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DIGITAL RECONSTRUCTION OF SOFT-TISSUE STRUCTURES IN FOSSILS

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ABSTRACT.—In the last two decades, advances in computational imaging techniques and digital visualization have created novel avenues for the study of fossil organisms. As a result, paleontology has undergone a shift from the study of fossilized bones, teeth, and other hard-tissues to using virtual computer models to study specimens in greater detail, restore incomplete specimens, and perform biomechanical analyses. The rapidly increasing application of these techniques further paved the way for the digital reconstruction of soft-tissue structures, which are rarely preserved in the fossil record. In this contribution, different types of digital soft-tissue reconstructions are introduced and reviewed. Examples include methodological approaches for the reconstruction of musculature, endocranial components (i.e., brain, inner ear, neurovascular structures), and other soft-tissues (e.g., whole-body and life reconstructions). Digital techniques provide versatile tools for the reconstruction of soft-tissues, but given the nature of fossil specimens some limitations and uncertainties remain. Nevertheless, digital reconstructions can provide new information, in particular if interpreted in a phylogenetically grounded framework. Combined with other digital analysis techniques, such as finite element analysis (FEA), multibody dynamics analysis (MDA) and computational fluid dynamics (CFD), soft-tissue reconstructions can be used to elucidate the paleobiology of extinct organisms and to test competing evolutionary hypotheses.

INTRODUCTION

Fossils form the only physical evidence of extinct life and our knowledge of past organisms and ecosystems almost entirely depends on their presence and preservation. The vast majority of fossils consist of bones and teeth in vertebrates, biomineralized shells and exoskeletons in invertebrates, trace fossils, and other diagenetically persistent structures (i.e., spores, pollen) (Schopf, 1975). In contrast, soft-tissues are only rarely preserved in the fossil record. Although a few examples of exceptional preservation have allowed remarkably detailed insights into the soft-tissue anatomy of extinct vertebrates (Sasso and Signore, 1998; Trinajstic et al., 2007; Schweitzer, 2011), invertebrates (Butterfield, 2003; Sutton et al., 2005), and plants (Gerrienne et al., 2006; Bernard et al., 2007), these cases generally form the exception rather than the rule (Allison and Briggs, 1993; Wilby and Briggs, 1997). However, detailed knowledge of soft-tissue structures is paramount to understanding the paleobiology of extinct organisms (Witmer, 1995): 1) Soft-tissues are responsible for a multitude of physiological functions, such as locomotion, breathing, or temperature regulation; 2) soft-tissues can drastically change the appearance of an organism in comparison to its preserved hard parts; 3) soft-tissue characters can provide important phylogenetic information; and 4) soft-tissues control the development and shaping of hard-tissues. As paleontologists, we are therefore challenged with the reconstruction of such anatomical components, which have not been mineralized and preserved, in order to understand fossils as living, functioning organisms.

As a consequence, soft-tissue reconstructions have a long history, in particular in vertebrate paleontology. Traditionally, the presence and arrangement of soft-tissues has been inferred on the basis of the preserved hard parts or in comparison with extant taxa, which form a phylogenetic bracket or a functional analogue (Bryant and Russell, 1992; Witmer, 1995). In the past, such soft-tissue reconstructions have generally been performed in a theoretical framework and in the form of two-dimensional drawings and schematics. This includes, for example, the reconstruction of musculature in different vertebrates (Adams, 1918; Romer, 1923; Miner, 1925; Barghusen, 1973; Sumida, 1989) and some invertebrate groups (Budd, 1998), pneumatic and pulmonary structures (Witmer, 1997; O'Connor, 2006), and other soft-tissues (Frey et al., 2003).

In recent years, the advent of novel computational techniques has dramatically changed the ways in which fossils can be studied and characterized (Cunningham et al., 2014). First and foremost, computed tomography (CT) now allows new insights into fossils, and the identification and visualization of internal structures (Sutton, 2008). Functional analyses, such as finite element analysis (FEA), multibody dynamics analysis (MDA), or computational fluid dynamics (CFD), based on digital models of fossils provide the means for biomechanical studies and to quantify fossil function (Rayfield, 2007; Curtis, 2011; Rahman et al., 2015). Digital techniques further provide powerful tools to restore the hard-tissue morphology of fossils and to remove taphonomic and preservational artefacts (Lautenschlager, 2012; Cunningham et al., 2014; Lautenschlager et al., 2014b). Similarly, the same methods have been used to reconstruct various soft-tissues in fossils (Fig. 1). However, as soft-tissue reconstructions rely greatly on the preserved hard-tissues, this approach has largely been restricted to vertebrate fossils in the past, but could easily be applied (with some limitations) to non-vertebrate fossils. This contribution provides an overview of existing examples of soft-tissue reconstructions and reviews applied techniques and methods.

DEFINITIONS

The popularity of digital methods to visualize and analyze fossils three-dimensionally has led to a variety of different terminologies – none of which, however, are clearly defined. As a result, the term "digital reconstruction" is often used ambiguously. This term has been used to describe the visualization of a physical specimen following its digitization; as such it is synonymous with the meaning of "digital representation" of the specimen, and the latter term is advocated here for this purpose. In contrast, digital reconstruction is used here in the context of recreating and visualizing anatomical structures, which are not preserved and directly observable. In addition, “digital restoration” is used as a further term to describe the process of removing preservational artefacts to restore the original morphology of a specimen as prior to fossilization.

MUSCULATURE

Examples

Muscles form an integral part of an animal's anatomy and play a fundamental role in feeding, locomotion, and other physiological activities. Unsurprisingly, numerous studies have focussed on the reconstruction of various parts of the musculature in fossils (e.g., Dilkes et al., 2012 and references therein) and the same is true for digital, three-dimensional reconstructions of musculoskeletal anatomy (Fig. 1A). The increased popularity of biomechanical modelling techniques, such as FEA or MDA, have further created demand and renewed interest in detailed and accurate muscle reconstructions to serve as input parameters for computational analyses (Bright, 2014). Driven by biomechanical studies, digital reconstructions have focussed mostly on the cranial jaw adductor musculature and the locomotory muscle complex in vertebrates.

Digital reconstructions of the jaw adductor muscles have been created for different vertebrate groups, including dinosaurs (Lautenschlager, 2013; Button et al., 2014; Cuff and Rayfield, 2015), pliosaurs (Foffa et al., 2014), and marsupials and fossil placental mammals (Wroe et al., 2013; Cherin et al., 2014; Sharp, 2014). However, variations exist as to how detailed the different muscle groups were reconstructed and to what further purpose. Similarly, reconstructions of postcranial muscles have been created to study dinosaurian locomotory capabilities (Hutchinson et al., 2005; Persons and Currie, 2011b; Sellers et al., 2013) and feeding behaviour (Snively et al., 2013).

Methodological approach

The identification of the muscle attachment sites forms the basis of all digital muscle reconstructions, regardless of whether they are performed on the cranial skeleton, postcranial elements, or in invertebrates (Lautenschlager, 2013) (Fig. 2A). Identification is performed either on the actual specimen (if available) or the digital model; ideally both, as some (osteological) correlates might only be visible on the physical specimen and vice versa. Correlates attributable to muscle attachment are usually preserved in the form of distinct surface features, such as bony ridges and projections, depressions, rugosities, and muscle scars. Further features constraining not only the position but also the extent of the muscle attachment may be consulted if present. In this, the digital approach is comparable to traditional muscle reconstructions (e.g., Dilkes, 1999; Holliday, 2009).

Following the identification of the muscle attachment sites, the three-dimensional muscle arrangement can be reconstructed. As the majority of muscles are suspended between their origin and insertion, a point-to-point connection will allow a simplified visualization of the muscle topology (Fig. 2B). In most instances, more than one muscle or muscle group attaches to the skeletal element of interest (e.g., the mandible) and the creation of simplified muscle connections will provide further constraints on the muscle arrangement. For example, between three and 10 jaw adductor muscles occupy the cranial skeleton in vertebrates. The different muscles will have to be accommodated within this bony housing without intersection, imposing further “packing-constraints”. For the digital reconstruction, this can mean that muscle attachments might have to be revisited in a recursive approach in order to produce a compatible muscle arrangement for the simplified muscles represented by point-to-point connections. However, the use of digital models usually permits rearranging these simplified muscles without too much effort and testing different configurations. The number of muscles to be reconstructed and hard-tissue constraints depend largely on the anatomical region and taxonomic group, thereby offering more or less information on the placement and muscle arrangement.

Depending on the type of subsequent analysis, the simplified muscle reconstruction may already be sufficient. For the investigation of muscle strain (Lautenschlager, 2015) (Fig. 2C) or muscle moment arms (Chapman et al., 2010), simplified muscle reconstructions have been used in the past. Similarly, studies involving multibody dynamics analysis rely largely on the position and orientation of muscles (Hutchinson et al., 2005; Curtis et al., 2008; Moazen et al., 2008; Bates and Falkingham, 2012) to calculate kinematic behavior. A similar approach has been applied for finite element analysis, an engineering technique, which calculates the magnitude and distribution of stress and strain in geometric objects in response to loading regimes, such as muscle forces. In the past, these muscle forces have mostly been applied to individual points (i.e., nodes) of the finite element (FE) models in the form of force vectors (Rayfield, 2007; Dumont et al., 2009). Information on the location and direction of these force vectors can be obtained from simplified muscle reconstructions. More recently, further techniques have been proposed to model muscles wrapping around bone to replicate actual muscle attachment in FE models (Grosse et al., 2007; Liu et al., 2012). However, this approach requires data on the three-dimensional muscle morphology. Furthermore, to calculate different muscle properties (volume, cross-section area, mass) and muscle forces, a more detailed “fleshed-out” reconstruction is necessary.

Different approaches exist to create a full muscle reconstruction and these depend largely on the type of models (surface-based vs tomographic) and the software used. For tomographic datasets, special segmentation software, such as Avizo (VSG, Visualization Science Group), Mimics (Materialise), or SPIERS (Sutton et al., 2012) can be used to increase the diameter of the simplified muscle connections isometrically until connections of the same muscle merge into another, or until other muscle groups or osteological/hard tissue boundaries are encountered (Lautenschlager, 2013) (Fig. 2D). This is based on the assumption that all muscles are increased by the same amount, but this can be adjusted if further information is available giving precedence of one muscle over the other. For surface-based data, it is possible to virtually sculpt muscles on top of digital skeletal elements, aided by cross-sectional guides. This method has been used, for example, to model the muscular components of the tails of different dinosaurs (Persons and Currie, 2011a, b; Persons et al., 2013). This forms the digital analogue to the creation and sculpting of physical (clay or polymer) models in order to obtain muscle forces (Rayfield et al., 2001; Mazzetta et al., 2009; Blanco et al., 2012).

The majority of muscle reconstructions are nearly entirely performed on the basis of preserved hard tissues, which might not be able to provide sufficient information for unusual muscle morphologies, such as muscle asymmetry, pathway curvature, or tendinous attachments. Similarly, fascia, tendons, and ligaments are rarely preserved in fossil taxa (Organ and Adams, 2005; Organ, 2006) but may form an important functional component. It is therefore advisable to interpret osteological correlates and emanating reconstruction in the context of living taxa (Fig. 2E). By employing an extant phylogenetic bracket approach (Witmer, 1995), homologies for muscle position and arrangement can be established. Furthermore, novel imaging techniques, such as contrast-enhanced CT scanning (Metscher, 2009; Lautenschlager et al., 2014a; Gignac et al., 2016), magnetic resonance imaging (Sharp and Trusler, 2015), or phase-contrast CT scanning (Walsh et al., 2013b), can provide further information and comparative data.

ENDOCRANIAL ANATOMY

Examples

The study of the endocranial anatomy, including the brain, inner ear, and neurovascular structures (i.e., nerves, blood vessels), of fossil animals has a long-standing history in paleontological research (Marsh, 1885; Edinger, 1929; Hopson, 1979; Buchholtz and Seyfarth, 1999, 2001). Due to the poor preservation potential of soft-tissue structures, however, early researchers had to rely on a few exceptionally preserved fossil endocasts – naturally occurring casts of the endocranial cavity, which are partially representative of the gross anatomy of the brain and associated structures – or to prepare artificial endocasts through serial grinding or casting techniques (Cunningham et al., 2014). The advent of non-destructive imaging techniques has revolutionized the field of paleoneurology and facilitated the acquisition and study of digital endocasts (Fig. 1B) to gain insight into brain anatomy, development, and neurosensory function.

Since one of the first applications of CT to reconstruct the endocranial anatomy of *Tyrannosaurus rex* (Brochu, 2000), the increasing availability of CT scanning technology and processing software has led to a surge of digital endocast reconstructions. In the past decade, digital endocasts have been created and studied for numerous fossil (and also extant) taxa across all vertebrate clades, including: jawless (Gai et al., 2011) and ray-finned fish (Giles and Friedman, 2014), dinosaurs (Witmer and Ridgely, 2009; Lautenschlager et al., 2012), pseudosuchians (Holloway et al., 2013; von Baczko and Desojo, 2016), crocodilians (Witmer et al., 2008), fossil flying and marine reptiles (Witmer et al., 2003; Marek et al., 2015), turtles (Carabajal et al., 2013), birds (Ksepka et al., 2012; Balanoff et al., 2013), mammals (Rowe et al., 2011; Racicot and Rowe, 2014; Ruf et al., 2016), and hominids (Zollikofer et al., 2005). These and comparable studies have consequently allowed the characterization of the endocranial anatomy of individual fossil taxa and provide a steadily increasing anatomical resource. Furthermore, they have paved the way for large-scale comparative studies, for example to shed light on the evolution of olfactory acuity in dinosaurs and birds (Zelenitsky et al., 2011), deducing auditory capabilities in reptiles and birds (Walsh et al., 2009, 2013a), and brain evolution across the cynodont-mammal transition (Rowe et al., 2011).

Methodological approach

Very recently, Balanoff et al. (2015) published a detailed guide on the digital reconstruction of endocasts and the reader is referred to this work for an in-depth step-by-step workflow. Here, a general overview on the methodological approach and potential applications is provided. More details on the tomographic segmentation processes and best practices can further be found in Abel et al. (2012) and Sutton et al. (2014).

Since digital endocasts are virtual casts of endocranial cavities enclosed by bone or cartilage, their reconstruction generally requires a tomographic dataset of the studied specimen. Although serial grinding methods have been used in the past and are still employed for specimens with poor internal contrast (Sutton, 2008; Cunningham et al., 2014; Balanoff et al., 2015), CT scanning is routinely used to obtain the necessary data. For disarticulated or broken specimens, surface-scanning methods can also be used (with limitations) to reconstruct parts of the endocranial anatomy (Lautenschlager and Hübner, 2013; Balanoff et al., 2015). Different approaches exist as to how the endocranial components can be reconstructed from the dataset. The most common one is the selection of features-of-interest (e.g., endocranial cavity, bony canals of nerves) in subsequent tomographic slices – a process known as segmenting or labelling (Fig. 3A). Depending on the quality of the dataset, this can be done semi-automatically on the basis of a specific greyscale value, which represents the cranial cavities and separates them from the bony housing. For fossil specimens, however, this is often not possible where sedimentary matrix has infilled the endocranial cavities and fossilization processes have remineralized the bone. As a result, the density of the matrix and (remineralized) hard tissues and their respective grey scale values are often too similar to define a distinct threshold. In such cases, segmentation has to be performed manually by tracing the boundary of the features-of-interest. Once the complete dataset or region-of-interest has been segmented, the individual slice labels are used to calculate a 3-D surface (Fig. 3B, C). A variety of software exists (Cunningham et al., 2014; Balanoff et al., 2015) for the segmentation and visualization of digital endocasts, ranging from freely available programs, such as SPIERS (Sutton et al., 2012) and Dristhi (http://sf.anu.edu.au/Vizlab/drishti/index.shtml), to commercial products, including Avizo (VSG, Visualisation Science Group), Mimics (Materialise) and VG Studio Max (Volume Graphics). The programs can differ considerably in the types of segmentation and image processing tools, import and export capabilities, and visualization quality, and the choice mostly depends on availability and personal preference.

OTHER CRANIAL SOFT TISSUES

Examples

Apart from muscles, the brain, and neurovascular structures, a number of other soft-tissues occupy the cranial skeleton. Of these, not all have been nor can be reconstructed in fossils as they leave no or only weak osteological correlates. A few examples exist for various other cranial soft tissue reconstructions.

Cranial pneumatic sinuses are among the most commonly reconstructed structures not pertaining to musculature or the endocranial anatomy (Fig. 1C). These sinuses represent pneumatic invasions of air-filled epithelial diverticula, leaving distinct cavities in the bone. They have been reconstructed for a number of dinosaurian taxa (Kundrát and Janáček, 2007; Tahara and Larsson, 2011; Gold et al., 2013) and other archosaurs (Witmer and Ridgely, 2008), marine mammals (Racicot and Rowe, 2014), and hominids (Zollikofer et al., 2008).

Similarly, the bony nasal cavity of vertebrates is filled by a number of different soft-tissue structures, such as cartilaginous conchae (turbinates) and epithelia. Although osteological correlates are rarely preserved, different conchae morphologies have been reconstructed in an ornithischian dinosaur using information from computational fluid dynamics modelling (Bourke et al., 2015).

Further examples include keratinous structures covering bony surfaces, such as beak-like rhamphothecae of theropod dinosaurs. Based on osteological inferences, such keratinous sheaths have been reconstructed in different theropods (Lautenschlager et al., 2013; Lautenschlager et al., 2014b; Cuff and Rayfield, 2015).

Methodological approach

Due to the variety of different cranial soft-tissues, reconstruction methods differ with and depending on the type of soft-tissue. The reconstruction process of cranial pneumatic sinuses is largely comparable to that of the endocranial anatomy. As many pneumatic sinuses are nearly completely enclosed by bone, tomographic datasets are necessary. An exception are the sinuses that occupy external regions, such as the antorbital sinus of archosaurs, for the reconstruction of which surface scans can suffice. Following the digitization (and if necessary conversion into a tomographic dataset) of the specimen, cavities representing sinuses are segmented and subsequently visualized. For pneumatic sinuses covering parts of the external surfaces, boundaries might not be clearly constrained. The recommended approach in these cases is to create a reconstruction flush with the margins of the surrounding bone structure.

For soft-tissues covering the external surface of bones, such as keratinous structures, a similar approach can be applied. Both tomographic and surface-scan datasets can be used, as no internal features are relevant for the reconstruction. However, this poses another problem. As surface features only constrain the location and extent of a keratinous sheath, its thickness and external boundaries are not constrained by hard-tissues. This information has to be obtained from comparisons with extant taxa forming a phylogenetic bracket. For example, data on the thickness and arrangement of the rhamphotheca of extant birds can be used to inform reconstructions in fossils (Soons et al., 2012; Lautenschlager et al., 2013).

In cases, where preserved hard-tissues do not offer any constraints on the shape and position of soft-tissues, a hypotheses-testing approach may be applied using computational models. To reconstruct the morphology and position of conchae within the nasal capsule of an ornithischian dinosaur, Bourke et al., (2015) used computational fluid dynamics to test airflow for varying configurations. Different models of conchae, as found in extant taxa, were created in the 3-D modelling and visualization software Maya (Autodesk Inc.) and their effect on inspiratory airflow were tested virtually. This allowed the identification of the most likely morphology and arrangement of the soft-tissue conchae in spite of the absence of osteological correlates.

WHOLE-BODY AND LIFE RECONSTRUCTIONS

Examples

As shown above, the majority of soft-tissue reconstructions are focussed on a particular anatomical structure or skeletal region. However, knowledge on the whole-body soft-tissue morphology can be necessary to address question about body mass evolution, locomotory performance, and paleoecology (e.g., Allen et al., 2013; Maidment et al., 2014). Virtual whole-body reconstructions have been created of placoderms (Béchard et al., 2014), early tetrapods (Nyakatura et al., 2015), various dinosaurs (Gunga et al., 2007; Hutchinson et al., 2007; Ősi and Makádi, 2009), fossil birds (Brassey et al., 2016), and mammals (Brassey and Gardiner, 2015), as well as invertebrates (Garwood and Dunlop, 2014). A large number of these reconstructions have been created on the basis of complete skeletons in order to obtain body mass estimates or to investigate locomotory behaviour.

In contrast, digital life reconstructions (Fig. 1D) have been created to provide hypotheses regarding the appearance of extinct organisms, including fossil cephalopods (Lukeneder, 2012), stegocephalians (Steyer et al., 2010), and mammals (Cherin et al., 2016). Although such models are often based on preserved hard-tissues, they include a large degree of interpretation and artistic license, and are mainly intended to supplement studies rather than act as the focus of scientific investigation.

Methodological approach

Virtual whole-body reconstructions are usually based on digitized skeletons, but can also be created using two-dimensional images as a template (see Rahman and Lautenschlager, in review). Due to the large size and number of individual skeletal elements, digitization is typically performed using surface-based methods such as laser scanning or photogrammetry (Gunga et al., 2007; Bates et al., 2009; Mallison and Wings, 2014). For fossil specimens, the digital removal of taphonomic artefacts and rearticulation of elements might be necessary, before the actual whole-body reconstruction can be performed (Gunga et al., 2007; Mallison, 2010).

Different methods exist for the subsequent reconstruction of the soft-tissue morphology. To aid in the reconstruction and to increase accuracy, the digitized model is usually subdivided into functional units (e.g., skull, torso, limbs). To generate the soft-tissue outline, simple geometric shapes (spheres, cylinders, ellipses) are superimposed onto each unit and adjusted to match and envelop the underlying shape of the skeletal elements (Hutchinson et al., 2007; Bates et al., 2009; Mallison, 2010), often informed by frontal or sagittal cross-section profiles (Liu et al., 2015). Additional components representing internal organs, such as lungs and air sacks, can be included to improve subsequent body mass estimates (Hutchinson et al., 2007; Bates et al., 2009). Similarly, variations of the individual components may be created to allow for “tight-fitting” or “loose” morphologies in order to provide minimum and maximum mass estimates.

The calculation of convex hull volumes presents an alternative to the manual adjustment of the soft-tissue outlines, which inevitably introduces a certain degree of interpretation into the model (Sellers et al., 2012; see also Brassey, in review). A convex hull is the smallest polygon, which contains a set of given points. As such a convex hull represents the minimum volume to envelop predetermined coordinates/points deemed important in three-dimensional space. As it is based on mathematical calculations, the convex hull method has the advantage that it can be automated using numerical computing tools such as MatLab (MathWorks Inc.) and is less prone to personal interpretation. The convex hull method has been applied to a variety of fossil taxa to provide body mass estimates (Brassey et al., 2015; Bates et al., 2015; Brassey et al., 2016).

Similar to whole-body reconstructions, life reconstructions are usually based on digitized fossil specimens. However, unlike the approach for whole-body reconstructions, no clear sets of standards or best practices have been formulated for the creation of life reconstructions. However, this is difficult to achieve considering that life reconstruction tend to be scientifically informed works of art, prone to subjectivity and artistic license. Existing examples (Steyer et al., 2010; Lukeneder, 2012) have used CAD and 3-D modelling programs, such as Maya (Autodesk Inc.) and ZBrush (Pixologic Inc.), to create soft-tissue morphologies. Several details, such as colouration, ornamentation, and the location of soft-tissues, such as the external naris or the eyeball, have been created subjectively, although results from other studies (e.g., Witmer, 2001; Hieronymus et al., 2009; Vinther, 2015) could potentially be included to inform future reconstructions. This could add additional value to life reconstructions as a useful tool for public understanding and outreach.

LIMITATIONS AND FUTURE DIRECTIONS

Digital approaches offer a huge potential to reconstruct soft-tissue structures of fossil organisms. However, their accuracy depends greatly on the presence and quality of preserved hard-tissues. Taphonomic artefacts, pathologies, ontogeny, and intraspecific variation can present major challenges during the reconstruction process. In addition, the method used to digitize specimens and the quality (e.g., scan resolution, model size, digital artefacts) of the resulting models can affect the ability to identify osteological correlates and other information necessary for the reconstruction process. It is therefore recommended to obtain and compare information from physical specimens and the corresponding digital representations. Furthermore, clear and traceable documentation of the digitization, hard-tissue restorations (if performed), and the soft-tissue reconstruction should be provided so that other researchers are in a position to evaluate the results or to adjust models, if new information comes to light.

In the past, concerns have been raised when reconstructing soft-tissues in fossils (McGowan, 1979; Brown, 1981; Bryant and Seymour, 1990). Not all soft-tissue structures, such as muscles, will necessarily leave osteological correlates, whereas other osteological correlates might not relate to the presence of the presumed soft-tissues (McGowan, 1982; Nicholls and Russell, 1985). This problem not only pertains to digital reconstruction in particular, but soft-tissue interpretations in paleontological studies in general. As suggested above, phylogenetically informed reconstructions making use of extant taxa can help minimize erroneous identifications (Bryant and Russell, 1992; Witmer, 1995). Similarly, information obtained from different sources, for example different specimens, analytical methods, and sensitivity tests can help to constrain and refine soft-tissue reconstructions.

Further limitations exist for the reconstruction of soft-tissues that are not or only partially constrained by hard-tissues, as for example the extent and external boundaries of muscles. Where possible, it is recommended to create such reconstructions flush with the surrounding hard-tissues to avoid unnatural bulges and extreme morphologies. For some soft-tissues, including the appendicular musculature, this approach can rarely be applied and the extant phylogenetic bracket approach is recommended here as the best solution.

Further concern has been raised that digital soft-tissue reconstructions are not reliable representations of the in-vivo condition (Jerison, 1973). In particular, the relationship between endocranial casts and the actual brain morphology has been discussed. Due to the presence of other soft-tissues, such as the dural meninges, vascular structures, and pneumatic sinuses, a cast of the endocranial cavity might not necessarily represent the actual brain. The degree to which an endocast reflects brain morphology can vary across different vertebrate clades (Hopson, 1979; Hurlburt et al., 2013; Balanoff et al., 2015). However, the combination of novel digital techniques and close comparisons with a range of extant taxa can provide an important step towards a solution to this problem. By using homologous osteological correlates, more accurate approximations of anatomical brain regions have been created (Morhardt et al., 2012). This offers a promising approach for future studies.

Similarly, the use of biomechanical modelling techniques, such as FEA, MDA, or CFD, provides future avenues to test soft-tissue reconstructions and competing hypotheses (e.g., Bourke et al., 2015). The integration of different soft-tissue structures could further be used to constrain and inform reconstructions. So far, soft-tissue reconstructions have mostly focussed on individual structures, such as muscles or the endocranial anatomy. However, using such existing reconstructions could provide additional information when reconstructing additional features (e.g., three-dimensional models of the cranial musculature can be used to constrain the position of the eyeball).

The surge of digital techniques has ushered in a large increase in digital soft-tissue reconstructions over the past decade. However, to date the largest disadvantages are the amount of time required to perform digital reconstructions, the financial cost involved to purchase hardware and software licenses, and the degree of interpretation and subjectivity introduced into the models due to the often manual approaches. A key prospect for the future will therefore lie in the automation of reconstructions. Methods, such as convex hull mass estimates (see above) or the use of geometric morphometrics to restore hominid crania (Gunz et al., 2009; Gunz, 2015; Senck et al., 2015) have incorporated automation into the reconstruction process, thereby minimizing individual subjectivity and providing increased reproducibility.

CONCLUDING REMARKS

Detailed knowledge on soft-tissue structures is paramount to understanding the paleobiology, paleoecology, and phylogeny of fossil organisms. Although rarely preserved, recent advances in digital imaging and modelling techniques provide versatile tools to reconstruct different soft-tissue structures. Using the methods presented and reviewed here, it is possible to reconstruct, for example, the cranial and postcranial musculature of dinosaurs, the endocranial (brain and inner ear) anatomy of early mammals and their kin, and the body mass of different tetrapods from whole-body reconstructions. Because such reconstructions are performed on the basis of preserved hard-tissues, they have nearly exclusively focussed on vertebrate fossils in the past, although many of the techniques are also applicable to invertebrate fossils. However, this also means that the quality and accuracy of the reconstructed soft-tissues depends to a considerable degree on the presence and preservation of hard-tissues. Consequently, the restoration of osteological-based models and the removal of preservational artefacts should be performed before any soft-tissue reconstructions are attempted. To avoid further uncertainties regarding the presence of osteological correlates and possible homologies, it is recommended that all reconstructions are performed in a phylogenetically ground framework using an extant phylogenetic bracket approach. Although some uncertainties and interpretation are inevitably introduced in the reconstruction process, soft-tissue reconstructions are nevertheless worthwhile as they allow researchers to gain useful approximations and estimates of fossil properties, which could not be assessed otherwise. Due to the digital nature of the reconstructions, it is possible to export the information to other applications (e.g., FEA, MDA) to test different competing hypotheses. It is anticipated that further technological advances will allow automation of certain steps, enabling large-scale comparative studies and increased objectivity.

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REFERENCES

ABEL, R. L., C. R. LAURINI, and M. RICHTER. 2012. A palaeobiologist's guide to 'virtual'micro-CT preparation. Palaeontologia Electronica, 15(2):6T.

ADAMS, L. A. 1918. A memoir on the phylogeny of the jaw muscles in recent and fossil vertebrates. Annals of the New York Academy of Sciences, 28(1):51–166.

ALLEN, V., K. T. BATES, Z. LI, and J. R. HUTCHINSON. 2013. Linking the evolution of body shape and locomotor biomechanics in bird-line archosaurs. Nature, 497(7447):104–107.

ALLISON, P. A., and D. E. G. BRIGGS. 1993. Exceptional fossil record: Distribution of soft-tissue preservation through the Phanerozoic. Geology, 21(6):527–530.

BALANOFF, A. M., G. BEVER, M. W. COLBERT, J. A. CLARKE, D. J. FIELD, P. M. GIGNAC, D. T. KSEPKA, R. C. RIDGELY, N. A. SMITH, C. R. TORRES, S. WALSH, and L. M. WITMER. 2015. Best practices for digitally constructing endocranial casts: examples from birds and their dinosaurian relatives. Journal of Anatomy: Online before print.

BALANOFF, A. M., G. S. BEVER, T. B. ROWE, and M. A. NORELL. 2013. Evolutionary origins of the avian brain. Nature, 501(7465):93–96.

BARGHUSEN, H. R. 1973. The adductor jaw musculature of *Dimetrodon* (Reptilia, Pelycosauria). Journal of Paleontology, 47:823–834.

BATES, K. T., and P. L. FALKINGHAM. 2012. Estimating maximum bite performance in *Tyrannosaurus rex* using multi-body dynamics. Biology Letters, 8:20120056.

BATES, K. T., P. L. FALKINGHAM, S. MACAULAY, C. BRASSEY, and S. C. R. MAIDMENT. 2015. Downsizing a giant: re-evaluating *Dreadnoughtus* body mass. Biology Letters, 11:20150215.

BATES, K. T., P. L. MANNING, D. HODGETTS, and W. I. SELLERS. 2009. Estimating mass properties of dinosaurs using laser imaging and 3D computer modelling. PloS one, 4(2):e4532.

BÉCHARD, I., F. ARSENAULT, R. CLOUTIER, and J. KERR. 2014. The Devonian placoderm fish *Bothriolepis canadensis* revisited with three-dimensional digital imagery. Palaeontologia Electronica, 17(1):1–19.

BERNARD, S., K. BENZERARA, O. BEYSSAC, N. MENGUY, F. GUYOT, G. E. BROWN JR, and B. GOFFÉ. 2007. Exceptional preservation of fossil plant spores in high-pressure metamorphic rocks. Earth and Planetary Science Letters, 262(1–2):257–272.

BLANCO, R. E., A. RINDERKNECHT, and G. LECUONA. 2012. The bite force of the largest fossil rodent (Hystricognathi, Caviomorpha, Dinomyidae). Lethaia, 45(2):157–163.

BOURKE, J. M., W. M. RUGER PORTER, R. C. RIDGELY, T. R. LYSON, E. R. SCHACHNER, P. R. BELL, and L. M. WITMER. 2015. Breathing life into dinosaurs: tackling challenges of soft-tissue restoration and nasal airflow in extinct species. The Anatomical Record, 297(11):2148–2186.

BRASSEY, C. A. In review. Body mass estimation in paleontology: a review of volumetric techniques. *In* L. TAPANILA and I. A. RAHMAN (eds.), Virtual Paleontology. The Paleontological Society Papers, XX.

BRASSEY, C. A., and J. D. GARDINER. 2015. An advanced shape-fitting algorithm applied to quadrupedal mammals: improving volumetric mass estimates. Royal Society Open Science, 2(8):150302.

BRASSEY, C. A., S. C. R. MAIDMENT, and P. M. BARRETT. 2015. Body mass estimates of an exceptionally complete *Stegosaurus* (Ornithischia: Thyreophora): comparing volumetric and linear bivariate mass estimation methods. Biology Letters, 11(3): 20140984

BRASSEY, C. A., T. G. O'MAHONEY, A. C. KITCHENER, P. L. MANNING, and W. I. SELLERS. 2016. Convex-hull mass estimates of the dodo (*Raphus cucullatus*): application of a CT-based mass estimation technique. PeerJ, 4:e1432.

BRIGHT, J. A. 2014. A review of paleontological finite element models and their validity. Journal of Paleontology, 88(4):760–769.

BROCHU, C. A. 2000. A digitally-rendered endocast for *Tyrannosaurus rex*. Journal of Vertebrate Paleontology, 20(1):1–6.

BROWN, D. S. 1981. The English Upper Jurassic Plesiosauroidea (Reptilia) and a review of the phylogeny and classification of the Plesiosauria. Bulletin of the British Museum (Natural History), Geology, 35:253–347.

BRYANT, H. N., and A. P. RUSSELL. 1992. The role of phylogenetic analysis in the inference of unpreserved attributes of extinct taxa. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 337(1282):405–418.

BRYANT, H. N., and K. L. SEYMOUR. 1990. Observations and comments on the reliability of muscle reconstruction in fossil vertebrates. Journal of Morphology, 206(1):109–117.

BUCHHOLTZ, E. A., and E.-A. SEYFARTH. 1999. The gospel of the fossil brain: Tilly Edinger and the science of paleoneurology. Brain Research Bulletin, 48(4):351–361.

BUCHHOLTZ, E. A., and E.-A. SEYFARTH. 2001. The study of "fossil brains": Tilly Edinger (1897–1967) and the beginnings of paleoneurology. BioScience, 51(8):674–682.

BUDD, G. E. 1998. Arthropod body-plan evolution in the Cambrian with an example from anomalocaridid muscle. Lethaia, 31(3):197–210.

BUTTERFIELD, N. J. 2003. Exceptional fossil preservation and the Cambrian explosion. Integrative and Comparative Biology, 43(1):166–177.

BUTTON, D. J., E. J. RAYFIELD, and P. M. BARRETT. 2014. Cranial biomechanics underpins high sauropod diversity in resource-poor environments. Proceedings of the Royal Society of London B: Biological Sciences, 281(1795):20142114.

CARABAJAL, A. P., J. STERLI, J. MÜLLER, and A. HILGER. 2013. Neuroanatomy of the Marine Jurassic Turtle *Plesiochelys etalloni* (Testudinata, Plesiochelyidae). PloS one, 8(7):e69264.

CHAPMAN, T., F. MOISEEV, V. SHOLUKHA, S. LOURYAN, M. ROOZE, P. SEMAL, and S. V. S. JAN. 2010. Virtual reconstruction of the Neandertal lower limbs with an estimation of hamstring muscle moment arms. Comptes Rendus Palevol, 9(6):445–454.

CHERIN, M., D. A. IURINO, R. SARDELLA, and L. ROOK. 2014. *Acinonyx pardinensis* (Carnivora, Felidae) from the Early Pleistocene of Pantalla (Italy): predatory behavior and ecological role of the giant Plio–Pleistocene cheetah. Quaternary Science Reviews, 87:82–97.

CHERIN, M., D. A. IURINO, G. WILLEMSEN, and G. CARNEVALE. 2016. A new otter from the Early Pleistocene of Pantalla (Italy), with remarks on the evolutionary history of Mediterranean Quaternary Lutrinae (Carnivora, Mustelidae). Quaternary Science Reviews, 135:92–102.

CUFF, A. R., and E. J. RAYFIELD. 2015. Retrodeformation and muscular reconstruction of ornithomimosaurian dinosaur crania. PeerJ, 3:e1093.

CUNNINGHAM, J. A., I. A. RAHMAN, S. LAUTENSCHLAGER, E. J. RAYFIELD, and P. C. J. DONOGHUE. 2014. A virtual world of paleontology. Trends in Ecology & Evolution, 29(6):347–357.

CURTIS, N. 2011. Craniofacial biomechanics: an overview of recent multibody modelling studies. Journal of Anatomy, 218(1):16–25.

CURTIS, N., K. KUPCZIK, P. O'HIGGINS, M. MOAZEN, and M. FAGAN. 2008. Predicting skull loading: applying multibody dynamics analysis to a macaque skull. The Anatomical Record, 291(5):491–501.

DILKES, D. W. 1999. Appendicular myology of the hadrosaurian dinosaur *Maiasaura peeblesorum* from the Late Cretaceous (Campanian) of Montana. Earth and Environmental Science Transactions of the Royal Society of Edinburgh, 90(2):87–125.

DILKES, D. W., J. R. HUTCHINSON, C. M. HOLLIDAY, and L. M. WITMER. 2012. Reconstructing the musculature of dinosaurs, p. 151–190. *In* M. K. BRETT-SURMAN, T. R. HOLTZ, and J. O. FARLOW (eds.), The Complete Dinosaur. Indiana University Press, Bloomington, IN.

DUMONT, E., I. R. GROSSE, and G. J. SLATER. 2009. Requirements for comparing the performance of finite element models of biological structures. Journal of Theoretical Biology, 256(1):96–103.

EDINGER, T. 1929. Die fossilen Gehirne. Ergebnisse der Anatomie und Entwicklungsgeschichte, 28:1–249.

FOFFA, D., A. R. CUFF, J. SASSOON, E. J. RAYFIELD, M. N. MAVROGORDATO, and M. J. BENTON. 2014. Functional anatomy and feeding biomechanics of a giant Upper Jurassic pliosaur (Reptilia: Sauropterygia) from Weymouth Bay, Dorset, UK. Journal of Anatomy, 225(2):209–219.

FREY, E., D. M. MARTILL, and M.-C. BUCHY. 2003. A new species of tapejarid pterosaur with soft-tissue head crest. Geological Society, London, Special Publications, 217(1):65–72.

GAI, Z., P. C. DONOGHUE, M. ZHU, P. JANVIER, and M. STAMPANONI. 2011. Fossil jawless fish from China foreshadows early jawed vertebrate anatomy. Nature, 476(7360):324–327.

GARWOOD, R., and J. DUNLOP. 2014. The walking dead: Blender as a tool for paleontologists with a case study on extinct arachnids. Journal of Paleontology, 88(04):735–746.

GERRIENNE, P., D. L. DILCHER, S. BERGAMASCHI, I. MILAGRES, E. PEREIRA, and M. A. C. RODRIGUES. 2006. An exceptional specimen of the early land plant *Cooksonia paranensis*, and a hypothesis on the life cycle of the earliest eutracheophytes. Review of Palaeobotany and Palynology, 142(3–4):123–130.

GIGNAC, P. M., N. J. KLEY, J. A. CLARKE, M. W. COLBERT, A. C. MORHARDT, D. CERIO, I. N. COST, P. G. COX, J. D. DAZA, and C. M. EARLY. 2016. Diffusible iodine-based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. Journal of Anatomy. Online before print.

GILES, S., and M. FRIEDMAN. 2014. Virtual reconstruction of endocast anatomy in early ray-finned fishes (Osteichthyes, Actinopterygii). Journal of Paleontology, 88(4):636–651.

GOLD, M. E. L., S. L. BRUSATTE, and M. A. NORELL. 2013. The cranial pneumatic sinuses of the tyrannosaurid *Alioramus* (Dinosauria: Theropoda) and the evolution of cranial pneumaticity in theropod dinosaurs. American Museum Novitates. 3790:1–46.

GROSSE, I. R., E. R. DUMONT, C. COLETTA, and A. TOLLESON. 2007. Techniques for modelling muscle-induced forces in finite element models of skeletal structures. The Anatomical Record, 290(9):1069–1088.

GUNGA, H.-C., T. SUTHAU, A. BELLMANN, A. FRIEDRICH, T. SCHWANEBECK, S. STOINSKI, T. TRIPPEL, K. KIRSCH, and O. HELLWICH. 2007. Body mass estimations for *Plateosaurus engelhardti* using laser scanning and 3D reconstruction methods. Naturwissenschaften, 94(8):623–630.

GUNZ, P. 2015. Computed tools for paleoneurology, p. 39–55, Human Paleoneurology. Springer International Publishing.

GUNZ, P., P. MITTEROECKER, S. NEUBAUER, G. W. WEBER, and F. L. BOOKSTEIN. 2009. Principles for the virtual reconstruction of hominin crania. Journal of Human Evolution, 57(1):48–62.

HIERONYMUS, T. L., L. M. WITMER, D. H. TANKE, and P. J. CURRIE. 2009. The facial integument of centrosaurine ceratopsids: morphological and histological correlates of novel skin structures. The Anatomical Record, 292(9):1370–1396.

HOLLIDAY, C. M. 2009. New insights into dinosaur jaw muscle anatomy. The Anatomical Record, 292:1246–1265.

HOLLOWAY, W. L., K. M. CLAESON, and F. R. O'KEEFE. 2013. A virtual phytosaur endocast and its implications for sensory system evolution in archosaurs. Journal of Vertebrate Paleontology, 33(4):848–857.

HOPSON, J. 1979. Paleoneurology, p. 39–146. *In* C. GANS (ed.), Biology of the Reptilia.Volume 9. Academic Press, London.

HURLBURT, G. R., R. C. RIDGELY, and L. M. WITMER. 2013. Relative size of brain and cerebrum in tyrannosaurid dinosaurs: an analysis using brain-endocast quantitative relationships in extant alligators, p. 1–21. *In* J. M. PARRISH, R. E. MOLNAR, P. J. CURRIE, and E. B. KOPPELHUS (eds.), Tyrannosaurid Paleobiology. Indiana University Press, Bloomington.

HUTCHINSON, J. R., F. C. ANDERSON, S. S. BLEMKER, and S. L. DELP. 2005. Analysis of hindlimb muscle moment arms in *Tyrannosaurus* rex *using* a three-dimensional musculoskeletal computer model: implications for stance, gait, and speed. Paleobiology, 31(4):676–701.

HUTCHINSON, J. R., V. NG-THOW-HING, and F. C. ANDERSON. 2007. A 3D interactive method for estimating body segmental parameters in animals: application to the turning and running performance of *Tyrannosaurus rex*. Journal of Theoretical Biology, 246(4):660–680.

JERISON, H. 1973. Evolution of the Brain and Intelligence. Academic Press, New York.

KSEPKA, D. T., A. M. BALANOFF, S. WALSH, A. REVAN, and A. HO. 2012. Evolution of the brain and sensory organs in Sphenisciformes: new data from the stem penguin Paraptenodytes antarcticus. Zoological Journal of the Linnean Society, 166(1):202–219.

KUNDRÁT, M., and J. JANÁČEK. 2007. Cranial pneumatization and auditory perceptions of the oviraptorid dinosaur *Conchoraptor gracilis* (Theropoda, Maniraptora) from the Late Cretaceous of Mongolia. Naturwissenschaften, 94(9):769–778.

LAUTENSCHLAGER, S. 2012. Paleontology 2.0 – A comprehensive protocol for the reconstruction of hard- and soft tissue structures in fossils. Geological Society of America Program and Abstracts, 44:372.

LAUTENSCHLAGER, S. 2013. Cranial myology and bite force performance of *Erlikosaurus andrewsi*: A novel approach for digital muscle reconstructions. Journal of Anatomy, 222(2):260–272.

LAUTENSCHLAGER, S. 2015. Estimating cranial musculoskeletal constraints in theropod dinosaurs. Royal Society Open Science, 2(11):150495.

LAUTENSCHLAGER, S., J. A. BRIGHT, and E. J. RAYFIELD. 2014a. Digital dissection–using contrast-enhanced computed tomography scanning to elucidate hard-and soft-tissue anatomy in the Common Buzzard *Buteo buteo*. Journal of Anatomy, 224(4):412–431.

LAUTENSCHLAGER, S., and T. HÜBNER. 2013. Ontogenetic trajectories in the ornithischian endocranium. Journal of evolutionary biology, 26(9):2044–2050.

LAUTENSCHLAGER, S., E. J. RAYFIELD, P. ALTANGEREL, L. E. ZANNO, and L. M. WITMER. 2012. The endocranial anatomy of Therizinosauria and its implications for sensory and cognitive function. PloS one, 7(12):e52289.

LAUTENSCHLAGER, S., L. M. WITMER, P. ALTANGEREL, and E. J. RAYFIELD. 2013. Edentulism, beaks, and biomechanical innovations in the evolution of theropod dinosaurs. Proceedings of the National Academy of Sciences, 110(51):20657–20662.

LAUTENSCHLAGER, S., L. M. WITMER, P. ALTANGEREL, L. E. ZANNO, and E. J. RAYFIELD. 2014b. Cranial anatomy of *Erlikosaurus andrewsi* (Dinosauria, Therizinosauria): new insights based on digital reconstruction. Journal of Vertebrate Paleontology, 34(6):1263–1291.

LIU, J., J. SHI, L. C. FITTON, R. PHILLIPS, P. O'HIGGINS, and M. J. FAGAN. 2012. The application of muscle wrapping to voxel-based finite element models of skeletal structures. Biomechanics and Modeling in Mechanobiology, 11(1–2):35–47.

LIU, S., A. S. SMITH, Y. GU, J. TAN, C. K. LIU, and G. TURK. 2015. Computer simulations imply forelimb-dominated underwater flight in plesiosaurs. PLoS Computational Biology 11(12):e1004605.

LUKENEDER, A. 2012. Computed 3D visualisation of an extinct cephalopod using computer tomographs. Computers & Geosciences, 45:68–74.

MAIDMENT, S. C., D. M. HENDERSON, and P. M. BARRETT. 2014. What drove reversions to quadrupedality in ornithischian dinosaurs? Testing hypotheses using centre of mass modelling. Naturwissenschaften, 101(11):989–1001.

MALLISON, H. 2010. The digital *Plateosaurus* I: body mass, mass distribution, and posture assessed using CAD and CAE on a digitally mounted complete skeleton. Palaeontologia Electronica, 13(13.2):1–26.

MALLISON, H., and O. WINGS. 2014. Photogrammetry in paleontology – a practical guide. Journal of Paleontological Techniques, 12:1–31.

MAREK, R. D., B. C. MOON, M. WILLIAMS, and M. J. BENTON. 2015. The skull and endocranium of a Lower Jurassic ichthyosaur based on digital reconstructions. Palaeontology, 58(4):723–742.

MARSH, O. C. 1885. The gigantic mammals of the Order Dinocerata, p. 34–302, Fifth Annual Report of the United States Geological Survey Washington (DC). US Government Printing Office.

MAZZETTA, G. V., A. P. CISILINO, R. E. BLANCO, and N. CALVO. 2009. Cranial mechanics and functional interpretation of the horned carnivorous dinosaur *Carnotaurus sastrei*. Journal of Vertebrate Paleontology, 29(3):822–830.

MCGOWAN, C. 1979. The hind limb musculature of the brown kiwi, *Apteryx australis mantelli*. Journal of Morphology, 160(1):33–73.

MCGOWAN, C. 1982. The wing musculature of the Brown Kiwi *Apteryx australis mantelli* and its bearings on ratite affinities. Journal of Zoology, 197:173–219.

METSCHER, B. D. 2009. MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. BMC physiology, 9(1):1–14.

MINER, R. W. 1925. The pectoral limb of Eryops and other primitive tetrapods. Bulletin of the American Museum of Natural History, 51(145):1924–1925.

MOAZEN, M., N. CURTIS, S. E. EVANS, P. O'HIGGINS, and M. J. FAGAN. 2008. Rigid-body analysis of a lizard skull: Modelling the skull of *Uromastyx hardwickii*. Journal of Biomechanics, 41(6):1274–1280.

MORHARDT, A. C., R. C. RIDGLEY, and L. M. WITMER. 2012. From endocast to brain: assessing brain size and structure in extinct archosaurs using gross anatomical brain region approximation (GABRA). Journal of Vertebrate Paleontology, Supplement, 32:145.

NICHOLLS, E. L., and A. P. RUSSELL. 1985. Structure and function of the pectoral girdle and forelimb of *Struthiomimus altus* (Theropoda: Ornithomimidae). Palaeontology, 28(4):643–677.

NYAKATURA, J. A., V. R. ALLEN, J. LAUSTRÖER, A. ANDIKFAR, M. DANCZAK, H.-J. ULLRICH, W. HUFENBACH, T. MARTENS, and M. S. FISCHER. 2015. A Three-dimensional skeletal reconstruction of the stem amniote *Orobates pabsti* (Diadectidae): analyses of body mass, centre of mass position, and joint mobility. PloS one, 10(9):e0137284.

O'CONNOR, P. M. 2006. Postcranial pneumaticity: An evaluation of soft-tissue influences on the postcranial skeleton and the reconstruction of pulmonary anatomy in archosaurs. Journal of Morphology, 267(10):1199–1226.

ORGAN, C. L. 2006. Thoracic epaxial muscles in living archosaurs and ornithopod dinosaurs. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology, 288A(7):782–793.

ORGAN, C. L., and J. ADAMS. 2005. The histology of ossified tendon in dinosaurs. Journal of Vertebrate Paleontology, 25(3):602–613.

ŐSI, A., and L. MAKÁDI. 2009. New remains of *Hungarosaurus tormai* (Ankylosauria, Dinosauria) from the Upper Cretaceous of Hungary: skeletal reconstruction and body mass estimation. Paläontologische Zeitschrift, 83(2):227–245.

PERSONS IV, W. S., and P. J. CURRIE. 2011a. Dinosaur speed demon: the caudal musculature of *Carnotaurus sastrei* and implications for the evolution of South American abelisaurids. PloS one, 6(10):e25763.

PERSONS IV, W. S., and P. J. CURRIE. 2011b. The tail of *Tyrannosaurus*: reassessing the size and locomotive importance of the m. caudofemoralis in non-avian theropods. The Anatomical Record, 294(1):119–131.

PERSONS IV, W. S., P. J. CURRIE, and M. A. NORELL. 2013. Oviraptorosaur tail forms and functions. Acta Palaeontologica Polonica, 59(3):553–567.

RACICOT, R. A., and T. ROWE. 2014. Endocranial anatomy of a new fossil porpoise (Odontoceti, Phocoenidae) from the Pliocene San Diego Formation of California. Journal of Paleontology, 88(04):652–663.

RAHMAN, I. A. and S. LAUTENSCHLAGER. In review. Applications of three-dimensional box modeling to paleontological functional analysis. *In* L. TAPANILA and I. A. RAHMAN (eds.), Virtual Paleontology. The Paleontological Society Papers, XX.

RAHMAN, I. A., S. ZAMORA, P. L. FALKINGHAM, and J. C. PHILLIPS. 2015. Cambrian cinctan echinoderms shed light on feeding in the ancestral deuterostome. Proceedings of the Royal Society of London B: Biological Sciences, 282(1818): 20151964.RAYFIELD, E. J. 2007. Finite Element Analysis and understanding the biomechanics and evolution of living and fossil organisms. Annual Review of Earth and Planetary Sciences, 35(1):541–576.

RAYFIELD, E. J., D. B. NORMAN, C. C. HORNER, J. R. HORNER, P. M. SMITH, J. J. THOMASON, and P. UPCHURCH. 2001. Cranial design and function in a large theropod dinosaur. Nature, 409(6823):1033–1037.

ROMER, A. S. 1923. The pelvic musculature of saurischian dinosaurs. Bulletin of the American Museum of Natural History, 49:605–617.

ROWE, T. B., T. E. MACRINI, and Z.-X. LUO. 2011. Fossil evidence on origin of the mammalian brain. Science, 332(6032):955–957.

RUF, I., V. VOLPATO, K. D. ROSE, G. BILLET, C. DE MUIZON, and T. LEHMANN. 2016. Digital reconstruction of the inner ear of *Leptictidium auderiense* (Leptictida, Mammalia) and North American leptictids reveals new insight into leptictidan locomotor agility. Paläontologische Zeitschrift:1–19. Online before print.

SASSO, C. D., and M. SIGNORE. 1998. Exceptional soft-tissue preservation in a theropod dinosaur from Italy. Nature, 392(6674):383–387.

SCHOPF, J. M. 1975. Modes of fossil preservation. Review of Palaeobotany and Palynology, 20(1):27–53.

SCHWEITZER, M. H. 2011. Soft tissue preservation in terrestrial Mesozoic vertebrates. Annual Review of Earth and Planetary Sciences, 39(1):187–216.

SELLERS, W., J. HEPWORTH-BELL, P. FALKINGHAM, K. BATES, C. BRASSEY, V. EGERTON, and P. MANNING. 2012. Minimum convex hull mass estimations of complete mounted skeletons. Biology Letters, 8:20120263.

SELLERS, W. I., L. MARGETTS, R. A. CORIA, and P. L. MANNING. 2013. March of the titans: The locomotor capabilities of sauropod dinosaurs. PloS one, 8(10):e78733.

SENCK, S., F. L. BOOKSTEIN, S. BENAZZI, J. KASTNER, and G. W. WEBER. 2015. Virtual reconstruction of modern and fossil hominoid crania: consequences of reference sample choice. The Anatomical Record, 298(5):827–841.

SHARP, A. C. 2014. Three dimensional digital reconstruction of the jaw adductor musculature of the extinct marsupial giant *Diprotodon optatum*. PeerJ, 2:e514.

SHARP, A. C., and P. W. TRUSLER. 2015. Morphology of the jaw-closing musculature in the common wombat (*Vombatus ursinus*) using digital dissection and magnetic resonance imaging. PloS one, 10(2):e0117730.

SNIVELY, E., J. R. COTTON, R. RIDGELY, and L. M. WITMER. 2013. Multibody dynamics model of head and neck function in *Allosaurus* (Dinosauria, Theropoda). Palaeontologia Electronica, 16(2):11A.

SOONS, J., A. HERREL, P. AERTS, and J. DIRCKX. 2012. Determination and validation of the elastic moduli of small and complex biological samples: bone and keratin in bird beaks. Journal of The Royal Society Interface, 9(71):1381–1388.

STEYER, J. S., M. BOULAY, and S. LORRAIN. 2010. 3D external restorations of stegocephalian skulls using ZBrush: The renaissance of fossil amphibians. Comptes Rendus Palevol, 9(6):463–470.

SUMIDA, S. S. 1989. The appendicular skeleton of the Early Permian genus *Labidosaurus* (Reptilia, Captorhinomorpha, Captorhinidae) and the hind limb musculature of captorhinid reptiles. Journal of Vertebrate Paleontology, 9(3):295–313.

SUTTON, M. D. 2008. Tomographic techniques for the study of exceptionally preserved fossils. Proceedings of the Royal Society of London B: Biological Sciences, 275(1643):1587–1593.

SUTTON, M., I. RAHMAN, and R. GARWOOD. 2014. Techniques for Virtual Palaeontology. John Wiley & Sons, Oxford, 208 p.

SUTTON, M. D., D. E. G. BRIGGS, D. J. SIVETER, and D. J. SIVETER. 2005. Silurian brachiopods with soft-tissue preservation. Nature, 436(7053):1013–1015.

SUTTON, M. D., R. J. GARWOOD, D. J. SIVETER, and D. J. SIVETER. 2012. SPIERS and VAXML; A software toolkit for tomographic visualisation and a format for virtual specimen interchange. Palaeontologia Electronica, 15(2):1–14.

TAHARA, R., and H. C. LARSSON. 2011. Cranial pneumatic anatomy of *Ornithomimus edmontonicus* (Ornithomimidae: Theropoda). Journal of Vertebrate Paleontology, 31(1):127–143.

TRINAJSTIC, K., C. MARSHALL, J. LONG, and K. BIFIELD. 2007. Exceptional preservation of nerve and muscle tissues in Late Devonian placoderm fish and their evolutionary implications. Biology Letters, 3(2):197–200.

VINTHER, J. 2015. A guide to the field of palaeo colour. BioEssays, 37(6):643–656.

VON BACZKO, M. B., and J. B. DESOJO. 2016. Cranial anatomy and palaeoneurology of the archosaur *Riojasuchus tenuisceps* from the Los Colorados Formation, La Rioja, Argentina. PloS one, 11(2):e0148575.

WALSH, S. A., P. M. BARRETT, A. C. MILNER, G. MANLEY, and L. M. WITMER. 2009. Inner ear anatomy is a proxy for deducing auditory capability and behaviour in reptiles and birds. Proceedings of the Royal Society of London B: Biological Sciences, 276(1660):1355–1360.

WALSH, S. A., A. N. IWANIUK, M. A. KNOLL, E. BOURDON, P. M. BARRETT, A. C. MILNER, R. L. NUDDS, R. L. ABEL, and P. D. STERPAIO. 2013a. Avian cerebellar floccular fossa size is not a proxy for flying ability in birds. PloS one, 8(6):e67176.

WALSH, S. A., Z.-X. LUO, and P. M. BARRETT. 2013b. Modern imaging techniques as a window to prehistoric auditory worlds, p. 227–261, Insights from Comparative Hearing Research. Springer.

WILBY, P. R., and D. E. G. BRIGGS. 1997. Taxonomic trends in the resolution of detail preserved in fossil phosphatized soft tissues. Geobios, 30:493–502.

WITMER, L. M. 1995. The extant phylogenetic bracket and the importance of reconstructing soft tissues in fossils, p. 19–33. *In* J. J. THOMASON (ed.), Functional morphology in vertebrate paleontology. Cambridge University Press.

WITMER, L. M. 1997. The Evolution of the Antorbital Cavity of Archosaurs: A Study in Soft-Tissue Reconstruction in the Fossil Record with an Analysis of the Function of Pneumaticity. Journal of Vertebrate Paleontology, 17(sup001):1–76.

WITMER, L. M. 2001. Nostril position in dinosaurs and other vertebrates and its significance for nasal function. Science, 293(5531):850–853.

WITMER, L. M., S. CHATTERJEE, J. FRANZOSA, and T. ROWE. 2003. Neuroanatomy of flying reptiles and implications for flight, posture and behaviour. Nature, 425(6961):950–953.

WITMER, L. M., and R. C. RIDGELY. 2008. The paranasal air sinuses of predatory and armored dinosaurs (Archosauria: Theropoda and Ankylosauria) and their contribution to cephalic structure. The Anatomical Record, 291(11):1362–1388.

WITMER, L. M., and R. C. RIDGELY. 2009. New insights into the brain, braincase, and ear region of tyrannosaurs (Dinosauria, Theropoda), with implications for sensory organization and behavior. The Anatomical Record, 292(9):1266–1296.

WITMER, L. M., R. C. RIDGELY, D. L. DUFEAU, and M. C. SEMONES. 2008. Using CT to peer into the past: 3D visualization of the brain and ear regions of birds, crocodiles, and nonavian dinosaurs, p. 67–87, Anatomical imaging. Springer.

WROE, S., U. CHAMOLI, W. C. H. PARR, P. CLAUSEN, R. RIDGELY, and L. WITMER. 2013. Comparative biomechanical modeling of metatherian and placental sabertooths: a different kind of bite for an extreme pouched predator. PloS one, 8(6):e66888.

ZELENITSKY, D. K., F. THERRIEN, R. C. RIDGELY, A. R. MCGEE, and L. M. WITMER. 2011. Evolution of olfaction in non-avian theropod dinosaurs and birds. Proceedings of the Royal Society of London B: Biological Sciences, 278(1725):3625–3634.

ZOLLIKOFER, C. P., M. S. P. DE LEÓN, D. E. LIEBERMAN, F. GUY, D. PILBEAM, A. LIKIUS, H. T. MACKAYE, P. VIGNAUD, and M. BRUNET. 2005. Virtual cranial reconstruction of *Sahelanthropus tchadensis*. Nature, 434(7034):755–759.

ZOLLIKOFER, C. P. E., M. S. PONCE DE LEÓN, R. W. SCHMITZ, and C. B. STRINGER. 2008. New Insights Into Mid-Late Pleistocene Fossil Hominin Paranasal Sinus Morphology. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, 291(11):1506–1516.

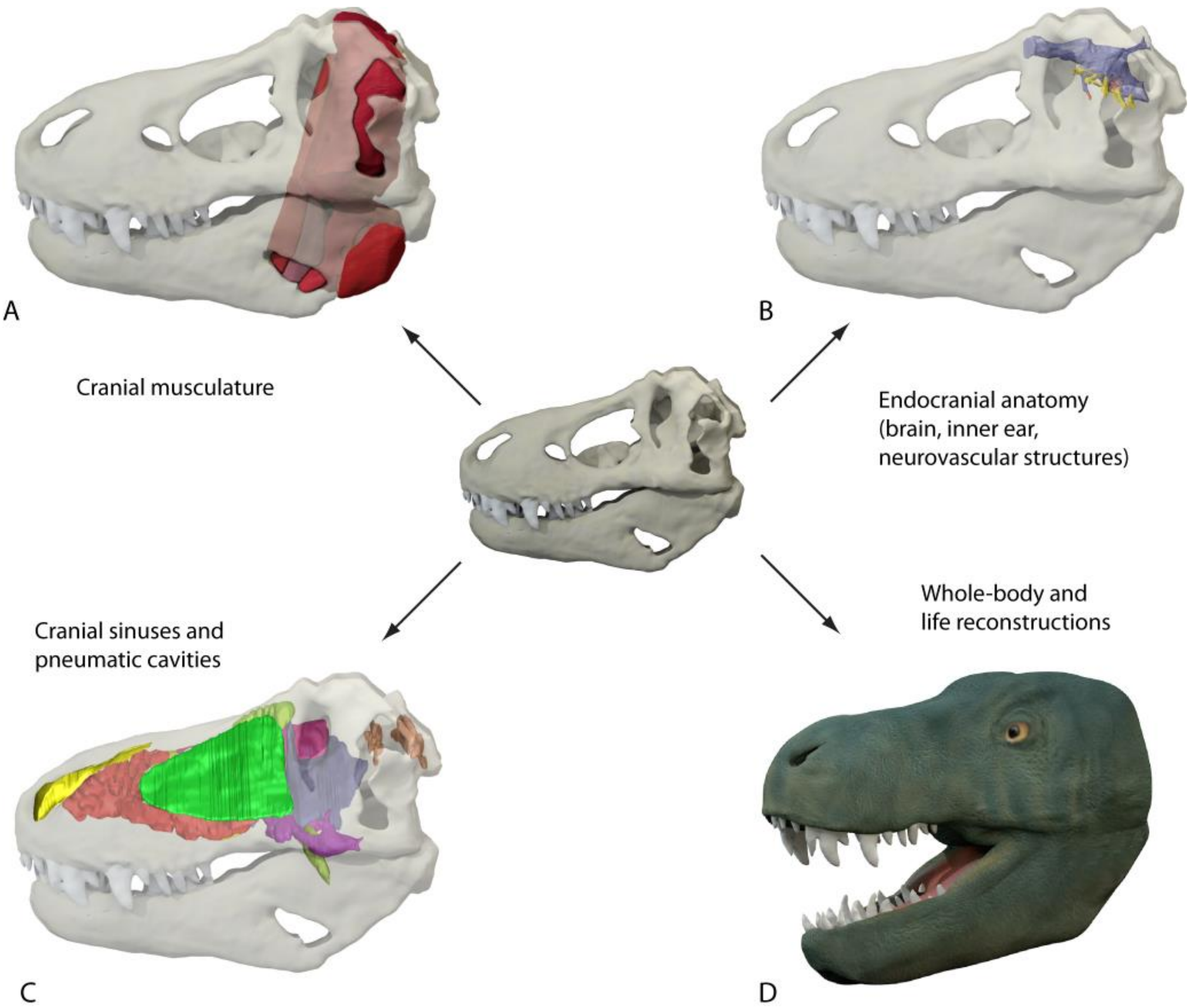

FIGURE 1.—Digital reconstruction of main cranial soft-tissue structures exemplified by *Tyrannosaurus rex*: (A) reconstructed jaw adductor musculature; (B) reconstructed endocranial components (brain, inner ear, and neurovascular structures) modified from Witmer and Ridgely (2009); (C) reconstructed paranasal sinuses and associated structures (airway, olfactory, and tympanic regions) modified from Witmer and Ridgely (2008); (D) life reconstruction based on osteological model.

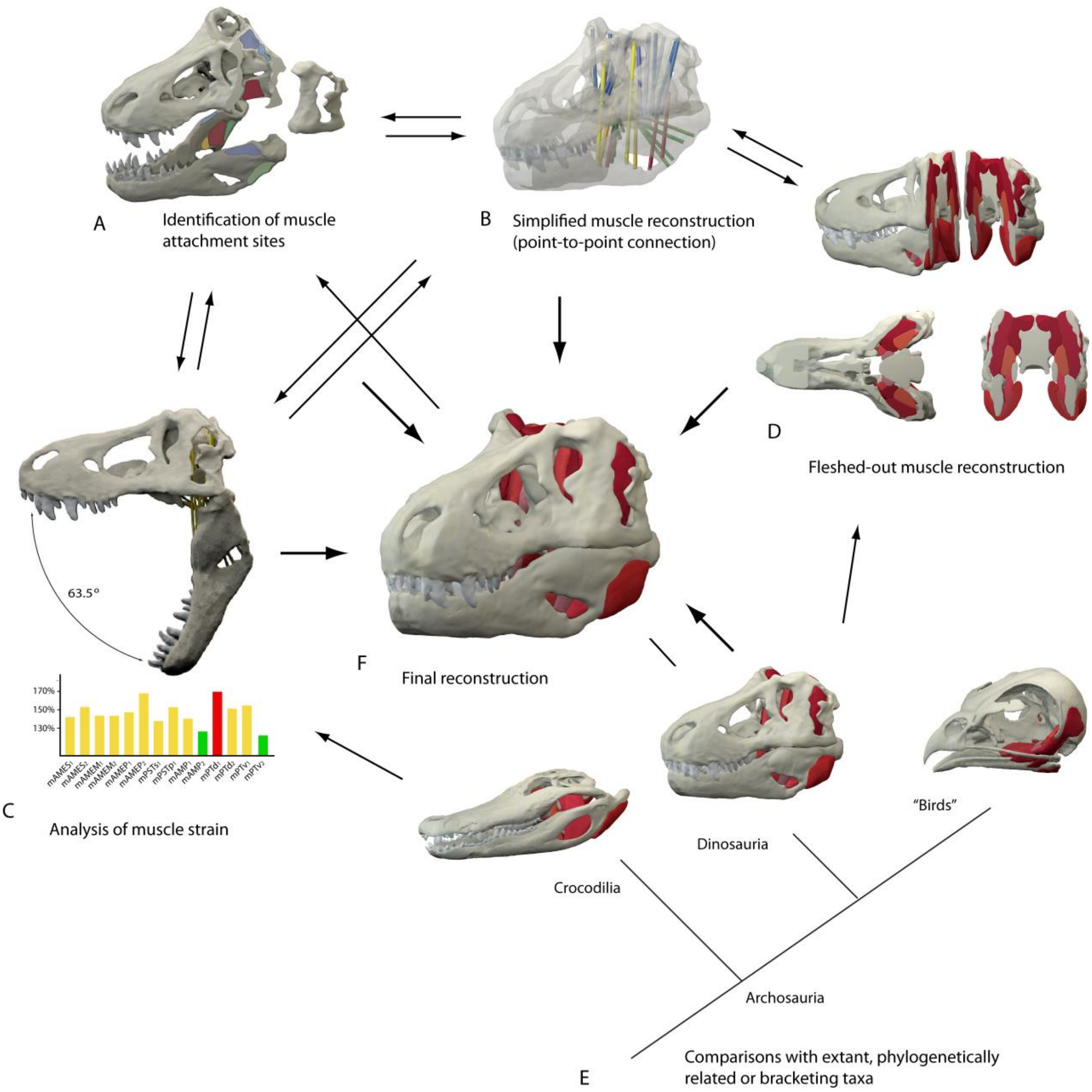

FIGURE 2.—Different procedures applicable to reconstruct the jaw adductor musculature exemplified by *Tyrannosaurus rex*: (A) identification of muscle origins and insertions based on osteological correlates; (B) simplified muscle reconstruction ("cylinder model") using point-to-point connections between corresponding muscle attachments (based on Lautenschlager, 2013); (C) analysis of muscle strain capabilities (Lautenschlager, 2015); (D) fleshed-out muscle reconstruction based on cylinder model and topological constraints (based on Lautenschlager, 2013); (E) comparisons with extant taxa, which are phylogenetically closely related or form an extant phylogenetic bracket; (F) final muscle reconstruction.

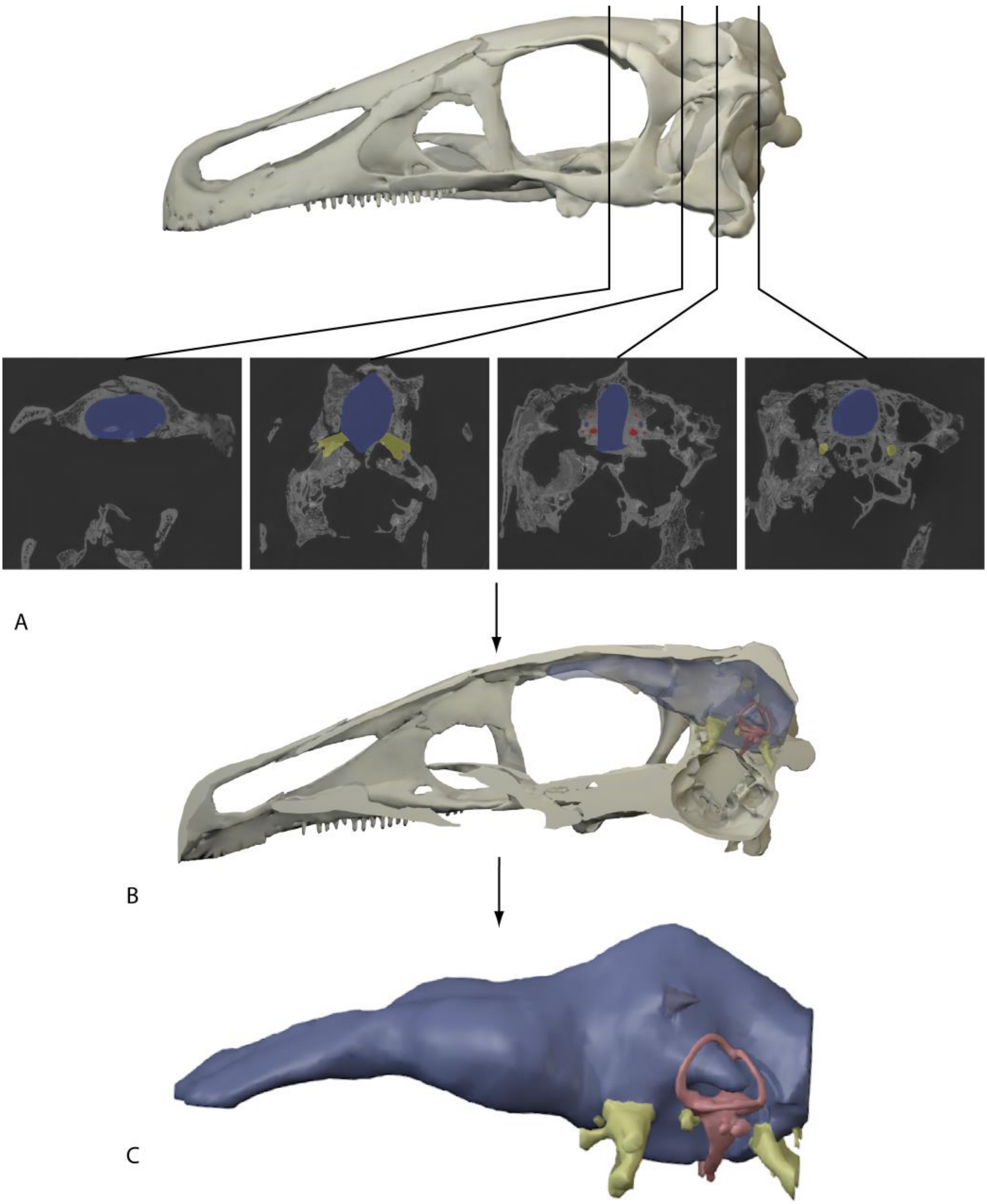

FIGURE 3.—Digital reconstruction of the endocranial anatomy exemplified by *Erlikosaurus andrewsi*: (A) examples of segmented CT slice data of the cranial skeleton of *Erlikosaurus andrewsi*; (B) endocranial components in-situ and rendered transparent; (C) reconstructed endocranial components.