

Monitoring muscle over three orders of magnitude: Widespread positive allometry among locomotor and body support musculature in the pectoral girdle of varanid lizards (*Varanidae*)

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Abstract

There is a functional trade-off in the design of skeletal muscle. Muscle strength depends on the number of muscle fibers in parallel, while shortening velocity and operational distance depend on fascicle length, leading to a trade-off between the maximum force a muscle can produce and its ability to change length and contract rapidly. This trade-off becomes even more pronounced as animals increase in size because muscle strength scales with area (length²) while body mass scales with volume (length³). In order to understand this muscle trade-off and how animals deal with the biomechanical consequences of size, we investigated muscle properties in the pectoral girdle of varanid lizards. Varanids are an ideal group to study the scaling of muscle properties because they retain similar body proportions and posture across five orders of magnitude in body mass and are highly active, terrestrially adapted reptiles. We measured muscle mass, physiological cross-sectional area, fascicle length, proximal and distal tendon lengths, and proximal and distal moment arms for 27 pectoral girdle muscles in 13 individuals across 8 species ranging from 64 g to 40 kg. Standard and phylogenetically informed reduced major axis regression was used to investigate how muscle architecture properties scale with body size. Allometric growth was widespread for muscle mass (scaling exponent >1), physiological cross-sectional area (scaling exponent >0.66), but not tendon length (scaling exponent >0.33). Positive allometry for muscle mass was universal among muscles responsible for translating the trunk forward and flexing the elbow, and nearly universal among humeral protractors and wrist flexors. Positive allometry for PCSA was also common among trunk translators and humeral protractors, though less so than muscle mass. Positive scaling for fascicle length was not widespread, but common among humeral protractors. A higher proportion of pectoral girdle muscles scaled with positive allometry than our previous work showed for the pelvic girdle, suggesting that the center of mass may move cranially with body size in varanids, or that the pectoral girdle may assume a more dominant role in locomotion in larger species. Scaling exponents for physiological cross-sectional area among muscles primarily associated with propulsion or with a dual role were generally higher than those associated primarily with support

against gravity, suggesting that locomotor demands have at least an equal influence on muscle architecture as body support. Overall, these results suggest that larger varanids compensate for the increased biomechanical demands of locomotion and body support at higher body sizes by developing larger pectoral muscles with higher physiological cross-sectional areas. The isometric scaling rates for fascicle length among locomotion-oriented pectoral girdle muscles suggest that larger varanids may be forced to use shorter stride lengths, but this problem may be circumvented by increases in limb excursion afforded by the sliding coracosternal joint.

KEYWORDS

locomotion, morphology, muscle, scaling, *Varanus*

1 | INTRODUCTION

Size is one of the most fundamental constraints which can change the shape and performance of animals. Geometric scaling predicts that if animals were to increase in size but keep the same shape, their linear dimensions would increase proportional to body mass (M)^{0.33} and area dimensions would increase proportional to M ^{0.67}. All else being equal, the strength of their muscles and bones increases only two-thirds as fast as their mass (M ^{0.67}) (Schmidt-Nielsen, 1984); thus, animals face a serious problem as they grow large. Force production relative to cross-sectional area can be increased by using pennation to arrange muscle fibers at an angle relative to the muscle line of action, but this necessitates employing shorter fascicle lengths, resulting in shorter muscle excursion ranges and slower contraction rates. Thus, there is a trade-off in the anatomical design of skeletal muscle—a muscle optimized to perform one task (e.g. strength) is likely limited in its ability to perform another (e.g. length change), a constraint which becomes even more important as animals increase in size.

Different groups of animals have developed a variety of strategies for overcoming this size-dependent muscle constraint. Mammals and birds typically adopt a more erect posture in order to increase the mechanical advantage of the limb muscles. This decreases peak limb stresses so that large animals can maintain performance within reasonable safety factors (Biewener, 1989). However, it is unclear how evolution might balance conflicting demands on muscle performance when posture does not change with increases in size.

These trade-offs can best be studied in a closely related group of animals that share similar body proportions despite great variation in body size such as varanid (*Varanus*) lizards. Varanids maintain a similar sprawled posture (Clemente *et al.*, 2011; Dick and Clemente, 2016; 2017) and body proportions both ontogenetically and interspecifically (Thompson and Withers, 1997) across body sizes ranging from 5 g to 100 kg (Dick and Clemente, 2016), with extinct forms having body mass estimates ranging from 100 to 600 kg (Wroe, 2002). Previous work reported positive allometry in the scaling of hindlimb musculature of varanids including knee flexors, as well as femur adductors and abductors (Dick and Clemente, 2016). Yet, it is unclear how muscles will scale in the pectoral girdle, where muscular anatomy is more complex and potentially specialized for climbing.

Previous studies on the muscle architecture in felids, which also maintain a similar posture over a substantial body size range (Day and Jayne, 2007), found that shoulder support muscles scaled with positive allometry, while other muscles became weaker with body size (Cuff *et al.*, 2016a). However, this is not the case in all groups of animals. In the Quenda, a fossorial bandicoot, pectoral muscles were also found to scale with positive allometry (Martin *et al.*, 2019), and an arboreal marten was found to have more powerful limb flexor and retractor muscles, and greater excursion lengths among adductor muscles than in a terrestrial species (Böhmer *et al.*, 2018). Among reptiles, crocodylids which have relatively longer proximal segments of forelimbs than in alligatorids (Iijima *et al.*, 2018) also display relatively longer fascicles and smaller physiological cross-sectional areas with increases in body size (Allen *et al.*, 2014). Crocodylids were also

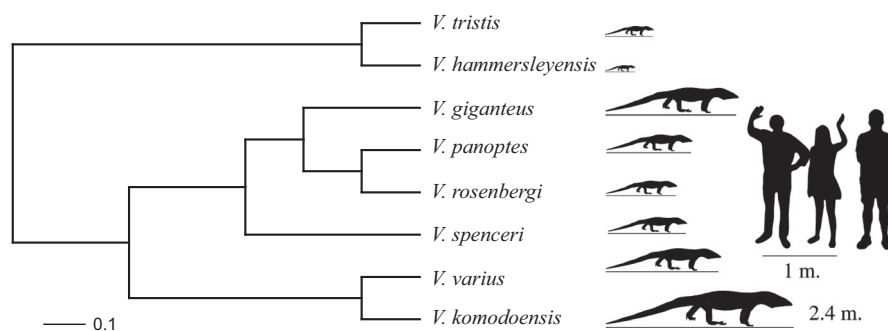


FIGURE 1 Cladogram of varanid lizards used in this study with outline of the authors shown to scale

TABLE 1 Anatomy of muscles including origin and insertion locations (Jenkins and Goslow, 1983; Russell and Bauer, 2008), known (Jenkins and Goslow, 1983) or presumed activity patterns, muscle functions (Jenkins and Goslow, 1983; Russell and Bauer, 2008), and abbreviations used

Muscle	Primary Function	Secondary Function	Abbreviation	Activity
Latissimus dorsi	Translate trunk cranially relative to scapulocoracoid and forelimb	Elevate Forelimb	LatDorsi	Anterior: transition from stance to swing, posterior: late swing to first 2/3 of stance
Trapezius posterior	Stabilizes scapulocoracoid		TrapPost	Swing
Trapezius anterior	Involved in raising the head		TrapAnt	Neither
Pectoralis anterior	Humerus protractor		PecAnt	Swing
Pectoralis medialis	Translate trunk cranially relative to scapulocoracoid and forelimb	Glenohumeral joint stabilizer	PecMed	Stance
Pectoralis posterior	Translate trunk cranially relative to scapulocoracoid and forelimb	Retracts scapulocoracoid	PecPost	Stance
Triceps lateralis	Elbow extensor		TriLat	Stance
Triceps longus	Elbow extensor	Extends glenohumeral joint	TriLong	Stance
Triceps medialis	Elbow extensor		TriMed	Stance
Biceps brachii (long, anterior)	Elbow Flexor	Glenohumeral joint stabilizer	BicL	Stance
Biceps brachii (short, posterior)	Elbow Flexor	Glenohumeral joint stabilizer	BicS	Stance
Muscle	Primary Function	Secondary Function	Abbreviation	
Deltoides Scapularis	Humerus protractor and elevator		DeltScap	Swing
Deltoides Clavicularis	Humerus protractor during swing	Glenohumeral joint stabilizer during stance	DeltClav	Both
Supracoracoideus	Glenohumeral joint stabilizer		Supracor	Stance
Scapho-humeralis anterior	Humerus protractor and elevator		ScaphHumAnt	Swing
Scapho-humeralis posterior	Humerus elevator during swing	Humerus rotator and glenohumeral joint stabilizer during stance	ScaphHumPost	Both
Brachialis	Elbow flexor		Brach	Swing
Coracobrachialis brevis	Glenohumeral joint stabilizer	Humerus retractor	CoracoBrev	Stance
Coracobrachialis longus	Humerus retractor		CoracoLong	
Serratus anterior	Shoulder stabilizer		SerrAnt	Stance
Extensor digitorum longus	Wrist extensor		ExtDigLong	Presumed stance
Extensor carpi ulnaris	Wrist extensor		ExtCarpUln	Presumed stance

Origin	Insertion
The aponeurosis from the spinous processes and intraspinous ligaments of C8-L3 vertebrae; hypaxial musculature and ribs along the lateral thorax	Linear sulcus on the dorsal aspect of the humeral shaft ₁
Aponeurosis between vertebrae C3-T3	Cranial half of the lateral aspect of the suprascapular cartilage ₁
Neural processes and supraspinous ligament between vertebrae C3-T3, as well as a dense layer of superficial nuchal fascia overlying the neck epaxials musculature	Along the cranial margin of the suprascapular cartilage and the dorsal/distal half of the clavicle ₁
Median process and lateral process of the interclavicle	Apex of the deltopectoral crest ₁
Posterior half of the median process of the interclavicle, the sternal midline, and sternocostal cartilages of 2-3 true ribs	Lateral surface of the deltopectoral crest by a broad tendon ₁
Along the linea alba and the thoracoabdominal fascia as far posterior as the level 3-4L vertebrae.	Distal margin of the deltopectoral crest by a narrow tendon ₁
Entire laterodorsal surface of the humeral shaft and an aponeurosis shared with brachialis	On the olecranon process directly and by an intramuscular tendon shared with the longus ₁
Two origins: posterior scapular portion from a stout tendon from the caudal border of the cranio-dorsal ligament and the posterolateral surface of the scapula; anterior scapular portion from a long tendon continuous with the sternoscapular ligament and a short tendon to the tendon of the latissimus dorsi	Onto the olecranon process via a flat tendon and an aponeurosis in common with the lateral head and to a tendon shared with the medial head ₁
Entire dorsomedial surface of the shaft of the humerus	The common triceps tendon to the olecranon ₁
Anterior, fleshy head from the lateral surface of the coracoid	Fascial expansion on the surface of the forearm flexors on the radial side ₁ a tendon to the proximal ulna shared with the brachialis ₁
Posterior, narrow head from the lateral surface of the coracoid	
Activity	Origin
From a narrow, linear area from the lateral surface of the suprascapula and scapula to the dorsal (distal) end of the clavicle.	Anterodorsal surface of the proximal end of the humerus ₁
Ventral head from the ventral surface of the proximal third of the clavicle and the ventral surface of the interclavicle. Dorsal head from the dorsal surface of the interclavicle at the junction of the median and transverse processes.	
Anterior margin and lateral surface of the coracoid cartilage to the coracoid proper.	Proximal margin of the deltopectoral crest of the humerus by a short, broad tendon ₁
First head originates from the anterolateral surface and anterior margin of the scapula; second head from the lateral surface and dorsal margin of the coracoid cartilage and the coracoid proper.	Dorsomedial surface of the humerus proximal to the tendon of the latissimus dorsi ₁
From the posterior half of the lateral surface of the scapula and suprascapula as well as the posterior margin of the scapula	Dorsal surface of the lesser tubercle by a robust tendon ₁
The lateral (anterior) surface of the humeral shaft—extending from the pectoral crest distally for two-thirds of its length—and a common aponeurosis shared with the triceps lateralis.	Biceps tendon to the proximal ulna and by a narrow tendon to the proximoposterior surface of the radius ₁
Posterior two-thirds of the ventral coracoid and the tissue spanning the posterior coracoid fenestra	The pectoral crest by a flat tendon and the proximal have of the humeral shaft over is anteroventral surface ₁
Superior head from the medial aspect of the posterior half of the coracoid; inferior head from the posterior terminus of the coracoid	Superior head inserts on a slender tendon to the entepicondyle, inferior head inserts to this tendon and to the ventral m directly ₁
Slips arise from cervical and thoracic ribs	Anterior margin and medial surface of the suprascapular ₁
Radial condyle of the humerus	Metacarpals via three tendons ₂
Humeral head by a flat tendon along the ectepicondyle; ulnar head from the anterior aspect of the olecranon	Pisiform and fifth metacarpal via a broad tendon ₂

(Continues)

TABLE 1 (Continued)

Muscle	Primary Function	Secondary Function	Abbreviations	Activity
Extensor carpi radialis	Wrist extensor		ExtCarpRad	Presumed stance
Pronator teres	Wrist flexor		ProTer	Presumed swing
Flexor digitorum longus	Wrist flexor		FlexDigLong	Presumed swing
Flexor carpi radialis	Wrist flexor		FlexCarpRad	Presumed swing
Flexor carpi ulnaris	Wrist flexor		FlexCarpUln	Presumed swing

found to have an increased number of pectoral girdle muscles that scale with positive allometry with body size compared with alligators, a difference that is hypothesized to account for the use of asymmetrical gaits in crocodylids but not alligators (Allen *et al.*, 2014).

Varanids are also an ideal group in which to investigate muscle trade-offs because their locomotor system is under strong selection for power and efficiency. Although maximum sprint speed is reduced in larger varanids (Clemente *et al.*, 2009b; Clemente *et al.*, 2012) and the largest extant species (*V. komodoensis*) is a sit-and-wait predator (Auffenberg, 1981), varanids are highly active and have enhanced endurance compared with other reptiles (Wang *et al.*, 1997; Clemente *et al.*, 2009a). Even larger varanids such as *V. varius* and *V. giganteus* are active foragers and can chase down prey (Pianka and King, 2004). Compared to other reptiles, varanids have slightly elevated standard and highly elevated maximal metabolic rates (Thompson *et al.*, 1997), as well as respiratory (Owerkovicz *et al.*, 1999; Schachner *et al.*, 2013; Cieri and Farmer, 2019) and circulatory (Burggren and Johansen, 1982; Munns *et al.*, 2004; Hanemaaijer *et al.*, 2019) adaptations that may increase active foraging capacity.

Finally, active foraging among larger varanids raises interesting questions about how muscle architecture responds to the competing demands of body support versus propulsion with increases in body size. Posture is largely independent of body size in varanids and hindlimb duty factor increases with body size to reduce peak limb bone stresses (Clemente *et al.*, 2011). This is likely why there is increased scaling of femur adductors and abductors compared with retractors in varanids (Dick and Clemente, 2016) and suggests that the pelvic girdle becomes increasingly specialized for body support over propulsion as body sizes increases. Thus, it is possible that as varanids increase in body size, the forelimb takes on a more propulsive role. Alternatively, gravitational demands on the pectoral girdle might become proportionally greater with increased size. The length of the head, neck, and tail all increase with body length in varanids (Thompson *et al.*, 2008), causing there to be no clear pattern among varanids for the antero-posterior movement of their body center of mass (Clemente, 2014). A comparison of how forelimb and hindlimb muscle properties scale with body size may provide some insight into the biomechanical roles of the pectoral and pelvic girdles in these sprawling animals.

The muscular system of varanids has been previously described (Russell and Bauer, 2008; Moritz and Schilling, 2013; Cieri, 2018),

particularly in the forelimb, where a study combining electromyography with cineradiography hypothesized functional roles for many pectoral muscles (Jenkins and Goslow, 1983). However, it is unknown how the architectural properties of these muscles scale with body size. This study investigated whether and how varanid pectoral muscles in different functional groups scale to respond to the increased locomotor and body support demands associated with increases in size. To do this, we performed a comprehensive analysis of muscle architecture in 27 muscles of the pectoral girdle in 13 individuals from 8 species of arboreal and terrestrial varanid lizards ranging in body mass from 63 g to 40 kg.

2 | METHODS

2.1 | Specimens

Measurements of 27 forelimb muscles were made from 13 individuals comprising 8 species ranging in body mass from 63 g to 40 kg. All animals were wild-caught using a variety of pit trapping, funnel trapping, and hand foraging techniques, with the exception of *V. komodoensis* and *V. giganteus*. Individuals that appeared to be sick, injured, or obviously malnourished were excluded from the study. *V. komodoensis* was obtained from the Queensland Museum (frozen specimens) and *V. giganteus* from the Museum of the Northern Territory (frozen specimens). Lizards were collected under permits WISP11435612 (QLD), SF009075 (WA), 61540 (NT), 08-001092-5 (WA), and WA0001919 (QLD) and ethics SBS/195/12/ARC (QLD), ANA16104 (QLD), and RA/3/100/1188 (WA). Animals included were as follows: *V. giganteus* (3.4 kg), *V. hammersleyensis* (0.06 kg), *V. komodoensis* (40 kg), *V. panoptes* (0.98, 1.06, 1.49, 4.15 kg), *V. rosenbergi* (0.28, 1.05 kg), *V. spenceri* (2.1 kg), and *V. varius* (0.83, 4.82 kg).

2.2 | Muscle architecture

As in Dick and Clemente (2016), muscle data were collected under a standard protocol (Calow and Alexander, 1973; Alexander and Ker, 1990; Payne *et al.*, 2005; Allen *et al.*, 2010) which included the measurement of muscle belly and external tendon lengths (proximal

Origin	Insertion
Ectepicondyle via a shared tendon with the extensor digitorum longus and from a fascial sheathe along the anterior aspect of the first head ₂	Medial and anterior aspect of the radius. ₂
Lateral aspect of the humeral entepicondyle via tendon ₂	Onto the anteromedial aspect of the distal half of the radius and the insertion tendon of the flexor carpi radialis. ₂
Two heads from the humeral entepicondyle and one from the posterior aspect of the ulnar shaft ₂	Ungual phalanxes of each digit via a broad palmar aponeurosis that passes deep to the annular ligament of the wrist. ₂
From the lateral aspect of the humeral entepicondyle via a broad tendon shared with the pronator teres ₂	First metacarpal via a stout tendon. ₂
Posterodistal aspect of the dorsal surface of the humeral entepicondyle ₂	Broad tendon toward the fifth metacarpal and pisiform. ₂

and distal, if present) for all muscles. Representative fascicles from the proximal, distal, and middle regions of the muscle were used to measure fascicle length (L_f) and pennation angle (θ) and were averaged to produce a single value for analysis. Each muscle was weighed individually after total dissection. Digital calipers were used to

measure muscle moment arms as the perpendicular distance from the joint axis of rotation to the line of action of the muscle when the limb was positioned in a neutral posture. An estimate of total muscle force based on physiological cross-sectional area (PCSA) was estimated as follows:

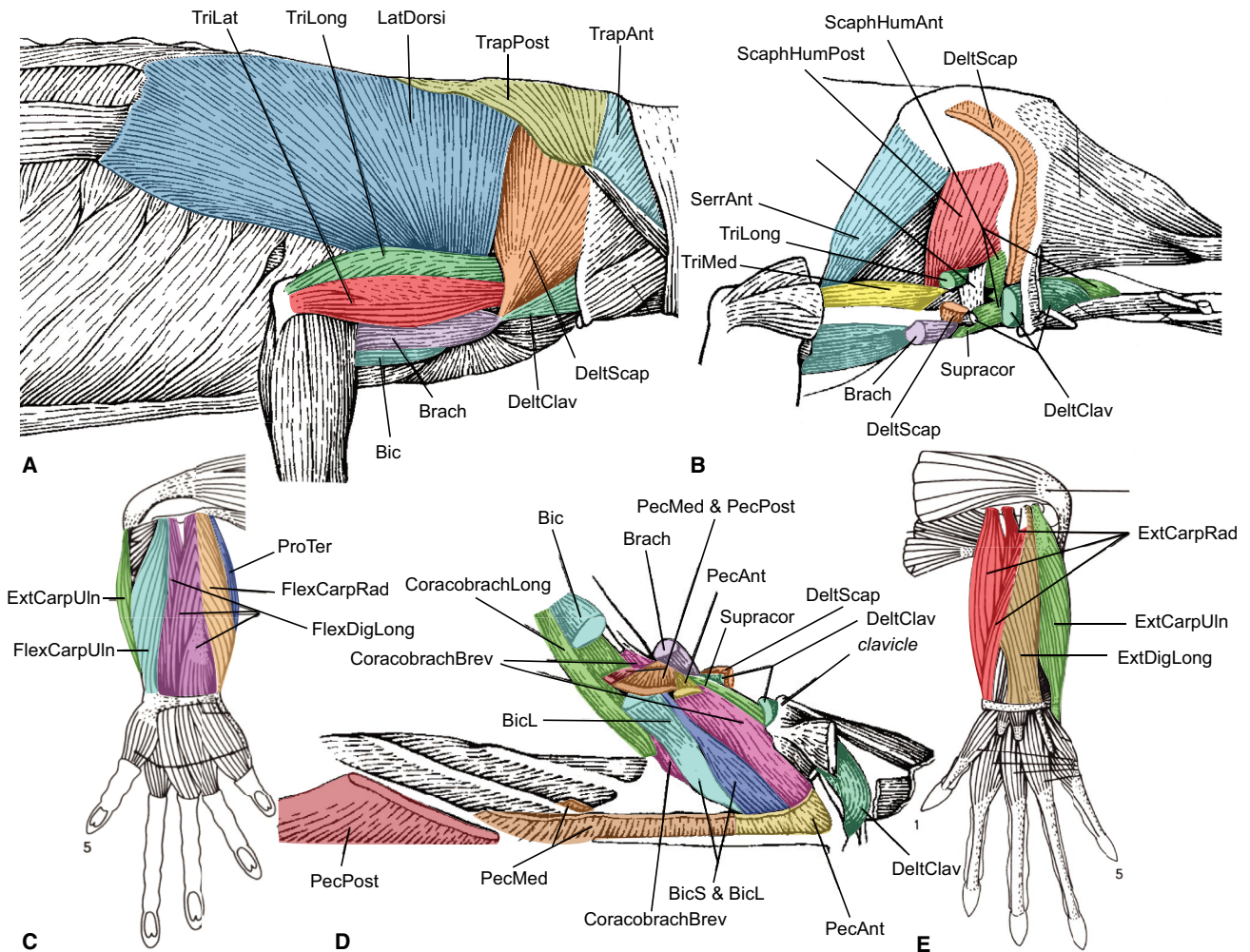


FIGURE 2 Forelimb muscular anatomy of varanid lizards. (a) Lateral view of the superior shoulder musculature. (b) Dorsal-lateral view of deeper shoulder musculature. (c) Caudal/ventral view of the superficial musculature of the left forearm. (d) Ventral-lateral view of the forelimb muscles. (e) Cranial/dorsal view of the superficial musculature of the left forearm. Illustrations in a, b, and d adapted from Jenkins and Goslow (1983), and c and e adapted from Russell and Bauer (2008)

$$PCSA = \frac{V_{\text{musc}}}{L_{\text{fasc}}}$$

where V_m is the volume of muscle defined as mass divided by estimated vertebrate muscle density (1.06 g/cm^3), and L_f is fascicle length (Narici *et al.*, 1992). Previous work on varanid hindlimb scaling divided PCSA by the cosine of pennation angle which would have slightly overestimated the PCSA of pennate muscles (Dick and Clemente, 2016). Others (Allen *et al.*, 2010; 2014) have calculated PCSA as the value above multiplied by the cosine of pennation angle, as suggested by Sacks and Roy (1982). This parameter is more indicative of the potential force along the tendon generated by the muscle (Sacks and Roy, 1982) rather than the physiological cross-sectional area *sensu stricto*.

2.3 | Statistical analysis

Raw values and species averages for each trait were reported, and two scaling analyses were used. First, individual muscle measurements were regressed against body mass ("individual means"), and then, species means were regressed in a phylogenetically informed context ("species means"). Because we did not have data for every muscle for each species at different sizes, our scaling analysis therefore consisted of two tests for each muscle, first including all individuals for all species and second including only species means. This approach represents a reasonable compromise between avoiding false negatives (type II errors) and not violating the independence of data points in our analyses.

Following previous investigators, reduced major axis (RMA) regression analysis was employed to determine the relationship of muscle

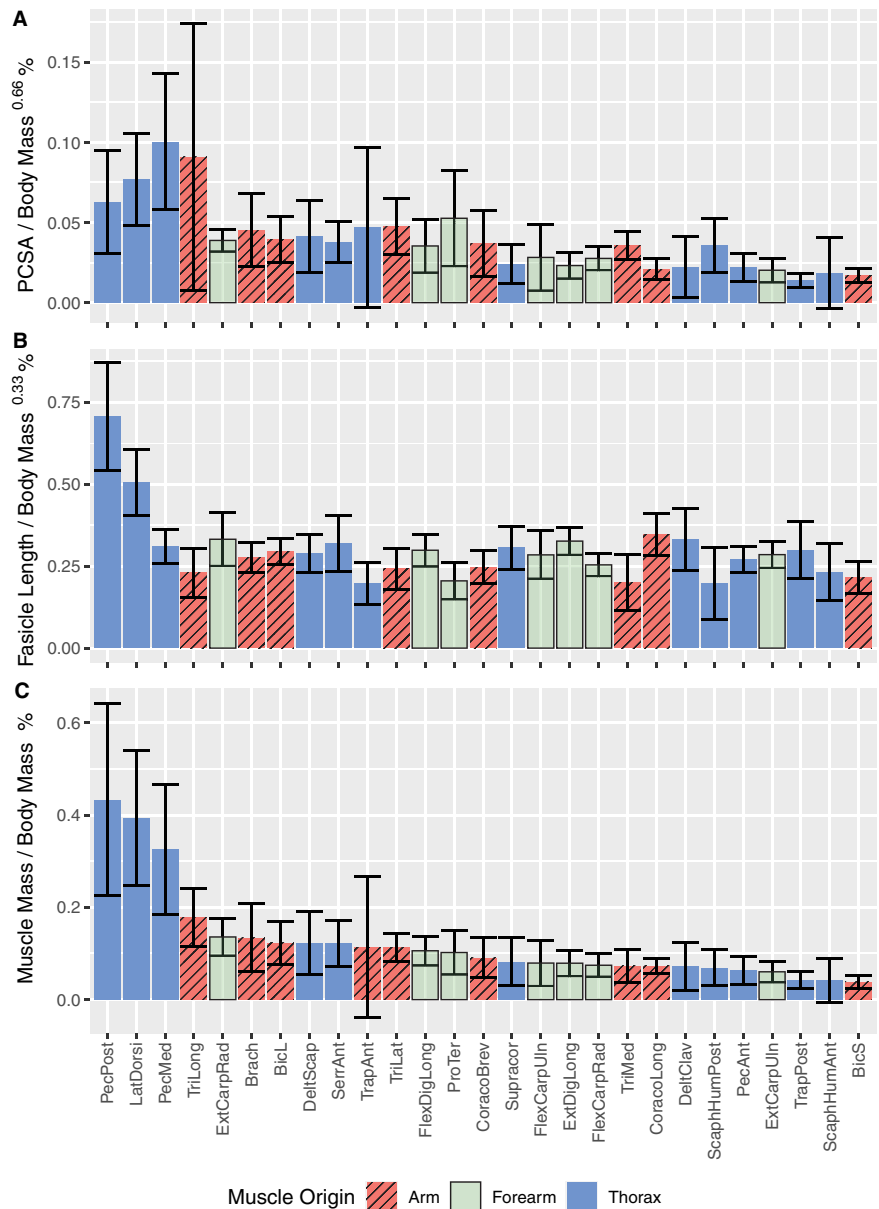


FIGURE 3 Bar plots showing relative PCSA (a) ($PCSA / \text{body mass [g]}^{0.66}$), relative fascicle length (fascicle length [m] / $\text{body mass [g]}^{0.33}$) (b), and relative muscle mass (muscle mass [g] / body mass [g]) (c) ordered by relative muscle mass averaged across species for each muscle. Bars are colored according to muscle origin on the thorax (extrinsic muscles), arm, or forelimb. All values are expressed in percentages (multiplied by 100)

TABLE 2 Average muscle values: Raw muscle parameters with averages and standard deviations for each muscle for relative muscle mass (muscle mass / body mass), fascicle length (fascicle length / body mass ^{0.33}), PCSA (physiological cross-sectional area / body mass ^{0.66}), distal and proximal tendon lengths (tendon length / body mass ^{0.33}), and pennation (angles in degrees). All values are expressed as percentages (raw values were multiplied by 100). NA indicates that this measurement was not made or could not be calculated due to sample size

Muscle	Function	Muscle mass		Fascicle length		PCSA		Dist tendon length		Prox tendon length		Pennation	
		Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
BicL	Elbow Flexor	0.1232	0.0465	0.2951	0.0404	0.0395	0.0144	0.0799	0.0627	0.2284	0.0280	7.70	10.14
BicS	Elbow Flexor	0.0387	0.0137	0.2163	0.0486	0.0170	0.0042	0.1409	0.1417	0.3276	0.0763	6.75	7.84
Brach	Elbow Flexor	0.1342	0.0735	0.2771	0.0461	0.0453	0.0229	0.0685	0.0373	NA	NA	2.52	3.71
CoracoBrev	Glenohumeral Joint Stabilizer	0.0910	0.0428	0.2465	0.0501	0.0369	0.0208	0.0360	0.0383	NA	NA	1.43	3.78
CoracoLong	Trunk Translator	0.0733	0.0157	0.3459	0.0635	0.0210	0.0065	0.0730	0.0662	0.0253	0.0045	1.08	3.06
DeltClav	Humerus Protractor	0.0723	0.0519	0.3321	0.0949	0.0222	0.0192	0.0382	0.0120	0.0466	NA	0	0
DeltScap	Humerus Protractor	0.1225	0.0676	0.2885	0.0571	0.0415	0.0225	0.0662	0.0377	0.0217	NA	0.27	0.84
ExtCarpRad	Wrist Extensor	0.1358	0.0407	0.3318	0.0813	0.0388	0.0070	0.0304	0.0332	0.0381	NA	0	0
ExtCarpUln	Wrist Extensor	0.0603	0.0226	0.2850	0.0405	0.0202	0.0075	0.0691	0.0121	0.0683	0.0044	0	0
ExtDigLong	Wrist Extensor	0.0789	0.0279	0.3262	0.0420	0.0232	0.0082	0.0976	0.0397	0.0458	0.0118	0	0
FlexCarpRad	Wrist Flexor	0.0745	0.0250	0.2537	0.0341	0.0276	0.0073	0.0871	0.0344	NA	NA	0	0
FlexCarpUln	Wrist Flexor	0.0792	0.0496	0.2842	0.0728	0.0282	0.0208	0.1001	0.0644	0.0452	0.0152	0	0
FlexDigLong	Wrist Flexor	0.1059	0.0316	0.2983	0.0493	0.0354	0.0167	0.0513	0.0085	NA	NA	0	0
LatDorsi	Trunk Translator	0.3937	0.1463	0.5052	0.1013	0.0768	0.0288	0.0844	0.0300	NA	NA	0	0
PecAnt	Humerus Protractor	0.0635	0.0301	0.2703	0.0401	0.0220	0.0089	0.0905	0.0310	NA	NA	0.27	0.90
PecMed	Trunk Translator	0.3251	0.1412	0.3096	0.0515	0.1005	0.0426	0.0532	0.0247	NA	NA	0	0
PecPost	Trunk Translator	0.4327	0.2081	0.7052	0.1642	0.0629	0.0321	0.1403	0.1530	NA	NA	0	0
ProTer	Wrist Flexor	0.1020	0.0474	0.2050	0.0559	0.0527	0.0298	0.0628	0.0174	0.0361	NA	7.33	8.15
ScaphHumAnt	Humerus Protractor	0.0424	0.0477	0.2313	0.0864	0.0185	0.0221	0.0691	0.0321	NA	NA	0	0
ScaphHumPost	Glenohumeral Joint Stabilizer	0.0689	0.0388	0.1974	0.1096	0.0356	0.0167	0.0405	0.0175	NA	NA	0	0
SerrAnt	Shoulder Stabilizer	0.1219	0.0493	0.3196	0.0857	0.0376	0.0128	0.0081	NA	0.0294	NA	1.79	4.64
Supracor	Glenohumeral Joint Stabilizer	0.0820	0.0518	0.3057	0.0657	0.0241	0.0124	0.0679	0.0547	NA	NA	0	0
TrapAnt	(Moves Head)	0.1138	0.1528	0.1965	0.0638	0.0469	0.0497	NA	NA	NA	NA	0	0
TrapPost	Shoulder Stabilizer	0.0428	0.0181	0.2987	0.0863	0.0138	0.0042	NA	NA	NA	NA	0	0
TriLat	Elbow Extensor	0.1134	0.0305	0.2425	0.0625	0.0477	0.0174	0.1119	0.0630	0.0645	0.0017	11.10	6.36
TriLong	Elbow Extensor	0.1780	0.0638	0.2296	0.0745	0.0909	0.0834	0.1042	0.0153	0.0983	0.0501	12.65	9.28
TriMed	Elbow Extensor	0.0736	0.0359	0.1999	0.0844	0.0356	0.0086	0.1251	0.0457	0.1464	NA	14.17	11.86

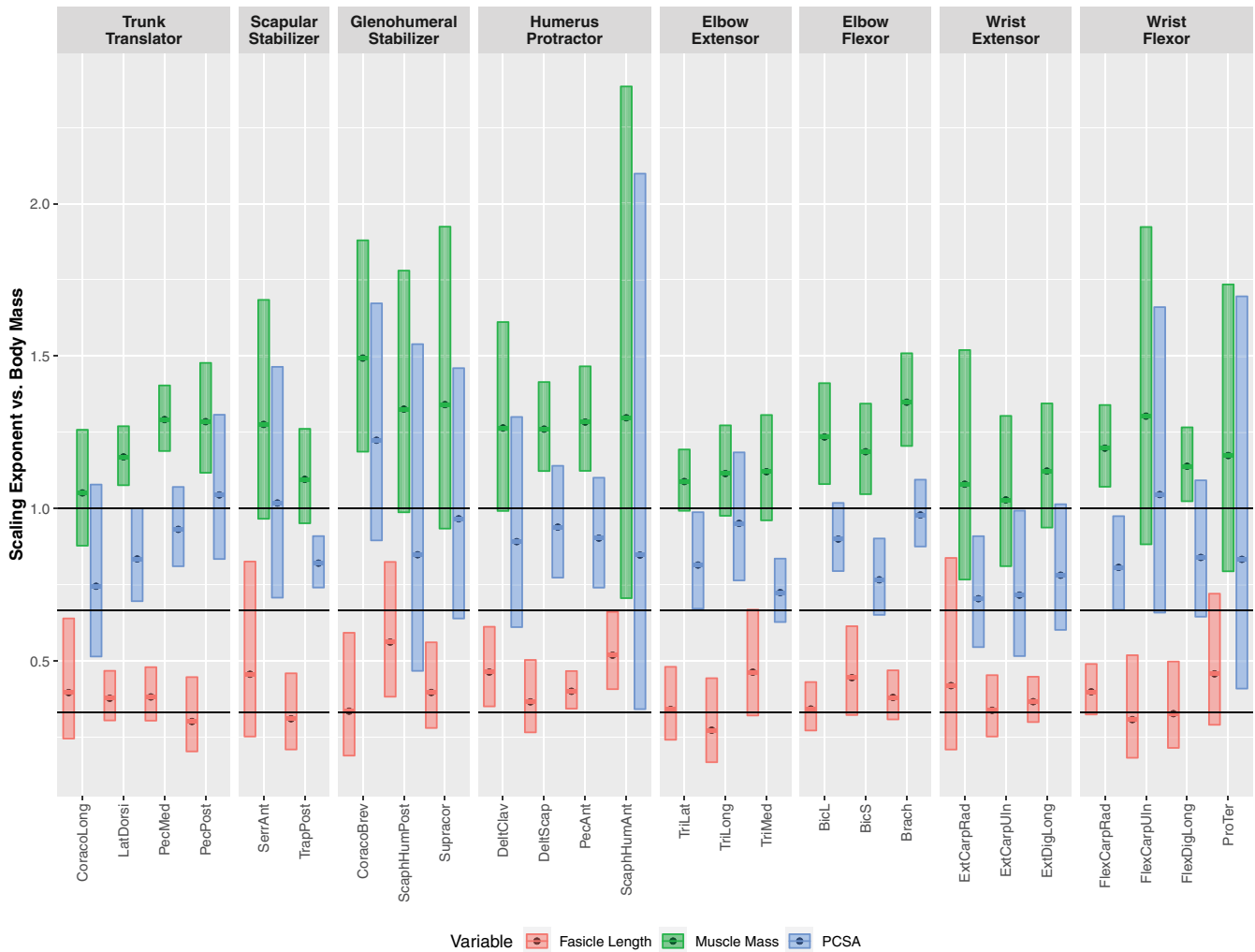


FIGURE 4 Scaling exponents for muscle properties versus body mass considering individual values. Boxes center on slopes and include 95% confidence intervals of the species mean RMA lines for log-transformed muscle properties: muscle mass (green), PCSA (blue), and fascicle length (red). Horizontal lines of isometry for length ($M^{0.33}$), area ($M^{0.66}$), and mass ($M^{1.0}$) are shown in black

properties with body mass. Although there is some debate about the utility of RMA compared with ordinary least squares regression (Seim and Sæther, 1983; Harper, 2014), it has the advantage of including variation in both the predictor and dependent variables in regression analysis. For the “individual means” approach, the *sma.R* function from the *SMATR* package was used (Warton *et al.*, 2012) in R version 6.3.6.3 (R Core Development Team 2012). The “species means” approach employed a phylogenetically informed analysis on species means using the *phyl.RMA.R* function from the *phytools* package (Revell, 2012). Muscles were considered to scale allometrically if the entire 95% confidence interval deviated from the expected isometric exponent. The maximum-likelihood tree built from 1038 bp of the NADH-2 gene for varanids (Thompson *et al.*, 2008) was used to build the phylogeny for this study (Figure 1). In the phylogeny, *V. hammsleyensis* was considered to be equivalent to *V. pilbarensis*, the two species only having been split in 2014 (Maryan *et al.*, 2014). Branch tips were set to unity using the *chronos.R* function and the tree was pruned using the *drop.tip.R* function, both from the *Ape* package (Paradis and Schliep, 2019) in R. Finally, the relative PCSA vs. relative fascicle length for each species

means of every muscle was plotted to assess differences in a muscle functional space.

2.4 | Average muscle properties

Twenty-seven pectoral girdle muscles (Table 1, Figure 2) were dissected from thirteen individuals representing eight species of Australian varanid lizards ranging from 63 to 40,000 g in body mass. The origin and insertions of the muscles were consistent with previous reports (Jenkins and Goslow, 1983; Russell and Bauer, 2008). Similar to Jenkins and Goslow (1983), we have grouped the forelimb muscles by their primary functions including translating the trunk relative to the forearm, stabilization of the scapulocoracoid, stabilizing the glenohumeral joint, protraction or retraction of the humerus, extension or flexion of the elbow, and extension or flexion of the wrist (Table 1). TrapAnt likely functions as a neck muscle (Jenkins and Goslow, 1983), but we report raw values for this muscle as they were collected and may be useful for other work. Majority of the forelimb

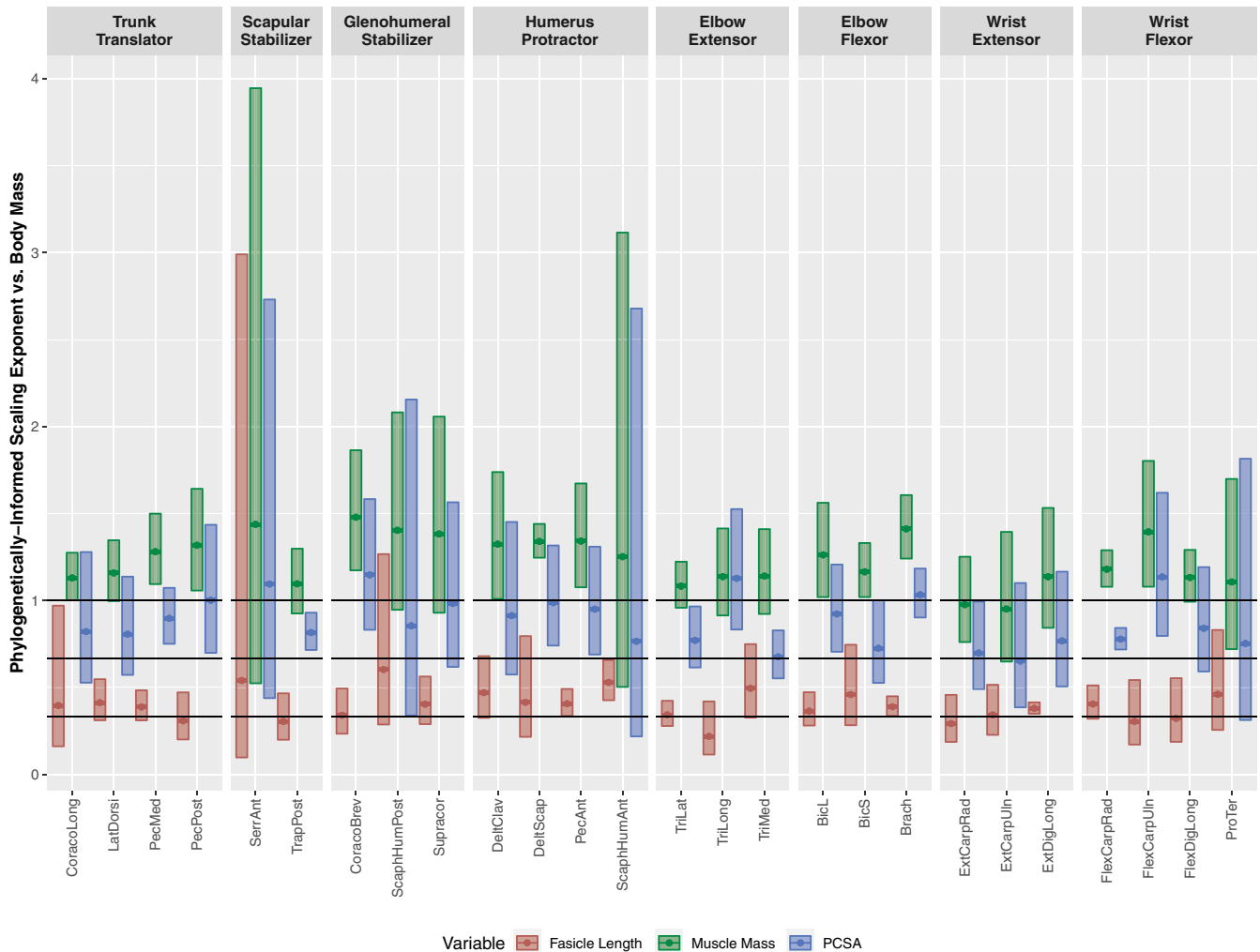


FIGURE 5 Scaling exponents for muscle properties versus body mass for a phylogenetically informed standard major axis regression of species means. Boxes center on slopes and include 95% confidence intervals of the species mean RMA lines for log-transformed muscle properties: muscle mass (green), PCSA (blue), and fascicle length (red). Horizontal lines of isometry for length ($M^{0.33}$), area ($M^{0.66}$), and mass ($M^{1.0}$) are shown in black

muscles have one or more secondary functions, but our analysis was organized around the primary function of the muscle estimated by Jenkins and Goslow (1983) from EMG and cineradiographic analysis. All raw muscle data included in subsequent analyses are presented in Table S1.

3 | RESULTS

We found a non-significant proximal to distal reduction in relative muscle mass shown by the relative muscle mass of muscles originating from the thorax ($0.171 \pm 0.18\%$), arm ($0.107 \pm 0.06\%$), and forearm ($0.089 \pm 0.04\%$) (Figure 3) mainly dominated by the large size of PecPost, LatDorsi, and PecMed. The relatively heaviest muscles (PecPost, PecMed, and LatDorsi) originate extrinsic to the forelimb (Figure 3a), but the next heaviest muscles included three muscles that originate from the arm (TriLong, BicL, and Brach) as well as the forelimb (ExtCarpRad). The relatively heaviest muscle of the forelimb

was ExtCarpRad ($0.1358 \pm 0.04\%$), and the relatively lightest muscle was ExtCarpUln ($0.0603 \pm 0.02\%$). In the arm, the relatively heaviest muscle was TriLong ($0.178 \pm 0.05\%$), and the lightest muscle was BicS ($0.0387 \pm 0.01\%$).

There was no clear distal reduction in relative fascicle length shown by the average relative fascicle lengths (fascicle length / body mass $^{0.33}$) of muscles originating from the thorax ($0.347 \pm 0.16\%$), arm ($0.253 \pm 0.07\%$), and forelimb ($0.287 \pm 0.06\%$). Indeed, the two shortest relative fascicle lengths were found in TrapAnt ($0.1965 \pm 0.06\%$) and ScaphHumPost ($0.1974 \pm 0.11\%$) which have origins extrinsic to the limb, and the third longest relative fascicle length was found in an intrinsic arm muscle CoracoLong ($0.3459 \pm 0.06\%$), while the longest fascicle lengths were in PecPost and LatDorsi, the two relatively heaviest muscles (Figure 3).

There was also no clear distal reduction in PCSA shown by the average relative PCSA (PCSA / body mass $^{0.66}$) of muscles originating in the thorax ($0.0437 \pm 0.04\%$), arm ($0.0442 \pm 0.5\%$), and forearm ($0.310 \pm 0.02\%$). PecMed had the highest ($0.1005 \pm 0.04\%$) and

TrapPost the lowest ($0.0138 \pm 0.004\%$) relative PCSA, and both of these muscles originate from the thorax (Figure 3).

Six of the twenty-seven muscles studied were pennate (pennation $>3^\circ$), and five others showed negligible amounts of pennation ($3^\circ > \text{pennation} > 0^\circ$) (Table 2). All of the truly pennate muscles were in the arm (TriMed, TriLong, TriLat, BicL, BicS, Brach) except for ProTer ($7.33 \pm 8.2^\circ$) which is a forearm muscle. The only thorax muscles with any pennation were SerrAnt ($1.79 \pm 4.6^\circ$), PecAnt ($0.27 \pm 0.9^\circ$), and DeltScap ($0.27 \pm 0.8^\circ$).

Five of the thirteen muscles that had reliably measurable proximal tendons (tendon length / body mass^{0.33}) originate from the arm (BicS, BicL, TriLong, TriLat, CoracobrachLong) (Table 2) with the first three having substantially longer tendons than the other muscles: BicS ($0.3276 \pm 0.08\%$), BicL ($0.2284 \pm 0.03\%$), and TriLong ($0.0983 \pm 0.05\%$). By comparison, all muscles investigated had measurable distal tendons except TrapAnt, TrapPost, and SerrAnt. Arm muscles generally had longer relative distal tendon lengths ($0.0978 \pm 0.07\%$) than either forearm muscles ($0.0759 \pm 0.04\%$) or thorax muscles ($0.0753 \pm 0.07\%$). The relatively longest distal tendons were found in BicS ($0.1409 \pm 0.14\%$) and PecPost ($0.1403 \pm 0.15\%$).

3.1 | Scaling regression analysis

The results of the scaling regression analyses for muscle mass, fascicle length, and PCSA are shown in Figures 4 and 5, and the slopes of the RMA lines for log-transformed muscle properties vs. body size are reported in Table 3 (individual means) and Table 4 (phylogenetically informed species means).

3.2 | Muscle mass

Scaling of muscle mass with body mass was well-correlated even using phylogenetically informed statistics ($R^2 > 0.85$ for all muscles, >0.95 for 23/27 muscles). Fourteen muscles scaled with positive allometry compared with body mass (exponent >1) including all muscles within the elbow flexor and trunk translator groups, and nearly all muscles within the humerus protractor, FlexCarpRad (slope: 1.079–1.289), and FlexCarpUln (1.079–1.802) groups (Figures 4 and 5). Two additional muscles scaled allometrically using individual means; however, the CIs trended into isometry considering species means: LatDorsi (species slopes: 0.997–1.158, individual slopes: 1.077–1.270), and FlexCarpUln (species slopes: 0.883–1.923, individual slopes: 1.079–1.802).

3.3 | Fascicle length

Considering species means, the only muscle that scaled with positive allometry (exponent >0.33) for fascicle length was ExtDigLong (slope: 0.349–0.414), although this muscle blended into isometry considering individual means (slope: 0.299–0.4486) (Figure 5). Scaling of

fascicle length with body mass again was well-correlated ($R^2 > 0.85$ for 21/27 muscles) excluding TrapPost, DeltScap, TriLong, TriMed, CoracobrachLong, and SerrAnt. Considering individual means, three other muscles scaled with positive allometry, but the CIs increased into isometry considering species means: DeltClav (slope species: 0.325–0.680), PecAnt (slope species: 0.3365–0.4915), and ScaphHumPost (slope species: 0.2872–1.266).

3.4 | Pennation angle

Pennation angle was not well-correlated with body size in any muscles using species means or individual means with the exception of one muscle: ProTer ($R^2 = 0.74$).

3.5 | PCSA

PCSA was well-correlated with body size in all muscles considering species means ($R^2 > 0.85$) with the exception of ScaphHumAnt ($R^2 = 0.72$) and ProTer ($R^2 = 0.71$). Several muscles, notably many trunk translators (PecMed, PecPost), humerus protractors (DeltScap, PecAnt), and elbow flexors (BicL, Brach), as well as CoracoBrev, TrapAnt, TrapPost, TriLong, FlexCarpRad, and FlexCarpUln, scaled with positive allometry (exponent >0.66) when considering species means (Figure 5). Considering individual means, LatDorsi (slope species: 0.696–1.007) scaled with positive allometry.

3.6 | Moment arms

Distal moment arms were well-correlated ($R^2 > 0.85$) with body size for only nine muscles considering species means and only two of these muscles showed positive allometric scaling for this trait (exponent >0.33): PecMed (slope species: 0.478–0.688) and Supracor (slope species: 0.432–0.732). Distal muscle arm scaled with positive allometry for several additional muscles considering individual means: TriLat (slope: 0.357–0.537), BicL (slope: 0.352–0.62), BicS (slope: 0.352–0.598), Brach (slope: 0.338–0.691), DeltScap (slope: 0.345–0.936), PecAnt (slope: 0.394–0.639), CoracobrachBrev (slope: 0.394–0.639), and ExtCarpUln (slope: 0.431–0.703). Proximal moment arms were not well-correlated with body mass for any muscles.

3.7 | Tendon lengths

Distal tendon lengths were only well-correlated ($R^2 > 0.85$) with body size for seven out a total of 23 muscles with measurable distal tendons considering species means. Out of these seven muscles, only two had distal tendon lengths that scaled with positive allometry vs. body mass (exponent >0.33): PecAnt (species slopes 0.453–0.712) and SerrAnt (species slopes 0.345–1.038). LatDorsi showed

TABLE 3 Results of standard ("individual means") RMA regression: Raw muscle parameters with averages and standard deviations for relative muscle mass (muscle mass / body mass), fascicle length (fascicle length / body mass ^{0.33}), PCSA (physiological cross-sectional area / body mass ^{0.66}), distal and proximal tendon lengths (tendon length / body mass ^{0.33}), and pennation (angles in degrees). All values are expressed as percentages (raw values were multiplied by 100)

A. Muscle Mass vs. Body Mass																
	LatDorsi	TrapPost	TrapAnt	PecAnt	PecMed	PecPost	BicL	BicS	Brach	DeltScap	TriLat	TriLong	TriMed	CoracolLong		
RMA	1.1693	1.0958	1.6712	1.2837	1.2918	1.2850	1.2348	1.1492	1.3488	1.2606	1.0886	1.1148	1.1209	1.0513		
lower	1.0766	0.9519	1.3144	1.1237	1.1887	1.1174	1.0803	1.0429	1.2053	1.1231	0.9927	0.9764	0.9614	0.8783		
upper	1.2701	1.2615	2.1249	1.4664	1.4037	1.4776	1.4112	1.2664	1.5093	1.4149	1.1937	1.2729	1.3068	1.2584		
r ²	0.9845	0.9649	0.9555	0.9687	0.9896	0.9704	0.9730	0.9928	0.9777	0.9798	0.9828	0.9644	0.9461	0.9672		
p	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		
CoracoBrev																
	DeltClav	ScaphHumAnt	ScaphHumPost	ExtCarpRad	ExtDigLong	ExtCarpUln	ProTer	FlexCarpRad	FlexDigLong	FlexCarpUln	SerrAnt	Supracor				
RMA	1.4934	1.2645	1.2976	1.3261	1.0799	1.0284	1.1740	1.1981	1.1388	1.3031	1.2759	1.3409				
lower	1.1866	0.9922	0.7063	0.9878	0.7673	0.8112	0.7944	1.0716	1.0241	0.8828	0.9665	0.9343				
upper	1.8796	1.6116	2.3842	1.7802	1.5198	1.3447	1.7349	1.3396	1.2663	1.9233	1.6843	1.9243				
r ²	0.9593	0.8945	0.8762	0.9537	0.9370	0.9425	0.9167	0.9935	0.9941	0.8794	0.8810	0.8634				
p	0.0001	0.0000	0.0192	0.0008	0.0015	0.0000	0.0027	0.0000	0.0000	0.0018	0.0001	0.0008				
B. Fascicle length vs. Body Mass																
	LatDorsi	TrapPost	TrapAnt	PecAnt	PecMed	PecPost	BicL	BicS	Brach	DeltScap	TriLat	TriLong	TriMed	CoracolLong		
RMA	0.3780	0.3108	0.4444	0.4009	0.3823	0.3018	0.3427	0.3935	0.3807	0.3662	0.3411	0.2734	0.4637	0.3966		
lower	0.3053	0.2101	0.2643	0.3440	0.3046	0.2037	0.2724	0.3154	0.3086	0.2663	0.2419	0.1684	0.3215	0.2459		
upper	0.4680	0.4598	0.7472	0.4672	0.4799	0.4473	0.4311	0.4910	0.4696	0.5037	0.4810	0.4438	0.6686	0.6398		
r ²	0.8949	0.7161	0.7766	0.9585	0.9209	0.7549	0.9192	0.9623	0.9214	0.8419	0.7526	0.4890	0.6820	0.7530		
p	0.0000	0.0010	0.0087	0.0000	0.0000	0.0011	0.0000	0.0001	0.0000	0.0002	0.0003	0.0114	0.0005	0.0052		
CoracoBrev																
	DeltClav	ScaphHumAnt	ScaphHumPost	ExtCarpRad	ExtDigLong	ExtCarpUln	ProTer	FlexCarpRad	FlexDigLong	FlexCarpUln	SerrAnt	Supracor				
RMA	0.3356	0.4639	0.5192	0.5622	0.4194	0.3385	0.4580	0.3991	0.3275	0.3081	0.4568	0.3969				
lower	0.1900	0.3514	0.4079	0.3831	0.2099	0.2525	0.2910	0.3248	0.2153	0.1828	0.2525	0.2806				
upper	0.5928	0.6124	0.6610	0.8251	0.8381	0.4538	0.7209	0.4904	0.4983	0.5192	0.8263	0.5614				
r ²	0.7275	0.8608	0.9824	0.9198	0.7089	0.9114	0.8857	0.9777	0.9031	0.7743	0.4066	0.8746				
p	0.0147	0.0000	0.0010	0.0025	0.0355	0.0002	0.0051	0.0002	0.0036	0.0090	0.0473	0.0006				

(Continues)

TABLE 3 (Continued)

C. Pennation vs. Body Mass															
	TriLat	TriLong	TriMed	BicL	BicS	Brach	TriLat	TriLong	TriMed	TriLat	TriLong	TriMed	ProTer		
RMA	0.4094	0.5498	0.4390	0.5525	0.6670	0.3356							-0.1410		
lower	0.2289	0.3407	0.2017	0.2489	0.2331	0.1070							-1.8402		
upper	0.7322	0.8871	0.9554	1.2263	1.9087	1.0523							-0.0108		
r ²	0.3355	0.5027	0.0777	0.4080	0.1812	0.4123							0.7395		
p	0.0618	0.0098	0.4676	0.1226	0.4000	0.2427							0.3410		
D. PCSA vs. Body Mass															
	LatDorsi	TrapPost	TrapAnt	PecAnt	PecMed	PecPost	BicL	BicS	Brach	DeltScap	TriLat	TriLong	TriMed	CoracoLong	
RMA	0.8348	0.8210	0.9319	0.9031	1.0447	0.8999	0.7665	0.9791	0.9391	0.8148	0.9515	0.7245	0.7453	0.8348	
lower	0.6964	0.7410	0.8109	0.7407	0.8346	0.7951	0.6514	0.8756	0.7735	0.6719	0.7644	0.6280	0.5149	0.6964	
upper	1.0007	0.9097	1.0709	1.1012	1.3077	1.0185	0.9019	1.0948	1.1402	0.9881	1.1843	0.8359	1.0787	1.0007	
r ²	0.9246	0.9814	0.9707	0.9299	0.9229	0.9768	0.9732	0.9780	0.9426	0.9241	0.9020	0.9533	0.8566	0.9246	
p	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010	0.0000	
	CoracoBrev	DeltClav	ScaphHumAnt	ScaphHumPost	ExtCarpRad	ExtDigLong	ExtCarpUln	ProTer	FlexCarpRad	FlexDigLong	FlexCarpUln	SerrAnt	Suprator		
RMA	1.2241	0.8916	0.8475	0.8486	0.7045	0.7815	0.7162	0.8336	0.8072	0.8398	1.0460	1.0182	0.9665		
lower	0.8957	0.6113	0.3423	0.4679	0.5457	0.6022	0.5164	0.4097	0.6682	0.6455	0.6588	0.7078	0.6396		
upper	1.6729	1.3004	2.0983	1.5391	0.9095	1.0142	0.9932	1.6959	0.9751	1.0926	1.6606	1.4647	1.4605		
r ²	0.9237	0.7373	0.6822	0.7933	0.9654	0.8955	0.8889	0.6911	0.9812	0.9632	0.8265	0.7922	0.8192		
p	0.0006	0.0007	0.0849	0.0173	0.0005	0.0000	0.0004	0.0403	0.0001	0.0005	0.0046	0.0006	0.0020		
E. Distal Tendon Length vs. Body Mass															
	TriLat	TriLong	TriMed	BicL	BicS	Brach	DeltScap	ScaphHumPost	ScaphHumAnt	ExtCarpRad	ExtDigLong	PecAnt	CoracoBrev	CoracoLong	LatDorsi
RMA	0.5212	0.3782	0.3832	0.5726	0.6423	-0.2308	0.6366	0.4664	0.4664	0.4318	0.5320	0.5320	1.2104	0.6155	0.5313
lower	0.3271	0.3247	0.2582	0.3057	0.2759	-0.4628	0.3692	0.0883	0.0883	0.2649	0.3938	0.3938	0.8586	0.2303	0.3714
upper	0.8305	0.4404	0.5688	1.0724	1.4951	-0.1151	1.0976	2.4640	2.4640	0.7040	0.7187	0.7187	1.7065	1.6450	0.7602
r ²	0.5301	0.9529	0.6270	0.3258	0.0974	0.0014	0.5903	0.2991	0.2991	0.6763	0.8360	0.8360	0.9992	0.0007	0.6960
p	0.0073	0.0000	0.0013	0.0849	0.4518	0.9133	0.0156	0.4531	0.4531	0.0065	0.0001	0.0001	0.0176	0.9559	0.0004
	PecMed	PecPost	Suprator	ExtCarpRad	ExtCarpUln	ExtDigLong	FlexCarpRad	FlexCarpUln	FlexDigLong	ProTer	FlexCarpUln	FlexCarpUln	FlexDigLong	ProTer	
RMA	0.3599	0.6575	-0.4330	-0.4330	0.3518	0.6642	0.2634	0.7325	0.4948	0.2796	0.7325	0.4948	0.2796	0.2796	
lower	0.2081	0.3909	-0.8604	-0.8604	0.1265	0.2491	0.0106	0.1278	0.3469	0.0135	0.1278	0.3469	0.0135	0.0135	
upper	0.6222	1.1061	-0.2179	-0.2179	0.9788	1.7716	6.5630	4.1995	0.7058	5.7776	4.1995	0.7058	5.7776	5.7776	

(Continues)

TABLE 3 (Continued)

	PecMed	PecPost	Supracor	ExtCarpRad	ExtCarpUln	ExtDigLong	FlexCarpRad	FlexCarpUln	FlexDigLong	ProTer				
r^2	0.5020	0.5551	0.4484	0.4484	0.5653	0.0061	0.0415	0.1655	0.9992	0.3418				
p	0.0218	0.0134	0.0693	0.0693	0.1427	0.8675	0.8694	0.5931	0.0182	0.6025				
F. Proximal Tendon Length vs. Body Mass														
	TriLong	BicL	BicS	CoracoLong	ExtCarpUln	ExtDigLong	FlexCarpUln	FlexCarpUln	FlexCarpUln					
RMA	0.3047	0.2639	0.2334	0.3337	0.3337	0.5042	0.5042	0.5042	0.5042					
lower	0.1626	0.2199	0.1365	0.1549	0.1549	0.1767	0.1767	0.1767	0.1767					
upper	0.5710	0.3167	0.3993	0.7190	0.7190	1.4390	1.4390	1.4390	1.4390					
r^2	0.0948	0.9578	0.8355	0.7883	0.7883	0.5359	0.5359	0.5359	0.5359					
p	0.3301	0.0000	0.0108	0.0443	0.0443	0.1597	0.1597	0.1597	0.1597					
G. Distal Moment Arm vs. Body Mass														
	TriLat	TriLong	TriMed	BicL	BicS	Brach	DeltScap	DeltClav	PecAnt	CoracobrachBrev	CoracoLong	LatDorsi	PecMed	PecPost
RMA	0.4379	0.4053	0.4177	0.4530	0.4586	0.4829	0.5678	0.4890	0.5013	0.5013	0.5013	0.4287	0.5737	0.4267
lower	0.3569	0.2765	0.3153	0.3313	0.3515	0.3377	0.3445	0.3117	0.3935	0.3935	0.3935	0.2959	0.4179	0.2766
upper	0.5373	0.5941	0.5535	0.6195	0.5983	0.6905	0.9361	0.7671	0.6387	0.6387	0.6387	0.6212	0.7877	0.6583
r^2	0.9254	0.7300	0.8154	0.8479	0.9274	0.7653	0.6602	0.7828	0.8949	0.8949	0.7506	0.6733	0.8437	0.6991
p	0.0000	0.0008	0.0000	0.0002	0.0001	0.0004	0.0078	0.0035	0.0000	0.0000	0.3329	0.0006	0.0002	0.0026
H. Proximal Moment Arm vs. Body Mass														
	Supracor	ExtCarpUln	ExtDigLong	FlexCarpUln	FlexCarpUln									
RMA	0.5502	0.5502	-1.1633	-1.1633	-1.1633									
lower	0.4308	0.4308	-28.7724	-28.7724	-28.7724									
upper	0.7027	0.7027	-0.0470	-0.0470	-0.0470									
r^2	0.9538	0.9538	0.0558	0.0558	0.0558									
p	0.0002	0.0002	0.8482	0.8482	0.8482									
I. Proximal Moment Arm vs. Body Mass														
	TriLong	BicL	ExtDigLong	ExtDigLong	ExtDigLong									
RMA	0.5101	1.3461	1.3461	1.3461	1.3461									
lower	0.2824	0.0543	0.0543	0.0543	0.0543									
upper	0.9214	33.3942	33.3942	33.3942	33.3942									
r^2	0.7965	0.0501	0.0501	0.0501	0.0501									
p	0.0167	0.8563	0.8563	0.8563	0.8563									

TABLE 4 Results of phylogenetically informed ("species mean") RMA analysis: Raw muscle parameters with averages and standard deviations for each muscle for relative muscle mass (muscle mass / body mass), fascicle length (fascicle length / body mass \wedge 0.33), PCSA (physiological cross-sectional area / body mass \wedge 0.66), distal and proximal tendon lengths (tendon length / body mass \wedge 0.33), and pennation (angles in degrees). All values are expressed as percentages (raw values were multiplied by 100)

A. Muscle Mass vs. Body Mass																
	LatDorsi	TrapPost	TrapAnt	PecAnt	PecMed	PecPost	BicL	BicS	Brach	DeltScap	TriLat	TriLong	TriMed	CoracoLong		
phyloRMA	1.158	1.096	1.735	1.342	1.281	1.318	1.262	1.173	1.412	1.340	1.083	1.137	1.141	1.130		
lower	0.997	0.925	1.334	1.076	1.094	1.057	1.020	1.027	1.241	1.246	0.958	0.914	0.923	1.002		
upper	1.346	1.298	2.256	1.673	1.499	1.642	1.562	1.340	1.606	1.440	1.223	1.414	1.410	1.275		
r^2	0.977	0.971	0.979	0.974	0.987	0.974	0.986	0.995	0.991	0.998	0.989	0.963	0.954	0.996		
p	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
CoracoBrev																
	DeltClav	ScaphHumAnt	ScaphHumPost	ExtCarpRad	ExtDigLong	ExtCarpUln	ProTer	FlexCarpRad	FlexDigLong	FlexCarpUln	SerrAnt	Suprator				
phyloRMA	1.479	1.324	1.252	1.404	0.976	1.136	1.106	1.179	1.132	1.395	1.438	1.383				
lower	1.173	1.009	0.503	0.947	0.761	0.843	0.721	1.079	0.993	1.079	0.524	0.929				
upper	1.864	1.738	3.115	2.081	1.252	1.532	1.699	1.289	1.291	1.802	3.946	2.057				
r^2	0.984	0.961	0.882	0.982	0.981	0.952	0.942	0.998	0.995	0.980	0.847	0.914				
p	0.000	0.000	0.009	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.009	0.000				
B. Fascicle length vs. Body Mass																
	LatDorsi	TrapPost	TrapAnt	PecAnt	PecMed	PecPost	BicL	BicS	Brach	DeltScap	TriLat	TriLong	TriMed	CoracoLong		
phyloRMA	0.4129	0.3050	0.4969	0.4067	0.3878	0.3081	0.3645	0.4316	0.3889	0.4146	0.3433	0.2190	0.4948	0.3961		
lower	0.3114	0.1995	0.2793	0.3365	0.3108	0.2012	0.2811	0.2990	0.3366	0.2161	0.2784	0.1143	0.3268	0.1617		
upper	0.5475	0.4662	0.8840	0.4915	0.4838	0.4717	0.4728	0.6230	0.4495	0.7955	0.4233	0.4198	0.7491	0.9703		
r^2	0.9181	0.8084	0.8903	0.9812	0.9742	0.9000	0.9795	0.9583	0.9891	0.8554	0.9663	0.6321	0.8176	0.6914		
p	0.1026	0.6737	0.0978	0.0311	0.1046	0.6827	0.2945	0.0847	0.0280	0.3558	0.6495	0.1833	0.0575	0.5980		
CoracoBrev																
	DeltClav	ScaphHumAnt	ScaphHumPost	ExtCarpRad	ExtDigLong	ExtCarpUln	ProTer	FlexCarpRad	FlexDigLong	FlexCarpUln	SerrAnt	Suprator				
phyloRMA	0.3401	0.4702	0.5296	0.6032	0.2924	0.3802	0.3424	0.4610	0.3220	0.3052	0.5401	0.4035				
lower	0.2342	0.3251	0.4266	0.2872	0.1870	0.3489	0.2274	0.2559	0.3201	0.1715	0.0976	0.2893				
upper	0.4940	0.6799	0.6575	1.2666	0.4573	0.4143	0.5155	0.8304	0.5119	0.5430	2.9900	0.5630				
r^2	0.9568	0.9262	0.9949	0.9288	0.9367	0.9962	0.9476	0.8850	0.9834	0.8903	0.2254	0.9403				
p	0.8137	0.0505	0.0017	0.0421	0.4515	0.0071	0.7943	0.1616	0.0516	0.8973	0.4751	0.1639				

(Continues)

TABLE 4 (Continued)

C. Pennation vs. Body Mass															
	BicL	BicS	Brach	TriLat	TriLong	TriMed	TriLong	TriMed	CoracoLong	CoracoBrev	TriLat	TriMed			
phyloRMA	0.5731	0.6284	0.3899	0.2479	0.5001	0.3351	0.5001	0.3351	0.8191	1.1472	0.2479	0.3351			
lower	0.1688	0.1711	0.0903	0.1170	0.2622	0.1196	0.2622	0.1196	0.5300	0.8388	0.1170	0.1196			
upper	1.9463	2.3081	1.6840	0.5251	0.9537	0.9388	0.9537	0.9388	1.2660	1.5691	0.5251	0.9388			
r ²	0.2877	0.1435	0.5487	0.6484	0.6383	0.2249	0.6383	0.2249	0.9402	0.9700	0.6484	0.2249			
p	0.3125	0.2841	0.7454	0.3785	0.1750	0.9732	0.1750	0.9732	0.0029	0.0002	0.3785	0.9732			
D. PCSA vs. Body Mass															
	LatDorsi	TrapPost	PecAnt	PecMed	PecPost	BicL	BicS	Brach	TriLat	TriLong	TriMed	TriLong	TriMed	CoracoLong	CoracoBrev
phyloRMA	0.8066	0.8158	0.8974	0.9501	1.0015	0.9155	0.7549	1.0293	0.7592	1.0028	0.6885	1.0028	0.6885	0.8191	1.1472
lower	0.5722	0.7156	0.7509	0.6894	0.6988	0.7480	0.5587	0.8968	0.6077	0.7017	0.5743	0.7017	0.5743	0.5300	0.8388
upper	1.1371	0.9301	1.0726	1.3095	1.4353	1.1205	1.0199	1.1815	0.9485	1.4333	0.8253	1.4333	0.8253	1.2660	1.5691
r ²	0.8771	0.9827	0.9833	0.9447	0.9299	0.9877	0.9724	0.9901	0.9749	0.8993	0.9667	0.8993	0.9667	0.9402	0.9700
p	0.0007	0.0000	0.0000	0.0004	0.0005	0.0001	0.0010	0.0000	0.0003	0.0004	0.0001	0.0004	0.0001	0.0029	0.0002
E. Distal Moment Arm vs. Body Mass															
	DeltClav	ScaphHumAnt	ScaphHumPost	ExtCarpRad	ExtCarpUln	ExtDigLong	ExtCarpUln	ProTer	FlexCarpRad	FlexCarpUln	SerrAnt	dSupracor			
phyloRMA	0.9131	0.7652	0.8547	0.6984	0.6984	0.7679	0.6516	0.7611	0.7779	1.1356	1.1052	0.9834			
lower	0.5744	0.2186	0.3389	0.4908	0.4908	0.5059	0.3858	0.3178	0.7184	0.7963	0.5566	0.6182			
upper	1.4515	2.6785	2.1556	0.9938	0.9938	1.1657	1.1006	1.8226	0.8423	1.6194	2.1942	1.5645			
r ²	0.8803	0.7209	0.8780	0.9616	0.9616	0.9042	0.9109	0.7105	0.9981	0.9611	0.9407	0.8799			
p	0.0023	0.0977	0.0248	0.0026	0.0026	0.0033	0.0164	0.0517	0.0000	0.0004	0.0041	0.0017			
E. Distal Moment Arm vs. Body Mass															
	LatDorsi	PecAnt	PecMed	PecPost	BicL	BicS	Brach	DeltScap	TriLat						
phyloRMA	0.4129	0.5219	0.5735	0.4439	0.4092	0.4458	0.4973	0.6356	0.4012						
lower	0.2246	0.3657	0.4779	0.3134	0.2197	0.2368	0.3146	0.2407	0.2919						
upper	0.7591	0.7450	0.6882	0.6287	0.7621	0.8395	0.7864	1.6783	0.5513						
r ²	0.5802	0.9315	0.9825	0.9345	0.8699	0.8646	0.8832	0.8618	0.9209						
p	0.4263	0.0189	0.0005	0.0713	0.3587	0.2281	0.0639	0.0783	0.1765						

(Continues)

TABLE 4 (Continued)

	TriLong	TriMed	CoracoLong	DeltClav	Supracor								
phyloRMA	0.3809	0.4155	-0.6697	0.5087	0.5625								
lower		0.2803	-0.0286	0.3222	0.4321								
upper	0.6443	0.6158	-15.6846	0.8030	0.7321								
r^2	0.8429	0.8367	0.1536	0.8841	0.9631								
p	0.5032	0.2103	1.0000	0.0540	0.0032								
F. Proximal Moment Arm vs. Body Mass													
	BicL	TriLat	TriLong	ExtDigLong	ExtCarpUln	FlexCarpUln	SerrAnt						
phyloRMA	0.5625	0.5625	0.4710	0.4710	0.4710	0.4710	0.4710						
lower	0.4321	0.4321	0.2132	0.2132	0.2132	0.2132	0.2132						
upper	0.7321	0.7321	1.0406	1.0406	1.0406	1.0406	1.0406						
r^2	0.9631	0.9631	0.7715	0.7715	0.7715	0.7715	0.7715						
p	0.0032	0.0032	0.2642	0.2642	0.2642	0.2642	0.2642						
G. Proximal Tendon Length vs. Body Mass													
	TriLong	BicL	ExtDigLong	ExtCarpUln	ExtDigLong								
phyloRMA	0.2843	0.2593	0.4252	0.4252	0.4252								
lower	0.1124	0.1939	0.1017	0.1017	0.1017								
upper	0.7187	0.3468	1.7780	1.7780	1.7780								
r^2	0.1396	0.9742	0.5803	0.5803	0.5803								
p	0.7302	0.0597	0.6129	0.6129	0.6129								
H. Distal Tendon Length vs. Body Mass													
	TriLat	TriLong	TriMed	BicL	Brach	DeltScap	DeltClav	PecAnt	CoracoBrev	CoracoLong	LatDorsi	PecMed	PecPost
phyloRMA	0.4774	0.3843	0.3700	0.5400	-0.1019	0.5808	0.4056	0.5706	1.3172	0.3601	0.5984	0.2661	0.4776
lower	0.2537	0.3063	0.2204	0.2709	-0.0345	0.2523	0.1963	0.4531	0.2082	0.0577	0.3449	0.1508	0.1995
upper	0.8982	0.4823	0.6213	1.0762	-0.3008	1.3371	0.8383	0.7186	8.3353	2.2479	1.0384	0.4696	1.1437
r^2	0.6552	0.9604	0.7059	0.8353	0.1131	0.7417	0.8145	0.9719	0.9411	0.0007	0.6635	0.8138	0.4930
p	0.2112	0.1435	0.6223	0.1015	1.0000	0.1231	0.4518	0.0016	0.0144	0.9078	0.0427	0.3656	0.3448
I. Distal Tendon Length vs. Body Mass													
	Supracor	ExtCarpUln	ExtDigLong	FlexCarpUln	ExtCarpUln								
phyloRMA	-0.4433	0.3965	-0.6107	0.4209	0.4209								
lower	-0.1806	0.1584	-0.0981	0.2254	0.2254								
upper	-1.0883	0.9926	-3.8016	0.7860	0.7860								
r^2	0.4562	0.9931	0.0068	0.9973	0.9973								
p	1.0000	0.1244	1.0000	0.0243	0.0243								

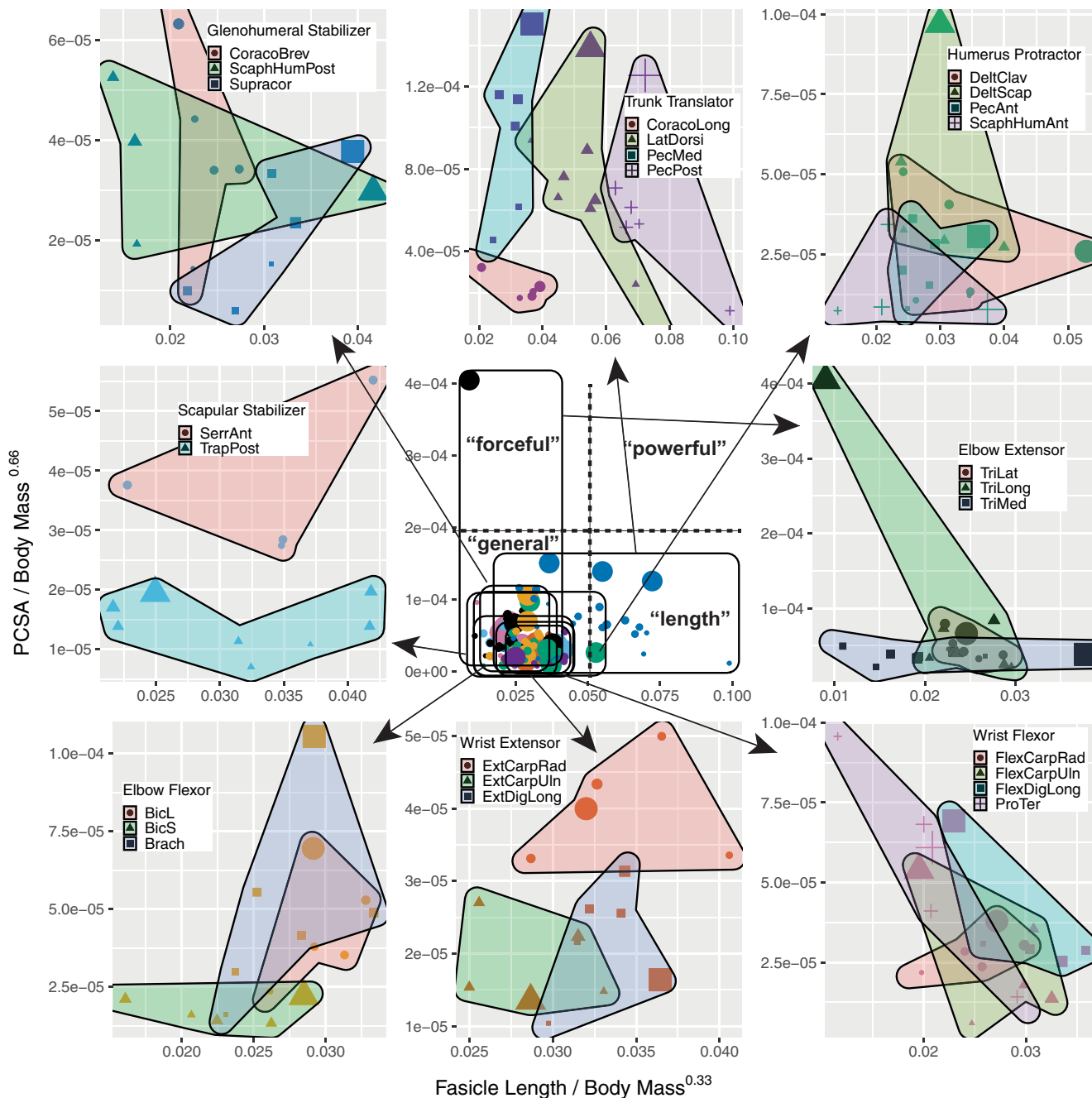


FIGURE 6 Performance space plot (fascicle length versus PCSA normalized by appropriate body mass scaling exponents) for each forelimb muscle. Species means are represented for each muscle where the size of the symbol indicates the mean body mass and color represents the functional group of each muscle. Rectangles highlight muscle groups with divergent properties. Detailed performance space plots for each functional group of muscles surround the summary plot for *all* muscles

some correlation ($R^2 > 0.5$) and allometric scaling: LatDorsi (species slopes 0.345–1.038). Considering individual means, the tendons of additional muscles showed some correlation ($R^2 > 0.5$) and positive allometric scaling: DeltScap (individual slopes 0.369–1.098), PecPost (individual slopes 0.391–1.106), FlexDigLong (individual slopes 0.347–0.706), and CoracoBrev (individual slopes 0.857–1.707). On average, distal tendon lengths (scaled to body mass^{0.33}) were greater

in the muscles of the arm (0.0924) than in the forearm (0.0712) or thorax (0.0658).

Proximal tendon length was well-correlated with body mass ($R^2 > 0.85$) for only one muscle (BicL) considering species means and scaled isometrically. Considering individual means, BicS also correlated well with body size and scaled isometrically. On average, proximal tendon lengths (scaled to body mass^{0.33}) were

greater in the muscles of the arm (0.148) than forearm (0.0466) or thorax (0.0326).

4 | DISCUSSION

We sought to investigate how forelimb muscle architecture responded to the biomechanical demands of increased body size in varanid lizards. One major finding of this study is that the pectoral muscles of varanid lizards generally scale with positive allometry with increases in body size, particularly for muscle mass and PCSA but less often for fascicle length. This suggests that muscles are becoming more force-specialized powerful with larger body sizes (Figure 6), presumably responding to the increases in biomechanical stress on bones and muscles caused by increased body mass without corresponding changes in posture (Clemente *et al.*, 2011; Cuff *et al.*, 2016a; 2016b; Dick and Clemente, 2016; 2017).

In terms of the distribution of muscle mass and pennation, we observed a proximal to distal gradient in muscle mass as seen in many other species (Allen *et al.*, 2010; 2014; Martin *et al.*, 2019). Unlike in the hindlimb, however, we did not observe a substantial proximal to distal gradient in relative fascicle lengths or PCSA. This may be explained by the mobile sternocoracoid joint (Jenkins and Goslow, 1983) in varanids which might enable them to achieve sufficient stride length without long fascicles and muscle excursions. Glenohumeral sliding also occurs in crocodylids, but it is thought to have only a minor impact on stride length (Baier and Gatesy, 2013). Pennate muscles were mostly localized into the arm muscles, consistent with scaling studies in the rat and alligator, and also the hindlimb of varanid lizards (Eng *et al.*, 2008; Allen *et al.*, 2010; Dick and Clemente, 2016).

Although tendon lengths in our dataset generally exhibit poor correlations with body mass and should therefore be interpreted with caution, our tendon length results are somewhat unexpected. Positive allometry for distal tendon length was observed in three proximal muscles: PecAnt, SerrAnt, and LatDorsi, indicating that these proximal muscles may better suited to benefit from elastic energy storage (Alexander, 1984), or maintain larger joint ranges of motion (Alexander *et al.*, 1981; Dick and Clemente, 2016) with increased body size. By contrast, distal tendon length was found to scale with negative allometry in several distal muscles (BicL, Brach, Supracor, and ExtCarpRad). In a study of ambulatory and cursorial mammals ranging from 0.04 kg to 338 kg, Pollock and Shadwick (1994) noted that positive tendon length allometry was found in muscles subject to high loading by ground impact forces (ankle extensors and digital flexors) but not in the digital extensors, muscles which cannot act as springs during stance phase. Although tendons from many of the forelimb muscles were not measured, negative allometry of distal tendon length in two elbow flexors (BicL and Brach), which should experience loading from ground impact forces, suggests that elastic energy storage may become less important with body size in varanids, at least with respect to body support.

Our results muddle the relationship between limb segment length and fascicle length. Previous studies in varanids have shown that limbs get relatively longer with body length (Christian and Garland, 1996; Thompson and Withers, 1997) or scale isometrically with body mass (Dick and Clemente, 2016). However, studies have also shown that varanid limbs get relatively thicker than they do longer with increases in body length (Christian and Garland, 1996) and fascicle lengths scale isometrically for all but a single varanid pectoral muscle. This is quite unlike reports for crocodylids which suggests a more direct relationship between limb segment and muscle fascicle length. In alligators, fascicle lengths scale with positive allometry with body size in nearly half of the measured pectoral muscles, even though limb segment scale with negative allometry (Allen *et al.*, 2010). Crocodylids, which have relatively longer limbs than alligators (Iijima *et al.*, 2018), also have higher-scaling muscle properties compared with alligatorids. Future studies on how locomotor kinematics scale with body size in varanids are needed to determine whether larger varanids are constrained by quick or long strides, or whether muscle architecture is decoupled from locomotor kinematics by lateral undulation and/or the mobile coracosternal joint.

Interestingly, many more muscles of the forelimb scale with positive allometry in terms of muscle mass vs. body mass (14-16/27) and PCSA vs. body mass (11-13/27) compared with hindlimb muscles of the same genus (4-7/22 and 4/22 for muscle mass and PCSA, respectively) (Dick and Clemente, 2016). More widespread positive allometry in forelimb than hindlimb muscles is consistent with previous studies in crocodylids (Allen *et al.*, 2010; 2014). A greater proportion of the measured pectoral muscles were found to scale with positive allometry in our study on varanids (11-13/27) and in crocodylids (16/36) than in that on alligatorids (9/36) (Allen *et al.*, 2010; 2014). In varanids, the forelimb muscles also scale with substantially higher exponents than hindlimb muscles in muscle mass (FL 1.23; HL 1.07) and PCSA (FL 0.881; HL 0.761), and with a less substantially higher slope for fascicle length (FL 0.39; HL 0.33).

More widespread and greater positive allometric scaling of muscle properties in the forelimb compared with the hindlimb may suggest that the center of mass moves cranially with body size in varanids, as is the case in crocodylians (Iijima and Kubo, 2019)—such that larger varanids support proportionally more weight on their forelimbs than smaller varanids. Head and neck length scale positively with body length in larger varanids (Thompson and Withers, 1997), and the associated proportionate increase in head mass may move the center of mass cranially with increases in body size. Alternatively, the fact that some larger varanids are specialized climbers (e.g. *V. varius* can reach 14 kg (Weavers, 2004)) may drive this increase in positive allometry when comparing the scaling of muscle architecture in the forelimb versus hindlimb. Climbing may also explain why many of the forelimb flexors scale with positive allometry, as some of the muscles that act as flexors during locomotion may also adduct the forelimb during climbing (e.g. *biceps brachii*; Jenkins and Goslow, 1983).

Changes in forelimb varanid muscle architecture also suggest a differing role in support versus propulsion when compared to the

hindlimbs. The equal or higher rates of scaling observed in forelimb muscle groups primarily responsible for forward locomotion (trunk translators, humerus protractors, elbow flexors) compared with muscle groups primarily associated with resisting gravity (glenohumeral stabilizers, elbow extensors, wrist flexors) suggest that the demands of forward locomotion may have just as important of an impact on muscle scaling as body support. Following this, greater scaling in the forelimb muscles may indicate that the forelimb contributes relatively more forward propulsive force at higher body sizes regardless of the center of mass. Studies comparing the ground reaction forces from forelimb and hindlimb in different sized varanids moving at different speeds are needed to test the influence of body size on center of mass and the relative contributions of the pelvic and pectoral girdle towards locomotion.

Varanids do not adopt a more erect posture as body size gets larger, as have been reported among mammals (Biewener, 1989; Biewener, 2005) and likely deal with the increased limb stresses associated with larger body sizes by increasing duty factor (Clemente *et al.*, 2011). However, increased duty factor likely imposes a trade-off against locomotor speed with increased size, supported by an observed reduction in sprint speed in larger varanids (Auffenberg, 1981; Clemente *et al.*, 2009b). Varanids could maintain higher speeds even with high duty factors by increasing stride length or by reducing swing-phase time. The scaling of muscle architecture properties among the forearm muscles makes sense in light of these demands and the mobile coracosternal joint of varanids as detailed below.

Among forelimb muscles primarily involved with body support against gravity tend to scale with lower exponents than those with dual roles in body support and propulsion, or primary roles in propulsion alone (Figures 4 and 5). Out of six muscles mainly involved in resisting gravity and maintaining posture during the stance phase (glenohumeral joint stabilizers and elbow extensors) only TriLong and CoracoBrev scale with positive allometry for PCSA. By contrast, two of three elbow flexors and two of four wrist flexors, muscles thought to make similar contributions to body support and locomotion, scale allometrically for PCSA. Finally, four of the eight muscles with primarily locomotor roles (translators of the trunk and protractors of the humerus) scale isometrically with regard to PCSA. Finally, muscles primarily active during swing phase show allometric increases in fascicle length as well as PCSA and muscle mass including DeltClav, PecAnt, and ScaphHumAnt (protractors of the humerus), ScaphHumPost, Brach, and TrapPost. These results suggest a strong link between musculoskeletal form and function as varanids increases in size.

Many muscles involved in the swing phase of gait also become more length specialized with increasing body size (Figure 6). These muscles must likely also generate increased force with body size, to translate the trunk forward around the scapulocoracoid joint (Jenkins and Goslow, 1983). The allometric increase in fascicle lengths, however, suggests they are also adapting to operate over longer excursion lengths and to contract more rapidly to allow for longer stride lengths and faster swing phases in larger animals. It seems that

selection for higher power output was greater than either excursion distance or contraction speed, as most of these swing-phase muscles show greater scaling for PCSA than fascicle length (DeltScap, Brach, PecAnt, TrapPost). This agrees with kinematic data that found no increase in the range of femoral protraction-retraction angles with body size in varanids (Clemente *et al.*, 2011). Wrist extensors prove an exception to the pattern by scaling mainly isometrically, presumably because hand size scales with lower allometric exponents than forearm or arm size (Christian and Garland, 1996).

With limb muscles becoming more specialized for force over excursion with increased body size (although the elbow flexors seem to scale toward power production) (Figure 6), the role of skeletal features on stride length might be quite important. The coracosternal joint translates cranially to increase stride length in *V. exanthematicus* (Jenkins and Goslow, 1983) and is also mobile in *V. gouldii* and *V. giganteus* (authors' observations), and presumably other large varanids. The degree to which coracosternal joint mobility and the lateral bending of the vertebral column (Cieri *et al.*, 2020) control stride length in varanids remains unknown, but both likely contribute.

Taken together, these facts may suggest an interesting suite of adaptations to maintain body support and forward propulsion in large-bodied varanids. Among larger-bodied varanids, duty factor increases to mitigate relatively higher limb stresses (Clemente *et al.*, 2011). In addition, gravity-resisting muscles become more force-specialized in the hindlimb (Dick and Clemente, 2016) and particularly the forelimb (shown here) to deal with increases in body mass (Dick and Clemente, 2017). The higher proportion of muscles that scale with positive allometry in the forelimb may suggest a general shift in the body center of mass cranially. These changes likely result in a reduction in speed, and to compensate, the mobile coracosternal joints as well as forelimb muscles with relatively larger PCSAs and longer fascicles may increase stride length. These changes in muscle properties may also be required to generate enough power to pull one side of the trunk forward at the coracosternal joint, as well as more powerful knee, ankle, and wrist flexors to provide more forward locomotive force. Future studies comparing force and kinematic data in varanids of different body sizes locomoting at different speeds is needed to test this intriguing scenario.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to data collection and analysis. CC and TD originally designed the study with later contributions from RC. RC wrote the manuscript and all authors contributed equally to manuscript revision and intellectual content.

DATA AVAILABILITY STATEMENT

Raw data is available in the supplementary files.

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REFERENCES

- Alexander, R.M. (1984) Elastic energy stores in running vertebrates. *Integrative and Comparative Biology*, 24, 85–94.
- Alexander, R.M.N., Jayes, A.S., Maloiy, G.M.O. and Wathuta, E.M. (1981) Allometry of the leg muscles of mammals. *Journal of Zoology*, 194, 539–552.
- Alexander, R.M. and Ker, R. (1990) The architecture of leg muscles. In: Winters, J. and Woo, W.-Y. (Eds.) *Multiple Muscle Systems*. New York: Springer-Verlag, pp. 568–577.
- Allen, V.R., Elsey, R.M., Jones, N., Wright, J. and Hutchinson, J.R. (2010) Functional specialization and ontogenetic scaling of limb anatomy in Alligator mississippiensis. *Journal of Anatomy*, 216, 423–445.
- Allen, V.R., Molnar, J., Parker, W., Pollard, A., Nolan, G. and Hutchinson, J.R. (2014) Comparative architectural properties of limb muscles in Crocodylidae and Alligatoridae and their relevance to divergent use of asymmetrical gaits in extant Crocodylia. *Journal of Anatomy*, 225, 569–582.
- Auffenberg, W. (1981) *The Behavioral Ecology of the Komodo Monitor*. Gainesville: University Press of Florida.
- Baier, D.B. and Gatesy, S.M. (2013) Three-dimensional skeletal kinematics of the shoulder girdle and forelimb in walking Alligator. *Journal of Anatomy*, 223, 462–473.
- Biewener, A.A. (1989) Scaling body support in mammals: limb posture and muscle mechanics. *Science*, 245, 45–48.
- Biewener, A.A. (2005) Biomechanical consequences of scaling. *Journal of Experimental Biology*, 208, 1665–1676.
- Böhmer, C., Fabre, A.C., Herbin, M., Peigné, S. and Herrel, A. (2018) Anatomical basis of differences in locomotor behavior in martens: a comparison of the forelimb musculature between two sympatric species of *Martes*. *Anatomical Record*, 301, 449–472.
- Burggren, W.W. and Johansen, K. (1982) Ventricular haemodynamics in the monitor lizard *Varanus exanthematicus*: pulmonary and systemic pressure separation. *Journal of Experimental Biology*, 343–354.
- Calow, L.J. and Alexander, R.M.N. (1973) A mechanical analysis of a hind leg of a frog (*Rana temporaria*). *Journal of Zoology*, 171, 293–321.
- Christian, A. and Garland, T. (1996) Scaling of limb proportions in monitor lizards (Squamata: Varanidae). *Journal of Herpetology*, 30, 219.
- Cieri, R.L. (2018) The axial anatomy of monitor lizards (Varanidae). *Journal of Anatomy*, 233, 636–643.
- Cieri, R.L. and Farmer, C.G. (2019) Computational fluid dynamics reveals a unique net unidirectional pattern of pulmonary airflow in the savannah monitor lizard (*Varanus exanthematicus*). *Anatomical Record*, 303, 1768–1791.
- Cieri, R.L., Hatch, S.T., Capano, J.G. and Brainerd, E.L. (2020) Locomotor rib kinematics in two species of lizards and a new hypothesis for the evolution of aspiration breathing in amniotes. *Scientific Reports*, 10, 1–10.
- Clemente, C.J. (2014) The evolution of bipedal running in lizards suggests a consequential origin may be exploited in later lineages. *Evolution (NY)*, 68, 2171–2183.
- Clemente, C.J., Withers, P.C. and Thompson, G.G. (2009a) Metabolic rate and endurance capacity in Australian varanid lizards (Squamata: Varanidae: *Varanus*). *Biological Journal of the Linnean Society*, 97, 664–676.
- Clemente, C.J., Thompson, G.G. and Withers, P.C. (2009b) Evolutionary relationships of sprint speed in Australian varanid lizards. *Journal of Zoology*, 278, 270–280.
- Clemente, C.J., Withers, P.C., Thompson, G.G. and Lloyd, D. (2011) Evolution of limb bone loading and body size in varanid lizards. *Journal of Experimental Biology*, 214, 3013–3020.
- Clemente, C.J., Withers, P.C. and Thompson, G.G. (2012) Optimal body size with respect to maximal speed for the yellow-spotted monitor lizard (*Varanus panoptes*; varanidae). *Physiological and Biochemical Zoology*, 85, 265–273.
- Cuff, A.R., Sparkes, E.L., Randau, M., Pierce, S.E., Kitchener, A.C., Goswami, A. et al. (2016a) The scaling of postcranial muscles in cats (Felidae) I: forelimb, cervical, and thoracic muscles. *Journal of Anatomy*, 229, 128–141.
- Cuff, A.R., Sparkes, E.L., Randau, M., Pierce, S.E., Kitchener, A.C., Goswami, A. et al. (2016b) The scaling of postcranial muscles in cats (Felidae) II: hindlimb and lumbosacral muscles. *Journal of Anatomy*, 229, 142–152.
- Day, L.M. and Jayne, B.C. (2007) Interspecific scaling of the morphology and posture of the limbs during the locomotion of cats (Felidae). *Journal of Experimental Biology*, 210, 642–654.
- Dick, T.J.M. and Clemente, C.J. (2016) How to build your dragon: Scaling of muscle architecture from the world's smallest to the world's largest monitor lizard. *Frontiers in Zoology*, 13, 1–17.
- Dick, T.J.M. and Clemente, C.J. (2017) Where have all the giants gone? How animals deal with the problem of size. *PLoS Biology*, 15, 1–10.
- Eng, C.M., Smallwood, L.H., Rainiero, M.P., Lahey, M., Ward, S.R. and Lieber, R.L. (2008) Scaling of muscle architecture and fiber types in the rat hindlimb. *Journal of Experimental Biology*, 211, 2336–2345.
- Hanemaaijer, J., Gregorovicova, M., Nielsen, J.M., Moorman, A.F.M., Wang, T., Planken, R.N. et al. (2019) Identification of the building blocks of ventricular septation in monitor lizards (Varanidae). *Development*, 146, dev177121.
- Harper, W.V. (2014) Reduced major axis regression: Teaching alternatives to least squares. ICOTS-9 Conference Proceedings held in July 2014 in Flagstaff, AZ and runs from pages 1-4 on a separate PDF on their website. <https://iaseweb.org/icots/9/proceedings/home.html>
- Iijima, M. and Kubo, T. (2019) Allometric growth of limb and body proportions in crocodylians. *Journal of Zoology*, 309, 200–211.
- Iijima, M., Kubo, T. and Kobayashi, Y. (2018) Comparative limb proportions reveal differential locomotor morphofunctions of alligatoroids and crocodyloids. *Royal Society Open Science*, 5, 171774.
- Jenkins, F.A. and Goslow, G.E. (1983) The functional anatomy of the shoulder of the savannah monitor lizard (*Varanus exanthematicus*). *Journal of Morphology*, 216, 195–216.
- Martin, M.L., Warburton, N.M., Travouillon, K.J. and Fleming, P.A. (2019) Mechanical similarity across ontogeny of digging muscles in an Australian marsupial (*Isoodon fusciventer*). *Journal of Morphology*, 280, 423–435.
- Maryan, B., Oliver, P.M., Fitch, A.J. and O'Connell, M. (2014) Molecular and morphological assessment of *Varanus pilbarensis* (Squamata: Varanidae), with a description of a new species from the southern Pilbara, Western Australia. *Zootaxa*, 3768, 139–158.

- Moritz, S. and Schilling, N. (2013) Fiber-type composition in the perivertebral musculature of lizards: Implications for the evolution of the diapsid trunk muscles. *Journal of Morphology*, 274, 294–306.
- Munns, S.L., Hartzler, L.K., Bennett, A.F. and Hicks, J.W. (2004) Elevated intra-abdominal pressure limits venous return during exercise in *Varanus exanthematicus*. *Journal of Experimental Biology*, 207, 4111–4120.
- Narici, M.V., Landoni, L. and Minetti, A.E. (1992) Assessment of human knee extensor muscles stress from in vivo physiological cross-sectional area and strength measurements. *European Journal of Applied Physiology and Occupational Physiology*, 65, 438–444.
- Owerkovicz, T., Farmer, C.G., Hicks, J.W. and Brainerd, E.L. (1999) Contribution of gular pumping to lung ventilation in monitor lizards. *Science*, 284, 1661–1663.
- Paradis, E. and Schliep, K. (2019) Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35, 526–528.
- Payne, R.C., Hutchinson, J.R., Robilliard, J.J., Smith, N.C. and Wilson, A.M. (2005) Functional specialisation of pelvic limb anatomy in horses (*Equus caballus*). *Journal of Anatomy*, 206, 557–574.
- Pianka, E.R. and King, D.R. (Eds) (2004) *Varanoid Lizards of the World*. Bloomington: Indiana University Press.
- Revell, L.J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223.
- Russell, A.P. and Bauer, A. (2008) The appendicular locomotor apparatus of lepidosaurs. In: Gaunt, A. and Adler, K. (Eds.) *Biology of the Reptilia*, vol. 21. Ithaca, NY: Society for the Study of Amphibians and Reptiles. pp. 1–466.
- Sacks, R.D. and Roy, R.R. (1982) Architecture of the hind limb muscles of cats: Functional significance. *Journal of Morphology*, 173, 185–195.
- Schachner, E.R., Cieri, R.L., Butler, J.P. and Farmer, C.G. (2013) Unidirectional pulmonary airflow patterns in the savannah monitor lizard. *Nature*, 506, 367–370.
- Schmidt-Nielsen, K. (1984) *Scaling: Why is Animal Size So Important?*. New York, NY: Cambridge University Press.
- Seim, E. and Sæther, B.E. (1983) On rethinking allometry: Which regression model to use? *Journal of Theoretical Biology*, 104, 161–168.
- Thompson, G.G. and Withers, P.C. (1997) Comparative morphology of Western Australian varanid lizards (Squamata: Varanidae). *Journal of Morphology*, 233, 127–152.
- Thompson, G.G., Withers, P.C. and Cowan, E. (1997) Standard and maximal metabolic rates of goannas (Squamata: Varanidae). *Physiological Zoology*, 70, 307–323.
- Thompson, G.G., Clemente, C.J., Withers, P.C., Fry, B.G. and Norman, J.A. (2008) Is body shape of varanid lizards linked with retreat choice? *Australian Journal of Zoology*, 56, 351.
- Wang, T., Carrier, D.R. and Hicks, J.W. (1997) Ventilation and gas exchange in lizards during treadmill exercise. *Journal of Experimental Biology*, 200, 2629–2639.
- Warton, D.I., Duursma, R.A., Falster, D.S. and Taskinen, S. (2012) smatr 3- an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution*, 3, 257–259.
- Weavers, B. (2004) *Varanus varius*. In: Pianka, E.R. and King, D.R. (Eds.) *Varanoid Lizards of the World*. Bloomington: Indiana University Press. pp. 488–515.
- Wroe, S. (2002) A review of terrestrial mammalian and reptilian carnivore ecology in Australian fossil faunas, and factors influencing their diversity: The myth of reptilian domination and its broader ramifications. *Australian Journal of Zoology*, 50, 1–24.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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