibiales (ilt), puboischiofemorales externus (pife), puboischiofemorales internus (pifi).

specimens were previously euthanized retired breeder females from the Reptile Breeding Facility at La Sierra University; *T. melleri* specimen was provided by California State University Northridge was chosen as the main representative of the type genus of the family Chamaeleonidae because it is easy to breed in a laboratory setting (Diaz et al., 2015b), and *Trioceros melleri* was chosen as a second representative of the distinctive chameleon phenotype, both because of its derived position within the chameleon phylogeny and its large size (for recent reviews, see Diaz & Trainor, 2015; Diaz et al., 2015a). The limbs were dissected with micro-dissection tools under a Nikon SMZ1500 stereomicroscope, and high-resolution photographs of each muscle were taken using a Nikon Digital Sight DS-Fi1 camera connected to the microscope. For clarity, illustrations of all musculoskeletal appendicular structures are included (Figures 1–4).

For comparison, we also dissected one set of forelimbs and hindlimbs in each of three adult specimens of the Desert Grassland Whiptail Lizard (Teiidae: *Aspidoscelis uniparens*) (Table 1) as a representative of anatomically more generalized, terrestrial lizards that do not display syndactyly or zygodactyly. Anatomical differences between *C. calytratus*, *T. melleri*, and *A. uniparens* are given in Tables 2 and 3. We use the nomenclature of Diaz and Trainor (2015) for skeletal elements and Diogo and Abdala (2010) and Diogo, Bello-Hellegouarch, Kohlsdorf,

Esteve-Altava, and Molnar (2016) for muscles. Other muscle names commonly used in the herpetological literature (e.g., Russell & Bauer, 2008) and those used by Mivart (1870) (one of the few previous detailed descriptions of chameleon musculature) are also provided in both Tables.

2.2 | Contrast enhanced micro-CTs

Adult female Veiled Chameleons (retired breeders) were used for contrast enhanced micro-computed tomography (micro-CT) imaging. Females were euthanized using the protocol of Conroy, Papenfuss, Parker, and Hahn (2009). Left forelimbs and hindlimbs were skinned, excised, and fixed at room temperature on a rocker in 4% formaldehyde diluted in 1X phosphate buffered saline (PBS) for five days (10% neutral buffered formalin or 4% phosphotungstic acid (PTA) work equally well). Samples were placed in in 3% PTA (3% PTA in PBS; chemicals obtained from VWR) solution in a volume that was at least eight times the specimen size. Samples were rocked at room temperature for three days. SCANCO QC (SCANCO MicroCT 50) were performed after 3 days as Quality Control (QC; 30 microns) scans to check on the progress of sample staining (lower resolution scans prior to scanning on high resolution in order to save time and

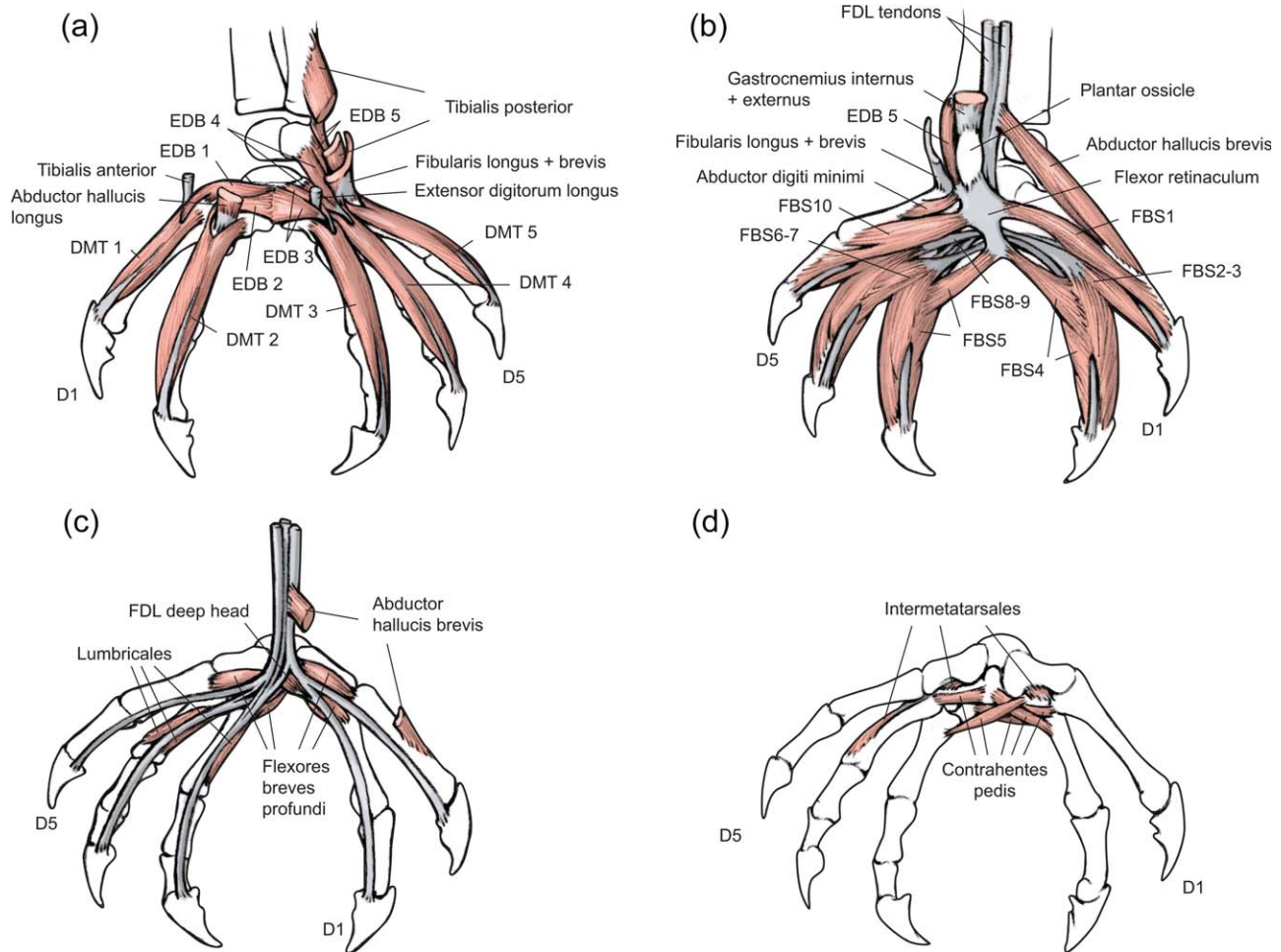


FIGURE 4 *Chamaeleo calytratus*, left pes. (a) Dorsal muscles of pes; (b) superficial ventral muscles of pes; (c) intermediate ventral muscles of pes; (d) deep ventral muscles of pes. Abbreviations: digit (d), dorsometatarsal (dmt), extensor digitorum brevis (edb), flexor brevis superficialis (fbs), flexor digitorum longus (fdl).

resources). If the sample was under-stained, it was placed in 5% PTA solution in a volume at least eight times the specimen size and rocked at room temperature for two days. If the sample was over-stained, it was placed back into PBS and rocked at room temperature and monitored every day or every other day by performing SCANCO QC scans. Once soft tissues had been optimally stained, we proceeded to high resolution scans (10 microns or less). Scanning parameters for our completed limbs were: 70 kVp, 114 uA, 8W, NO Filter, 1,000 projections, NO averaging. Raw DICOM files were imported into Amira 6.0 (FEI) for volume and orthoslice rendering. Three-dimensional animations of fully rendered limbs with contrast enhanced soft tissue were produced in Amira 6.0 and are included as two Supporting Information Movies. Two-dimensional figures showing surface anatomy (Figures 5–8) were exported from Amira and compiled in Adobe Creative Suite 6.

3 | RESULTS

The limb muscle anatomy of *C. calytratus* and its differences from that of *A. uniparens* are summarized in Tables 2 and 3 and Figures 1–4.

Below, we compare our results with the musculoskeletal anatomy of other lizards and chameleons, including the *T. melleri* specimen we dissected for this work and the results of previous studies. Except where explicitly noted, our observations agree with those of Mivart (1870) for the Parson's Chameleon (*Calumma parsonii*). However, we provide much greater detail about the autopodial muscles of chameleons than any previous study. Peterson (1973) described only the shoulder and proximal forelimb muscles of two chameleon species, *Chamaeleo dilepis* and *Chamaeleo senegalensis*. Mivart (1870) only briefly discussed the myology of the autopodia and described many fewer autopodial muscles than we do. Moreover, as was common practice at the time of its publication, Mivart's (1870) paper used names of human muscles to describe lizard muscles that are (as we know now) not homologous (see Tables 2 and 3). The nomenclature used in this manuscript, which mainly agrees with Russell and Bauer (2008), takes into account the evolution and homology of the appendicular muscles within all major tetrapod groups, facilitating direct comparisons between chameleons and other tetrapods (Diogo & Abdala, 2010; Diogo & Molnar, 2014) that are crucial for the establishment of chameleons for broader studies on development, macroevolution, and human teratology.

TABLE 2 Attachments of muscles of the pectoral appendage of *Chamaeleo calypttratus*, *Trioceros melleri*, and *Aspidoscelis uniparens* according to our dissections

Muscle and synonyms	<i>Chamaeleo calypttratus</i> (Veiled Chameleon)	Differences from <i>Trioceros melleri</i> (Meller's Chameleon)	Differences from <i>Aspidoscelis uniparens</i> (Desert Grassland Whiptail)
Serratus anterior (serratus magnus ¹ , serratus superficialis ⁶)	From ribs to posterior margin of scapula (2 dorsal and 1 ventral bellies)	No differences	No differences; (12) described 3 dorsal and 3 ventral bellies
Latissimus dorsi	From fascia of erector spinae muscles and ribs to latissimus dorsi process on medial aspect of head of humerus; lies posterior to trapezius	No differences	Lies mainly deep to trapezius; no origin from ribs
Costocoracoideus (costoscapularis ^{3,4} , costosternocoracoideus ^{4,5})	Not present as a distinct muscle	No differences	From anterior ribs to scapula, mainly via the sternoscapular ligament
Levator scapulae (upper part of levator scapulae ² ; part of levator clavicularis ¹)	From basioccipital and atlas to anterior margin of scapula and suprascapula	No differences	No origin from basioccipital
Levator clavicularis (lower part of levator scapulae ² ; part of levator clavicularis ¹)	Fused with levator scapulae	No differences	From atlas to suprascapula and scapula (and possibly also to clavicle)
Deltoideus scapularis (scapulodeltoideus ² ; suprascapularis 1 and 2 ¹ , scapulodeltoideus and suprascapularis ⁶)	Part 1 from anterior lateral scapula to head of humerus, and part 2 from lateral scapula and suprascapula to proximal portion of deltopectoral crest	No differences	One part only, from suprascapula and scapula to proximal humerus
Deltoideus acromialis et clavicularis (clavodeltoideus ^{2,6} ; deltoid 1 and 2 ¹ , sternocoracohumeralis ⁶)	Part 1 from coracoid and part 2 from sternum; both parts - usually named coracohumeralis anterior and sternohumeralis anterior in chameleons ² - insert on deltopectoral crest	No differences	Single head, mainly from clavicle but also from scapula/coracoid
Sternocoracoideus (sternocoracoid superior and inferior ³ ; sternocoracoideus externus and internus ²)	Only sternocoracoideus internus is present, from internal sternum to anterior medial coracoid	No differences	Additional origin from first ribs; also has an "externus" head, ventral to the internus head, from sternum to coracoid
Supracoracoideus (subclavius ¹ ; coracohumeralis ³)	From anterolateral coracoid and part of scapula to proximal humerus	No differences	No differences
Scapulo-humeralis anterior	From lateral scapula just dorsal to glenoid to proximal humerus between medial and lateral heads of triceps	No differences	No differences
Triceps coracoideus (coraco-triceps ⁶)	Not present as a distinct head of the triceps brachii, being either lost or fused with the other heads	No differences	From coracoid to proximal ulna
Triceps scapularis (triceps part 1 ¹ , scapulotriceps ⁶)	From posterior scapula just above glenoid and scapulohumeral ligament to olecranon of ulna	No differences	No differences
Triceps humeralis lateralis and medialis (part 2 and part 3 + internus, respectively ¹)	Medial head from whole lateral surface and part of anterior and posterior surfaces of humerus, internal head from humerus just distal to latissimus dorsi insertion, and lateral head from internal humerus at level of latissimus dorsi insertion; unite with triceps scapularis and insert on olecranon of ulna	No differences	No internus head
Pectoralis	From sternum, fascia of external oblique muscles, and sternal ribs 1–3 to deltopectoral crest of humerus	From sternal ribs 2 and/or 3	No differences

(Continues)

TABLE 2 (Continued)

Muscle and synonyms	<i>Chamaeleo calytratus</i> (Veiled Chameleon)	Differences from <i>Trioceros melleri</i> (Meller's Chameleon)	Differences from <i>Aspidoscelis uniparens</i> (Desert Grassland Whiptail)
Subcoracoscapularis (subscapularis plus subcoracoideus ^{4, 5} ; subscapularis 1 and 2 ¹)	Subscapular head (subscapularis 1 ¹) from medial scapula and subcoracoid head (subscapularis 2 ¹) from internal coracoid; both insert on medial tuberosity of humerus	No differences	No differences
Scapulo-humeralis posterior	Not observed	Not observed	Not observed
Biceps brachii	Via tendon from anterolateral coracoid beneath supracoracoideus which passes through fascial sling associated with pectoralis insertion, splits into medial and lateral bellies (blended with triceps); part inserts directly onto the proximal radius via a tendon, and part unites with brachialis to insert on radius and ulna	No differences	Single muscle belly
Brachialis (brachialis inferior ^{4, 5})	From entire anterolateral humerus distal to deltopectoral crest, in 2 or even 3 layers; unites with biceps brachii and inserts on proximal radius and ulna	No differences	Inserts mainly on ulna; only one layer
Coracobrachialis longus and brevis (parts 1 and 2, respectively ¹)	Brevis from external coracoid to proximal humerus between brachialis and triceps, and longus from posterior inferior corner of coracoid to distal humerus just above its medial condyle	No differences	No differences
Extensor carpi radialis (part 1 of extensor ossis metacarpi pollicis ¹ ; extensor radialis longus + extensor radialis brevis ¹¹)	From distal humerus to lateral MC1; blends distally with abductor pollicis longus	No differences	Fused with brachioradialis
Brachioradialis (supinator longus ¹)	From humeral lateral epicondyle to distal 2/3 of ventral radius with pronator teres; fused with extensor digitorum	No differences	No differences
Supinator (supinator brevis ²)	Not present as a distinct muscle, corresponding to part of the "supinator longus" of (1)	No differences	Not present as a distinct muscle
Extensor antebrachii et carpi ulnaris	Radial portion of flexor carpi ulnaris; sends tendon to MC5; not divided into antebrachial and carpal parts	No differences	From humerus to proximal MC5, partially fused with flexor carpi ulnaris
Extensor digitorum (extensor carpi radialis longior and brevoir ¹)	Via two bellies, from humeral lateral epicondyle and shaft of radius to MC3 and MC4; connected to brachioradialis	No differences	Single belly inserts on MC2–5
Epitrochleoanconeus (flexor antebrachii ulnaris ⁹ ; part of flexor carpi ulnaris ¹)	Not described as distinct muscle by (1) but described in chameleons by Gasc (1963); it runs from humeral medial condyle to proximal 1/4 of posterior ulna	No differences	No differences
Flexor digitorum longus (flexor pollicis longus + flexor profundus digitorum ¹)	Part 1 from medial epicondyle of humerus and tendon of pronator accessorius, connected to flexor carpi ulnaris, and inserting on distal phalanges of D2–5; part 2 from proximal proximal 1/2 of flexor surface of ulna and inserting on distal	Part 1 inserts on D4–5 only	Two bellies form a common tendon that splits to insert on the distal phalanx of D1–5; sesamoid in insertion tendon before split; deep head from carpals to tendon to D2

(Continues)

TABLE 2 (Continued)

Muscle and synonyms	<i>Chamaeleo calytratus</i> (Veiled Chameleon)	Differences from <i>Trioceros melleri</i> (Meller's Chameleon)	Differences from <i>Aspidoscelis uniparens</i> (Desert Grassland Whiptail)
	phalanges of D1–4		
Flexor carpi ulnaris (part of flexor antebrachii et carpi ulnaris ⁸ ; part of flexor carpi ulnaris ¹)	Via 3 heads, from distal ventral humerus via long tendon, posterior ulna, and humeral medial condyle to pisiform and flexor retinaculum; fused with flexor digitorum longus	No differences	Medial epicondyle of humerus to pisiform, mainly
Flexor carpi radialis	From humeral medial epicondyle via 2 tendons surrounding origin of pronator teres to MC1	Arises via a single tendon	Arises via a single tendon
Pronator accessorius	Via 2 heads, from humerus medial condyle and proximal 1/2 of radial border of ulna to distal 2/3 of flexor aspect of radius and radiale (the palmaris profundus 1 is probably present but fused with the pronator accessorius and/or the pronator quadratus)	No differences	No insertion on radius
Pronator quadratus (pronator profundus ^{7,8})	From distal 1/4 of radial flexor surface of ulna to distal 1/4 of ulnar flexor surface of radius	No differences	No differences
Pronator teres (flexor antebrachii radialis ¹⁰ ; part of the flexor carpi radialis ^{4,5})	From humeral medial epicondyle to distal 2/3 of radius, connected to biceps	No differences	No differences
Extensores digitorum breves (extensores III–IX ¹ ; extensores digitores breves superficiales ²)	Five muscles: extensor 1 (=extensor III ¹) from styloid process of radius to the proximal phalanx of D1; extensor 2 (=extensor IV ¹) from styloid process of radius and MC3–4 to the proximal phalanx of D2; extensor 3 (=extensor V ¹) from distal ulna, ulnare, and MC3–4 to the proximal phalanges of D2 and D3; extensor 4 (=extensor VI ¹) from MC3–4 to the proximal phalanx of D4; and extensor 5 (=extensor VII+VIII+IV ¹) with various bundles from distal ulna, ulnare, and MC3–4 to MC5 and the proximal phalanx of D5	No differences	Five muscles from carpal region to the proximal phalanx of each digit
Abductor pollicis longus (part 2 of extensor ossis metacarpi pollicis ¹)	From shaft of radius and ulna to MC1	No differences	Origin from ulna and ulnare
Dorsometacarpales (extensores phalangorum ¹ ; extensores digitores breves profundi ²)	Five muscles, from MC1–5 to the distal phalanx of respective digits	No differences	No differences
Intermetacarpales (part of interossei ¹ ; interossei dorsalis ²)	Two muscles: one from MC2 to MC1 and the proximal phalanx of D1; one from MC3 to MC2 and the proximal phalanx of D2	Three muscles: one from MC2 to MC1 and the proximal phalanx of D1; one from MC3 to MC2 and the proximal phalanx of D2; 1 from MC5 to MC4 and the proximal phalanx of D4	Four muscles between metacarpals, also going to the distal phalanx of at least some digits
Flexores breves superficiales (flexor brevis digitorum and adductor digiti tertii and adductor digiti quarti ¹ ; flexores digitores breves ²)	Ten muscles (two for each digit, to radial and ulnar sides of the digit) from flexor retinaculum and plantar aponeurosis to proximal, middle, and distal phalanges of D1–5	No differences	Ten muscles that insert on the proximal phalanx of D1–5 (2 muscles for each digit), and then fuse with tendons of flexor digitorum longus, thus inserting also on the middle and distal phalanges

(Continues)

TABLE 2 (Continued)

Muscle and synonyms	<i>Chamaeleo calytratus</i> (Veiled Chameleon)	Differences from <i>Trioceros melleri</i> (Meller's Chameleon)	Differences from <i>Aspidoscelis uniparens</i> (Desert Grassland Whiptail)
Lumbricales (palmar heads of flexor digitorum longus ²)	Two, from tendons of flexor digitorum longus to D3 and D4 to ulnar sides of the first phalanx of D3 and D4, respectively	Two, from tendon of flexor digitorum longus to D4 to radial and ulnar sides of the first phalanx of D4	Five, from tendons to D2–4 to radial sides of D3–4 and ulnar sides of D2–4
Contraahentes (probably correspond to part of interossei ¹ ; part of lumbricales ²)	Five muscles: four from contraahens fascia and ligaments connecting MC3–4 to the proximal phalanx of D1, D2, and D3 (2 muscles), plus one from MC5 to the proximal phalanx of D4	No differences	Mainly from contraahens fascia to D1–5
Flexores breves profundi (part of interossei ¹ ; flexores digiti brevis profundus ⁷ ; interossei ventrales and lumbricales ²)	Five muscles from MC1–5 to proximal phalanx of the respective digit	No differences	No differences
Abductor pollicis brevis (flexor brevis pollicis ¹)	From radiale and flexor retinaculum to radial margin of the proximal phalanx of D1	No differences	No differences
Abductor digiti minimi (flexor brevis minimi digiti ¹ ; abductor digiti quinti ²)	From pisiform to MC5	No differences	No differences

Aspidoscelis uniparens represents anatomically more generalized, terrestrial lizards that have no syndactyly or zygodactyly in the limbs (N.B., to make these tables simpler, only differences between the latter species and *T. melleri* are described in the right-hand column). Abbreviations: D = digit/digits, MC = metacarpal(s), MT = metatarsal(s). References: 1 (Mivart, 1870); 2 (Russell & Bauer, 2008); 3 (Howell, 1936); 4 (Holmes, 1977); 5 (Dilkes, 1999); 6 (Peterson, 1973); 7 (Abdala & Moro, 2006); 8 (Diogo & Abdala, 2007); 9 (Jouffroy & Lessertisseur, 1971); 10 (Ribbing, 1907); 11 (Straus, 1941a, 1941b); 12 (Fisher & Tanner, 1970).

3.1 | Shoulder and arm

The dorsal shoulder muscles in chameleons (exemplified by *Chamaeleo calytratus*) are highly modified compared to those of most other lizards (exemplified by *Aspidoscelis uniparens*), and these modifications extend to the muscles anchoring the girdle to the body wall (Figures 1 and 5; Supporting Information Movie 1; Table 2). The *m. serratus anterior* in *Chamaeleo calytratus* has only three bellies compared with the six described by Fisher and Tanner (1970) in teiid lizards such as *A. uniparens* (however, we only observed two bellies of this muscle in our specimens). The *m. latissimus dorsi* lies posterior to rather than deep to the reduced *m. trapezius*, and its area of origin includes not only the vertebrae but several thoracic ribs as well. The *m. costocoracoideus* in *C. calytratus* is reduced to a membrane between the first rib and the shoulder girdle. Because adult chameleons normally lack a clavicle (Skinner, 1958), the shoulder muscles that attach to the clavicle in *A. uniparens*, the *m. levator claviculae* and *m. deltoideus acromialis et clavicularis*, usually attach to the coracoid and/or sternum in chameleons. The *m. levator scapulae* in *C. calytratus* and *T. melleri* is fused with the *m. levator claviculae* and originates from both the atlas and basioccipital, whereas it originates from the atlas only in most other lizards (Russell & Bauer, 2008) and from the basioccipital only according to Mivart's (1870) description of *C. parsonii*. Furthermore, as pointed out by Peterson (1973), this muscle has longer, more diagonal fibers in arboreal lizards such as chameleons and shorter, more horizontal fibers in terrestrial lizards such as *Agama* and *Aspidoscelis*.

In *C. calytratus*, the *m. deltoideus scapularis* is divided into two parts, posterior/superficial and anterior/deep, which insert adjacent to each other on the humerus. Likewise, the *m. deltoideus acromialis et clavicularis* has dorsal and ventral parts, but they are only separable at their origins. Peterson (1973) noted that these muscles tend to insert more proximally on the humerus in arboreal lizards than terrestrial ones, and the specimens we examined also follow this trend. The *m. sternocoracoideus* in *C. calytratus* has a single internal head that originates from the internal sternum, but in most other lizards it has both internal and external heads and originates from both sternum and ribs (Russell & Bauer, 2008). The *m. scapulohumeralis anterior* is present in *C. calytratus* and *T. melleri*, contra Mivart's (1870) description of *C. parsonii*, originating from the lateral scapula just dorsal to the glenoid and passing underneath the scapulohumeral ligament to insert on the proximal humerus between the medial and lateral heads of triceps. This muscle has a similar configuration in *A. uniparens*. Moving more distally in the arm, the configuration of the *m. triceps* in chameleons is also different from that in other lizards. In chameleons the coracoid head of *m. triceps* is either absent or completely fused with other heads; the single long head of *m. triceps*, which originates from the posterior scapula just above the glenoid and the scapulohumeral ligament, corresponds mainly to the scapular head of other lizards (Peterson, 1973). The chameleons we studied also possess a third humeral head of *m. triceps* (triceps internus sensu Mivart, 1870) that originates from the humerus just distal to the insertion of *m. latissimus dorsi*.

TABLE 3 Attachments of muscles of the pelvic appendage of *C. calyptratus*, *T. melleri*, and *A. uniparens* according to our dissections

Muscles and synonyms	<i>Chamaeleo calyptratus</i> (Veiled Chameleon)	Differences from <i>Trioceros melleri</i> (Meller's Chameleon)	Differences from <i>Aspidoscelis uniparens</i> (Desert Grassland Whiptail)
Ischiocaudalis	From ventral part of caudal vertebrae 4–12 to tuberosity of ischium	No differences	No differences
Iliocaudalis	From the sacro-iliac attachment and sacrum to caudal vertebrae	No differences	No differences
Iliotibialis (rectus femoris 2 and 3 ¹)	Via two heads, from anterior ilium to cnemial crest of tibia via common extensor tendon	No differences	Via a single head
Iliofemoralis (gluteus primus, secundus, and tertius and pectineus ¹)	Part 1 from ilium between two heads of iliotibialis to lateral femur; part 2 from posterolateral ilium to proximal part of greater trochanter of femur; part 3 from ilium just dorsal to acetabulum to distal part of greater trochanter of femur	No differences	A single muscle from ilium to femur
Tenuissimus (ilioperoneal ¹ ; iliobularis ²)	From external ilium to middle portion of lateral fibular shaft	No differences	No differences
Puboischiofemoralis internus (iliacus ¹)	Part 1 from anterior pubis, wraps around ventral aspect of femoral head, to posterior proximal 1/2 of femur; part 2 from anterior internal pubis to proximal lateral femur; part 3 from internal anterior pubis and ischium to proximal lateral femur	No differences	No differences
Femorotibialis (vastus externus, vastus internus, and crureus ¹)	Via three heads, from medial femur and lateral femur to patella with other extensors	No differences	No differences
Sartorius (rectus femoris ¹ ; ambiens ²)	From ventral margin of acetabulum to common extensor tendon	No differences	No differences
Caudofemoralis longus (femoro-caudal ¹)	From caudal vertebrae 1–5 to greater trochanter and, via long tendon, to posterior surface of knee joint	No differences	From caudal vertebrae 1–16
Caudofemoralis brevis (gluteus maximus ¹)	From caudal vertebrae to ilio-ischiatic ligament	No differences	Inserts on greater trochanter
Ischiotrochantericus (obturator internus ¹ ; puboischiofemoralis posterior ³)	From internal pelvis to posterior proximal femur	No differences	No differences
Puboischiofemoralis externus (quadratus femoris and obturator externus ¹ ; includes iliacus, pectineus, obturator internus, obturator externus and adductor brevis of various authors ²)	Part 1 from ischium to head of femur; part 2 from ischio-pubic symphysis, deep to adductor femoris, to greater trochanter	No differences	No differences
Pubotibialis (tibial adductor ¹)	From anterior pubis between parts 2 and 3 of puboischiofemoralis to insertion tendon of part 1 of flexor tibialis internus	No differences	No differences
Adductor femoris (adductor ¹)	From ventral midline of pubis-ischium and associated ligament to middle third of posterior femur	No differences	From pubo-ischiadic ligament
Gracilis (puboischiotibialis ²)	From ventral midline of pubis-ischium to proximal medial tibia	No differences	From pubo-ischiadic ligament
Flexor tibialis internus (semitendinosus and semimembranosus ¹)	Part 1 (semitendinosus ¹) from ilio-ischiatic ligament to posterior aspect of inter-articular cartilage of knee and proximal tibia with pubotibialis; part 2 (semimembranosus ¹) via tendon from internal ischium to proximal tibia	No differences	No differences
Flexor tibialis externus (biceps ¹); includes flexor cruris et tarsi tibialis	From tendinous arch to fibula with tenuissimus and, via long tendon, to plantar ossicle	No differences	No differences

(Continues)

TABLE 3 (Continued)

Muscles and synonyms	<i>Chamaeleo calyptatus</i> (Veiled Chameleon)	Differences from <i>Trioceros melleri</i> (Meller's Chameleon)	Differences from <i>Aspidoscelis uniparens</i> (Desert Grassland Whiptail)
Extensor digitorum longus	Via two heads, from anterior distal femur via tendon and from tibia to MT3	No differences	Origin from femur only; inserts on MT2 and MT3
Tibialis anterior (tibialis anticus ¹)	From anteromedial tibia, fused with extensor digitorum longus, to MT1	No differences	Not fused with extensor digitorum longus
Fibularis brevis (part of peroneus ¹)	Not present as a distinct muscle, i.e. it is completely fused with the fibularis longus	No differences	From proximal fibula to MT5
Fibularis longus (part of peroneus ¹)	Via two heads, from middle 1/3 of anterolateral fibula and proximal 1/2 of lateral tibia to MT4–5	No differences	From distal femur
Gastrocnemius internus (femorotibial gastrocnemius ²)	From proximal half of posterior tibia to plantar ossicle and plantar aponeurosis	No differences	Origin from both femur and tibia; no sesamoid
Gastrocnemius externus (femoral gastrocnemius ²)	From lateral condyle of femur via long tendon to plantar ossicle, base of MT5, and plantar aponeurosis, with flexor tibialis externus tendon	No differences	Originates from proximal tibia; no sesamoid
Flexor digitorum longus (flexor digitorum longus, flexor hallucis longus, and flexor tertius digiti ¹)	Part 1 from fibular epicondyle and fibula to D3–5; part 2 from anterior and medial fibula to D1–2 and D4; part 3 from femur and proximal tendon of gastrocnemius externus to D3–4; plantar head from MT4 to tendon of FDL to D2	Part 1 goes to D3–4; part 2 goes to D1–3; part 3 goes to D3–5	Single muscle from femur, fibula and fibulare +intermedium to D1–5; large sesamoid bone proximal to tendon split; plantar heads to D2–5
Tibialis posterior (tibialis posticus ¹ ; pronator profundus ²)	From lateral distal fibula to proximal MT4 and MT5	No differences	Inserts on MT1; small sesamoid bone embedded in insertion tendon
Popliteus	From proximal fibula to most of medial tibia	No differences	No differences
Interosseus cruris (peroneotibial ¹)	From distal 1/3 fibula to distal 1/4 tibia	No differences	No differences
Abductor hallucis longus (extensores I and II ¹ ; adductor et extensor hallucis et indicus ²)	From distal 2/3 of anteromedial fibula to MT2	No differences	Single belly from distal end of fibula; inserts on MT1
Extensores digitorum breves (extensores III–IX ¹)	Five muscles: extensor 1 (extensor III ¹) from MT3 to proximal phalanx of D1; extensor 2 (extensor IV ¹) mainly from MT2–3 to proximal phalanx of D2; extensor 3 (extensor V ¹) mainly from MT2–3 to proximal phalanx of D3; extensor 4 (extensor VI ¹) mainly from fibulare +intermedium and MT3 to proximal phalanx of D4; extensor 5 (extensores VII+VIII+IX ¹) via several bundles from fibula and fibulare +intermedium to MT5 and flexor retinaculum	No differences	Mainly from carpal region, insert onto the distal phalanx of each digit with dorsometatarsals
Dorsometatarsales (extensores phalangorum ¹)	Five muscles from distal portion of MT1–5 via 2 heads to the distal phalanx of D1–5	No differences	No differences
Abductor hallucis brevis (flexor brevis hallucis ¹ , flexor hallucis ²)	From tendons of flexor digitorum longus to MT1 and the proximal phalanx of D1	No differences	Not observed
Abductor digiti minimi (flexor brevis minimi digiti ¹ , abductor digiti quinti ²)	From plantar ossicle to the proximal phalanx of D5	No differences	No differences
Flexores breves superficiales (flexor brevis digitorum, adductor digiti secundi, and adductor digiti tertii ¹)	Ten muscles from flexor retinaculum and plantar aponeurosis, ligament between MT2–3 and surrounding connective tissues to medial and lateral aspects of proximal, middle, and distal phalanges of D1–5	No differences	From aponeurosis of the deep head of the gastrocnemius externus to D1–4 and possibly also D5
Lumbricales	Three: 2 from tendon of FDL 4 to tibial and fibular sides of D4; 1 from tendon of FDL 3 to tibial side of D3	Four: additional lumbrical to fibular side of D3	Five: additional lumbrical to D2

(Continues)

TABLE 3 (Continued)

Muscles and synonyms	<i>Chamaeleo calytratus</i> (Veiled Chameleon)	Differences from <i>Trioceros melleri</i> (Meller's Chameleon)	Differences from <i>Aspidoscelis uniparens</i> (Desert Grassland Whiptail)
Contraahentes pedis	Three muscles from contraahens fascia/metatarsals to the proximal phalanx of D1–2 and 4	No differences	Four muscles to digits 1–4
Flexores breves profundi (part of interossei ¹)	Five muscles from MT1–5 to proximal phalanges of respective digits	No differences	No differences
Intermetatarsales (part of interossei ¹)	Three muscles, reduced in size, mainly between adjacent metatarsals except for the one between MT3–4 which inserts on the proximal phalanx of D3	No differences	Four muscles between four metatarsals, inserting mainly onto the proximal phalanx of D1–5

Abbreviations as in Table 1. References: 1 (Mivart, 1870); 2 (Russell & Bauer, 2008); 3 Gregory & Camp, 1928.

The ventral shoulder muscles are similar between *C. calytratus* and *A. uniparens*, but the ventral arm muscles are noticeably different. The origin and insertion of the *m. biceps brachii* in *C. calytratus* are the same as they are in *A. uniparens*, but its architecture is different. In *A. uniparens*, the *m. biceps brachii* has a tendinous origin and a single belly that inserts on the radius. However, the probable ancestral condition in lepidosaurs is a fleshy origin and an intermediate tendon that divides the muscle into proximal and distal bellies (contrary to the condition in mammals, which often have medial and lateral heads that are separate proximally) (Russell & Bauer, 2008). As in *A. uniparens*, the *biceps brachii* in *C. calytratus* and *T. melleri* has a tendinous origin and lacks an intermediate tendon, but it has distinct medial and lateral bellies that are separate distally, near the muscle's insertion onto the forearm bones. The *m. brachialis*, which blends distally with the *m. biceps brachii*, has distinct superficial and deep layers in *C. calytratus* but only a single layer in *A. uniparens*. Two

layers of the *m. brachialis* were also described in *Gekko* (Lecuru-Renous, 1968).

3.2 | Dorsal (extensor) forearm

Many of the muscles in the dorsal forearm of *C. calytratus* are divided into multiple distinct bellies (Figures 1 and 5; Supporting Information Movie 1; Table 2). The *extensor carpi radialis* and *brachioradialis* are fused in *A. uniparens* and in many other lizards as well (Russell & Bauer, 2008), but in *C. calytratus* they are well separated: the former muscle arises from the humerus as usual but blends distally with the *m. abductor pollicis longus*. Also, in *C. calytratus*, the *m. extensor antebrachii et carpi ulnaris* lacks an individual origin, consisting of a second belly that splits from the *flexor carpi ulnaris* in the distal forearm and inserts onto metacarpal 5. The *m. extensor digitorum* in *A. uniparens* consists of a single belly that originates from the ulnar epicondyle of the humerus and

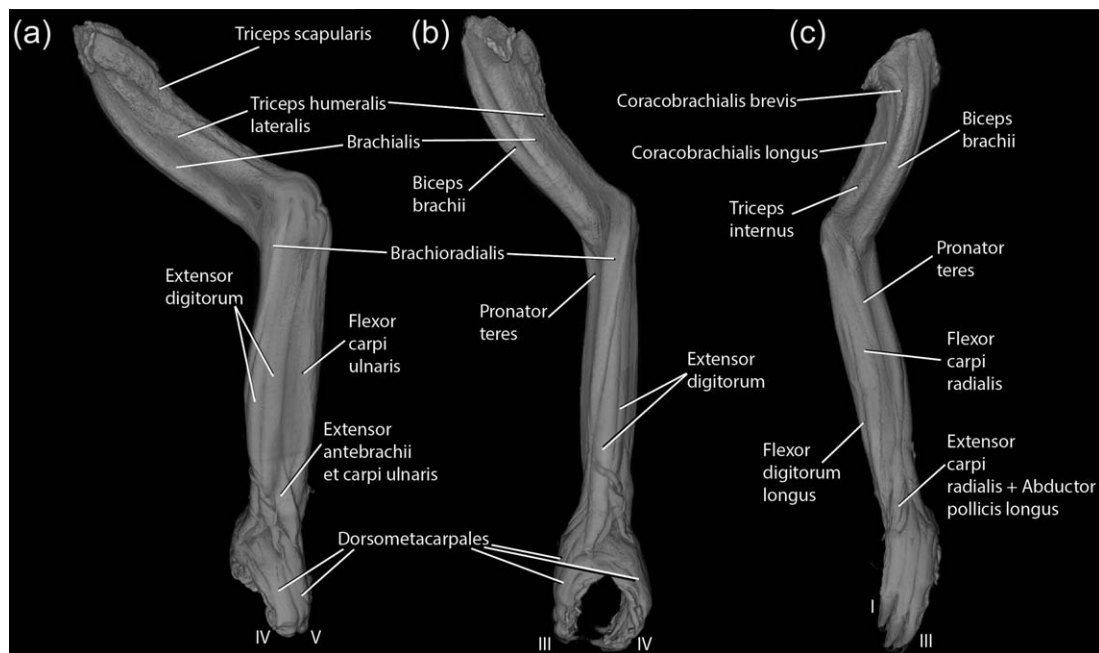


FIGURE 5 (a) Lateral, (b) anterior, and (c) medial views of Phosphotungstic Acid (PTA) contrast enhanced forelimb of an adult female Veiled Chameleon (*Chamaeleo calytratus*; left forelimb, skinned)

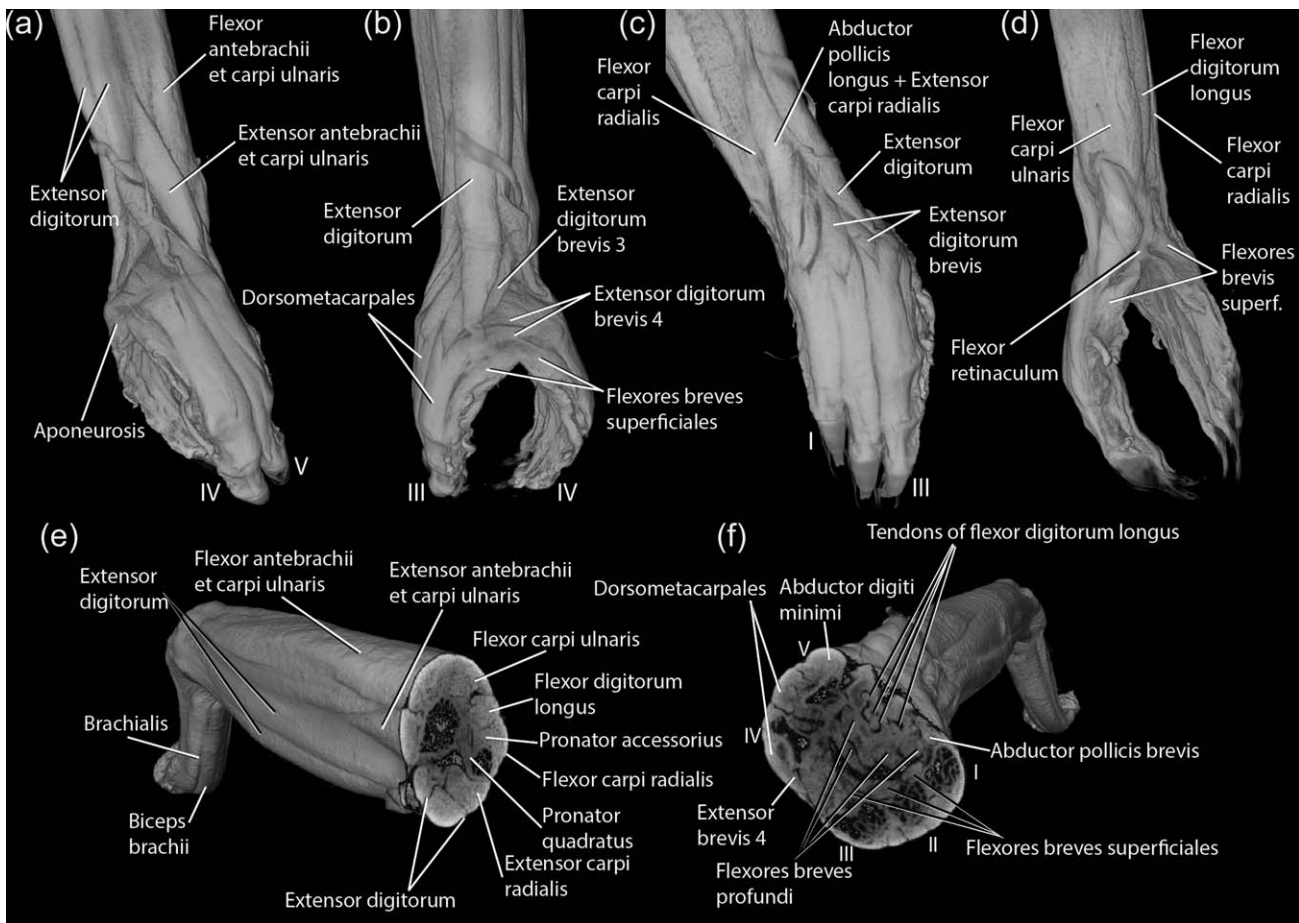


FIGURE 6 (a) Lateral, (b) anterior, (c) medial, and (d) posterior views of the left forelimb autopodium of the same female *Chamaeleo calyptatus* from Figure 1. (e) is a volume rendering/orthoslice (Amira 6.0) of the contrast enhanced limb through the anterior zeugopod (radius/ulna), while (f) is a transverse slice at the level of the proximal metacarpals

inserts onto metacarpals 2–5. In *C. calyptatus*, this muscle has two distinct parts (extensor carpi radialis longior and brevior sensu Mivart, 1870) that originate together from the medial condyle of the humerus and split in the proximal forearm, where they are connected to the shaft of the radius. Both parts pass beneath an extensor retinaculum before inserting separately onto metacarpals 3 and 4. Russell and Bauer (2008) referred to an additional insertion onto metacarpal 5 in chameleons, but this was not the case in the chameleons dissected by us (*T. melleri* and *C. calyptatus*) or by Mivart (1870) (*C. parsonii*).

3.3 | Ventral (flexor) forearm

Like its counterpart on the dorsal forearm, the *m. flexor digitorum longus* consists of two distinct bellies (flexor pollicis longus and flexor profundus digitorum sensu Mivart, 1870) whose tendons split and exchange slips in the wrist (Figures 1 and 5; Supporting Information Movie 1; Table 2). In *C. calyptatus*, the tendons from more radial belly (part 1) insert on the distal phalanges of digits 2–5, while those from the more ulnar belly (part 2) insert on digits 1–4. In *C. parsonii*, however, the more radial belly serves digits 4–5 only (Mivart, 1870), and the same pattern appeared to be present in *T. melleri*. The more

radial belly is fused with the *m. flexor carpi ulnaris*. It originates from the radial epicondyle, the origin tendon of the *m. pronator accessorius* (Mivart, 1870 did not describe this connection in *C. parsonii*, but it was present in both chameleon species we dissected), and the ulnar shaft. The more ulnar belly originates from the proximal ulna. The *m. flexor carpi radialis* arises via two tendons that surround the origin of the *m. flexor digitorum longus* in *C. calyptatus* and in *C. parsonii* according to Mivart (1870), but in both forelimbs of our specimen of *T. melleri* (and in *A. uniparens*) the ulnar tendon was absent. In *A. uniparens*, a palmar head joins the tendon of *m. flexor digitorum longus* to digit 2, but we did not observe this head in either chameleon we dissected.

The *m. flexor carpi ulnaris* has multiple heads of origin in *C. calyptatus*: in addition to the normal origin from the medial epicondyle of the humerus, it also attaches via a long tendon to the elbow joint capsule and fleshily to the posterior ulna. In addition to inserting on the pisiform, as it does in *A. uniparens*, the muscle also sends a slip to the *m. flexor digitorum longus* and attaches to the flexor retinaculum. The *m. pronator accessorius* in *C. calyptatus* inserts not only onto the radiale, as it does in *A. uniparens* and in many other lizards (Russell & Bauer, 2008), but also extensively onto the distal 2/3 of the radius.

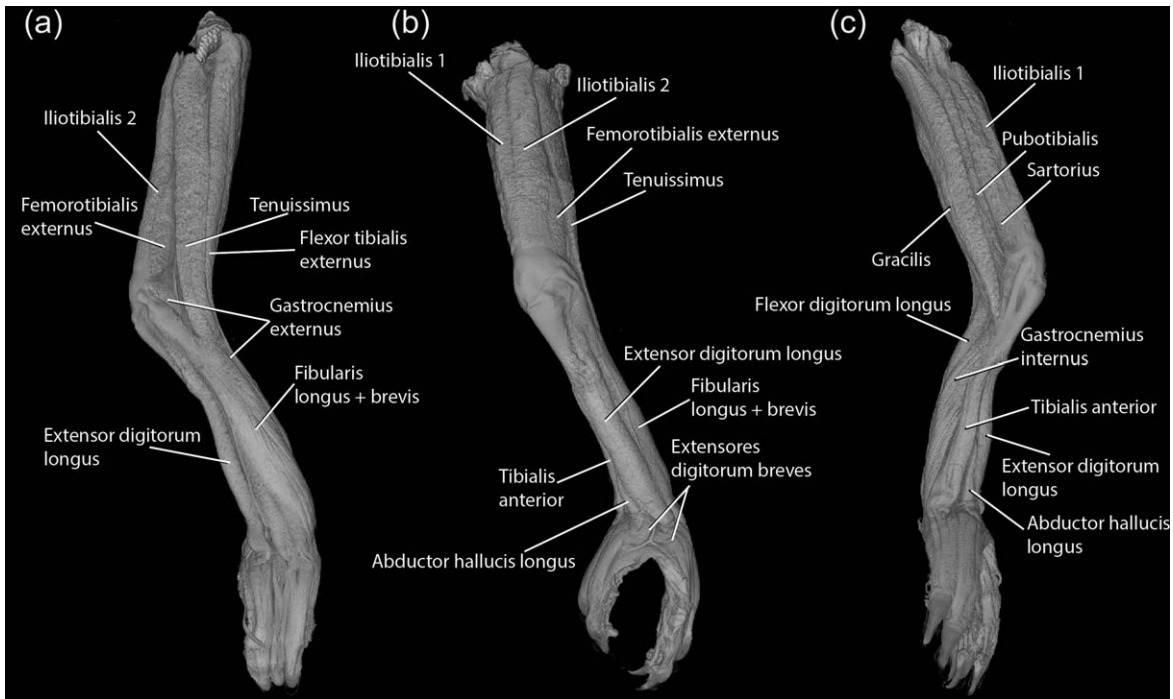


FIGURE 7 (a) Lateral, (b) anterior, and (c) medial views of a PTA Contrast Enhanced left hindlimb (skinned) from the same adult female *Chamaeleo calypttratus* used in Figures 1 and 2

3.4 | Dorsal wrist and manus

Five *mm. extensores digitorum breves* are present in *C. calypttratus*, as in other lizards, but several have multiple divisions that could be counted separately (Figures 1, 2, 5, and 6; Supporting Information Movie 1; Table 2). For example, Mivart (1870) describes three muscles inserting on digit 5, but as they are fused with each other we count them as divisions of a single short extensor to digit 5. Mivart (1870) described the *mm. extensores digitorum breves* (his “extensores III-IX”) as inserting on the metacarpals, but in our specimens they inserted mainly on the proximal phalanges, as in *A. uniparens*, between the two heads of the *mm. dorsometacarpales* of the respective digits. The exception is the deep portion of the muscle to digit 5, which also inserts on metacarpal 5. The muscle to digit 1 (extensor III sensu Mivart, 1870) originates from the styloid process of the radius. The muscles to digits 2, 3, and 5 (extensores IV, V, and VII + VIII + IX sensu Mivart, 1870) originate via three heads each from the distal ulna, ulnare, and metacarpals 3–4. The muscle to digit 4 (extensor VI sensu Mivart, 1870) originates solely from metacarpals 3–4. The *m. abductor pollicis longus* in *C. calypttratus* originates from the radius and ulna rather than the ulna and ulnare as in *A. uniparens*. The *m. intermetacarpales* are present as usual, except that the muscles between metacarpals 3–4 (where the manus is cleft) and 4–5 are missing in *C. calypttratus* (the latter muscle is present in *T. melleri*).

3.5 | Ventral (palmar) manus

The normal complement of ten *mm. flexores breves superficiales* are present in *C. calypttratus*, but their configuration is somewhat different from that in *A. uniparens* (Figures 1, 2, 5, and 6; Supporting Information

Movie 1; Table 2). First, rather than inserting onto the tendons of the *m. flexor digitorum longus*, these muscles insert directly onto the radial and ulnar aspects of proximal, middle, and distal phalanges. Second, the flexors that lie between syndactylous digits (2–3, 4–5, and 8–9) are fused to each other and arise via shared tendons and split at the level of the proximal phalanges. Third, the *m. flexor retinaculum* from which the *mm. flexores breves superficiales* originate (annular ligament sensu Russell & Bauer, 2008) extends much further distally and dorsally than in *A. uniparens* or other lizards, forming a structure that resembles an extensive V-shaped palmar aponeurosis. The retinaculum is connected to the ligaments between metacarpals 3–4 on the dorsum of the manus and divides the palm into the medial and lateral portions related to the zygodactyl and opposable manus of chameleons. The two *mm. flexores breves superficiales* on either side of the cleft between digits 3 and 4 (FBS 6 and 7) originate from the proximal extreme of this palmar aponeurosis, so their proximal portions are oriented obliquely, almost transversely, in fact, to the axis of the limb.

While five *mm. lumbricales* are present in *A. uniparens*, originating from the insertion tendons of *m. flexor digitorum longus* to digits 2–5 and inserting on the respective digits, only two or three *mm. lumbricales* appear to be present in chameleons. In *C. calypttratus*, two muscles are present, originating from the tendons to digits 3 and 4 and inserting on the ulnar aspects of the proximal phalanges of the respective digits. In *T. melleri*, both *mm. lumbricales* were associated with digit 4, and in *C. parsonii* an additional *m. lumbricalis* inserting on the radial aspect of digit 3 was described (Mivart, 1870). Mivart (1870) did not describe *contrahentes* muscles in *C. parsonii* (probably because he did not focus on the autopodia), but these muscles were present in all the chameleons we dissected. Their configuration is very similar to that of other

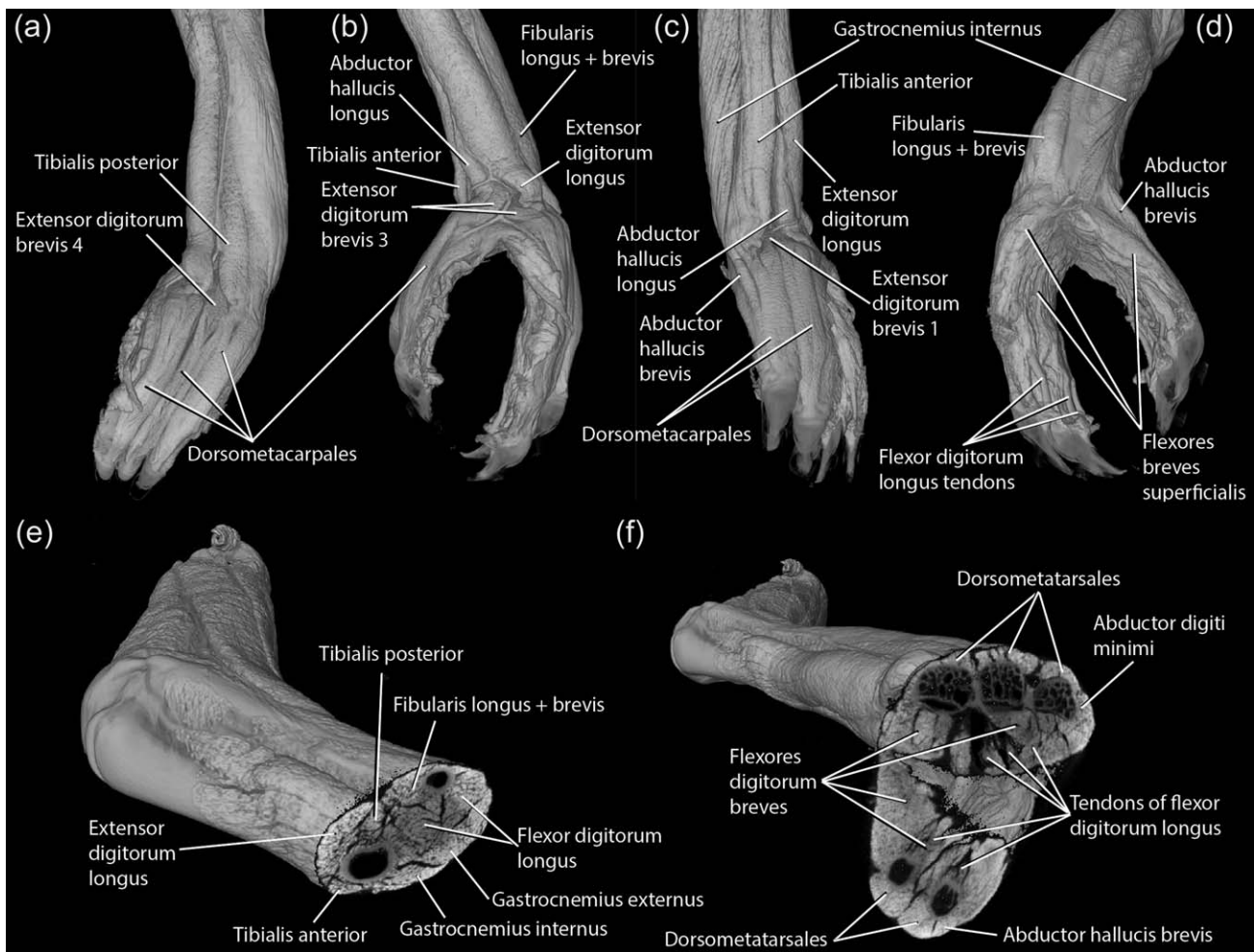


FIGURE 8 (a) Lateral, (b) anterior, (c) medial, and (d) posterior views of the hindlimb autopodium of the adult female *Chamaeleo calypttratus* used for Figures 1–3. (e) is a volume and orthoslice rendering through the mid zeugopod (tibia, fibula), while (f) is a section through the proximal metatarsals

lizards and tetrapods in general: they arise mainly from the *contrahens* fascia and insert onto the proximal phalanges. However, in the chameleons we dissected, the *contrahens* to digit 4 originates from metacarpal 5, and in the place of a *contrahens* to digit 5 is a second *contrahens* to digit 3, also originating from metacarpal 5.

3.6 | Hip and thigh

The muscles of the hip are not as modified as those of the shoulder in *C. calypttratus*, but those in the distal region of the hindlimb are modified in very similar ways as those in the distal region of the forelimb (Figures 3 and 7; Supporting Information Movie 2; Table 3). Notably, as in the forelimb (see above), in terms of the number and identity the hindlimb muscles in *C. calypttratus* are much more similar to those of in other lizards than might be expected in the face of the functional peculiarities of chameleons. The main difference between the hip muscles in *C. calypttratus* versus *A. uniparens* and other lizards (as described by Russell & Bauer, 2008 and references therein) is that in the former the larger muscles have a greater number of distinct divisions. The *m. iliobtibialis* in *C. calypttratus* arises via two heads rather than one (as is the case in *A.*

uniparens and most other lizards). Between the origins of the two heads is the origin of part 1 of the *m. iliofemoralis*, which lies between the two bellies until it inserts on the middle portion of the lateral aspect of the femur. The *m. iliofemoralis* (which comprises a single undivided muscle in *A. uniparens*) has two additional parts that originate posterior to the *m. iliobtibialis* and insert near the head of the femur. The *m. puboischiofemoralis internus*, like its counterpart in other lizards, has three parts that originate from the medial aspect of the pelvic girdle and insert on the proximal femur and femoral trochanter. Mivart (1870) described a partial origin from the ilium in *C. parsonii*, but Russell and Bauer's (2008) review of the literature on other chameleons and our observations of *T. melleri* and *C. calypttratus* show an origin from the pubis and ischium only.

Regarding the ventral musculature of the hip and thigh, the origins of the *m. adductor femoris* and *m. gracilis* in *C. calypttratus* encompass not only the pubo-ischiadic ligament, as in other lizards, but also part of the pubo-ischiadic symphysis. The extent of origin of the *m. caudofemoralis longus* spans only the first five caudal vertebrae in *C. calypttratus*, whereas in *A. uniparens* it originates from the first 16 (Oldham, 1975).

3.7 | Dorsal (extensor) leg

The *m. extensor digitorum longus* in *C. calypttratus* has a secondary fleshy head originating from the tibia in addition to the tendon attaching to the distal femur that is found in *A. uniparens* (Figures 3 and 7; Supporting Information Movie 2; Table 3). It inserts onto metatarsal 3 only, not metatarsal 2–3 as in *A. uniparens*. This muscle is extensively fused with the *m. tibialis anterior* in *C. calypttratus*. As in many other lizards (Russell & Bauer, 2008) (but not *A. uniparens*), the *m. fibularis longus* and *brevis* are not well separated in *C. calypttratus*, but unlike in most other lizards the *m. fibularis* complex is not attached to the femur. The *m. fibularis* complex in *C. calypttratus* inserts partially onto metatarsal 4 as well as metatarsal 5.

3.8 | Ventral (flexor) leg

The origins of the ventral leg muscles are similar between *C. calypttratus* and *A. uniparens*, but their insertions are very different (Figures 3 and 7; Supporting Information Movie 2; Table 3). The *m. gastrocnemius internus* lacks its usual origin from the femur and arises from the proximal tibia only. It inserts, with the *m. gastrocnemius externus* and tendon of *m. flexor tibialis externus*, onto a sesamoid bone that we designate the “plantar ossicle” (following Mivart, 1870; see Discussion), as well as onto the plantar aponeurosis and the base of metatarsal 5 as in *A. uniparens*. The *m. flexor digitorum longus* in the hindlimb of *C. calypttratus*, like its counterpart in the forelimb, is composed of multiple bellies whose tendons branch and recombine in the ankle region. Unlike in *A. uniparens*, there are no additional sesamoid bones in any of these tendons. The complex consists of three parts: one from the fibular epicondyle and fibula, the second from the fibular shaft, and the third from the distal femur, fibula, and the proximal part of *m. gastrocnemius externus*. In *C. calypttratus*, the first part inserts on the distal phalanges of digits 3–5, the second on digits 1–2 and 4, and the third on digits 3–4. In *T. melleri*, part 1 serves digits 3–4, part 2 serves digits 1–3, and part 3 serves digits 3–5, as described for *C. parsonii* (Mivart, 1870). There is no connection between the *m. flexor digitorum longus* and the tarsal bones in the chameleons we dissected. *Musculus flexor digitorum longus* receives multiple plantar heads in *A. uniparens*, but only a single head from metatarsal 4 to the tendon to digit 2 in *C. calypttratus* and *T. melleri* (Mivart, 1870 did not describe any plantar heads of this muscle). The insertion of the *m. tibialis posterior* is strikingly different in *C. calypttratus*: it inserts onto metatarsals 4–5, whereas in *A. uniparens* it inserts mainly onto metatarsal 1 via a tendon in which a small sesamoid is embedded. This muscle often also inserts onto metatarsals 1–3 and distal tarsal 4 in other lizards (Russell & Bauer, 2008).

3.9 | Dorsal ankle and pes

The *m. abductor hallucis longus* in *C. calypttratus* inserts not onto metatarsal 1, as the name would indicate and as it does in *A. uniparens*, but on metatarsal 2 instead (Figures 3, 4, 7, and 8; Supporting Information Movie 2; Table 3). This muscle is partially divided and has a larger and more proximal area of origin in *C. calypttratus*: from the distal 2/3 of the fibula rather than the distal end of the fibula as in *A. uniparens*. The

mm. extensores digitorum breves in the pes of *C. calypttratus* resemble their topological equivalents in the manus. As in *A. uniparens*, these five muscles originate from the tarsal region and insert on the digits. However, the origins and insertions of the individual muscles are modified, and the hindlimb short *mm. extensores* in chameleons (both those we dissected and that described by Mivart, 1870) tend to originate and insert more proximally than those in other lizards. In *C. calypttratus*, the *m. extensor* to digit 1 originates from metatarsal 3 and inserts on the first phalanx. The muscles to digits 2 and 3 originate from the proximal ends of metatarsals 2–3 and insert on the proximal phalanges. The muscle to digit 4 originates from the fibulare + intermedium and the abovementioned connective tissue and inserts on the proximal phalanx. The muscle to digit 5 originates from the fibula and fibulare + intermedium and inserts on metatarsal 5 and wraps around to the ventral aspect of the ankle to insert on the plantar aponeurosis.

3.10 | Ventral (plantar) pes

The *m. abductor hallucis brevis* is absent in *A. uniparens* but present in *C. calypttratus*, originating from the insertion tendons of the *m. flexor digitorum longus* proximal to the ankle and inserting onto the metatarsal and proximal phalanx of digit 1 (Figures 3–7, and 8; Supporting Information Movie 2; Table 3). As in the manus, ten *mm. flexores breves superficiales* are present in the pes of *C. calypttratus*, and the muscles flanking the cleft (*mm. flexores breves superficiales* 4–5, in the pes) have similarly oblique fiber directions associated with the proximal and dorsal extension of the flexor retinaculum, from which they originate. The retinaculum forms a broad, V-shaped plantar aponeurosis similar to the palmar aponeurosis of the manus. In *A. uniparens*, the *m. flexores breves superficiales* originate from the aponeurosis of the gastrocnemius muscles. The number of *mm. lumbricales* and their distribution varies somewhat between chameleon species. In *C. calypttratus*, three *mm. lumbricales* are present: two from the tendon of *m. flexor digitorum longus* to digit 4 to insert on that digit, and one from the tendon to digit 3. In *T. melleri*, a second *m. lumbricalis* to digit 3 is present. Mivart (1870) also described four *mm. lumbricales* in *C. parsonii* inserting on digits 3 and 4, but the muscle to the fibular side of digit 4 originates from the tendon of *m. flexor digitorum longus* to digit 5. *Musculi contrahentes pedis* appear to be present in both chameleons we dissected, although they were not described by Mivart (1870). Their configuration is similar to the *mm. contrahentes* in the manus except that the muscle to digit 3 is absent. The *mm. intermetatarsales* are even less developed than the *mm. intermetacarpales*. They lie mainly between adjacent metatarsals, as in *A. uniparens*, but while in the latter lizard (and in most other lizards) all *mm. intermetacarpales* extend to the proximal phalanges, in the chameleons we dissected only the muscle between digits 3 and 4 could be seen inserting onto the proximal phalanx of digit 3. Thus, as in the manus, the short autopodial muscles lying between syndactylous digits are often either fused with other muscles (e.g., the short flexors and extensors) or reduced in size (e.g., the *mm. intermetatarsales*).

4 | DISCUSSION

4.1 | Functional morphology and the evolution of prehensile and opposable appendages in chameleons

The appendicular muscle anatomy of chameleons, particularly in terms of the number and identity of the muscles, is surprisingly conservative considering the remarkable structural and functional modifications of the limb skeleton. The most striking and unusual soft tissue feature that we found in the limbs of chameleons was the configuration of the flexor retinacula and the short superficial digital flexors attached to them. However, even in this unusual configuration, no muscles commonly present in other lizards were absent and no additional muscles were present. In fact, the number and identities of the autopodial muscles in chameleons are surprisingly plesiomorphic (Figures 2 and 4; Tables 2 and 3), not only among squamates, but also among reptiles and even tetrapods in general (see, e.g., Diogo & Abdala, 2010; Diogo, Molnar, & Smith, 2014).

Chameleons are specialized for arboreal locomotion, and many of their locomotor adaptations parallel those of mammals (Fischer et al., 2010). The functional morphology of the chameleon shoulder was characterized by Peterson (1973). In terms of kinematics, the shoulder of chameleons differs from that of other lizards in two main ways: (1) increased anterior-posterior movement of the pectoral girdle relative to the body wall, and (2) greater anterior-posterior arc of movement of the limb, primarily achieved through increased protraction of the humerus (Peterson, 1984). These differences are reflected in muscle anatomy as well; for example, the *serratus anterior* has relatively longer bellies in chameleons, which may facilitate the movement of the shoulder girdle on the body wall (Peterson, 1973). Other differences in muscle anatomy, including the loss or fusion of the coracoid head of the *triceps* and of an associated coracoid arm of the sternoscapular ligament and the more proximal location of the *pectoralis* and *deltoideus* insertions, have been interpreted as adaptations for greater shoulder mobility (Peterson, 1973; Russell & Bauer, 2008). The more proximal insertion of the glenohumeral muscles translates into greater shoulder mobility because the same amount of muscle shortening produces a larger arc of movement of the humerus, at the expense of leverage (Peterson, 1973). Likewise, the decreased role of humeral rotation due to the more sagittal orientation of the limbs in arboreal forms such as chameleons is reflected in the insertion of muscles such as *deltoideus scapularis* closer to the long axis of the humerus (Peterson, 1973).

Movements of the hindlimb are not as strictly parasagittal as are those of the forelimb in chameleons, and undulation of the trunk is greater toward the pelvic girdle (Fischer et al., 2010). However, the skeletal architecture of the chameleon pelvic girdle is very different from that of other lizards (Russell & Bauer, 2008). Compared with *A. uniparens*, the ilium is longer and oriented almost vertically rather than caudally, and the entire girdle is compressed mediolaterally. A narrower pelvis allows the leg to move closer to the sagittal plane (Russell & Bauer, 2008), and chameleons use this ability during pes touchdown when the hindlimb is almost parasagittal (Fischer et al., 2010). The dorsal expansion of the ilium would provide greater leverage for the dorsal

hip muscles such as the *iliofemoralis*, *iliotibialis*, and *tenuissimus*. The *iliotibialis* extends the knee during swing phase of gait, and the *tenuissimus* ("iliofibularis") may flex the knee and elevate the limb (Higham, 2004). A cartilaginous supra-ilium, which would have further extended the ilium dorsally, has been reported in one species of *Chamaeleo* (Baur, 1886), but we did not observe this feature in our *T. melleri* or *C. calyptratus* specimens. Uniquely among lizards, the femoral condyles in chameleons are symmetrical and the axis of the knee joint is perpendicular to the femur, as it is in mammals (Russell & Bauer, 2008). This arrangement would allow parasagittal flexion and extension of the knee.

Chameleons possess all of the pelvic and thigh muscles commonly found in other lizards, and they do not possess any additional muscles. However, some minor differences in attachments suggest clear biomechanical consequences. For example, the area of origin of *caudofemoralis longus* is much smaller in *C. calyptratus* and *T. melleri* than in *A. uniparens*, reflective of slower locomotor speeds (Russell & Bauer, 2008). Also, the insertion of the *extensor digitorum longus* onto metatarsal 3 only (as opposed to both metatarsals 2 and 3 in *A. uniparens*; Table 3; Figures 3 and 4) may aid rotation of the pes on the proximal tarsals as the foot leaves the ground (Russell & Bauer, 2008), reflecting the extensive rotation of the ankle joints in chameleon locomotion.

One of the most important adaptations for arboreal locomotion in both reptiles and mammals is prehensile appendages (Fischer et al., 2010). Several adaptations of the autopodial musculature of chameleons would appear to enhance grasping ability. For example, due to the distal and dorsal expansion of the flexor retinaculum from which they originate, the orientation of the *mm. flexores breves superficiales* that insert on the digits adjacent to the cleft (i.e., 3 and 4 in the manus; 2 and 3 in the pes) is more transverse than proximo-distal (Figures 2b and 4b). Without changing the number of muscles or their main attachments, this configuration would allow muscles that in most lizards act as flexors to instead adduct and thus oppose the two "super-digits" of each autopod. This change would presumably enhance the animal's prehensile ability (fittingly, Mivart, 1870 designated these muscles "adductor digiti tertii" and "adductor digiti quarti"). The relatively large cross-sectional area of these muscles and their insertion directly onto the phalanges may also increase grasping ability. Not surprisingly, chameleons can generate substantially greater grasping forces than other lizards (Abdala et al., 2009). In contrast, the muscles lying between the individual digits within each "super-digit" are often either fused with other muscles (e.g., *flexores digitorum breves profundi* and *extensores digitorum breves*) or reduced in size (e.g., *intermetatarsales*). In fact, reduction of the *intermetacarpales* and *intermetatarsales* is the only change in number of muscles clearly related to a particular functional feature in chameleons (cleft and related zygodactyly). While most pentadactyl tetrapods have four of these muscles in each autopod occupying the spaces between the five digits, in all chameleon taxa in which the myology has been analyzed so far the muscles between the digits affected by the cleft are lost, and there are normally only three intermetacarpales/intermetatarsales in each autopod.

In summary, the most obvious musculoskeletal features related to the peculiar opposable autopodia of chameleons are (1) syndactyly and

zygodactyly of the autopod; (2) shortened metacarpals and modification of the carpal and tarsal bones; (3) peculiar broad, V-shaped plantar and palmar aponeuroses between the digits separated by a cleft; (4) oblique orientation of the superficial short flexors originating from these aponeuroses, which probably allows these muscles to act as powerful adductors of the two “super-digits” leading to their opposition; (5) absence of *intermetacarpales* and *intermetatarsales* between the digits separated by a cleft; and (6) well-developed *abductor digiti minimi* and *abductor pollicis/hallucis brevis* muscles, which could act as powerful abductors of the “super-digits,” leading to their reposition.

4.2 | Comparisons between forelimb and hindlimb

Comparisons between the muscles and skeleton of the forelimb and hindlimb pave the way for studies examining whether the structures that topologically correspond to each other develop from similar anlagen/share similar developmental mechanisms (indicating evolutionary parallelism) or not (indicating evolutionary convergence) (Diogo, Linde-Medina, Abdala, & Ashley-Ross, 2013; Miyashita & Diogo, 2016). Skeletal structures of the chameleon manus and pes are very similar (Diaz & Trainor, 2015), showing an almost perfect match between their adult topological correspondence and development. The single exception is that the carpal bone intermedium seems to be developmentally more closely related to the radius, while the tarsal bone intermedium seems to be developmentally more closely related to the fibula (which topologically corresponds to the ulna, not to the radius). Likewise, the chameleon pisiform seems to correspond to the plantar ossicle. The pisiform is segmented from the ulnare and the plantar ossicle from the fibulare (Diaz & Trainor, 2015), which corresponds topologically to the ulnare. Because recent studies in mice have shown that the pisiform shares similar developmental mechanisms with the calcaneum (e.g., Reno, Kjosness, & Hines, 2016), the “plantar ossicle” *sensu* Mivart (1870) should be designated the calcaneum as it probably corresponds to the mammalian calcaneum. Therefore, the synonymy between the calcaneum and the complex formed by the fibulare + intermedium that is often used in the squamate literature (e.g., Russell & Bauer, 2008) is probably inaccurate. In fact, the muscles that are developmentally associated with the pisiform and the plantar ossicle, that is, *m. flexor carpi ulnaris* and *m. gastrocnemius externus*, respectively (Diaz & Trainor, 2015), also correspond to each other topologically (see below).

Mivart's (1870) description of the *C. parsonii* noted the striking resemblance between the forelimb and hindlimb muscles of chameleons in the context of the question of serial homology of the limbs and included a detailed list of “correspondences” between specific hindlimb and forelimb muscles. We have argued that the striking similarity between the distal regions of the forelimb and hindlimb of tetrapods is not due to serial homology (in the historical morphological sense of this term), but is instead the result of derived similarity (homoplasy), very likely related to the co-option of similar genes/molecular mechanisms (e.g., Diogo & Molnar, 2014; Diogo et al., 2013; Sears, Capellini, & Diogo, 2015). As the cited studies explain, the phylogenetically older regions of these appendages, that is, the pelvic and pectoral girdles and associated soft tissues, probably have remained morphologically differ-

ent since the origin of paired appendages in gnathostomes. The muscle anatomy of chameleons supports these ideas because, as Mivart (1870) noted, these lizards display a striking number of clear topological correspondences between forelimb and hindlimb muscles, but these correspondences concern only the leg-pes and forearm-manus muscles, and not the more proximal regions of the limbs. That is, the pattern observed in chameleons reinforces the idea that the pectoral and pelvic girdles and the muscles related to them have been, and continue to be, markedly different since the origin of paired appendages.

One example of a clear topological correspondence between muscles of the forearm-leg and manus-pes that is evident in adult chameleons but only clear during embryonic development in other tetrapods (e.g., Diogo & Molnar, 2014) is between *m. pronator teres/flexor carpi radialis* and part of *m. gastrocnemius internus* (compare Figures 1 and 3). In addition, it is clear that the *m. extensor digitorum longus* corresponds to the forelimb *m. extensor digitorum*; the *m. tibialis anterior* to the forelimb *mm. extensor carpi radialis + brachioradialis; fibularis longus* and *brevis* to the forelimb *m. extensor antebrachii et carpi ulnaris*; the *m. gastrocnemius externus* to the *m. flexor carpi ulnaris* and *m. epitrochleoanconeus*; the *m. popliteus, m. interosseous cruris* and *m. tibialis posterior* to the forelimb muscles *mm. pronator accessorius, pronator quadratus*, and *palmaris profundus 1* (the latter muscle is probably fused with the former two forelimb muscles: Table 2); and *mm. extensores digitorum breves, abductor hallucis longus, flexor digitorum longus, flexores breves profundi, intermetatarsales, abductor digiti minimi, abductor hallucis brevis, flexores breves superficiales, lumbriales, contrahentes*, and *dorsometatarsales* to the forelimb muscles that have the same, or similar, names.

These correspondences match those listed by Mivart (1870), with three main exceptions. First, the *m. brachioradialis* (“supinator longus”) is a dorsal (extensor) muscle, so it cannot correspond to part of *m. gastrocnemius externus*, as proposed by Mivart (1870). Second, the *m. tibialis anterior* (“*tibialis anticus*”) appears to correspond to part of the *m. extensor carpi radialis* complex of the forelimb, whereas Mivart (1870) listed a correspondence with the *m. extensor digitorum* of the forelimb (“*extensor carpi radialis longus et brevis*”). Third, Mivart (1870) suggested that the *m. tibialis posterior* (“*tibialis posticus*”) corresponds to the *m. flexor carpi radialis* of the forelimb, while the available data shows that it likely corresponds instead to *m. palmaris profundus I* (Diogo & Molnar, 2014). Many other studies published around the same time also recognized the latter correspondence (e.g., Humphry, 1872; Owen, 1866). Mivart (1870) probably did not recognize it because, as noted above, in chameleons the *m. palmaris profundus I* is fused with the *m. pronator accessorius*, obscuring its topological correspondence to the *m. tibialis posterior* of the hindlimb.

4.3 | Teratology of the chameleon autopodium and the split-hand/split-foot framework: Chameleons as model organisms for studies on limb evolution, development, and teratology

Arboreality has led to extensive modification of tetrapod limbs and, more importantly, to the arrangements of digits (dactyly) in response to

a species' adaptive requirement for climbing and/or perching and grasping onto a surface with a narrower diameter than the body of the organism. Many birds display zygodactyly in the pes, the second most common arrangement amongst birds, with digits I and IV opposing digits II and III (Nupen, 2016), allowing for grasping of branches and twigs when perching. Recent work (Botelho et al., 2015) underscores the importance of muscular activity in spatially shifting digit I opposite to digits II–IV of the feet to increase avian foot grasping ability. Despite these avian digit rearrangements, digits remain free from one another due to loss of interdigital tissue (except in cases where II–III remain in syndactyly, such as in Kingfishers; Nupen, 2016). Although chameleons are described as “zygodactylous” due to their ability to oppose two “super digits,” as discussed above, their autopodia differ significantly from the feet of birds (e.g., in number of digits and prevalence of syndactyly). Separation of the two “super digits” in chameleons occurs through major interdigital cell death at the clefting site. Thus, chameleons are unique amongst tetrapods in their overall autopodial arrangement (Diaz & Trainor, 2015).

Phenotypically, the most similar autopodial morphology to that found in chameleons is found in tetrapods with the Split Hand/Split Foot malformation (Ectrodactyly) (Barsky, 1964; Blauth & Borisch, 1990; Flatt, 1977; Tani, Ikuta, & Ishida, 2000). While highly variable, this abnormal hand/foot phenotype is generally characterized by a reduction or loss of mid-digital skeletal elements (from single phalanges to entire digital rays) with anterior and posterior rays less affected. Remaining digits (complete or proximally truncated) are generally found in soft (soft tissue) or hard (bone) syndactyly with the remaining adjacent digits. While ectrodactyly is superficially similar to the chameleon phenotype, chameleons retain a pentadactyl complement of digits and a typical number of phalanges for squamate lizards (Diaz & Trainor, 2015). Thus, chameleons only share the formation of a midline cleft in the hands and feet reaching proximally between the metacarpals/metatarsals and the associated loss of interdigital muscles facing the cleft (intermetacarpales/intermetatarsales). These features that mirror human malformations may have arisen over an extended period of time as chameleons began to explore locomotion away from a terrestrial environment. Alternatively, chameleons might represent “hopeful monsters”; that is, a taxon in which relatively rapid transitions leading to major morphological changes were possible and viable due to facilitated evolution/homeorhesis (reviewed in Diogo, 2017; Diogo, Guinard, et al., 2016).

Several studies from human patients and on lab models for ectrodactyly point to a destabilization of the Apical Ectodermal Ridge (AER; ectodermal thickening along the anterior-posterior margin of the embryonic limb bud along the dorsoventral border) as the primary cause for more proximal cell death and loss of limb elements (Duijf, van Bokhoven, & Brunner, 2003; Klar, 2016; Naruse, Takahara, Takagi, Oberg, & Ogino, 2007; Sowińska-Seidler, Socha, & Jamsheer, 2014; Spielmann et al., 2016). Data from Diaz and Trainor (2015) support an increased amount of cell death associated with cleft formation in the chameleon autopodia, despite the AER being robust and still expressing Fibroblast Growth Factor 8 (*Fgf8*), which is both necessary and suffi-

cient to maintain limb bud outgrowth. Thus, the chameleon autopodia express aspects of cell death common to cleft formation in human patients with ectrodactyly, but they retain their skeletal elements because cell death occurs only between digits. Cleft formation also appears to follow a unique path in chameleons relative to what has been observed in human clinical cases, other tetrapods, and model lab species and should continue to be studied as a potential system to identify factors associated with limb cleft formation and syndactyly, the latter phenotype being one of the most common limb malformations in humans. This is the first detailed study of a tetrapod limb expressing distal autopodial clefting; no such descriptive study has been performed on humans or mouse laboratory models with similar phenotypes. While case studies exist for humans and in the veterinary literature (reviewed in Duijf et al., 2003; Harasen, 2010; Rahal et al., 2012), these descriptions are primarily on the osteology and reconstructive approaches and lack any information about musculature.

4.4 | Future directions

As part of our current project, we plan to address this scarcity of comparative data by undertaking similar descriptive studies of other taxa, including species of all extant chameleon genera and humans with congenital malformations. Comparative anatomical studies can shed light on the evolution of morphological and functional innovations, including phenotypes such as syndactyly and cleft autopodium that closely resemble congenital malformations of the human limb. By doing so, these studies also pave the way for developmental experimental works to investigate the mechanisms that lead to those phenotypes. While many studies have described the anatomical diversity of chameleons (e.g., Anderson & Higham, 2014; Mivart, 1870), few have focused on the development and morphogenesis of their unique anatomical traits (e.g., Diaz & Trainor, 2015; Rieppel, 1993). Given that there are more than 200 species of chameleons which vary greatly in body size, complement of wrist/ankle bones, and limb proportions, our understanding of the musculoskeletal system of the limbs and girdles in chameleons is limited. In combination with future work on chameleon development and genetics, this comparative approach may help to explain the processes behind their remarkable anatomy, as well as the mechanisms that lead to human limb malformations.

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AUTHOR CONTRIBUTIONS

RD, RED, and JLM designed the study. RD and JLM performed the dissections. TS and GD stained and scanned the chameleon limbs for MicroCT imaging. RED provided the specimens. JLM and RD

wrote the manuscript. JLM produced the illustrations. All authors reviewed and edited the manuscript.

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SUPPORTING INFORMATION

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

Supplementary Movie 1. Phosphotungstic Acid (PTA) contrast enhanced female *Chamaeleo calyptratus* forelimb.

Supplementary Movie 2. Phosphotungstic Acid (PTA) contrast enhanced female *Chamaeleo calyptratus* hindlimb.

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