SYMPOSIUM

Reaction Forces and Rib Function During Locomotion in Snakes

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Synopsis Locomotion in most tetrapods involves coordinated efforts between appendicular and axial musculoskeletal systems, where interactions between the limbs and the ground generate vertical (G_V), horizontal (G_H), and mediolateral (G_{ML}) ground-reaction forces that are transmitted to the axial system. Snakes have a complete absence of external limbs and represent a fundamental shift from this perspective. The axial musculoskeletal system of snakes is their primary structure to exert, transmit, and resist all motive and reaction forces for propulsion. Their lack of limbs makes them particularly dependent on the mechanical interactions between their bodies and the environment to generate the net G_H they need for forward locomotion. As organisms that locomote on their bellies, the forces that enable the various modes of snake locomotion involve two important structures: the integument and the ribs. Snakes use the integument to contact the substrate and produce a friction-reservoir that exceeds their muscle-induced propulsive forces through modulation of scale stiffness and orientation, enabling propulsion through variable environments. XROMM work and previous studies suggest that the serially repeated ribs of snakes change their cross-sectional body shape, deform to environmental irregularities, provide synergistic stabilization for other muscles, and differentially exert and transmit forces to control propulsion. The costovertebral joints of snakes have a biarticular morphology, relative to the unicapitate costovertebral joints of other squamates, that appears derived and not homologous with the ancestral bicapitate ribs of Amniota. Evidence suggests that the biarticular joints of snakes may function to buttress locomotor forces, similar to other amniotes, and provide a passive mechanism for resisting reaction forces during snake locomotion. Future comparisons with other limbless lizard taxa are necessary to tease apart the mechanics and mechanisms that produced the locomotor versatility observed within Serpentes.

Introduction

Limbless locomotion in snakes is one of the most versatile locomotor mechanisms in tetrapods. Within the confines of an elongate and limbless body plan, snakes have managed to radiate to every continent besides Antarctica and invade almost every possible habitat. There are marine snakes that cruise the open ocean (Lillywhite 2014), arboreal snakes that cantilever through complex canopies (Astley and Jayne 2009), desert dwelling snakes that ascend slippery sand dunes (Marvi et al. 2014), fossorial snakes that burrow through the earth (Greene 1997), and even flying snakes that glide through the sky (Socha 2011). In comparison to mammalian equivalents, many of which have evolve exaggerated, specialized morphologies for a particular habitat, such as marine whales, arboreal monkeys, desert camels, fossorial moles, or flying bats, the conserved body form of snakes is equally adept in almost all environments and even capable of rapid transition between multiple locomotor modes (Gans 1974; Jayne 1986). While our understanding of the mechanisms and mechanics that govern terrestrial limbed locomotion abound, the mechanisms and properties of limbless snake locomotion are less well understood.

Propulsion results from the combination of the force exerted from the animal into the environment and the equivalent "reaction force" exerted from the environment to the animal (Biewener and Patek
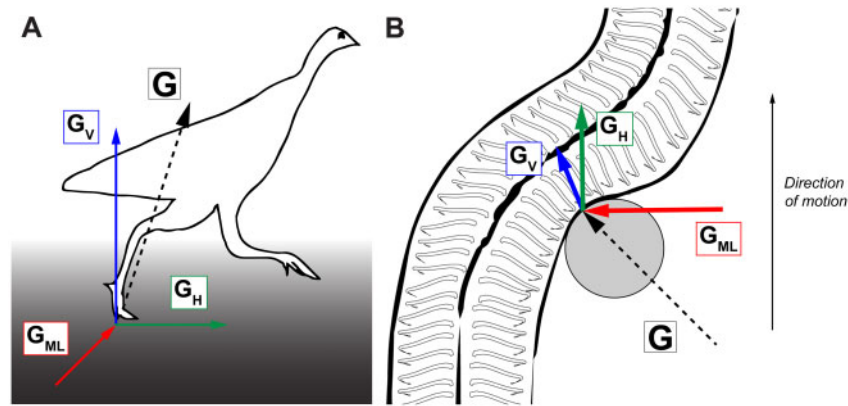
Fig. 1 Reaction forces during terrestrial locomotion in limbed tetrapods and limbless snakes. Propulsion is a result of the motive force exerted from the animal being resisted by an equivalent reaction force from the environment. This three-dimensional reaction force (**G**) can be described as the force vector components relative to the animal: the vertical reaction force (**G$_V$**), the horizontal reaction force (**G$_H$**), and the mediolateral reaction force (**G$_{ML}$**). (**A**) Lateral view of a bipedal bird. In typical limbed terrestrial locomotion, when the limb presses against the ground, the ground inherently pushes back and generates requisite reaction forces for motion. (**B**) Dorsal view of a limbless snake. In limbless locomotion in snakes, the axial musculoskeletal system functions similarly to the appendicular musculoskeletal system of limbed tetrapods and is the structure used to exert, transmit, and resist reaction forces. The direction and style of snake locomotion used is determined by mechanical properties of the environment and the direction in which the snake can exert force, as the environment does not necessarily provide enough reaction force in the required orientation for each locomotor mode.

2018). In terrestrial animals with limbs, these forces are the force of the feet against the ground and the reaction force of the ground back to the feet. These three-dimensional ground reaction forces can be described with their force vector components: vertical (**G$_V$**), horizontal (**G$_H$**), and mediolateral (**G$_{ML}$**) (Fig. 1). Typically, the vertical reaction force (**G$_V$**) results from a combination of the organism's mass, the normal force of gravity, and any vertical propulsion components; the horizontal reaction force (**G$_H$**) is attributed to the craniocaudal propulsive force minus any frictional interactions; and the mediolateral reaction force (**G$_{ML}$**) is attributed to sideways forces, which can be associated with maneuverability (Fig. 1). As a limbed terrestrial animal moves through the environment, these reaction forces are first experienced via individual limbs and then transmitted into, and experienced by, the axial musculoskeletal system (Carrier 1991; Ritter 1995; Schilling 2011).

In snakes, the axial system is all that remains and this musculoskeletal framework becomes the primary structure with which snakes exert, transmit, and experience all locomotor forces (Jayne 1988a, 1988b; Ritter 1995; Schilling 2011). As a snake exerts force into the environment, locomotor forces are generated through epaxial activity and then transmitted into the substrate through the body wall in contact with the environment (Cundall 1987; Capano et al. 2019a, 2019b). As such, the body wall of snakes is their sole locomotor structure and contains two key

components that are likely intrinsic to the locomotor versatility observed in these organisms: the ribs and the integument. These two structures are of crucial importance to how snakes experience locomotor reaction forces and their functional roles vary depending on the specific locomotor mode the snake uses.

Snake locomotion: A continuum of propulsive kinematics and mechanics

Terrestrial snake locomotion is a continuum of movement patterns often categorized into discrete locomotor modes. Classically, the four predominant modes are considered to be lateral undulation, rectilinear crawling, sidewinding, and concertina locomotion and most species are capable of some variant of all four (Gans 1974; Jayne 1986). However, most snakes can simultaneously employ two different modes in disparate body regions or combine the mechanics from multiple modes, blurring the lines between these discrete categories (Gray 1946; Greene 1997; Marvi and Hu 2012; Gart et al. 2019; Jayne 2020). Moreover, while these four locomotor patterns share similar characteristics of force exertion and kinematics, there can be large differences in how a snake executes each particular mode, and even variation within that mode.

In a broad context, we can decouple snake locomotion into two distinct patterns of body contact. When snakes move, each body region can either be (1) in static contact or (2) non-static contact with

the environment (Gans 1974; Cundall 1987). In static contact modes, force is exerted with regions of the body that do not move relative to the surrounding environment, enabling other body regions to move toward or away from the anchored zone. Rectilinear crawling, concertina locomotion, and sidewinding all employ these mechanics, where single or multiple body regions provide a static base through which locomotor forces are exerted and transmitted (Gans 1974; Marvi and Hu 2012; Marvi et al. 2013, 2014; Newman and Jayne 2018). In non-static contact modes, the entirety of the snake is in constant motion and body segments that generate propulsive force move relative to spatially constant contact zones, sliding past them. Lateral undulation is the primary example of this, as the traveling waves of muscle contraction continuously propel the whole animal forward, while force is exerted at environmentally stationary push points (Jayne 1988b; Moon and Gans 1998; Lillywhite 2014). With most species of snakes capable of such an extensive repertoire of locomotor modes, it is important to understand why an individual may select one mode over another.

In typical terrestrial locomotion in limbed tetrapods, the gait used is related to energy efficiency, locomotor speed, and movement of the center of mass (Cavagna et al. 1976; Hildebrand 1989; Farley and Ko 1997; Farley and Ferris 1998; Biewener and Patek 2018). A limbed tetrapod may use a different gait, for example, run instead of walk, to change the ratios of kinetic to gravitational potential energies and capitalize on inertia to increase energetic efficiency (Biewener and Patek 2018). In contrast, the specific locomotor style a snake uses in any given situation is primarily determined by mechanical properties of the environment and the direction in which the snake can exert propulsive forces (Gans 1962; Jayne 1986). In legged locomotion, when the limb presses against the ground, the ground inherently pushes back and the animal is able to generate G_V to counter gravity, G_H to move forward, and G_{ML} to maneuver. This is not the case in snakes. Although the mass of the snake does generate G_V, this single dimension does not necessarily provide enough reaction force for the direction the snake is attempting to travel, as forward motion often requires G_H and G_{ML}. For example, in generalized lateral undulation, the sweeping bends of a snake exert force laterally and caudally. The multiple opposite lateral forces cancel each other out and the vector sum of the forces propels the snake forward. This requires more than the ground below, however, as the snake must be able to exert force caudally

against objects in the environment that provide adequate G_H. Hence, these push points are essential for resisting the caudally directed forces required for forward motion.

If the environment does not provide push points against which the snake can exert force in the necessary direction, the snake cannot move and becomes more heavily reliant on friction-induced reaction forces. While limbed locomotion also depends on friction between the foot and the ground for G_H and G_{ML}, friction is generally not in short supply due to the large G_V in limbed locomotion, where friction against a surface is proportional to the normal force. Therefore, a fundamental difference between legged and limbless terrestrial locomotion is that it imparts particular importance on friction to generate the requisite reaction forces for propulsion. This is evident from the consistently low Froude numbers generated during snake locomotion, a measurement of the relative importance of inertial to frictional forces, which indicate that frictional and gravitational forces are an order of magnitude larger than inertial forces in snake locomotion (Hu et al. 2009). Hence, relative to legged locomotion, where the single plane ground provides enough reaction force for motion, snakes rely more heavily on three-dimensional force exertion, particularly the frictional interactions that produce the G_H and G_{ML} they struggle to achieve in the absence of limbs.

The importance of snakeskin

As limbless organisms that locomote on their bellies, the integument of snakes is integral to the frictional interactions these organisms are reliant upon to generate much of their locomotor reaction forces. Each locomotor mode of snakes uses the integument to contact the substrate and produce a friction-reserve that exceeds their muscle-induced propulsive forces, otherwise they experience slippage rather than force transmission. As such, the amount of force a snake can exert is limited by the maximum amount of friction they can produce. Snakes also appear capable of modulating the amount of friction they generate and often produce a static-friction reservoir or reserve (Gans 1970) that exceeds the minimum amount necessary for propulsion. The ventral scutes, the enlarged belly scales snakes most often locomote upon, are of particular importance because of the direction-dependent frictional anisotropy these scales exhibit; measurements of their coefficients of friction in the forward (μ_f), backward (μ_b), and transverse (μ_t) directions indicate that the ventral scutes most easily slide forward, strongly resist sliding backward,

and most strongly resist sliding sideways or transversely (Hazel et al. 1999; Berthé et al. 2009; Hu et al. 2009; Abdel-Aal et al. 2010). In contrast, the lateral and dorsal scales of snakes exhibit significantly less frictional anisotropy, although these regions have significantly higher absolute frictional coefficients than the ventral scales (Berthé et al. 2009; Abdel-Aal et al. 2010; Marvi et al. 2016). The magnitude of the anisotropic frictional properties of the ventral scales, related to the micro- and nanostructure properties of the scales (Hazel et al. 1999; Abdel-Aal et al. 2010), also appears to be under partial active control. Relative to unconscious snakes, conscious snakes exhibit nearly 30% higher sliding friction coefficients and almost double their static friction coefficients (Hu et al. 2009; Marvi and Hu 2012). These results, combined with quantitative observations of scale actuation during locomotion (Marvi et al. 2016) and intrinsic cutaneous muscle activation associated with skin stiffening for locomotor force transmission (Newman and Jayne 2018), demonstrate that snakes actively control their ventral scutes to modulate the reaction forces they experience and therefore, the locomotor forces they can transmit. The mechanics of the various locomotor modes each use the integument differently to exert motive force and likely synergize with and involve another often-overlooked component of snakes: their ribs.

Biological roles of ribs in snakes

In the absence of limbs, the hundreds of repeating pairs of ribs in snakes are their primary skeletal structures to interact with the environment, although their functional role during locomotion is unclear. While electromyography experiments have demonstrated that the epaxial muscles produce much of the propulsive forces during most serpentine locomotor modes (Jayne 1988a; Moon and Gans 1998; Newman and Jayne 2018), our understanding of the locomotor contributions of the over fifteen serially repeated hypaxial muscles, most of which attach to the ribs, is nowhere near as complete (Mosauer 1935; Gasc 1981; Gasc et al. 1989; Moon and Gans 1998; Newman and Jayne 2018; Penning 2018; Martins et al. 2019). As organisms that locomote on their bellies, however, the locomotor forces a snake exerts must inherently be transmitted through the ribs, to the integument, and into the substrate (Cundall 1987; Capano et al. 2019a, 2019b). This intrinsic costal involvement during snake locomotion was first noted over three decades ago but has received little attention since (Cundall 1987), likely due in part to the difficulty of quantifying and comparing *in vivo*

rib kinematics in any animal, let alone snakes. The advent of XROMM, X-ray Reconstruction of Moving Morphology, (Brainerd et al. 2010; Gatesy et al. 2010) and the expanded use of biplanar fluoroscopy in the field of comparative biomechanics, have since provided the necessary techniques to begin teasing apart how ribs operate, including their contribution to snake locomotion (Brainerd et al. 2016; Brocklehurst et al. 2017, 2019; Cieri et al. 2018; Capano et al. 2019a, 2019b).

Although the exact role of the ribs during snake locomotion remains elusive, it is abundantly clear that snakes use their ribs for a wide array of behaviors. Rib rotations contribute to cross-sectional body shape changes that help generate lift and enable gliding between trees (Socha 2011), mediolateral compression to increase swimming performance (Pattishall and Cundall 2008), production of an edge to dig into the sand for crypsis (Young and Morain 2003), dorsoventrally flattening to increase surface area during basking (Greene 1997), threatening body inflation during hissing (Lillywhite 2014), defensive signaling during hooding (Greene 1997; Young and Kardong 2010; Lillywhite 2014), and the volume changes associated with an ancestral function of ribs for all amniotes, lung ventilation (Rosenberg 1973; Brainerd and Owerkowicz 2006). Moreover, as squamates, snakes' ribs are highly mobile and have the capacity to rotate about three potential axes of rotation, colloquially described as (1) "bucket handle" rotation about a dorsoventral axis (Fig. 2A and D) (Supplementary Video S1), (2) "caliper" rotation about a craniocaudal axis (Fig. 2B and E) (Supplementary Video S2), and (3) "pump handle" rotation about a mediolateral axis, a rotation that can also be thought of as long-axis rotation about the neck of the rib (Fig. 2C and F) (Supplementary Video S3) (Jordanoglou 1970; Osmond 1985). Within the variety of behaviors for which snakes use rib motions, the axes each rib rotates about appear to differ depending on the desired output; for example, the axes a rib rotates about during hooding, a probable combination of caliper and pump handle rotations (Young and Kardong 2010), are likely quite different from those for the lateral compression for swimming, presumed to be primarily bucket and pump handle rotations (Pattishall and Cundall 2008). Additionally, snakes may exhibit further rotational permissiveness because of their complete lack of a sternum and absence of sternal ribs; an individual snake's hundreds of pairs of ribs are all unipartite (composed of a single costal element homologous with the vertebral ribs of other amniotes; Hoffstetter and Gasc 1969). This unipartite
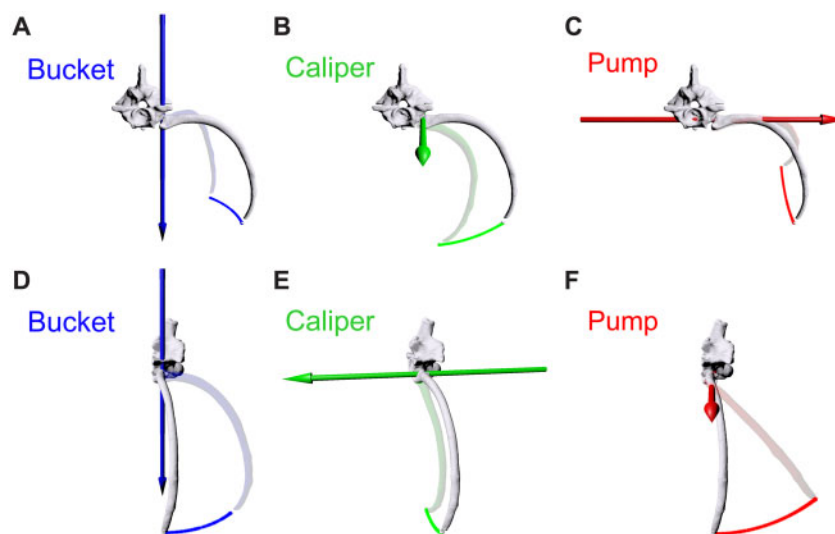
Fig. 2 Three-dimensional rotations about each costovertebral joint. Rib motions are conventionally described relative to three anatomical axes; (**A**) and (**D**) "Bucket handle" rotation about a dorsoventral axis (blue arrow; see Supplementary Video S1); (**B**) and (**E**) caliper rotation about a craniocaudal axis (green arrow; see Supplementary Video S2); and (**C**) and (**F**) Pump handle rotation about a mediolateral axis (red arrow; see Supplementary Video S3), that is, long-axis rotation about the neck of the rib.

Note: (A), (B), and (C) are cranial (frontal) views of vertebrae and left rib; (D), (E), and (F) are lateral views of the same vertebrae and left rib (cranial is to the left); colored tracers indicate path of distal rib tip; transparent colored ribs indicate rib position after rotation about the corresponding axis.

morphology may therefore permit an even wider range of motion due to the absence of potentially constraining ventral connections.

Regional control of rib motion in snakes

The serially repeated ribs of snakes are present almost the entire length of all snake species and rib motions do not appear to be restricted to specific regions. Rib rotations can be spatially localized, such as during the hooding display of cobras, or present throughout the length of the animal, such as the rib motions during lateral compression for swimming or horizontal compression during gliding and basking (Greene 1997; Pattishall and Cundall 2008; Young and Kardong 2010; Socha 2011). Furthermore, snake locomotion often involves simultaneous activity of disparate body segments, with the function of each region, and likely their underlying ribs, fluctuating throughout a locomotor cycle (Cundall 1987; Greene 1997; Gart et al. 2019).

As in all squamates, a critical function of rib rotations in snakes is for lung ventilation. Within the elongate bodies of snakes are similarly elongate lungs, where the left lung is reduced or entirely vestigial and the right lung extends ~30–80% of snout-vent length (Wallach 1998; Lillywhite 2014). Numerous anecdotal observations have suggested that lung ventilation can shift throughout the lung, typically in response to external stimuli, such as

distension during prey ingestion or force exertion during constriction (McDonald 1959; Rosenberg 1973; Wallach 1998). In these scenarios, the ribs are presumably unable to rotate because they are either displaced by the bolus or pressed against the ribcage of the prey. Recent XROMM experiments of lung ventilation in *Boa constrictor* have confirmed this capacity to spatially relocate (Capano et al. 2019a, 2019b). In these experiments, rib kinematics were measured (1) during normal ventilation at rest and (2) while an inflated blood pressure cuff was applied to this same region, in an attempt to preclude rib motion and simulate constriction. The findings were that each individual tested ceased rib rotations in regions where the cuff was applied and shifted the location of ventilation posteriorly, a clear demonstration of active control of rib rotations throughout the body (Capano et al. 2019a, 2019b).

Reaction-force perspective on terrestrial locomotion in snakes

For locomotion, the extensive mobility and independent control of rib motions in snakes suggest the ribs may have different mechanical functions depending on the locomotor style used and the reaction forces experienced. Each of the four predominant modes of snake locomotion provides examples of the multiple mechanisms used to generate reaction forces and plausible functional roles of the ribs that combine

to produce the versatility and variation observed in the continuum of snake locomotor styles.

Rectilinear crawling

Of all the forms of snake locomotion, rectilinear crawling is the only one that does not use axial bending to generate motion. Instead, this mode produces unidirectional propulsion through movement of the external skin relative to the internal skeleton (Lissmann 1950; Newman and Jayne 2018; Jayne 2020). The ventral scutes interact with the substrate to simultaneously produce numerous, at times upwards of 100 (Newman and Jayne 2018), static contact zones along the body and generate G_V and G_H via weight of the animal and friction of the scales (Marvi et al. 2013). Regions of skin between these zones are then simultaneously lifted and pulled forward toward the static areas in alternating waves to produce propulsive and recovery phases. Two muscles known to effect this form of locomotion are the costocutaneous superior (CCS) and costocutaneous inferior (CCI) (Lissmann 1950; Gasc 1981; Newman and Jayne 2018). From each rib, a CCS courses posteriorly from approximately one-third the length down the rib from the costovertebral joint to connect to multiple caudal lateral and ventral scales. The CCI runs anteriorly from each ventral rib tip for approximately six intercostal spaces to attach to more cranial lateral and ventral scales (Gasc 1981; Newman and Jayne 2018). As the animal progresses, the CCI shortens, pulling the skeleton forward toward anchored zones, while the CCS shortens and pulls posterior scutes anteriorly, to generate a new anchored zone (Lillywhite 2014; Newman and Jayne 2018). The result is that the skeleton moves forward at a nearly constant rate while regions of skin either slide faster than the entirety of the animal or remain stationary to transmit locomotor forces.

Over two centuries ago it was proposed that rectilinear crawling involved literal "rib-walking," with the snake crutching and crawling on its ribs similar to the legs of a caterpillar (Home 1812; Sibson 1846; Sood 1946). Numerous experimental methods including X-ray fluoroscopy, electromyography, and marker-based XROMM have since overturned this hypothesis and shown that the ribs do not move relative to the vertebrae during rectilinear progression (Supplementary Video S4; Lissmann 1950; Newman and Jayne 2018). However, this presents an interesting conundrum: if musculature is shortening to either physically pull the entire skeleton forward by the ribs or retract the skin toward the

ribs, and the ribs are highly mobile, why do they not rotate or fold under these contractile forces? Moreover, the CCS and CCI are functional antagonists that both attach to the ribs with no overlapping or counterbalancing activation (Newman and Jayne 2018). As such, the lack of motion suggests a new potential hypothesis: that each rib is actively immobilized by other hypaxial musculature to enable anterior or posterior costocutaneous musculature to pull against the rib, depending on the locomotor phase.

Isometric contractions of numerous costal muscles could provide the necessary stabilization for costocutaneous contraction, and the architecture of these muscles suggests tractable hypotheses to explain the observed rib immobility during rectilinear crawling. For example, costal muscles with posterior fiber orientation, relative to the vertebrae, such as the levator costae, tuberculocostalis, or intercostalis quadrangularis, may hold the ribs steady while the CCS pulls caudal scales cranially toward static contact zones; while anteriorly directed costal muscles such as the iliocostalis, obliquus internus ventralis, obliquus internus dorsalis, or transversus dorsalis stabilize the ribs as the CCI pulls the entire skeleton toward static scales. This new active stabilization hypothesis could be thoroughly tested through a combination of XROMM of the ribs, along with electromyography of the CCS, CCI, and potential stabilizing muscles mentioned above. A lack of rib motion, combined with activity of the CCS and CCI, would suggest the ribs are actively immobilized, while activity of other costal muscles may provide evidence for how this immobilization occurs. Nevertheless, the absence of rib motion is likely an active mechanism in this behavior and presents an interesting line of inquiry to better understand the ribs' role in this specialized form of vertebrate locomotion.

Concertina

Concertina locomotion is generally described as "accordion style" movement, where the snake folds itself like the pleats of an accordion for ratcheted progression (Jayne 1988a; Marvi and Hu 2012; Xiao and Chen 2013). In this mode, at least one portion of the body is statically anchored within the environment, which enables anterior or posterior regions to move toward or away from the anchored zone. Generally speaking, the reaction forces in this mode are produced by the weight of the animal (G_V), the frictional coefficient and activation of the scales (G_H), and any muscle-induced laterally directed forces (G_{ML}) (Marvi and Hu 2012;

Lillywhite 2014). These general principles govern most concertina mechanics, but variations in body contact, mechanisms of friction generation, and rib function produce a spectrum of concertina styles.

Although rarely observed, concertina may be used on flat surfaces. In the absence of lateral contact zones such as tunnel walls or push points, snakes using concertina mechanics on flat surfaces must generate locomotor reaction forces solely through their mass and frictional interactions of their ventral scales (Gray and Lissmann 1950; Hirose 1993; Hazel et al. 1999; Lillywhite 2014). This form is therefore inherently reliant on the direction of force application and will generate the highest static friction per surface area of substrate contact when the ventral scutes are oriented sideways or backward, due to the relatively high friction coefficients (μ_t and μ_b) in these directions. This suggests that the S-shape often observed during flat concertina may increase frictional forces, as it orients more scales sideways rather than backward to generate higher G_H and increase the reserve of friction-induced reaction force required for locomotor force exertion. Evidence of snakes modulating scale orientation, contact time, and stiffness (Marvi and Hu 2012; Newman and Jayne 2018) to increase friction and force transmission also suggests that snakes may actively regulate reaction force production, although robust measurements of reaction forces and correlation with scale orientation during snake locomotion are lacking.

Typically, however, snakes employ concertina locomotion in narrow tunnels and burrows (Gray and Lissmann 1950; Jayne 1986, 1988a). In these circumstances, the lack of cranially oriented contact zones prevents the animal from experiencing sufficient G_H that enables caudal propulsive force exertion because the snake is unable to create the cranially directed force vectors needed for lateral undulation. Forward propulsion is, therefore, a stepwise process of anchoring and sliding, where the animal bends the body to contact the straight-sided walls and produce a static contact zone. Transverse forces are exerted across the channel width, predominantly with the robust epaxial muscles (Jayne 1988a), and these forces are often in excess of the force required to prevent sliding out of contact. Snakes have been shown to push against sidewalls with forces up to nine times their own body weight, a safety factor of 400% (Marvi and Hu 2012). This inherently involves force transmission through the lateral body wall and, by proxy, the ribs, that are in contact with the tunnel. Considering the rotational mobility of the ribs and substantial G_{ML} experienced, this presents another interesting dilemma: How do the ribs not

fold under these forces? How do they transmit force? Undesired rib rotation would represent a substantial energy dissipation and although concertina is not particularly efficient, the metabolic demand is attributed to the transitions of acceleration and deceleration associated with this mode (Marvi and Hu 2012). Moreover, observations of snakes using concertina do not suggest high degrees of body deformation that would be associated with rib folding. Potential explanations might be that (1) the costal muscles stabilize the ribs to immobilize them for force transmission, similar to rectilinear crawling or (2) the ribs actively rotate laterally and therefore contribute to force exertion during contact. Stabilization against G_{ML} or mediolateral exertion would likely involve lateral rib rotations and muscles such as the levator costae, tuberculocostalis, or intercostalis quadrangularis are possible candidates with mechanical orientations to provide such function.

Additionally, the anchor-slide mechanics of concertina locomotion also enable snakes to climb relatively steep and cylindrical surfaces such as those found in arboreal habitats. In the absence of discrete push points, snakes are able to generate static contact zones by coiling with their ventrum against the branch-like object to periodically grip a surface through medial force exertion (Astley and Jayne 2007, 2009). These kinematics are remarkably similar to those of constriction, although constriction coils are applied with ventral and lateral body walls, dependent upon species and number of loops (Mehta 2005; Mehta and Burghardt 2008; Moon et al. 2019). As the slope of the incline increases, the animal's mass contributes less to the production of normal forces and contact becomes more reliant on muscular effort and friction, the latter aided by the μ_t and μ_b of the scales. A similar question remains, however: how are reaction forces resisted by the snake and what role may the ribs play? Snake ribs are capable of dorsal caliper rotations, as observed in hooding displays, basking behaviors, and large prey ingestion (Greene 1997; Lillywhite 2014), but uncontrolled ventral displacement during concertina locomotion would dissipate locomotor energy and place undue pressure on viscera. It is possible that the ribs maintain their posture and position similarly as proposed in tunnel-based concertina: through active rotation or stabilization to exert or transmit force during climbing. Muscles of interest may include those active during exhalation, including the transversus dorsalis, obliquus internus dorsalis, transversus ventralis, and obliquus internus ventralis, as these produce medially directed rotations and would provide favorable orientations to avoid the ribs from

splaying dorsally (Rosenberg 1973; Gasc 1981). Nevertheless, a more concentrated focus on the re-action forces generated during concertina locomo-tion, and the implicit function of the ribs and associated muscles, would provide an opportunity to more deeply understand the mechanics of this serpentine mode.

Sidewinding

Sidewinding, a mesmerizing testament to the incred-ible neuromuscular control of snake locomotion, is a specialized mode particularly effective for traversing granular and slippery substrates (Gans 1970). It is most commonly and effectively executed by stout, desert dwelling viper species adapted to navigate steep sand dunes, and aquatic snake species that reg-ularly negotiate exposed mud flats (Jayne 1986; Marvi et al. 2014). When a snake side winds, indi-vidual body segments maintain static contact while other regions simultaneously bend and lift; in effect, the static segments "roll over," are lifted out of con-tact, and are then placed down at the next static contact zone, producing distinct, discontinuous tracks. The track orientation and the snake's body, however, are oblique relative to the snake's direction of travel, a peculiar pattern that appears crucial to this mode's stability on granular substrates.

The propulsive force a sidewinding snake can ex-ert is constrained by the maximum static-friction reservoir generated through body contact. As a snake ascends a sand dune, the oblique orientation of the body directs any slippage laterally, such that sliding frictional forces are converted into static friction. A sliding sidewinder merely digs into the sand more, with slippage producing an accumulation of material posterior to the long axis of the body and giving them a deeper trough to push against. This com-bined with active twisting and torsion of the body that directs the posterior edge of their scales into the sand, increases and maintains the potential reaction forces that enable their ascent (Gans 1970). The three-dimensional nature of sidewinding is of equal importance, as lifting behavior reduces sliding con-tact, which reduces frictional drag and lessens the yielding of sand that will diminish available reaction forces (Marvi et al. 2014; Astley et al. 2015).

A sidewinder employs numerous mechanisms to increase reaction force generation, many of which may involve participation of the ribs. With the body oriented obliquely relative to the direction of travel, force is applied by regions with sideways-oriented scales with high frictional coefficients (μ_t). This may increase absolute reaction forces generated,

although measurements of the frictional anisotropy in sidewinders and their influence on this locomotor mode are underexplored. In instances where in-creased reaction forces are required, however, such as inclined sand dunes, sidewinders do not increase sand penetration depth but rather increase the rela-tive proportion of their body in contact with the substrate, in effect increasing G_H, relative to the di-rection of travel (Marvi et al. 2014). It is also possi-ble that these snakes actuate their scales to increase frictional coefficients, using active rib motions to increase body width and surface area contact, or contract musculature attached to their ribs to sharpen the lateral keel of their scales to dig into the sand (Jayne et al. 2015; Marvi et al. 2016). Similar to other bending forms of locomotion, the motive forces of sidewinding are generated with epaxial muscles and transmitted into the substrate with the lateral side of the body and highly mobile ribs. Effective force transmission therefore likely involves active stabilization of these skeletal ele-ments, although another potential is that individual ribs rotate to exert propulsive force or press into the trough of the trackway, similar to the rib motions used for digging in other desert-dwelling vipers (Young and Morain 2003).

Lateral undulation

Terrestrial lateral undulation is the fastest, most ubiquitous form of snake locomotion and is the characteristic slithering associated with snakes (Jayne 1986; Greene 1997; Lillywhite 2014). Unlike other forms of snake locomotion, lateral undulation involves non-static contact with the environment, such that body segments that exert propulsive force are in constant motion relative to spatially fixed con-tact zones, sliding past them. The snake bends from side-to-side to exert force caudally and laterally (Gans 1962; Jayne 1988b), and when these arcing bends exert force at multiple locations, the lateral forces cancel each other out and the vector sum of the forces propels the snake forward.

Slithering, however, is more than mere side-to-side bending, and the presence or absence of discrete push points changes the fundamental mechanisms used for motive force production. In the presence of push points, such as rocks, branches, or the "pegs" often used in controlled experiments (Jayne 1988a; Lillywhite 2014), snakes press their bodies against each push point and exhibit differential body curvature, where the vertebral column bends widely around the peg and the body wall asymmet-rically deforms on either side of the peg (Moon and

Gans 1998). This produces higher curvature on the anterior side of the peg relative to the posterior side. As the snake bends, force is then differentially applied to the region of higher curvature, causing the contact zone to be displaced from the area of higher curvature, anterior to the peg, to the area of lower curvature, posterior to the peg (Moon and Gans 1998). In effect, this results in forward translation of the whole animal with a locomotor mechanism analogous to a modified cam follower (Gasc et al. 1989; Moon and Gans 1998).

Although curvature is essential to push-point lateral undulation, it is not entirely clear how snakes produce these body shape adjustments. Electromyography experiments in *Python regius* suggest anterior curvature about the peg is associated with activity of the supracostalis ventralis and dorsalis, two superficial hypaxial muscles that course between two ribs numerous interspaces apart, and that this musculature produces bulging to increase curvature (Gasc et al. 1989). In this scenario, however, contraction of the supracostalis to produce bulging would require active stabilization of the corresponding ribs to prevent interspace collapse and instead enable body wall deformation. It is also possible that the supracostalis or other musculature may actively displace ribs laterally to produce asymmetrical curvature. The large levator costae, a hypaxial muscle with substantial physiological cross-sectional area (PCSA) and already hypothesized to participate in snake axial bending (Moon and Gans 1998; Penning 2018), is mechanically oriented to move ribs as it does during lung ventilation and defensive hooding (Rosenberg 1973; Young and Kardong 2010) and may contribute to body wall deformations at contact sites. Additional muscles such as the tuberculocostalis or intercostalis quadrangularis have similar architecture to produce such deformation through rib motion, and future studies of muscle function in this mode would provide useful information into these complex mechanics.

Unlike most other serpentine modes, the mechanics of push-point lateral undulation do not necessarily require friction to function. Experimental manipulations with a snake slithering in a nearly frictionless system composed of a smooth lubricated surface with fixed, rotatable vertical pegs, found that the snake moved faster than through a standard pegboard (Gans 1970). These observations and the μ_f of scales demonstrate that sliding friction detracts from propulsion, static friction is not required to produce motion, and the pegs merely enable the requisite G_H and G_{ML} to enable force exertion. At the point where the body exerts force into the peg, the mobile ribs are either (1) stabilized to prevent folding and enable force transmission, as this mode is driven at least in part by the large epaxial muscles (Jayne 1988a), or (2) rotated laterally via hypaxial musculature to contribute to force exertion (Moon and Gans 1998).

In contrast, when no discrete push points are available, the mechanics of lateral undulation are entirely reliant on frictional interactions. A snake on a flat surface can still laterally undulate by using the asperities of the surface to function as microscopic push points. These irregularities enable the anisotropy of the ventral scales to generate sufficient G_H and G_{ML} to prevent slippage and enable force transmission (Hu et al. 2009; Hu and Shelley 2012). As the snake moves, the μ_f of the scales in contact with the ground produces frictional resistance that must be overcome with additional muscular effort. When snakes slither at high speeds using this method, they often raise sections of the body off of the substrate. This has two important consequences: (1) it reduces frictional resistance due to μ_f and (2) redistributes weight to areas still in substrate contact (Hu et al. 2009). This phenomenon of "sinus-lifting" has been observed and noted in the literature since the late 1800s, although the mechanical underpinnings are only now beginning to be understood (Stradling 1882; Hirose 1993).

Sinus-lifting, therefore, appears to be a mechanism that allows snakes to slither faster without slipping. Body lifting occurs in regions that exhibit the highest degree of curvature and appears to be a mechanism to modulate frictional forces (Hu et al. 2009). The three-dimensionality of this lifting behavior means that the normal force exerted by the snake is a function of mass distribution and torque, not necessarily curvature (Hirose 1993). Furthermore, the normal force exerted is concentrated in regions with the lowest curvature, the same regions where tangential force is lowest, indicating that this is where slippage is most likely to occur (Hirose 1993; Hu et al. 2009; Hu and Shelley 2012). In order to modulate those normal forces, however, theoretical modeling suggests that body lifting non-uniformly distributes the animal's weight to load less curved regions and changes the directionality of frictional forces (Hirose 1993; Hu et al. 2009; Hu and Shelley 2012). This increases the normal force, effectively increasing the magnitude of friction against which the snake can push and enabling the snake to exert more motive force. While, admittedly, the non-uniform weight distribution model did not account for non-uniform body contact or craniocaudal variation in cross-sectional width and mass, researchers were still unable to account for the high range of speeds observed in their slithering trials, discrepancies

potentially related to the intricacies of snake skin, the modulation of weight distribution, and the forces associated with body lifting (Hu et al. 2009).

In the sinus-lifting configuration, the snake distributes weight to and exerts force in body regions oriented obliquely relative to their direction of travel. Theoretical models of lateral undulation and mass distribution (Hu et al. 2009; Hu and Shelley 2012) demonstrated that μ_b had little effect on locomotor speed, suggesting that snakes rarely exerted force directed backward relative to their continually reorienting body axis. As the snake bends from side to side, however, it is evident that the scales continuously reorient with respect to one another and the direction of travel, potentially to optimize the use of the scales' high μ_t. It has also been observed that snakes actuate individual scales to modify their frictional coefficients (Marvi et al. 2016), contract cutaneous musculature to stiffen the scales for force transmission (Newman and Jayne 2018), and modify body width and surface area contact, often through rib motions (Greene 1997; Pattishall and Cundall 2008; Socha 2011; Lillywhite 2014). Therefore, ventral scale contact and friction generation is not constant, and snakes may preferentially distribute weight to specific regions, potentially to those with the most advantageous scale or body axis configuration, in an attempt to optimize force output. Furthermore, lifting may also enhance force optimization, as hypothesized in other studies, through increased torsion, which may increase the normal force more than anticipated by models and enable higher overall force exertion and locomotor speed (Hirose 1993; Moon and Gans 1998). Nevertheless, although it is clear that snakes modulate the directionality of frictional forces, it is not clear what reaction forces are modified and through what mechanisms this occurs. Continued study of lateral undulation is necessary to understand the extent to which slithering snakes distribute weight non-uniformly, the interactions of scale actuation and stiffness on overall speed, and how snakes modulate these variables in relation to the frictional coefficients of their own scales and the substrate. The use of small pressure sensors, X-ray videography, and electromyography would prove useful in teasing apart the intricacies of flat lateral undulation and likely provide a wealth of inspiration for the next generation of biomimetic applications.

Biomechanics of ribs during locomotion in snakes

In terms of locomotion, the extensive mobility of costovertebral joints in snakes means the ribs may provide multiple functions depending on how the snake moves. As described above, locomotor propulsion in snakes often includes mediolateral reaction forces, activity of musculature with one or multiple costal attachment sites, differential weight distribution along the body, and active modulation of friction (Gans 1962, 1974; Jayne 1988a; Moon and Gans 1998; Hu and Shelley 2012; Marvi et al. 2016; Newman and Jayne 2018). In each instance, the ribs and their associated musculature likely provide different roles that, given the modularity in snake design and body control, plausibly fluctuate between functions throughout a locomotor cycle or in response to environmental variables. Ribs may (1) exert forces, through muscle-induced active rotation or (2) transmit forces, through muscular stabilization to prevent rib cage collapse and energy dissipation during substrate contact. They also (3) provide an attachment site for locomotor musculature and may be actively held still as antagonists for other muscles to pull toward or against, particularly cutaneous musculature likely involved in friction modulation (Marvi et al. 2016). Additionally, rib motions may (4) change body shape to contribute to the generation or maintenance of frictional reserves. Therefore, ribs may actively rotate or be stabilized to contribute to overall force production and optimization and present a wealth of opportunities for tractable hypotheses about the function of these structures during locomotor behaviors (Cundall 1987; Gasc et al. 1989; Moon and Gans 1998; Penning 2018). All of these proposed functions involve active muscular control, whereas the intrinsic morphology of the rib heads and costovertebral joints of snakes suggest a passive mechanism of equal importance.

Functional morphology of costovertebral joints in snakes

An important consideration when thinking about skeletal kinematics are the joints about which the elements move, and costovertebral joints are no exception. The ancestral costovertebral joint morphology for amniotes is two-headed ribs that have a tuberculum and capitulum on each rib that articulate with a diapophysis and parapophysis on each vertebra, respectively. This bicapitate condition is retained in almost all extant amniotes except squamates, that are notably different in having unicapitate ribs which articulate with each vertebrae at a single articular surface, the synapophysis (Romer 1956; Hoffstetter and Gasc 1969). Snakes, however, have double-headed ribs that articulate with the vertebrae at two distinct articular surfaces (Fig. 3A–C).
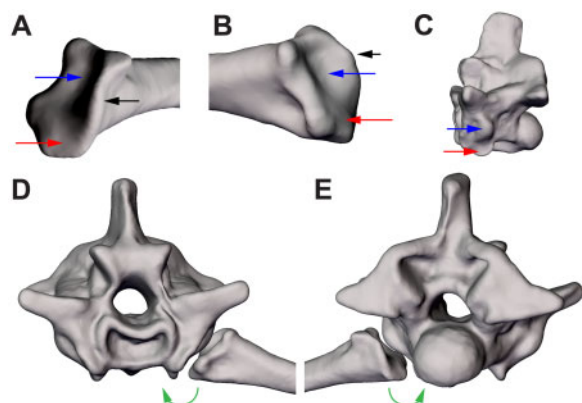
Fig. 3 Biarticular costovertebral morphology in *B. constrictor.* Unlike most squamates, the costovertebral joints in snakes have two distinct articular surfaces. (**A**) Oblique lateral view of a left rib head and (**B**) a cranial view of the same left rib head. The rib heads of *B. constrictor* have a concave, dorsal articular facet (blue arrow), a slightly convex and flattened ventral articular facet (red arrow), and a prominent caudal ridge (black arrow). (**C**) Lateral view of the left side of the corresponding vertebra shows the convex dorsal prominence (blue arrow) that fits with the dorsal facet of the rib similar to a ball-and socket (blue arrow) and the broad, slightly concave ventral articular surface (red arrow) that is medially recessed and articulates with the ventral facet of the rib. (**D**) A cranial view and (**E**) caudal view of the costovertebral joint showing the oblique orientation of the costovertebral joint. Note the ventral facet of the rib buttressed against the ventral articular surface of the vertebra; in this configuration, medial rotation about a craniocaudal axis, that is, caliper rotation (green arrow), would be precluded by this joint articulation.

This biarticular morphology has been noted in the literature for over two centuries, and the distinct articular surfaces have been described as "capitulum," "tuberculum," "diapophysis," or "parapophysis," although there is no developmental evidence that suggests such homology (Home 1812; Sibson 1846; Sood 1946; Hoffstetter and Gasc 1969; Pattishall and Cundall 2008; Young and Kardong 2010). Rather, the synapophyses of squamates have been shown to be a developmental fusion of the parapophysis and diapophysis of each vertebrae (Romer 1956; Hoffstetter and Gasc 1969). This evidence, combined with snakes' phylogenetic nesting within Squamata (Fig. 4) (Streicher and Wiens 2017), suggests the biarticular condition in snakes is a convergent and derived secondary evolution of multiple articular surfaces from the plesiomorphic unicapitate morphology for Squamata.

The two articular surfaces on each rib and vertebrae of snakes have distinct anatomical features likely related to their function. The dorsal aspect of each costovertebral joint has (1) a concave articular surface on the rib (Fig. 3A, B, and E; blue arrow), analogous to a tuberculum, which articulates with a convex prominence on the vertebra (Fig. 3C; blue arrow), analogous to a diapophysis. The ventral aspect of each costovertebral joint has (2) a slightly convex and flattened articular surface on the rib (Fig. 3A, B, and E; red arrow), analogous to a capitulum, which articulates with a broad, slightly concave articular surface on the vertebrae (Fig. 3C; red arrow), analogous to a parapophysis. This ventral portion is also obliquely oriented and medially recessed relative to the dorsal prominence (Fig. 3D and E; Sood 1946; Hoffstetter and Gasc 1969; Pattishall and Cundall 2008; Young and Kardong 2010). The dorsal articular concavity of the rib also exhibits a conspicuous ridge along the caudal edge of the joint (Fig. 3A and B; black arrow) that is suggested to preclude cranial translation and enable the dorsal articulation to function similar to a ball-and-socket (Hoffstetter and Gasc 1969; Pattishall and Cundall 2008). Anatomical observations also demonstrate that, at rest, the ribs' ventral flattened articular surface is flush against the similarly flattened ventral vertebral surface and permits craniocaudal shear, while rotation occurs primarily about the dorsal facet (Pattishall and Cundall 2008). These generalized morphological characteristics have been documented in numerous phylogenetically diverse taxa, including Pythonidae (Sood 1946; Hoffstetter and Gasc 1969), Viperidae (Hoffstetter and Gasc 1969), Elapidae (Hoffstetter and Gasc 1969; Young and Kardong 2010), and Colubridae (Sood 1946; Hoffstetter and Gasc 1969; Pattishall and Cundall 2008), and parsimoniously suggest this trait originated at the latest within Alethinophidia (Hsiang et al. 2015; Streicher and Wiens 2017). Contrary descriptions exist for blind snakes, for example, *Typhlops* spp., with some suggesting unicapitate morphology, while other accounts and diagrams denote similar biarticular morphology to all other snakes (Sood 1946; Hoffstetter and Gasc 1969). These conflicting descriptions and the variable morphology present throughout Serpentes suggest that a more robust morphological analysis of these traits and their phylogenetic variation would be productive directions for future inquiry. Nevertheless, the ubiquity and prominence of this biarticular morphology within snakes beg the question: what functional role does this morphology serve?

One of the most notable characteristics of snakes is their lack of limbs, and at first glance one might presume that this trait evolved in relation to limblessness. An elongate, limbless, "snake-like" body plan has convergently evolved within Squamata at least 25 separate times and provides a phylogenetically and ecologically diverse set of comparisons
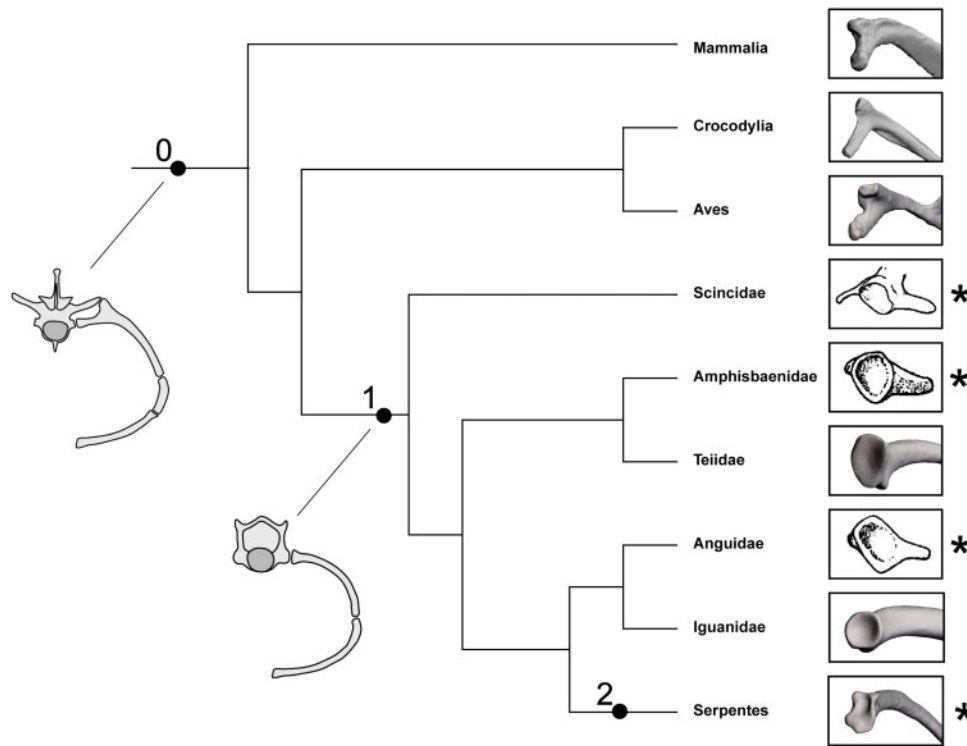
Fig. 4 Phylogenetic distribution and evolution of costovertebral joint morphology in Amniota. The plesiomorphic costovertebral condition for Amniota (0), inherited from early tetrapods, is bicapitate ribs articulating with two articular facets on the vertebrae, the diapophysis and parapophysis. Bicapitate morphology is retained in many extant amniote taxa including Mammalia, Crocodylia, and Aves. Squamata shows a derived condition (1) in which unicapitate ribs articulate with a single articular facet, the synapophysis. Serpentes (2) have re-evolved biarticular costovertebral joints as a modification of the ancestral squamate uniarticular condition. (Asterisk denotes images from limbless squamate taxa.) Images after Hoffstetter and Gasc (1969).

(Brandley et al. 2008). In no other instance, however, do we see a similar biarticular costovertebral morphology arise (Fig. 4) (Hoffstetter and Gasc 1969). Even in the second-most specious and widespread group of limbless squamates, amphisbaenians, unicapitate joints are retained (Fig. 4) (Hoffstetter and Gasc 1969; Gasc 1981). This suggests that biarticular morphology is not necessarily correlated with a mere lack of limbs and may instead be associated with character traits present only in snakes.

Of the numerous derived features of snakes, there are two important traits that may be linked to the evolution of biarticular costovertebral morphology: locomotion and constriction. Unlike snakes, most limbless lizards are capable of only a few forms of locomotion, often "simple undulation" and concertina, and display little to no localized body deformation (Gans 1986; Gans and Gasc 1990; Gasc and Gans 1990). Of the two limbless lizard species extensively tested, Anguis fragilis and Ophisaurus apodus, A. fragilis do exert a higher degree of regionalized control than O. apodus (Gasc and Gans 1990), but neither species is capable of the full continuum of limbless locomotor mechanics observed in snakes,

and often slip into and out of contact with push points (Moon and Gans 1998; Marvi and Hu 2012; Gart et al. 2019). In contrast, snakes are capable of numerous modes of locomotion that may be simultaneously performed in disparate body segments, each of which is capable of propulsive force exertion in multiple dimensions with magnitudes much higher than their own body weight (Gans 1970; Marvi and Hu 2012). This suggests that snakes, clearly capable of regionalized body deformation and local control, have finer neuromuscular control of each segment and overall force modulation.

In addition to complex locomotor mechanics, constriction is a behavior found only within Serpentes and is proposed to be a major innovation in snake evolution that contributed to their extensive radiation (Greene and Burghardt 1978; Mehta 2005). In this prey subjugation mode, the snake wraps one or more loops of its body around the prey to form a constriction coil (Greene and Burghardt 1978; Greene 1983; Mehta and Burghardt 2008). The snake exerts pressure at two or more of these points of contact, effectively using its own rib cage to compress and pressurize the ribcage of its prey, thereby

disrupting the prey's cardiovascular blood flow, and inducing death likely via cardiac arrest (Boback et al. 2015; Moon et al. 2019). Although this clearly involves tremendous force, this behavior does not substantially pressurize the systemic blood pressure of the snake exerting the force; systemic blood pressures reach higher levels throughout hissing and prey ingestion than during constriction (Wang et al. 2001).

The key component that these two traits have in common is the medially directed reaction force experienced by the ribs. In many of the various modes of snake locomotion described, the ribs experience substantial G_{ML}, such as when the body wall and associated ribs transmit force into a tunnel with concertina (with up to 400% of the snake's body weight) or push points throughout lateral undulation (Gasc et al. 1989; Moon and Gans 1998; Hu et al. 2009; Hu and Shelley 2012; Marvi and Hu 2012). When a snake constricts, it generates pressure through activity of the epaxial musculature that is transmitted through the ribs in contact with the prey, with force transmission only possible due to the G_{ML} of the prey pushing back against the snake (Moon 2000). This direct transmission and application of force with the ribs is remarkably similar to concertina mechanics, where snakes often exert large forces with their lateral body wall and rib cage (Jayne 1986; Marvi and Hu 2012). In these scenarios, if the mobile ribs reactively rotate under the requisite G_{ML} forces, this represents a substantial dissipation of energy that would require active rib rotation and muscular stabilization to prevent, thereby increasing the metabolic demands of constriction and locomotion. It is, therefore, possible that the biarticular costovertebral morphology of snakes is passive mechanism to enable force transmission in the absence of muscular effort and would be similar to the function of costovertebral joints in other amniotes.

In limbed amniotes, there is considerable variation in costovertebral morphology that has been suggested to functionally resist locomotor reaction forces. Crocodilians, birds, and some lizard species have convergently evolved dorsoventrally elongated costovertebral joints, particularly near the pectoral girdle regions of the thorax that experiences high magnitudes of locomotor forces (Claessens 2015; Brocklehurst et al. 2017; Capano et al. 2019a, 2019b). This alignment has been hypothesized to function as a skeletal strut that dissipates locomotor forces away from the rib cage. In the absence of this morphology, axial musculature typically used for ventilation is co-opted to stabilize limbed locomotor reaction forces, constraining ventilation during locomotion (Carrier 1990, 1991; Brainerd and Owerkowicz 2006). This negative influence of locomotion on ventilation is thought to be reduced by strut-like costovertebral joints, transferring energy into the axial skeleton rather than requiring muscular stabilization (Claessens 2015; Capano et al. 2019a, 2019b). While the reaction forces experienced during snake locomotion are quite different than those in limbed locomotors, the biarticular costovertebral morphology of snakes may serve a similar functional role. As previously suggested, muscle-induced stabilization would enable the transmission of locomotor forces via the ribs, although considering the number and likely involvement of ribs to various snake behaviors, this stabilization would represent a non-trivial use of energy. A plausible alternative is that the biarticular joints in snakes serve as a passive buttress; as the ribs are pressed into the environment, the flattened ventral facet of the rib may abuts the corresponding ventral articular surface of the vertebrae (Fig. 3D and E; curved arrows), thereby constraining medial-directed caliper rotation and instead transmitting force into the environment or prey.

Functionally, a buttress mechanism may specifically constrain medially directed caliper rotation while enabling rotation about other axes. Precise XROMM measurements of rib rotations during costal ventilation in B. constrictor suggest rotational permissiveness about axes other than caliper. In normal lung ventilation and hissing, B. constrictor rotates their ribs primarily about bucket and pump handle axes, with only small amounts of caliper rotation (Fig. 5) (Capano et al. 2019a, 2019b). In the >55 individual rib rotations analyzed for that study, the average medial caliper rotation, including standard deviations, never exceeded 5 degrees (Fig. 5), and no individual rib ever rotated medially in excess of 11 degrees (Supplementary Fig. S1). Physical manipulations of anesthetized and deceased specimens used in that study further corroborate a buttress mechanism. When the ribs are postured almost orthogonal to the body axis and force is mediolaterally exerted on them, they are unable to rotate toward the midline; when the ribs are postured more cranially or caudally, however, mediolateral pressure exertion produces pronounced medial caliper rotations.

While these observations do not conclusively demonstrate that medial caliper rotations are prevented rather than not performed, they do provide the framework for tractable hypotheses as to the function of this perplexing biarticular costovertebral morphology in snakes. This morphology does appear to vary among major snake taxa and morphometric analyses, *ex vivo* range of motion studies, and
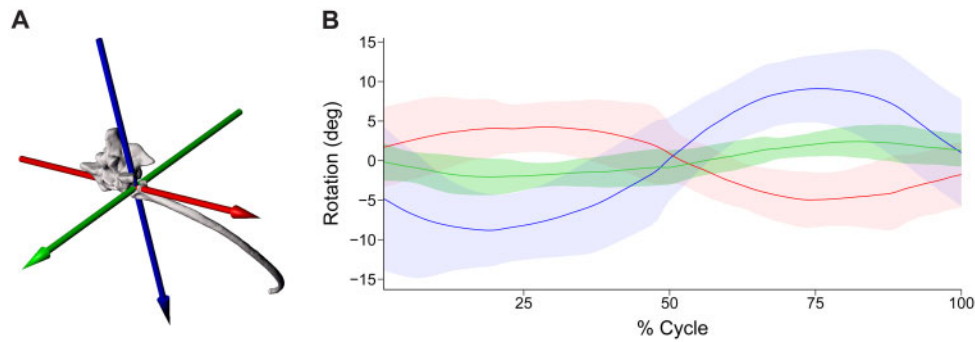
Fig. 5 Joint coordinate systems for lung ventilation in *B. constrictor* and mean rotations. (**A**) Joint coordinate system orientation relative to a left rib of i > *B. constrictor*; all rotations measured used the right-hand rule, that is, positive bucket (blue) = cranial rotation, positive caliper (green) = dorsal rotation, positive pump handle (red) = caudal rotation. (**B**) Mean rib rotation about each of the three anatomical axes during lung ventilation (*n* = 56 individual rib rotations; three individuals; shaded region shows ± 1 SD); each breath was defined as exhalation followed by inhalation (maximum exhalation at 25% breathing cycle; maximum inhalation at 75% breathing cycle; all rotations were zeroed and normalized to 100% cycle duration. Note that the average caliper rotations never exceeded 5 degrees in either direction; combined with morphology in Fig. 3 this suggests that costovertebral morphology may preclude medial caliper motions. See Fig. 3 for costovertebral morphology and Supplementary Fig. S1 for non-averaged rotational traces.

phylogenetic analyses would provide invaluable insight into the evolution of this trait. These data, coupled with *in vivo* kinematic work concentrated on rib motions throughout each locomotor mode, including the rib rotations and posture during concertina locomotion, and also constriction, could provide evidence for the function of biarticular joint and yield interesting results as to how snakes integrate rib motions into their complex movement patterns. While ribs are of clear importance to snake locomotion, similar *in vivo* work could be extended to better understand the role of the integument. The measurement of three-dimensional reaction forces and scale orientation in response to changes in substrate friction, or species-specific frictional anisotropy, could attempt to decouple how snakes modulate their exertion of motive forces and generation of requisite reaction forces. On the whole, snake locomotion presents numerous perplexing mechanisms and mechanics, and to understand these fully, we need to learn more about the mechanical roles of both the integument and the ribs across the all locomotor modes and major groups of these versatile limbless locomotors.

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Supplementary data

Supplementary data available at *ICB* online.

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