

RESEARCH ARTICLE

Pelvic girdle mobility of cryptodire and pleurodire turtles during walking and swimming

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ABSTRACT

Movements of the pelvic girdle facilitate terrestrial locomotor performance in a wide range of vertebrates by increasing hind limb excursion and stride length. The extent to which pelvic movements contribute to limb excursion in turtles is unclear because the bony shell surrounding the body presents a major obstacle to their visualization. In the Cryptodira, which are one of the two major lineages of turtles, pelvic anatomy indicates the potential for rotation inside the shell. However, in the Pleurodira, the other major suborder, the pelvis shows a derived fusion to the shell, preventing pelvic motion. In addition, most turtles use their hind limbs for propulsion during swimming as well as walking, and the different locomotor demands between water and land could lead to differences in the contributions of pelvic rotation to limb excursion in each habitat. To test these possibilities, we used X-ray reconstruction of moving morphology (XROMM) to compare pelvic mobility and femoral motion during walking and swimming between representative species of cryptodire (*Pseudemys concinna*) and pleurodire (*Emyda subglobosa*) turtles. We found that the pelvis yawed substantially in cryptodires during walking and, to a lesser extent, during swimming. These movements contributed to greater femoral protraction during both walking and swimming in cryptodires when compared with pleurodires. Although factors related to the origin of pelvic-shell fusion in pleurodires are debated, its implications for their locomotor function may contribute to the restriction of this group to primarily aquatic habits.

KEY WORDS: Locomotion, Biomechanics, XROMM, Kinematics, Long-axis rotation

INTRODUCTION

Limb excursion is essential to the locomotor performance of a wide range of animals, contributing to stride length, speed, distance traveled and ultimately impacting survival through effects on functions such as resource acquisition and escape from predators (Irschick and Jayne, 1999; Calsbeek and Irschick, 2007). One mechanism used by a variety of vertebrates to increase limb excursion (and stride length) during terrestrial locomotion is the incorporation of pelvic motion into the stride (Jenkins, 1971; Pridmore, 1992; Reilly and Delancey, 1997; Russell and Bels, 2001). Frequently evaluated via kinematic measurements of external markers filmed with standard light videos (although see Gatesy, 1991; Nyakatura et al., 2014), pelvic rotation, particularly

yaw, can increase the anteroposterior arc of hind limb excursion, thereby lengthening terrestrial strides.

Although locomotor pelvic movements are widespread across many groups of vertebrates, novel body plans that have evolved in some taxa might impede the extent to which such motion is an effective mechanism for lengthening strides. For example, in turtles, the dorsal vertebrae and sacrum are fused to a bony shell that surrounds the body (Walker, 1973; Gilbert et al., 2001). Not only does such enclosure of the pelvis potentially limit its motion in turtles, but it also obscures visualization of potential pelvic movements. In an early study of turtle locomotor function that sought to overcome this technical challenge, Walker (1971) collected dorsal-perspective X-ray films of walking in the painted turtle (*Chrysemys picta*), a semi-aquatic species from the cryptodire lineage. These images did not show evidence of pelvic girdle movements (Walker, 1971). However, they were collected while animals were suspended in the field of view by a cloth sling, potentially impeding natural movement patterns. In addition, Walker's later landmark study of musculoskeletal anatomy in turtles (Walker, 1973) noted the possibility for motion between the pelvis and shell in cryptodires, as the ilium is connected to the sacrum via a sliding joint, and the pubis and ischium are suspended above the ventral portion of the shell via the puboischiadic ligament. This anatomical evidence suggests that pelvic rotation or translation might yet contribute to hind limb excursion in some turtle taxa under certain conditions.

Many species of turtle make frequent use of both aquatic and terrestrial habitats (Ernst and Lovich, 2009). Compared with many other semiaquatic vertebrates, however, turtles are distinctive in that the fusion of their vertebrae to the shell means that they must propel themselves with their limbs, rather than their body axis, in water as well as on land (Pace et al., 2001; Rivera et al., 2006). As a result, pelvic girdle motion provides the only potential contribution to hind limb excursion during swimming and walking in turtles beyond the motions of the limbs themselves. However, without contact of the foot with solid ground, reduced substrate reaction forces acting on the hind limb during swimming (Blob et al., 2003; Butcher and Blob, 2008) might decrease any contribution of pelvic rotation or translation to limb excursion in water, relative to on land.

Girdle structure is a major factor in the evolutionary diversification of turtles (Joyce et al., 2013a) and may carry functional consequences that distinguish the locomotor performance of clades (Renous et al., 2008). In contrast to cryptodires, in the other main clade of turtles, the pleurodires, the pelvis exhibits a derived, bony fusion to both the dorsal (carapace) and ventral (plastron) portions of the shell (Walker, 1973; Joyce et al., 2013b). Such fusion would be expected to preclude any pelvic motion relative to the shell, limiting hind limb excursion. As a result of the potential differences in girdle movements between cryptodires and pleurodires, turtles represent an excellent system for examining the effects of pelvic girdle mobility in vertebrate locomotion. However, no quantitative locomotor kinematics have

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been measured for any pleurodire species, making it difficult to assess the impact of the structural novelty of their pelvis on locomotor function.

To evaluate the potential contributions of pelvic girdle rotation to locomotor performance in turtles, and to assess its variation across habitats and taxa with different pelvic structures, we used marker based X-ray reconstruction of moving morphology (XROMM; Brainerd et al., 2010) to measure the movements of the pelvis and femur relative to the shell in representative species of cryptodire and pleurodire turtles during both walking and swimming. We predicted that the pelvis would rotate in cryptodires during both behaviors, with rotation being greater on land than in water. We also predicted that fusion to the shell in pleurodires would prevent pelvic movements relative to the shell both in water and on land. By measuring motion of the femur relative to the pelvis, we also obtained new measurements of long-axis rotation of the femur in both swimming and walking that are difficult to obtain through other techniques (Kambic et al., 2014, 2015), and which inform understanding of how limb function and skeletal loading change between environments (Butcher and Blob, 2008; Young and Blob, 2015). These findings may have a bearing on differences in locomotor function and habitat between the cryptodire and pleurodire clades.

MATERIALS AND METHODS

Experimental subjects

XROMM analyses were performed for both walking and swimming in three adult males (mass, 660, 1050 and 1200 g; carapace length, 185, 207 and 223 mm) of the cryptodire *Pseudemys concinna* (LeConte 1830), the river cooter, and two adult males (mass, 610 g and 675 g; carapace length, 171 mm and 184 mm) of the pleurodire *Emydura subglobosa* (Krefft 1876), the Jardine river turtle. *P. concinna* specimens were collected with hoop traps from a spillway of Lake Hartwell, Pickens County, SC, USA (South Carolina Scientific Collection Permit #29-2014). *E. subglobosa* were obtained from a commercial supplier (Turtles and Tortoises, Brooksville, FL). Prior to data collection, turtles were separated by species and housed at Clemson University in plastic tub enclosures that were half-filled with water and fitted with dry areas for basking. To collect XROMM data, turtles were transported to Brown University and housed in similar enclosures for the days over which data were collected. Turtles were fed commercial reptile pellets daily. All procedures and animal care were approved by the Institutional Animal Care and Use Committees (IACUC) of Clemson University (protocol 2015-001) and Brown University (protocol 1105990018).

Surgical procedures

Prior to data collection, 1 mm radio-opaque tantalum bead markers (Bal-Tec, Los Angeles, CA) were implanted into the pelvis, femur

and shell of each individual turtle (3–5 markers per bone, Fig. 1), using aseptic technique. In both turtle species studied, the carapace and plastron are firmly connected together by a bony bridge, so the shell was treated as a single rigid body. Surgeries were conducted at Clemson University, following published protocols (Brainerd et al., 2010). To induce analgesia and a surgical plane of anesthesia, doses of 1 mg kg⁻¹ butorphanol, 90 mg kg⁻¹ ketamine and 1 mg kg⁻¹ xylazine were injected into the muscles of the forelimbs. To expose the pelvis and femur, single incisions were made on the ventral and dorsal aspects of the proximal region of the left hind limb. Muscles were separated along fascial planes to expose surfaces of the bones (Butcher et al., 2008). At each site of marker implantation, a small ‘window’ of periosteum was removed to expose the bone cortex by gently scraping with a periosteal elevator. Each marker then was implanted by hand-drilling a 1-mm-diameter hole into the bone with a pin vise, and pressing the bead into the hole with the stick handle of a cotton-tipped applicator. Markers were located in each of the three fused, tri-radiate bones that comprise the pelvis (ilium, pubis and ischium), maximizing the distance of markers in this element from each other. Femoral markers were placed in proximal and midshaft regions as well as the distal condyles. Incisions were sutured closed once all markers were implanted. Each turtle was then allowed to recover on land for 24 h before being placed in an individual aquatic enclosure with a basking platform.

Experimental data collection

Turtles were allowed to recover from surgery for 1–2 weeks before collection of XROMM data. Turtles were filmed during walking and swimming using biplanar X-ray video. At the beginning, middle and end of each day, X-ray images of standard grid and calibration objects were taken at the settings used for experimental trials.

During walking, a hand-powered treadmill was used to stimulate locomotion while minimizing magnetic interference with images. Two X-ray generators (Imaging Systems and Service, Painesville, OH, USA) were positioned in dorsal and lateral views. X-ray settings were 100 mA for both views, with kVp between 68 (laterally) and 85 (dorsally). X-ray images were recorded using Phantom v.10 high-speed cameras (Vision Research, Wayne, NJ, USA) at a 1760×1760 pixel resolution. Data were recorded at 100 frames s⁻¹ with a 1/500 s shutter speed. Turtles were allowed to rest for approximately 10 min between trials, and 10–15 total steps were collected per turtle.

Swimming trials were filmed in a 161×61 cm acrylic aquarium filled to a depth of no more than 10 cm to minimize the amount of water through which X-rays had to pass before reaching animals. Turtles were stimulated to swim by placing them at the opposite end of the tank from a dark shelter, toward which they swam when released. X-ray generators were positioned over the left and right

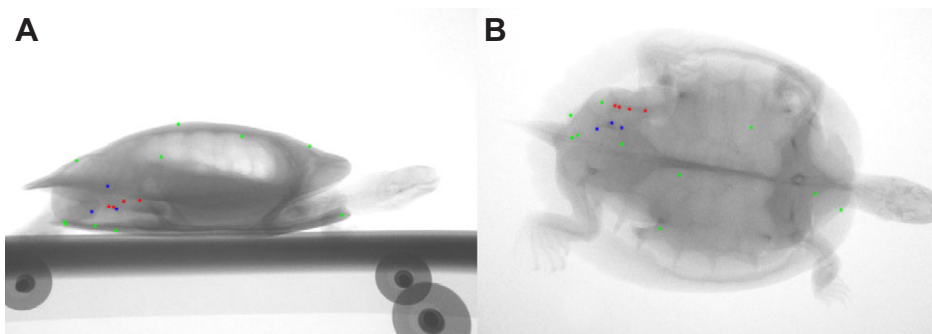


Fig. 1. X-ray images of a walking turtle. Lateral (A) and ventral (B) views. Blue dots indicate markers on the pelvis, red dots are marker locations on the femur, and green dots are markers located on the shell (carapace and plastron).

sides of the tank, angled at 45 deg to the plane of animal movement and 90 deg from each other. X-ray settings were 100 mA for both views, with kVp between 90 (left view) and 100 (right view). Images were recorded as above, with data recorded at 150 frames s⁻¹ and 1/1000 s shutter speed. Turtles were allowed to rest for approximately 10 min between trials, and 10–15 total strokes were collected per turtle.

Following data collection, turtles were sedated with an intramuscular injection of ketamine (30 mg kg⁻¹) and computed tomography (CT) scans were taken of each individual with an Animage Fidex veterinary scanner with an 8–15 cm field-of-view and 0.2–0.3 mm isotropic voxels. These CT scans were used to generate polygonal mesh models of the shell, pelvis and femur in OsiriX (v.3.9.2 64 bit, Pixmeo, Geneva, Switzerland) and Geomagic Design X64 (v. 2016 0.1, Geomagic, Triangle Park, NC, USA).

Data processing

The X-ray video and CT scan data collected and analyzed for this study are available from the X-ray Motion Analysis Portal (xmaportal.org). Following data collection, X-ray videos were processed using XMA Lab v.1.2.12 (open source; bitbucket.org/xromm/xma-lab). Standard grid images were used to correct for distortions in the video that are introduced by X-ray image intensifiers (Brainerd et al., 2010). Calibration objects of known geometry (cubes with 64 radio-opaque markers) were used to calibrate the 3D space (Brainerd et al., 2010). After tracking markers (3–5 per bone, Fig. 1), rigid body motions of the femur, pelvis and shell were filtered with a Butterworth low-pass filter (10 Hz cut off for walking and 15 Hz for swimming) and exported. Animations were constructed by applying these motions to polygonal mesh models of the bones in Autodesk Maya 2014 (San Rafael, CA, USA).

To describe the 3D movements of the pelvis (Fig. 2A) and femur (Fig. 2B), we created anatomical coordinate systems (ACSs) for each of these elements, as well as for the shell, which we then used to create joint coordinate systems (JCSs) to describe the motions of one distal element relative to another more proximal element (Brainerd et al., 2010; Menegaz et al., 2015). These JCS systems were established using Autodesk Maya and XROMM Maya Tools (xrommwiki.org) and the rotation order for calculating the Euler angles for all JCSs was ZYX in Maya. We measured pelvic girdle

movements relative to the more proximal shell, and measured femoral movements relative to both the shell and the pelvis as more proximal elements. For each movement in each individual, we chose a neutral posture that we defined as a zero point.

For pelvic girdle movements, we defined our neutral posture as when the anterior tip of the pelvic midline pointed directly cranially. We created the pelvic ACS to be centered at the mediolateral midpoint of the pelvis, at the level of the center of the acetabulum. We aligned this ACS so that its *x*-axis was perpendicular to the plastron, its *y*-axis passed through the acetabulum on both sides of the pelvis, and its *z*-axis pointed directly craniocaudally. We then created a second ACS in the same location and orientation that we parented to the shell, allowing the construction of a pelvic JCS to measure relative movement of the distal pelvic ACS relative to the proximal shell ACS. We arranged the pelvic JCS (Fig. 2A) so that roll, pitch and yaw of the pelvis were measured as rotations about the *x*, *y* and *z* axes of the JCS, respectively, and craniocaudal, mediolateral and dorsoventral translations were measured as translations along the *x*, *y* and *z* axes of the JCS, respectively.

We defined our reference pose for femoral movements to be when the long axis of the femur was perpendicular to the pelvis. We first created an ACS centered on the femoral head at the acetabulum. The *x*-axis of this ACS was aligned through the long axis of the femur, with the *y*-axis parallel to the ventral plane of the shell. At this position, the femur was depressed by 8–10 deg relative to the body and the trochanters were aligned perpendicular to the ground. We then created a second ACS in the same location and orientation and parented it to the shell, allowing the construction of a femoral JCS to measure relative movement of the distal femoral ACS to the more proximal shell ACS. To measure femoral movements relative to the pelvis, we also created a third ACS in the same location and orientation, but parented it to the pelvis to provide an alternative proximal ACS. We oriented our femoral JCS so that rotations about the *x*-axis correspond to long-axis rotation, rotations about the *y*-axis correspond with elevation and depression, and rotations about the *z*-axis correspond to femoral protraction and retraction (Fig. 2B).

To measure the translation of the dorsal tips of the ilia relative to the sacrum, we created an ACS on the sacrum in which the *x*-axis was aligned craniocaudally, the *y*-axis was aligned mediolaterally, and the *z*-axis was aligned dorsoventrally. We then attached a locator at the dorsal tip of the left ilium and output the motion of the

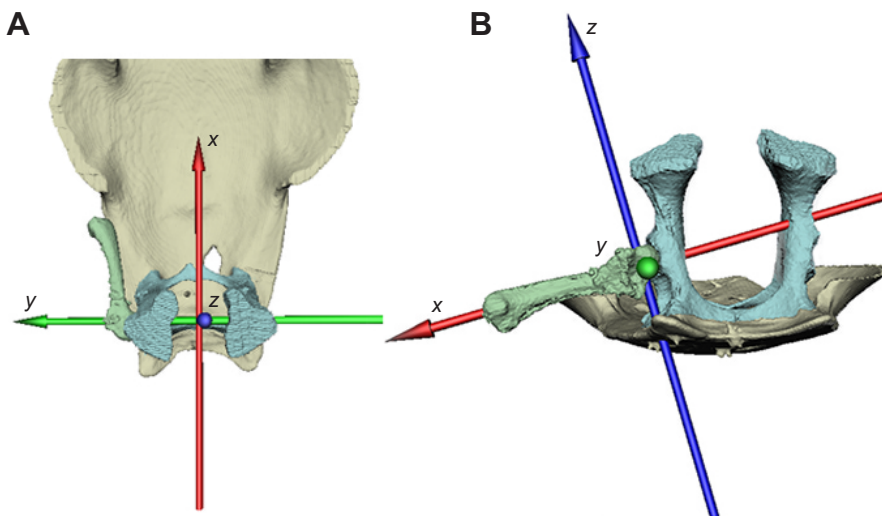


Fig. 2. Joint coordinate systems used in this study from the pleurodire turtle *Emydura subglobosa*. (A) Pelvic girdle joint coordinate system (dorsal view) for measuring motion of the pelvis relative to the shell. Axis orientations were set so that rotation about the *x*-axis (red) is roll, rotation about the *y*-axis (green) is pitch, and rotation about the *z*-axis (blue) is yaw. (B) Femoral joint coordinate system (posterior view). Axis orientations were set so that rotation about the *x*-axis (red) is long-axis rotation, rotation about the *y*-axis (green) is elevation–depression, and rotation about the *z*-axis (blue) is protraction–retraction. Femoral (pale green) motion was measured relative to the pelvis (in turquoise) and relative to the shell (in cream).

locator relative to the body axes defined by the sacrum ACS. To visualize translations of the pubis and ischium over the interior surface of the plastron, we created motion trails for each bone and then measured the major and minor axes of these ovoid paths of motion.

Precision analysis

Precision of both marker tracking and animation was calculated following published protocols (Brainerd et al., 2010; Camp et al., 2014; Menegaz et al., 2015). Following data collection from live animals, we collected X-ray footage from a previously euthanized, frozen turtle that had been implanted with a similar set of markers, moving the specimen on the treadmill and through water at similar speeds to *in vivo* locomotion. Because the animal was frozen, any deviations from zero in distance between markers, and any apparent movement measured with JCSs could be considered a threshold for identifying noise in measurements for *in vivo* trials. Precision of marker tracking was obtained by calculating the standard deviation of mean inter-marker distance, while precision of animation was

calculated as the average standard deviation of apparent angular movement for each bone (femur and pelvis).

Statistical analyses

Unless noted, all statistical analyses were performed in R (v.3.2.1, www.r-project.org). For both walking and swimming, pelvic girdle movements were compared between species using linear mixed-effects models (lmer4; Bates et al., 2015) with species as a fixed effect and individual as a random effect. Within each species, comparisons of motions between walking and swimming were performed using linear mixed-effects models with locomotor behavior as a fixed effect and individual as a random effect. Effect sizes based on mixed-effects models were calculated following published methods (Xu, 2003). Femoral movements were further compared between species and environments using a canonical discriminant function analysis (CDA) in JMP (v.11, SAS Institute, Cary, NC 1989–2007). Variables included in the CDA were maximal and minimal long-axis rotation (LAR), maximal elevation and depression, and maximal protraction and retraction. A

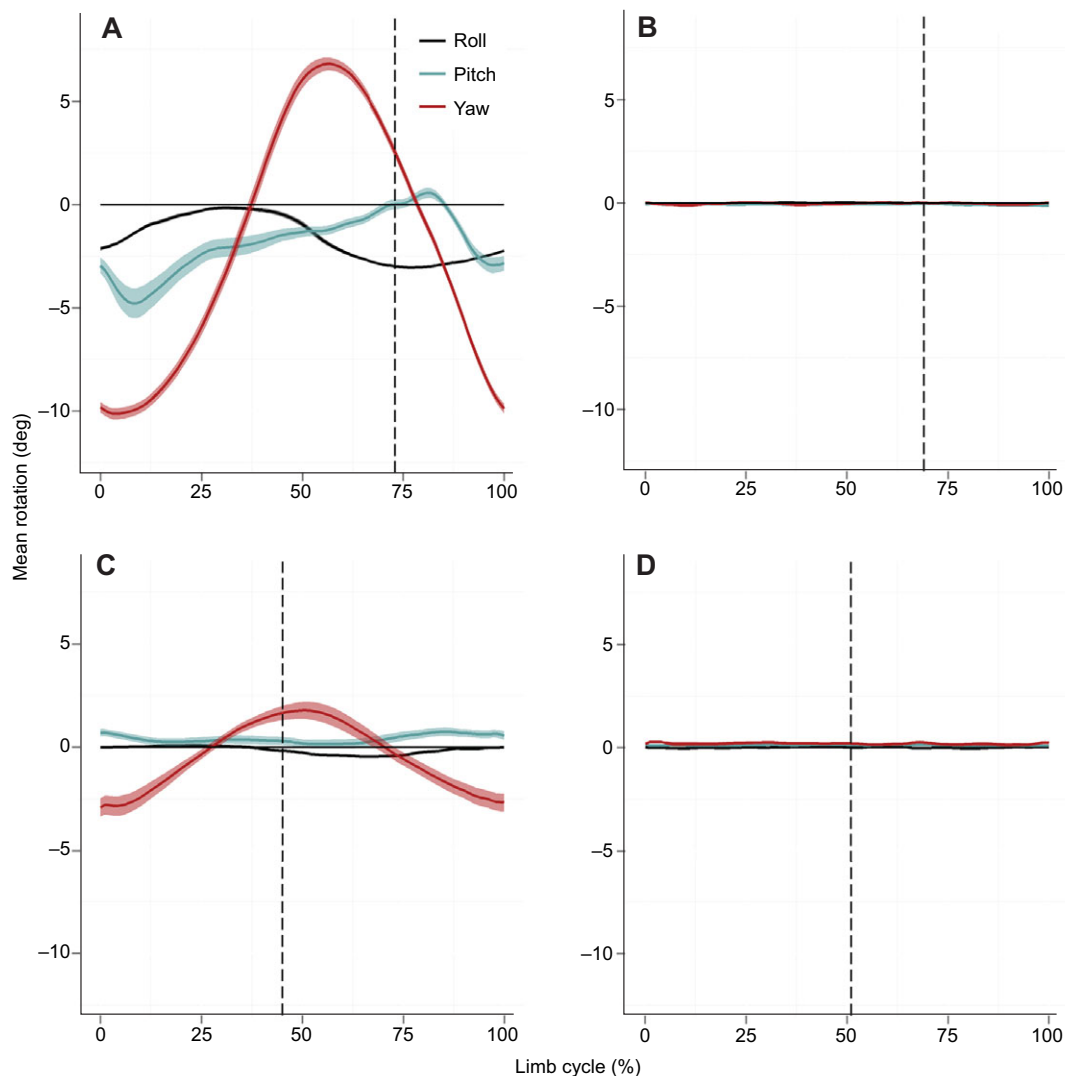


Fig. 3. Pelvic girdle rotations in cryptodire (*Pseudemys concinna*) and pleurodire (*Emydera subglobosa*) turtles during walking and swimming. Solid lines represent mean traces for each motion, shading represents standard errors for each motion and colors indicate different axes of rotation. Traces were normalized to the same duration. Vertical dashed lines represent the transition from stance to swing (walking) or from stroke to recovery (swimming). (A) Cryptodire pelvic rotations while walking ($N=3$ individuals, 54 cycles). (B) Pleurodire pelvic rotations while walking ($N=2$ individuals, 35 cycles). (C) Cryptodire pelvic rotations while swimming ($N=3$ individuals, 30 cycles). (D) Pleurodire pelvic rotations while swimming ($N=2$ individuals, 18 cycles).

Table 1. Pelvic girdle rotations for cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles during walking and swimming, with mixed-effects model significance and effect size (Ω^2)

	Cryptodire turtles		Pleurodire turtles		Between-species walking		Between-species swimming		Cryptodire between environment	
	Walking (3, 54)	Swimming (3, 30)	Walking (2, 35)	Swimming (2, 18)	<i>P</i>	Ω^2	<i>P</i>	Ω^2	<i>P</i>	Ω^2
Roll	3.49±0.05	1.17±0.06	0.29±0.02	0.48±0.05	<0.001	0.97	<0.001	0.59	<0.001	0.91
Pitch	2.57±0.09	1.18±0.18	0.31±0.02	0.39±0.04	<0.001	0.86	<0.001	0.7	<0.001	0.45
Yaw	18.2±0.45	8.75±0.38	0.61±0.04	0.92±0.09	<0.001	0.98	<0.001	0.91	<0.001	0.87

Rotation values are means±s.e. in deg. Numbers in parentheses indicate sample sizes of (individuals, cycles) for each category.

Wilks' lambda test was performed to determine whether the differences explained by the discriminant variables were significant. A multiple regression was conducted to evaluate the effect of stride frequency and femoral excursion on pelvic rotation of cryptodire turtles in each environment, using the degrees of femoral retraction per second and degrees of femoral excursion as predictor variables and pelvic yaw as a response variable.

RESULTS

Precision

Mean marker tracking precision was 0.1 mm for swimming and for walking. JCS translation precision was 0.4 mm for the femur and 0.5 mm for the pelvis while walking, and 0.8 mm for the femur and 0.9 mm for the pelvis while swimming. Angular precision for JCS data for the femur was 0.22 deg during walking and 0.32 deg during swimming. Pelvic girdle rotation precision was 0.25 deg for walking and 0.27 deg for swimming.

Pelvic motion

Pelvic motion differed substantially between the two species (Fig. 3, Table 1). The greatest difference was for yaw movements, in which the cryptodire pelvis rotated by 18.20±0.45 deg and 8.75±0.38 deg (means±s.e.) during walking and swimming, respectively (Movie 1). In contrast, the pleurodire pelvis did not yaw during walking or swimming (Table 1, Movie 2). The noise in mean pleurodire pelvic motions was approximately 0.1 deg (Fig. S1), which is less than the 0.3 deg precision of our measurements, indicating that any motion that could be occurring was less than our ability to detect it. The pelvis also pitched and rolled in cryptodires, especially on land (pitch, 2.57±0.09 deg; roll, 3.49±0.05 deg); however, the pleurodire pelvic girdle did not rotate in either direction in either environment (Fig. 3, Fig. S1). Translations of the cryptodire pelvis were small, less than 1 mm in the craniocaudal and mediolateral directions, and less than 0.1 mm in the dorsoventral direction (Fig. 4). In general, all movements of the cryptodire pelvis were smaller during swimming than during walking (Table 1, $P<0.001$, $\Omega^2>0.45$).

Yawing of the pelvis inside the shell in cryptodires caused craniocaudal sliding of the dorsal tips of the ilia relative to their articulations with the sacrum. Craniocaudal ilium translation relative to the sacrum was 3.4 mm per step while walking and 1.9 mm per stroke while swimming ($P<0.001$, $\Omega^2=0.833$). Pelvic yaw also caused the ventral processes of the pubis and ischium to slide in elliptical patterns relative to the plastron (Movie 3). The pubis during walking circumscribed the largest ellipses, with major and minor axes of 7.4 and 2.4 mm. Smaller, but still substantial, sliding motions occurred between pubis and plastron during swimming and between ischium and plastron during walking and swimming (Table 2).

Increased speeds in cryptodires led to increased pelvic girdle rotation while walking (multiple regression $P<0.001$, $r^2=0.58$,

$F_{2,51}=35.49$) and swimming ($P=0.001$, $r^2=0.39$, $F_{2,27}=8.28$) (Fig. 5). During walking, pelvic yaw increased from 14 deg at the slowest speeds to 22 deg at the fastest speeds and, during swimming, yaw increased from 6 deg at slow speeds to 14 deg at faster speeds (Fig. 5).

Femoral kinematics

In cryptodires, we measured the contribution of pelvic girdle rotations to femoral kinematics by comparing the movements of the femur relative to the shell with movements of the femur relative to the pelvis. This effectively allowed us to 'subtract' pelvic girdle rotations from femoral kinematics. Pelvic movements contributed little to either elevation/depression of the femur or its axial rotation. However, pelvic yaw had a strong effect on femoral protraction/retraction excursions, contributing 9.86±1.49 deg on land and 7.64±3.57 deg in water (paired Student's *t*-test, $P<0.001$, Cohen's $d=0.90$).

Canonical discriminant function analysis identified two primary axes that cumulatively explained 95.16% of the variation in femoral kinematics between species and across environments. Canonical 1

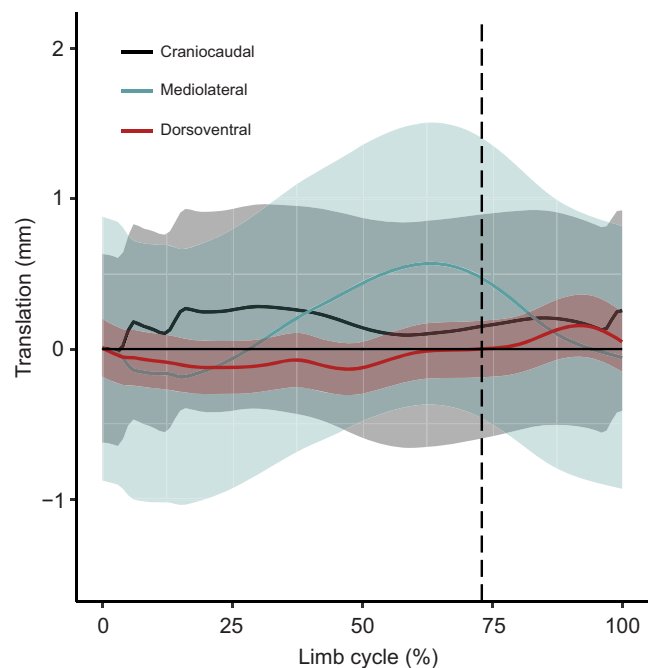


Fig. 4. Cryptodire turtle pelvic girdle translations during walking. Solid lines represent mean traces for each motion, shading represents standard errors for each motion and colors indicate different axes of translation. Vertical dashed line represents the transition from stance to swing. Traces from all trials were normalized to the same duration for calculation of mean kinematic profiles ($N=3$ individuals, 54 cycles). Note that whole-pelvis translations are small, generally <1 mm.

Table 2. Major and minor axes of the elliptical path of motion of the cryptodire pubis and ischium relative to the plastron during walking and swimming

	Walking		Swimming	
	Major	Minor	Major	Minor
Pubis	7.4±1.5	2.4±0.3	3.7±0.4	1.1±0.2
Ischium	5.4±0.5	1.9±0.3	2.9±0.5	1.2±0.3

Values are means±s.e. in mm ($N=3$ individuals, 54 cycles for walking; $N=3$ individuals, 35 cycles for swimming).

(C1, 68.86% of variance) was defined primarily by differences between swimming and walking, whereas canonical 2 (C2, 26.25% of variance) distinguished cryptodires from pleurodires (Fig. 6). Walking cycles were characterized by positive scores on C1, reflecting larger retraction angles and larger pronation values. Swimming cycles were characterized by negative scores on C1, reflecting low magnitudes of maximum elevation and supination (Table 3). On C2, pleurodires exhibit lower values of protraction (by 12.89 deg on land and 8.03 deg in water) and depression (by 4.84 deg on land and 2.56 deg in water) than cryptodires (Table 3). A Wilks' lambda test indicated that the differences explained by the discriminant variables were significant (Wilks' lambda=0.16, $P<0.001$).

Both taxa showed similar long axis rotation (LAR) excursions of the femur while walking (cryptodire, 34.32 ± 1.30 deg; pleurodire,

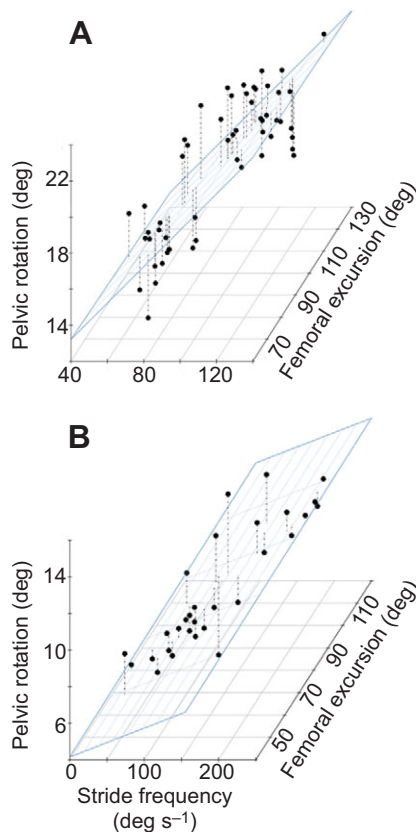


Fig. 5. Multiple regression results for walking and swimming in the cryptodire turtle *Pseudemys concinna*. The effect of increasing femoral excursion and stride frequency on pelvic rotation during walking (A) and swimming (B). Points represent individual locomotor cycles on the regression surface (blue planes). Pelvic rotation increases significantly as excursion and frequency increase in both environments (Table 3).

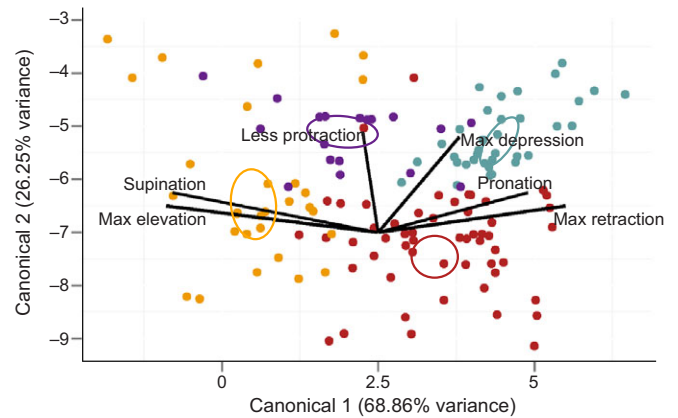


Fig. 6. Canonical discriminant function analysis of femoral movements of cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles. Colors of points indicate different categories of cycles: red, cryptodire walking; orange, cryptodire swimming; turquoise, pleurodire walking; purple, pleurodire swimming. Colored ovals represent 95% confidence limit for the mean of each of these groups. Black lines indicate the magnitude and direction of each variable (Youngerman et al., 2014). Locomotor behavior (canonical 1) is loaded most strongly by increased retraction and pronation as well as negatively loaded by increased elevation and supination. Canonical 2 discriminates species, with decreased protraction and increased depression loading most heavily and separating pleurodires from cryptodires.

31.72 ± 1.13 deg; $P=0.747$, $\Omega^2=0.75$). However, marked differences in LAR emerged between the two taxa during swimming (Table 3, Fig. 7). Pleurodires retained LAR during swimming that was only marginally lower (27.87 ± 1.93 deg; $P=0.038$, $\Omega^2=0.25$) than during walking; in contrast, cryptodires exhibit a substantial decrease in LAR by 12.5 deg (to 18.4 ± 1.08 deg; $P<0.001$, $\Omega^2=0.65$) as they shift from walking to swimming (Fig. 7).

DISCUSSION

Functional implications of pelvic movement for turtle locomotion

Movements of the pelvic girdle play a crucial role in locomotion for a variety of tetrapods, improving locomotor performance by increasing femoral excursion and stride length (Jenkins, 1971; Pridmore, 1992; Reilly and Delancey, 1997; Reilly and Elias, 1998). Using XROMM, we were able to observe pelvic rotation during locomotion in a species of cryptodire turtle in the directions of pitch, roll, and, most dramatically, yaw (Fig. 3). These results provide new insight into the locomotor function of turtles, as the pelvis had been considered immobile relative to the shell during earlier observations using standard X-ray visualization (Walker, 1971). Our data indicate that, despite being surrounded by a bony shell, cryptodire turtles move the pelvis in a fashion that is quite similar to many other vertebrates, with pelvic yaw in particular contributing to femoral excursion in both walking and swimming. Pelvic movements for cryptodires were greater during terrestrial walking than swimming, probably as a result of the greater reaction forces acting on the limb during the support of body weight on land (Blob et al., 2003; Butcher and Blob, 2008). However, in both environments, pelvic yaw for cryptodires increased with increasing speed. To the extent that greater speeds reflect efforts to achieve maximal performance, pelvic movements may make their greatest contribution to cryptodire locomotion under conditions in which animals face the highest performance demands.

With a joint-coordinate system located at the center of the pelvis (Fig. 2A), we found that the motion of the cryptodire pelvis was

Table 3. Femoral rotation in each direction, relative to the neutral reference pose for cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles during swimming and walking with discriminant analysis loadings

	Cryptodire turtles		Pleurodire turtles		Canon 1 (68.86% var.)	Canon 2 (26.25% var.)
	Walking (3, 54)	Swimming (3, 30)	Walking (2, 35)	Swimming (2, 18)		
Supination (LAR)	−20.94±0.88	−12.47±1.90	−16.47±0.78	−19.09±3.63	−0.98	0.26
Pronation (LAR)	13.01±1.43	5.92±1.26	15.24±0.79	8.78±2.85	0.81	0.18
Max. depression	−10.26±0.50	−6.87±0.80	−5.42±0.34	−4.32±0.82	0.39	0.60
Max. elevation	4.89±0.37	6.08±1.43	5.76±0.41	6.84±1.05	−1.09	0.18
Max. protraction	−83.77±1.41	−75.95±1.78	−70.88±0.85	−67.92±2.09	−0.08	0.58
Max. retraction	15.76±0.89	1.87±2.63	26.96±2.03	6.33±2.91	1.04	0.11

Values are means±s.e. in deg. Numbers in parentheses for each of the first four columns indicate samples sizes of (individuals, cycles) for each category. Primary loadings for each canonical axis are in bold (Chan, 2003); canonical 1 distinguishes locomotor habitat, whereas canonical 2 distinguishes the two lineages of turtles (Fig. 3). LAR, long-axis rotation; var., variance.

nearly pure rotation, with less than 1 mm of whole-pelvis translation. These rotations caused the dorsal tips of the ilia to slide relative to the sacrum, and the ventral tips of the ischia and pubis to move in elliptical patterns relative to the plastron (Movie 3). Some of these movements were quite substantial, typically more than 3 mm and up to 7 mm in the case of the pubis (Table 2). Many hip muscles originate on the cryptodire pubis (e.g. puboischiofemoralis internus) and ischium (e.g. the ventral head of the flexor tibialis complex) (Walker, 1973), and understanding how these muscles and their tendons interact with and influence the sliding of the pelvis relative to the shell at these joints is a promising area of future research.

In contrast to the prominent pelvic movements we observed in our cryptodire species, the derived fusion of the pelvis to both the carapace and plastron in pleurodire turtles appears to render their pelvis motionless relative to the shell during locomotion, because any movements in the pleurodire pelvis were smaller than our ability to detect them (<0.3 deg). This limitation of pelvic movement is correlated with smaller femoral protraction, in both walking and swimming, for pleurodires compared with cryptodires over the range of speeds that we observed. Whereas limits to limb excursion might typically be viewed as detrimental, they might not be disadvantageous for all types of locomotion. For example, during the limb-propelled rowing that most non-marine turtles use to swim (Rivera et al., 2011, 2013), greater protraction of the hind limb could reduce the aquatic stability of turtles by exposing them to higher lateral forces during locomotion (Blob et al., 2003). Although more species need to be examined to test the generality of the patterns of femoral motion that we observed, if our measurements are representative of both lineages, pleurodires could be predicted to be more stable swimmers than cryptodires, potentially providing them with energetic and sensory advantages (Dougherty et al., 2010; Rivera et al., 2011). Evaluations of the differences in swimming performance between pleurodires and cryptodires could help to inform understanding of the differences that have been recognized in the ecological distributions of these clades: whereas cryptodires have radiated onto land multiple times, pleurodires are all primarily aquatic (Bonin et al., 2006). Although factors that led to the derived fusion of the pelvis to the shell in pleurodires are unresolved, data on swimming performance could provide a new context for considering the origin of their morphological novelty by indicating potential locomotor costs and benefits that might accompany any structural advantages of fusion of the pelvis to the shell.

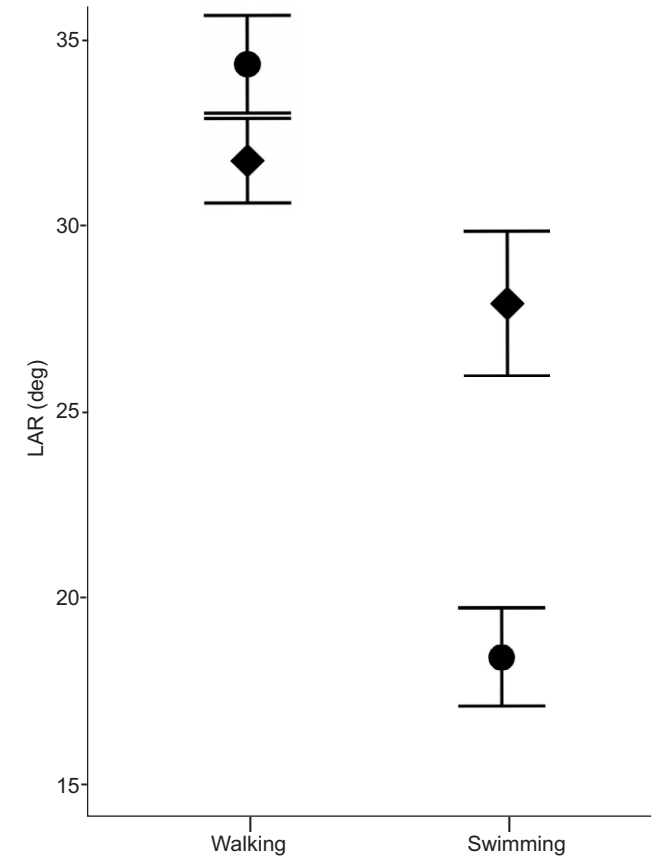


Fig. 7. Mean femoral long axis rotation (LAR) excursions of cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles during walking and swimming. Error bars indicate ±1 s.e. Pleurodires (circles) show substantially less femoral LAR during swimming compared with walking, whereas cryptodires (diamonds) show more similar femoral LAR excursions between behaviors.

Implications of pelvic and femoral movements for femoral loading mechanics

Our data on femoral movements also indicated long axis rotation (LAR) of the femur that varied between taxa and habitats. Whereas pleurodires exhibited similar levels of LAR between swimming and walking, cryptodires showed a marked decrease in LAR during swimming (Fig. 6, Table 3). The functional role of limb bone LAR during locomotion is poorly understood. In birds, it has been shown to increase working space and maneuverability while walking (Kambic et al., 2014, 2015), whereas in taxa with a sprawling gait it has been suggested as a mechanism for increasing stride length (Rewcastle, 1983; Ashley-Ross, 1994; Reilly and Delancey, 1997). Regardless of its function, a consequence of femoral LAR identified during sprawling locomotion has been an elevation of torsional loads (Blob and Biewener, 1999, 2001; Butcher and Blob, 2008;

Butcher et al., 2008; Sheffield et al., 2011; Blob et al., 2014). In this context, our data on pelvic and femoral movement in turtles are noteworthy in two regards. First, previous measurements of shear stresses (Butcher and Blob, 2008) and strains (Butcher et al., 2008) from *P. concinna* showed some of the highest torsional loads on the femur that have been found in walking vertebrates. This high torsion was attributed, in part, to the presumed immobility of the pelvis within the shell (Walker, 1971), which might have required any twisting imposed on the body during locomotion to be accommodated by the limb (Butcher and Blob, 2008). However, our finding that the pelvis is mobile in cryptodires indicates an additional structure beyond the limbs that can help to accommodate locomotor twisting, at least to some degree, suggesting a greater role for other mechanisms (e.g. immobile body axis, limb muscles; Butcher et al., 2008) that might impose high torsion on cryptodire femora. In contrast, the lack of pelvic movements in pleurodires might lead to elevated torsional loads on the femur for this clade on land, perhaps limiting their terrestriality and contributing to their predominant use of aquatic habitats.

A second insight into femoral loading mechanics in turtles provided by our XROMM data relates to changes in loads between walking and swimming. *In vivo* strain measurements from the femur of swimming cryptodires indicate a dramatic reduction in shear strains during aquatic rowing compared with terrestrial walking, outpacing reductions in bending loads (Young and Blob, 2015) and indicating that an additional factor, beyond just the overall reduction in loads that accompanies aquatic body support from buoyancy, is likely to be responsible. Our XROMM measurements provide evidence for such a factor, as our cryptodires reduced femoral LAR during swimming by almost half in comparison to walking (Fig. 6). The reduction of torsional loading in the proximal limb segments of rowing cryptodires may have facilitated the evolution of flattened limb bones that are typical of hyperspecialized flapping swimmers, such as sea turtles (Young and Blob, 2015). Beyond this possibility, our data indicate that the significant rotation of the foot that occurs during the rowing strokes of turtles (Blob et al., 2008) must be largely achieved through rotations of the distal limb segments – a prediction that could be tested through additional XROMM measurements.

Concluding remarks

Within the constraints of a bony shell, turtles exhibit considerable diversity in body plan and locomotor habits. Among cryptodire turtles in particular, species range from fully terrestrial taxa with high-domed shells, such as tortoises and box turtles, to highly aquatic species with flattened bodies, such as the trionychids (softshells). XROMM has, essentially, allowed the identification of a previously unknown mobile component in the locomotor apparatus of at least some members of the turtle lineage. The extent to which this component contributes to variation in the locomotor performance and ecology of these taxa remains to be explored, but such analyses carry strong potential to give insight into the functional diversity of this distinctive clade of animals and the diversity of pelvic girdle function across vertebrates more broadly.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

C.J.M. and R.W.B. conceived the study and performed surgical procedures; C.J.M., E.L.B. and R.W.B. collected data; C.J.M. and E.L.B. analyzed data. All authors contributed to manuscript preparation and approved the manuscript.

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Supplementary information

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