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Supplemental information

Musculoskeletal modeling of sprawling and parasagittal forelimbs provides insight into synapsid postural transition

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Table S1. Scan parameters for the tegu, echidna and opossum, related to STAR Methods.

	Echidna	**Tegu**	**Opossum**
Specimen	SEP 42	SEP 74	SEP 87
Original Publication	**Regnault S, Fahn-Lai P, Norris RM, Pierce SE.** 2020 Shoulder Muscle Architecture in the Echidna (Monotremata: *Tachyglossus aculeatus*) Indicates Conserved Functional Properties. *J Mammal Evol* **27**, 591–603. (doi:10.1007/s10914-020-09498-6)	**Fahn-Lai P, Biewener AA, Pierce SE.** 2020 Broad similarities in shoulder muscle architecture and organization across two amniotes: implications for reconstructing non-mammalian synapsids. *PeerJ* **8**, e8556. (doi:10.7717/peerj.8556)	**Fahn-Lai P, Biewener AA, Pierce SE.** 2020 Broad similarities in shoulder muscle architecture and organization across two amniotes: implications for reconstructing non-mammalian synapsids. *PeerJ* **8**, e8556. (doi:10.7717/peerj.8556)
Body Mass (kg)	3.31	0.68	2.0
Humerus Length (mm)	54	37	64
Humerus Volume (mm^3)	9452	800	3181
Treatment	Whole	Head and lower body removed	Whole
Stain Duration (days)	91	7	63
Iodine Concentration (%)	3	10	3
Voltage (kV)	130	130	115
Current (µA)	120	61	130
Exposure (ms)	2000	1000	2000
Filter	Cu 1mm	Cu 0.5mm	Cu 0.5mm
Voxel Size (microns)	110	127	127

Figure S1. Global coordinate system used to determine the reference pose, related to STAR Methods and Figure 1.

Top row shows forelimb in dorsal view. **Bottom row** shows forelimb in cranial view. The blue Z-axis = retraction-protraction, the green Y-axis = depression-elevation and red X-axis = long-axis rotation or pronation-supination. See main text for a full description of the reference pose.

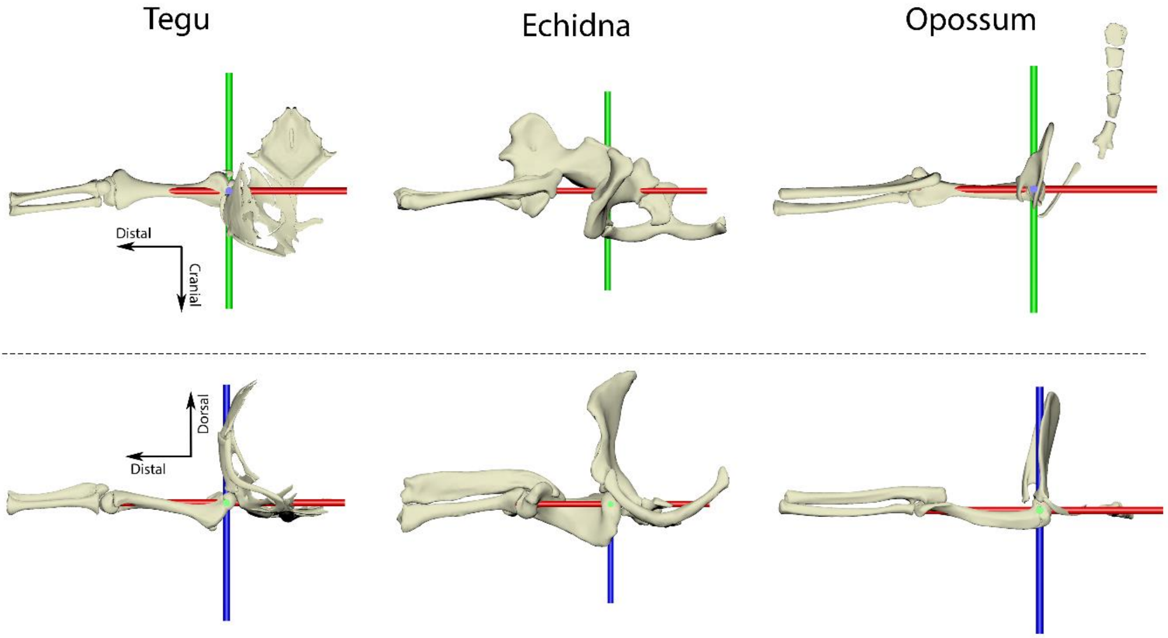


Figure S2. Anatomical coordinate system used to determine neutral pose, related to STAR Methods and Figure 1.

Top row shows scapulae and proximal joint coordinate systems; view axis set perpendicular to the glenoid. For the proximal shoulder joint, plane primitives were fit to the glenoid and the X-axis (red) was oriented normal to the fitted plane. The Y and Z-axes (green and blue, respectively) were then aligned so that, when articulated, the flexor surface of humerus faced dorsally. **Middle row** shows humeri in reference pose with distal shoulder joint coordinate systems; view axis set perpendicular to the humeral head. For the distal shoulder joint, convex hulls were fit to the articular surface of the humeral head, which approximated hemi-spheres or hemi-ellipsoids and the X-axis (red) was oriented such that it passed through the 'pole'. The Y-axis (green) was then aligned with the cranio-caudal axis of the humeral head, and the Z-axis (blue) was perpendicular to both. **Bottom row** shows scapulae and humeri in neutral pose after the two joint coordinate systems were superimposed. Black anatomical axes indicate global orientations. These anatomical axes were used solely to align the humerus to the glenoid and create the neutral pose; all rotation angles were calculated using the globally aligned reference coordinate system.

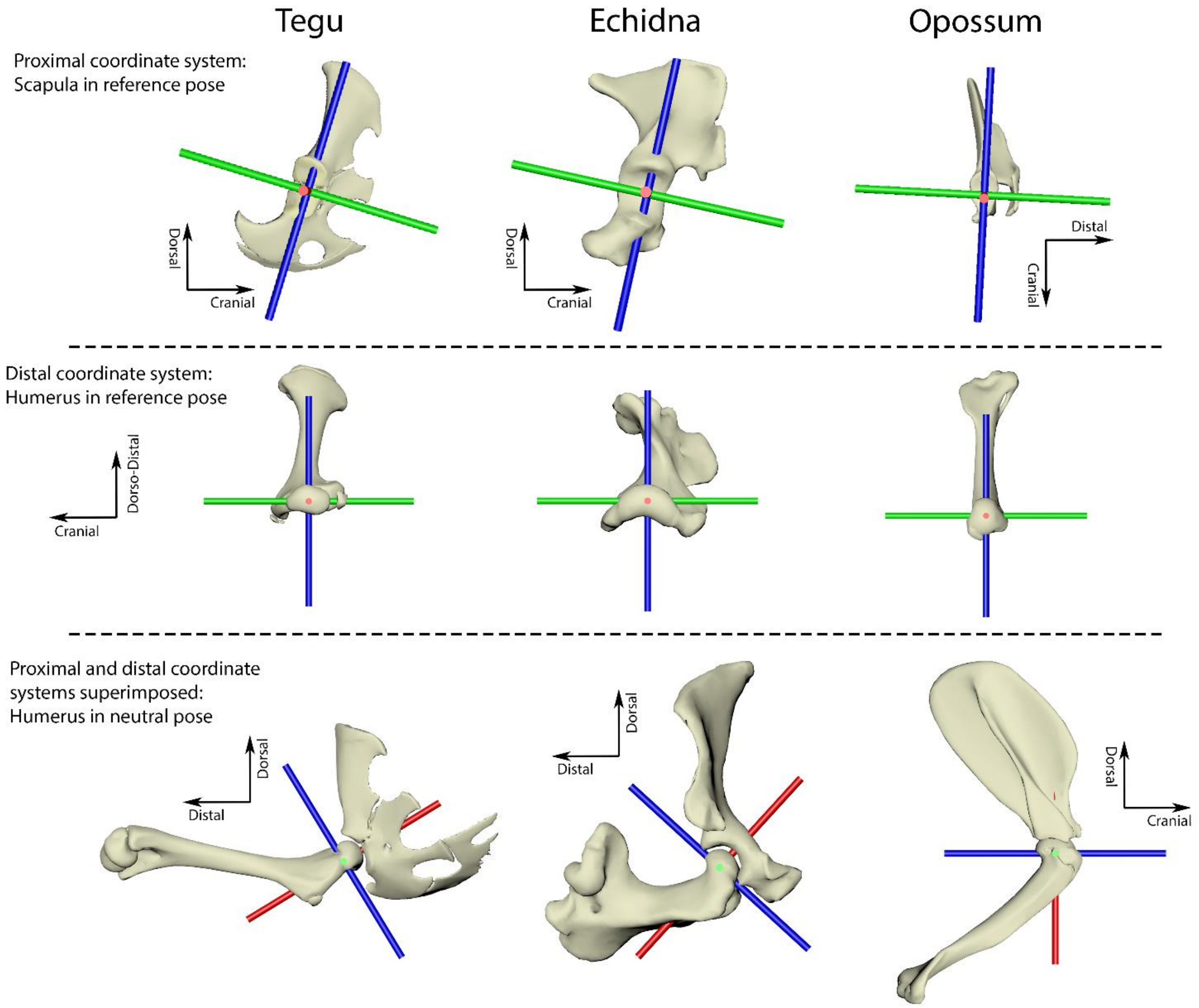


Figure S3. Example of Boolean intersection to determine viable poses, related to STAR Methods.

Left: Viable poses have the joints in articulation and no bony intersections. Joint articulation was assessed via a Boolean mesh intersection on the articular surface of the humeral head and a convex hull generated from the concave articular facet of the glenoid joint (purple). **Middle**: Joint disarticulation, where the humeral head no longer articulates with the glenoid, is recorded is an inviable pose. **Right**: Bone-to-bone interpenetration, where the bones intersect one another (bones rendered transparent, intersection in red), is recorded as an inviable pose. Bone-to-bone interpenetration was assessed via a Boolean intersection mesh operation on the humerus and scapula.

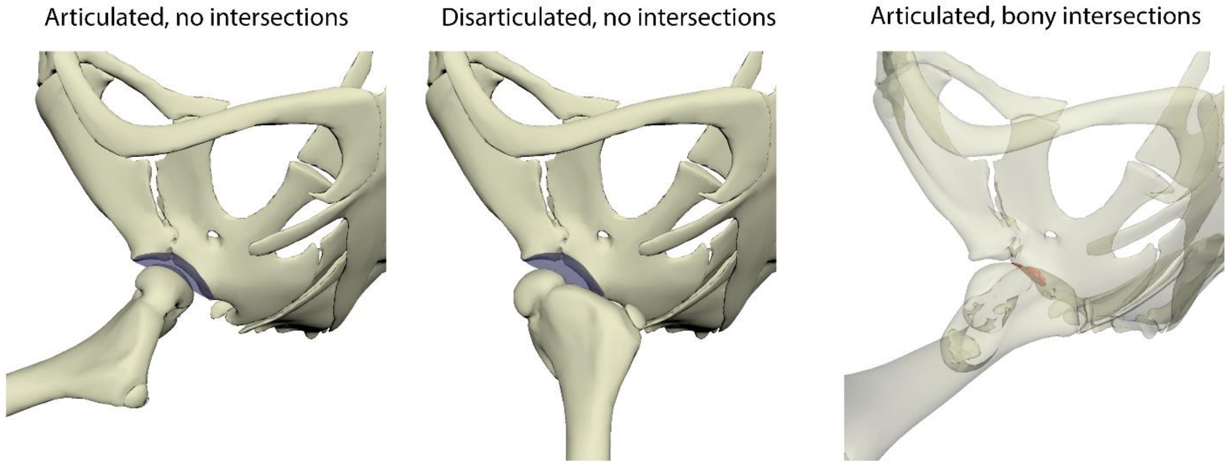


Figure S4. 2D Projections of 3D Range-of-motion envelopes, related to Figure 2.

Range of motion (ROM) envelopes for the glenohumeral joint in the tegu, echidna, and opossum, projected onto 2D planes. ROM envelopes are shown plotted in both uncorrected and cosine-corrected Euler space. Projected points are color coded according to the two axes of rotation which make up the plane (cyan on the ZY plane, magenta on the ZX plane, gold on the XY plane).

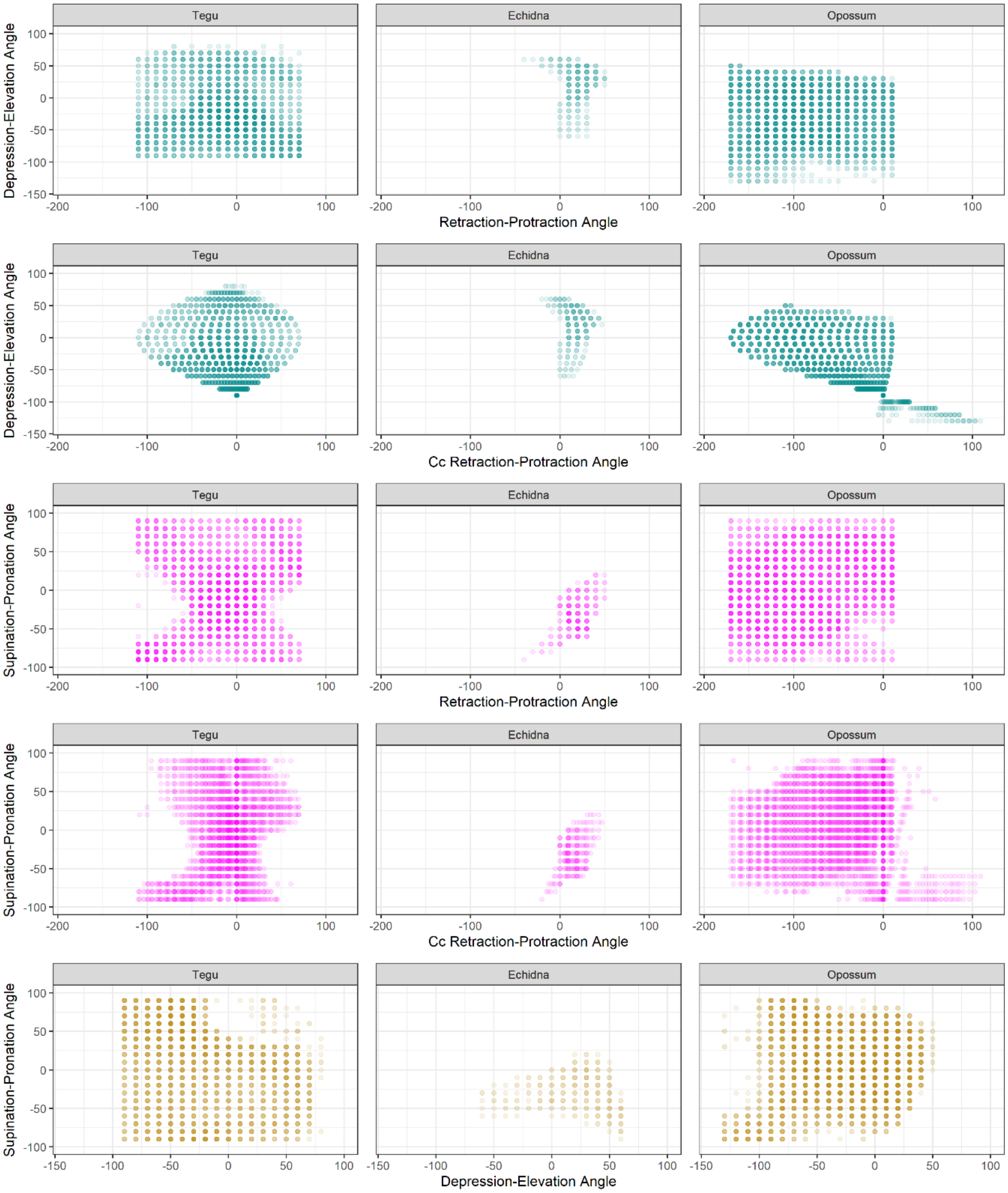
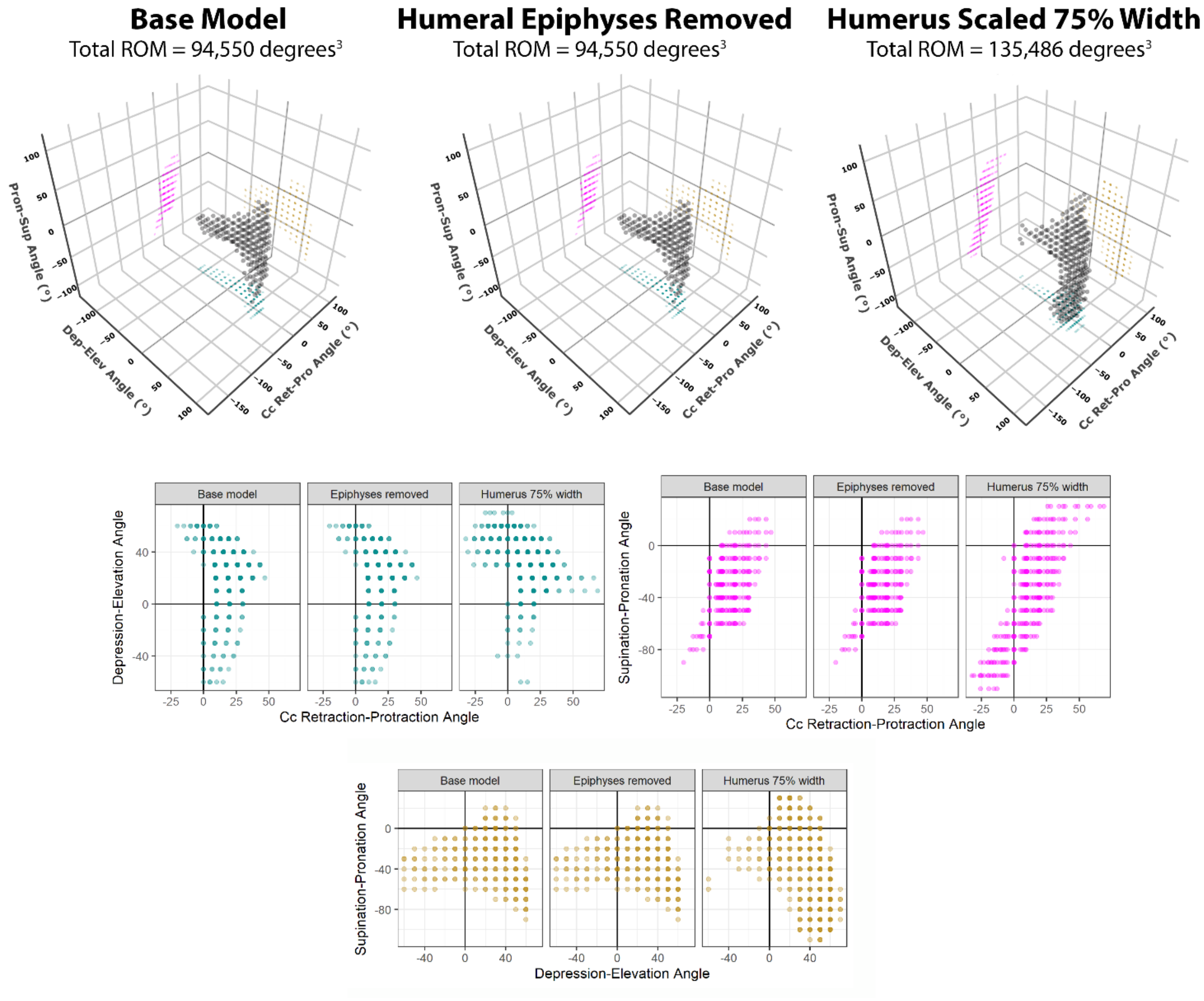


Figure S5. Echidna range of motion sensitivity analysis, related to Figure 2.

3D Range of motion (ROM) envelopes and 2D projections of ROM envelopes for the glenohumeral joint in the echidna base model, without epiphyses, and with a narrowed humerus (humerus scaled to 75% width). ROM envelopes are plotted in cosine-corrected Euler space. Projected points are color coded according to the two axes of rotation which make up the plane (cyan on the ZY plane, magenta on the ZX plane, gold on the XY plane).



Base Model
Total ROM = 94,550 degrees^3

Humeral Epiphyses Removed
Total ROM = 94,550 degrees^3

Humerus Scaled 75% Width
Total ROM = 135,486 degrees^3

Figure S6. Change in m. pectoralis action with shoulder joint retraction angle, related to Figure 4.

Pose space projected onto the ZY plane (retraction-protraction and depression-elevation), with points color coded according to the MMA values for the m. pectoralis in the tegu (top row) and opossum (bottom row). Points are color coded according to values. Note how particularly in the m. pectoralis (M + P) the tegu has a similar change in function to the opossum from depression MMAs (blue, -ve) to elevation MMAs (red, +ve) with more retracted (-ve) and depressed (-ve) shoulder joint angles.

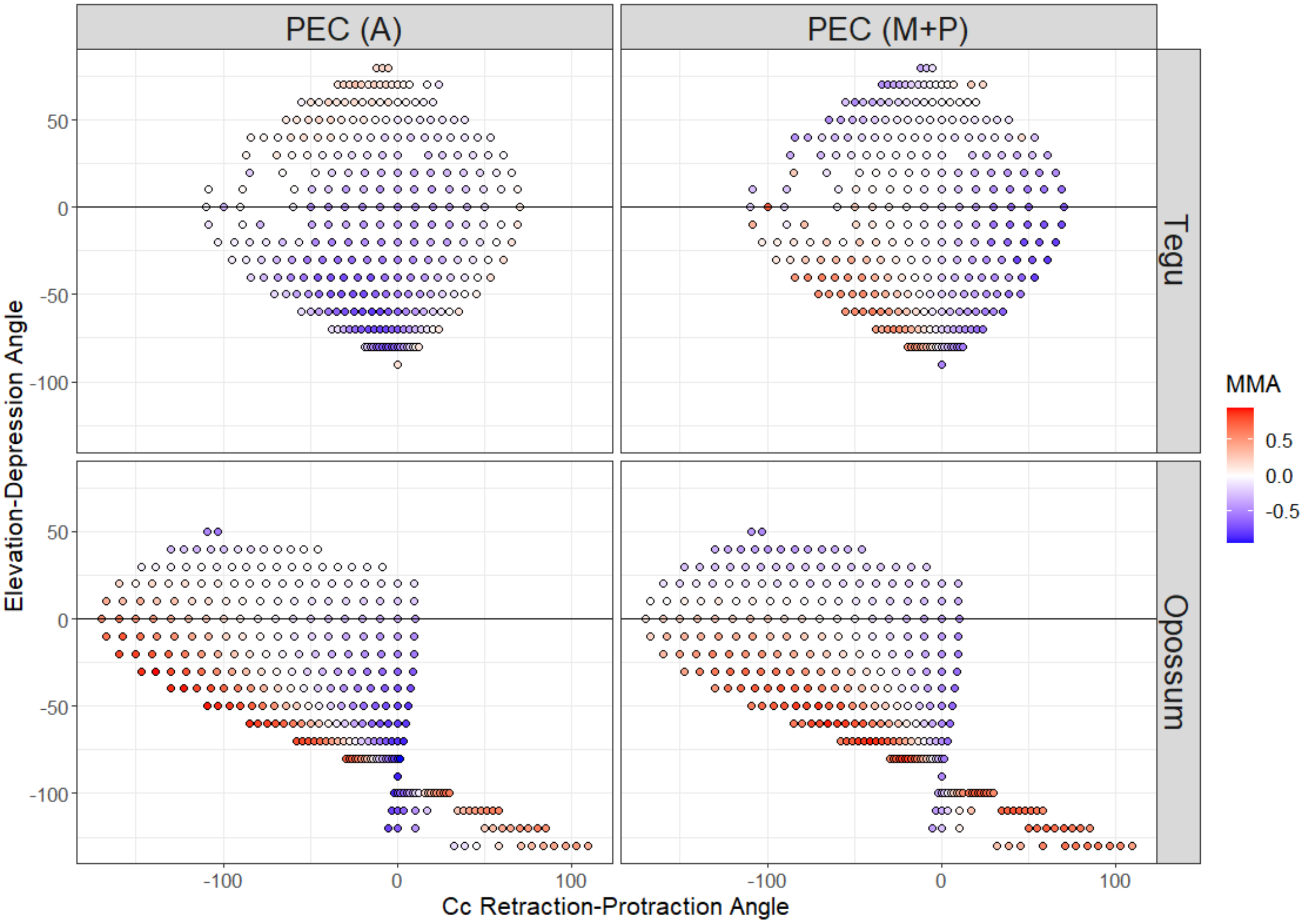
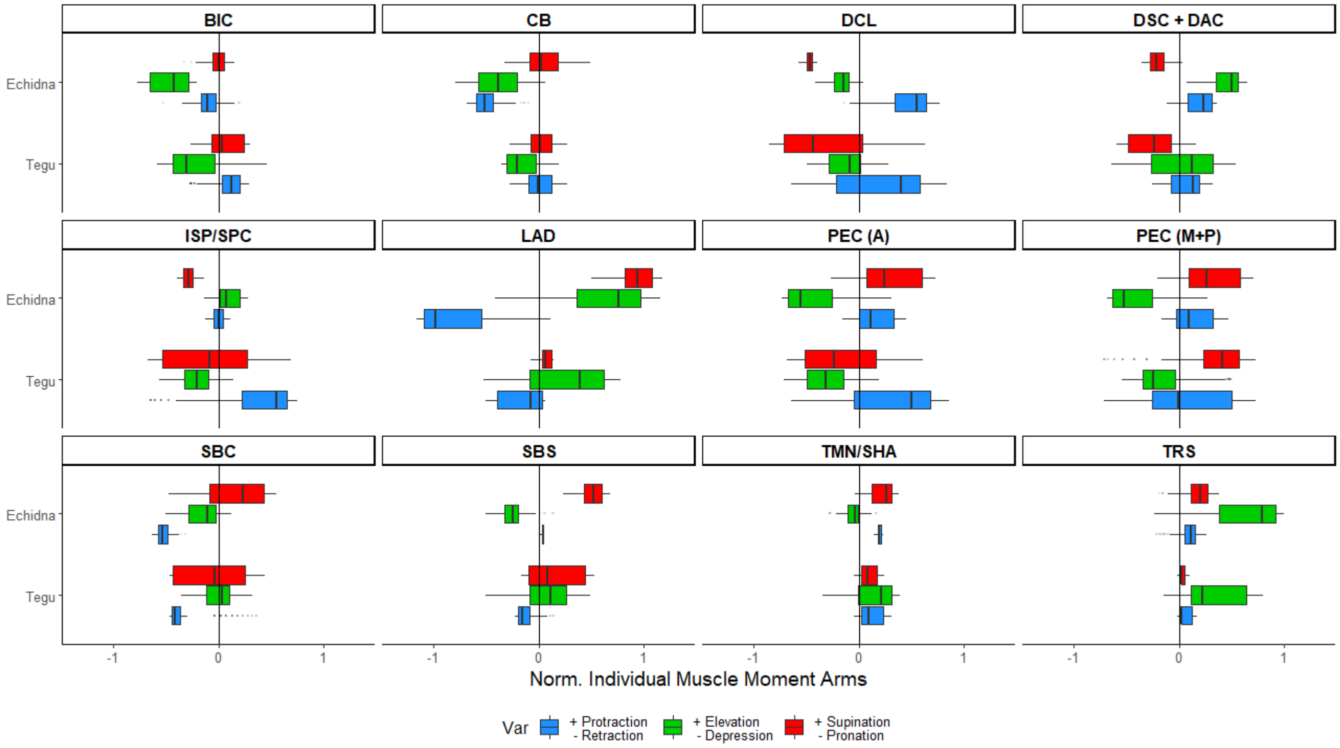


Figure S7. Muscle moment arms of the tegu and echidna in shared pose-space, related to Figure 4.

Individual muscle moment arm results for the tegu and echidna, but only those muscles and shoulder joint poses shared by both taxa. For muscle abbreviations see Figure 1 of the main text.



Methods S1. Locally running the accompaying shiny app to view results as interactive 3D plots, related to Figures 2, 3 and 4.

Please download and install RStudio onto your computer, as well as the following R packages – shiny, tidyverse, png and plotly. Then download the .7zip file called “ROM and MMA App” from Harvard Dataverse (link in the main text of the paper) and unzip it on your computer. Open RStudio, and type the following lines of code to open the app in an RStudio window:

```
library(shiny)
setwd("File path to the unzipped folder")
runApp()
```