1 Introduction

The availability of increasingly powerful computers has transformed the ways in which paleontologists study fossils, opening up a plethora of opportunities for processing, analyzing, and simulating paleontological data. This has included the development of an entirely new approach, termed virtual paleontology (Sutton et al. 2014, 2017), which entails using modern computational methods to generate three-dimensional digital representations of fossil specimens. These "virtual fossils" have enabled the characterization and reassessment of form, function, and phylogeny in greater detail than previously possible, and the techniques involved are broadly applicable across a wide range of taxonomic groups, specimen sizes, and modes of preservation (Cunningham et al. 2014; Rahman & Smith 2014; Sutton et al. 2014, 2017).

The study of 3D structures by creating a series of 2D parallel "slices" through an object, or tomography, enables exploration of internal anatomy and gross morphology in different ways. Tomographic techniques are among the most powerful methods in virtual paleontology. Traditional methods relied on creating physical sections through fossils using serial grinding or sawing, with images captured by manual tracing or acetate peels (e.g. Sollas 1904, Stensiö 1927) and more recently digital photography (e.g. Sutton et al. 2001); although potentially informative, these approaches are laborious and destructive, restricting their utility for studying fossil samples (Cunningham et al. 2014). However, in the past two decades, non-destructive X-ray computed tomography (CT) and its variants (e.g. micro-CT and synchrotron tomography) have rapidly grown in prominence, due in part to the increasing accessibility of suitable facilities (Sutton et al. 2014, 2017). This has driven the rise of tomography in paleontology.

Vertebrates were an initial focus of study in virtual paleontology (e.g. Tate & Cann 1982; Conroy & Vannier 1984; Haubitz et al. 1988; Rowe et al. 2001), but in recent years invertebrate fossils have been the subject of a growing number of tomographic investigations. Echinoderms are an invertebrate group well-suited to these techniques; they have an excellent fossil record, comprised of numerous three-dimensionally preserved specimens that are amenable to tomography. Moreover, the phylum represents a diverse, disparate, and geographically and temporally widespread clade, providing an ideal model system for addressing complex ecological and evolutionary questions through time (e.g. Sheffield et al. 2018; Cole et al. 2019; Deline et al. 2020). Research using tomographic techniques has addressed aspects of echinoderm morphology, function, preservation, and phylogeny with three-dimensional virtual fossils (e.g. Stock & Veis 2003; Sutton et al. 2005; Schmidtling & Marshall 2010; Rahman et al. 2015). These studies have led to a better understanding of, for example, homology,

informing on evolutionary relationships and transitions among major echino-
derm groups.

Herein, we summarize advances in understanding the anatomy, function,
taphonomy, and evolutionary history of extinct echinoderms obtained through
the generation and study of virtual fossils. In addition, we review the methods
used in this work and discuss possible future developments with particular
relevance to echinoderm paleobiology. We focus on the applications of tomo-
graphic techniques, especially physical–optical and X-ray computed tomog-
raphy; surface-based methods (e.g. laser scanning and photogrammetry) also
have great potential for studying fossil echinoderms (e.g. Hendricks et al. 2015),
but are not detailed herein.

2 Methods

Tomographic techniques have a long history of use in paleontology, dating back
more than a century (e.g. Sollas 1904), but it is only in the last 10–20 years that
they have become a routine part of the paleontologist's tool kit. In the following
section, we introduce physical–optical and X-ray tomography, which are the
two methods most commonly applied to fossil echinoderms. For more detailed
treatments of these and other tomographic techniques, with a focus on their
applications in paleontology, see Sutton et al. (2014, 2017).

2.1 Physical–Optical Tomography

This method involves physically exposing surfaces of a fossil specimen, which
are then optically imaged to create a dataset of slice images (Figure 1, parts 1–3).
Surfaces are typically exposed by serial grinding, with the exposed surfaces
captured at regular intervals through manual tracing, acetate peels, or photog-
raphy (Sutton et al. 2014, 2017). These two-dimensional slices can be used to
generate physical or digital models of the specimens, enabling three-dimensional
visualization of fossil morphology (virtual fossils; Sollas 1904; Stensiö 1927;
Schmidtling and Marshall 2010; Huynh et al. 2015). The major drawbacks of this
process are that it is at least partially destructive and very time consuming,
although the speed of imaging has been greatly improved by the integration of
digital photography (Sutton et al. 2014, 2017). Physical–optical tomography
remains an especially useful approach for studying fossil specimens that cannot
be physically or chemically prepared out of the host rock and which do not
provide sufficient internal density contrast for X-ray imaging techniques (e.g.
some calcite fossils preserved in calcareous sediments).

Physical–optical tomography has been extensively used to study fossil inver-
tebrates from the Herefordshire Lagerstätte of England (Briggs et al. 1996;
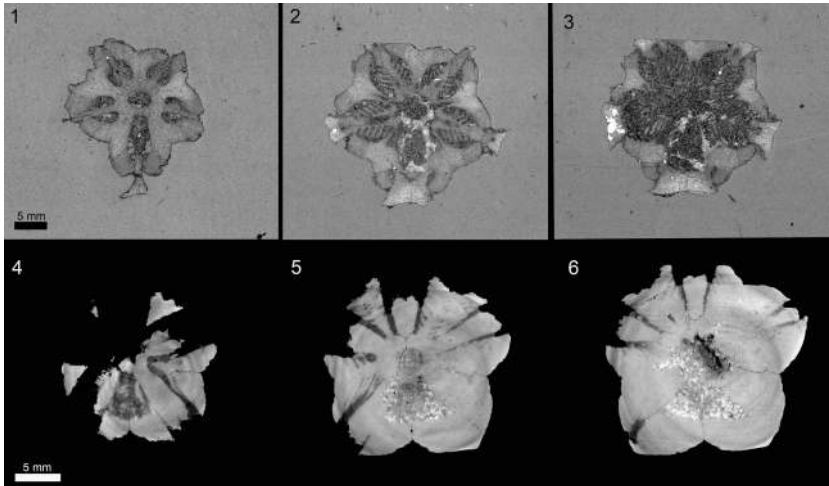
Figure 1 Comparison of destructive and non-destructive tomography applied to the blastoid *Cryptoschisma*. (1–3) Acetate peels generated by A. Breimer and digitized by J. A. Waters, housed physically and digitally at the Naturalis Biodiversity Center RGM.986207. (4–6) Synchrotron X-ray tomographic slices. Slices are available in the Supplemental Material and the specimen is housed at Museo Geominero (MGM), Instituto Geológico y Minero de España (MGM-3384D).

Sutton et al. 2001; Siveter et al. 2020), including echinoderms (e.g. Sutton et al. 2005; Briggs et al. 2017; Rahman et al. 2019). This deposit is early Silurian in age (Wenlock Series, ca. 425 Ma) and preserves fossils three-dimensionally as calcite infills in calcareous nodules, sometimes with details of soft tissue anatomy (Orr et al. 2000; Sutton et al. 2001, 2005; Siveter et al. 2020). Because the fossils are typically very similar in chemical composition to the host rock, non-destructive X-ray tomography has generally not proved effective (although see Nadhira et al. 2019). Consequently, specimens have been studied by necessity using destructive techniques. Sutton et al. (2001) developed a protocol for serially grinding and digitally photographing specimens, which has subsequently been applied to an assortment of Herefordshire fossils (see Siveter et al. 2020 and references therein).

2.2 X-Ray Tomography

The most widely used tomographic techniques in paleontology rely on X-rays for non-destructive imaging of fossil samples (i.e. X-ray computed tomography or CT). These methods trace their roots back to the end of the nineteenth century, when X-rays were first used to capture images of fossils (Branca

1906). CT involves penetrating the specimen of interest with an X-ray beam and capturing a set of radiographic images at multiple angles, which are then used to reconstruct a tomographic dataset that maps X-ray attenuation within the specimen (Sutton et al. 2014, 2017). Such datasets can be visualized in three dimensions using a multitude of free (e.g. Drishti, SPIERS) and commercial (e.g. Avizo, Mimics, VG Studio Max) software packages (see Cunningham et al. 2014 and Sutton et al. 2014 for more detailed treatments), thereby virtually extracting the fossil from the surrounding rock. While medical CT scanners have proved useful for studying some large vertebrates, laboratory-based, high-resolution variants (i.e. micro-CT and nano-CT) are better suited for most fossil specimens (e.g. University of Texas High-Resolution X-ray Computed Tomography Facility, UTCT). Micro-CT, in particular, has broad utility in paleontology; it can be used to penetrate dense samples while at the same time resolving fine anatomical details for a range of specimen types. Additionally, micro-CT equipment is now more widely available and accessible to many researchers.

Micro-CT scanners can typically achieve much higher resolutions than medical systems, down to a few microns or less, and are not restricted by the need to minimize radiation dosage (as is the case for medical CT) (Sutton et al. 2014, 2017). Settings such as the energy of the source X-rays can be adjusted to provide a balance between penetration and contrast (according to the composition of the sample). The need to minimize artifacts, which can obscure anatomical details, is another consideration when determining the optimal scan settings. Beam hardening is an especially common artifact when scanning large and dense fossils with micro-CT and has the effect of artificially darkening the center of the slices; this can be partly addressed through the use of filters, thin pieces of metal that are positioned between the source and sample to increase the average X-ray energy (Sutton et al. 2014, 2017).

Conventional CT techniques require that the fossil and matrix be composed of different materials, as chemically homogenous samples do not normally provide sufficient X-ray attenuation contrast for imaging in this way (Sutton et al. 2014, 2017). A common example of this problem is calcitic fossils embedded in calcareous rock, which can be very difficult to differentiate with lab-based CT scanners. Synchrotron tomography provides a potential solution to this problem (Figure 1, parts 4–6). This method uses a particle accelerator to generate extremely bright X-rays, producing beams that consist of X-rays of a single energy (i.e. monochromatic X-rays). Synchrotron tomography beamlines are optimized for different sample sizes and resolutions, and are capable of enhancing contrast between similar materials through phase-contrast imaging, which exploits X-ray refraction at material interfaces (Cunningham et al. 2014;

Sutton et al. 2014). Some lab-based nano-CT scanners can be used to improve contrast in a similar way (e.g. Dunlop et al. 2012).

3 Anatomy and Function

For many groups of fossil echinoderms, the most detailed descriptive studies remain those published in the mid- to late-twentieth century, particularly the *Treatise on Invertebrate Paleontology* and a handful of other seminal works (e.g. Moore et al. 1952; Sprinkle 1973). However, this work was frequently restricted to the exposed surfaces of fossil specimens, with key details hidden in the surrounding rock. Moreover, internal anatomy proved difficult to describe using traditional methods. Serial sectioning of specimens was undertaken in some cases (e.g. Breimer & Macurda 1972) but was typically incomplete due to the internal structure of interest (e.g. gonads or respiratory structure) terminating within the sample, the time required for such work, and the difficulty in visualizing the results, which were often hand-drawn or traced from the sections. Consequently, virtual paleontology has much potential for improving knowledge of both the external and internal anatomy of extinct echinoderms, with implications for functional morphology, and this has been a growing area of research in echinoderm paleobiology over the past two decades.

3.1 External Morphology

3.1.1 Carpoidea

Carpoids are an enigmatic and controversial extinct group, most likely paraphyletic or polyphyletic, that includes ctenocystoids, stylophorans, solutes, and cinctans (David et al. 2000; Smith 2005). Representatives of the group are weakly to strongly asymmetrical or bilaterally symmetrical, unlike many modern echinoderms, which exhibit pentaradial symmetry as adults (Zamora & Rahman 2014). Commonly, specimens are three-dimensionally preserved as molds with no original skeletal material remaining. Traditionally, latex casts have been prepared from such fossils, allowing surface anatomical features to be described, but this process risks damaging fragile specimens. An alternative approach is to digitally reconstruct fossils non-destructively using X-ray computed tomography. One example of such work is the study of the Cambrian echinoderm *Ctenoimbricata spinosa* Zamora et al. 2012. Two specimens were collected, both preserved three-dimensionally as molds with part and counterpart. Micro-CT was used to image and digitally reconstruct these fossil specimens. This allowed *C. spinosa* to be virtually extracted from the rock surrounding it, described as a new genus and species, and used as a basis for inferring homologies with ctenocystoids and cinctans (Figure 2). Rahman et al.
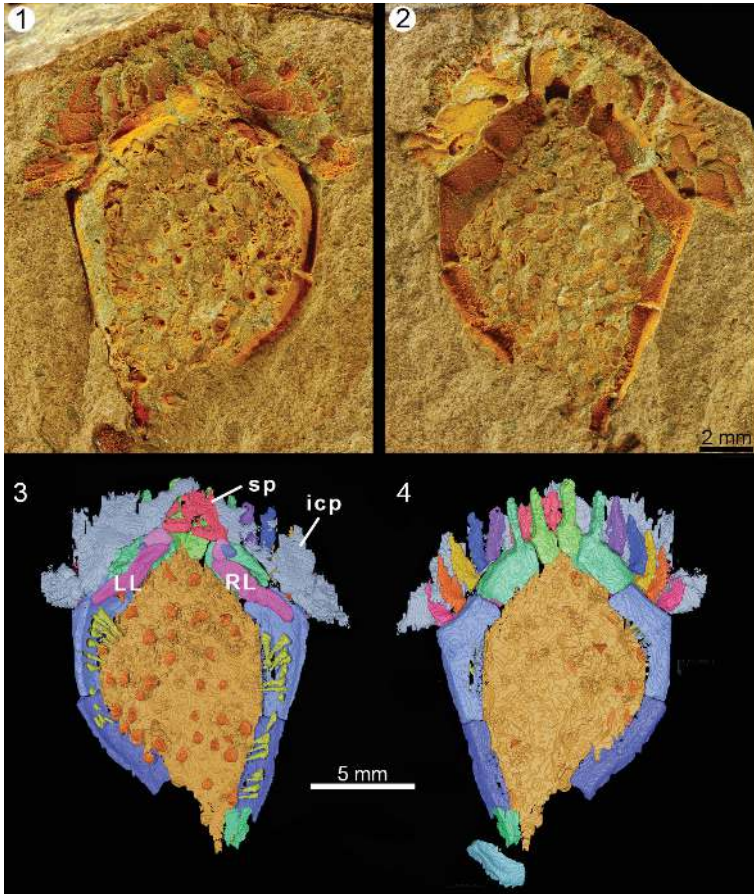
Figure 2 An example showing how our understanding of the external morphology of fossil echinoderms has been improved through the application of X-ray computed tomography. Specimens preserved as part and counterpart can be digitally extracted from the surrounding matrix to produce a complete three-dimensional model. (1–2) Natural mold of *Ctenoimbricata spinosa* (MPZ 2011/93), (1) dorsal and (2) ventral views. (3–4) Virtual reconstructions of the same specimen in (3) dorsal and (4) ventral views. Modified from Zamora et al. (2012).

(2015) used micro-CT to image a slab of siltstone preserving multiple moldic specimens of the Ordovician ctenocystoid *Conollia sporranoides* Rahman et al. 2015. This revealed a number of fossils that were previously hidden within the rock, providing anatomical details that informed a description of the new species and a redescription of the genus. Lastly, Rahman et al. (2010) described the Cambrian stylophoran *Ceratocystis prosthiakida* Rahman et al. 2010,

preserved as a mold in shale, with the aid of micro-CT. Using the resulting virtual fossil, they were able to digitally restore the original orientations and articulations of thecal plates, thereby providing a more accurate three-dimensional model of the animal's external morphology and helping to identify it as a new species.

More rarely, carpoid fossils are preserved three-dimensionally as recrystallized calcite, embedded in matrix. Historically, this material was studied through serial sectioning (e.g. Jefferies & Lewis 1978). However, such specimens are also well suited for micro-CT, as long as there is sufficient density contrast between fossil and rock. Domínguez et al. (2002) were among the first to apply micro-CT to fossil echinoderms, digitally reconstructing a three-dimensional specimen of the Carboniferous stylophoran *Jaekelocarpus oklahomensis* Kolata et al. 1991 and redescribing its anatomy. The oldest cinctan yet discovered, *Protocinctus mansillaensis* Rahman and Zamora 2009 from the Cambrian of Spain, was described as a new genus and species based on micro-CT scans of three specimens preserved as recrystallized calcite in siltstone (Rahman & Zamora 2009). The resulting digital reconstructions clearly showed the posterior appendage in an oblique orientation, suggesting it functioned as an anchor in the living animal. Rahman and Clausen (2009) used micro-CT to image a specimen of the Cambrian ctenocystoid *Ctenocystis utahensis* Robison and Sprinkle 1969, which is preserved three-dimensionally as a mold coated in recrystallized calcite. This allowed the species to be described in greater detail than previously possible, shedding new light on the morphology and function of key ctenocystoid characters, such as the anterior ctenoid apparatus (a series of distinctive bladelike plates), a structure considered to be used for feeding and/or respiration.

3.1.2 Blastozoa

Blastozoan anatomy was thoroughly described by Sprinkle (1973). Much of this work was restricted to the exposed surfaces of fossil specimens, augmented with serial sections revealing internal details (Sprinkle 1973). As with carpoids, three-dimensionally preserved fossil specimens are known, many of which are potentially amenable to investigation using tomographic techniques. However, a major challenge is differentiating between fossils preserved as calcite and the infilling sedimentary matrix, often rich in calcium carbonate, which can provide insufficient internal density contrast for conventional micro-CT. In such cases, synchrotron tomography can be effective (see Section 2.2). Bauer et al. (2019) applied this technique to two specimens of the oldest recorded blastoid, *Macurdablastus uniplicatus* Broadhead 1984 (Late Ordovician of eastern Tennessee). They scanned specimens using propagation-based phase-contrast synchrotron

tomography on the TOMCAT beamline at the Swiss Light Source. This allowed them to better resolve the boundaries between plates in the oral area and thereby identify homologous structures shared with other blastozoans (including coronoids and other conservatively plated species; see Section 5 on phylogeny).

If fossil specimens are moldic, micro-CT is often the method of choice because suitable instruments are widely available (synchrotron tomography relies on access to a small number of paleontologically suitable beamlines). Zamora and Smith (2012) used this technique to image two specimens of the Cambrian blastozoan *Dibrachicystis purujoensis* Zamora and Smith 2012. This helped them elucidate the morphology of the paired feeding appendages, indicating that these structures arose as thecal extensions of the body, with plating changing from proximal to distal. These were interpreted as key characters and supported a description of the fossils as a new genus and species.

3.1.3 Crinoidea

Combining traditional paleontological methods and non-destructive micro-CT, Zamora et al. (2015) thoroughly described a new species of iocrinid crinoid, *Iocrinus africanus* Zamora et al. 2015 (Middle Ordovician of Morocco). This specimen is preserved as a mold with no remaining skeletal material. Micro-CT imaging enabled the digital reconstruction of the specimen in three dimensions, bringing to light key anatomical features of the column, distal arms, and aboral cup that were recorded as impressions in matrix. These details allowed Zamora et al. (2015) to properly assign the material to *Iocrinus* and recognize it as a new species.

3.1.4 Cyclocystoidea

The anatomy of a new cyclocystoid genus and species, *Moroccodiscus smithi* Reich et al. 2017, from the Middle Ordovician of Morocco, was described with the aid of micro-CT scanning (Reich et al. 2017). Specimens are preserved three-dimensionally as part and counterpart molds in concretions. Details gained from the virtual reconstructions of *Moroccodiscus*, including the round flattened shape, domed central disk, and lack of an enclosed body cavity, suggest that cyclocystoids most likely lived with their flat marginal surface down, resting on or attached to the ocean floor (Reich et al. 2017).

3.1.5 Ophiuroidea

Many Paleozoic ophiuroids, or brittle stars, possessed anatomy different from that of their modern counterparts, which makes inferences of life mode difficult. Clark et al. (2020a) presented six virtual ophiuroid fossils from the Lower

Devonian Hunsrück Slate, with some taxa displaying typical Paleozoic arm structures and others with arm morphologies more similar to modern taxa. The use of computed tomography promoted the visualization of the delicate arms in situ and allowed for detailed descriptions of the arms. This work allowed for the inference of different locomotion strategies in ophiuroids.

3.2 Respiratory and Water Vascular System

The water vascular system (WVS) is a network of fluid-filled coelomic canals that is unique to Echinodermata (Nichols 1972; Zamora & Rahman 2014). This system has been hypothesized to perform a variety of functions in fossil echinoderms, including feeding, locomotion, and respiration. Although typically inferred in fossil specimens indirectly based on skeletal features such as the hydropore and surrounding plates (e.g. Dean 1999; Sprinkle & Wilbur 2005), in some groups direct evidence of the water vascular system is preserved in the form of calcified structures within a tightly sutured theca (e.g. Paul 1967, 1968; Sprinkle 1973; Sumrall & Waters 2012). Even more rarely, soft parts of the water vascular system, such as the tube feet, can be preserved in fossil specimens (e.g. Glass 2006; Ausich et al. 2013; Lefebvre et al. 2019); these parts have typically been identified through elemental analyses and scanning electron microscopy. Below, we summarize key advances in understanding the water vascular system enabled by virtual paleontology.

3.2.1 Eublastoidea

Traditionally, characteristics of the respiratory system of blastozoan echinoderms have been taken as synapomorphies for defining taxonomic groups and delineating species (Sprinkle 1973; Bauer et al. 2017; see Sheffield et al. forthcoming in this series for a full discussion on this topic). Endothecal, or internal, respiratory structures of blastozoans exist as infoldings of the body wall, and as lightly calcified structures they are often preserved when thecae are intact (Paul 1968; Sprinkle 1973; Sumrall & Waters 2012). These structures have been examined and described by serially sectioning specimens (e.g. Beaver et al. 1967; Breimer 1988a, 1988b; Dexter et al. 2009; Schmidtling & Marshall 2010). Recent efforts have focused on digitally reconstructing eublastoid hydrospire structures from serial sections (e.g. Schmidtling & Marshall 2010; Waters et al. 2015, 2017; Huynh et al. 2015; Bauer et al. 2017).

Schmidtling and Marshall (2010) provided the first attempt to create three-dimensional hydrospires from serial sections of *Pentremites rusticus* Hambach 1903, an early Pennsylvanian species from Oklahoma, USA. The specimen was serially sectioned across several sessions at regular intervals, and the hydrospire

groups of ambulacra C and B were digitally reconstructed. This confirmed that these two hydrospire groups merged to form a single spiracle. This work also included an analysis of how fluid was able to flow through the hydrospires, and helped show the amount of space these structures occupy within the theca (Schmidtling & Marshall 2010). The work of Schmidtling and Marshall (2010) spurred the efforts of Huynh et al. (2015), who examined fluid flow through the same reconstructed hydrospires to test hypotheses about the function of the hydrospires (see Sheffield et al. forthcoming in this series).

Waters et al. (2015) were the first to digitally reconstruct complete eublastoid hydrospires in three dimensions, using a serial acetate peel dataset produced by Breimer in the 1960s (e.g. Breimer & Macurda 1972), thus providing an incredibly detailed look at these anatomically complex structures (Figure 3, parts 1–2). This work served as a baseline for reconstructing hydrospires in different species spanning Eublastoidea, informing descriptions of the morphology as a whole rather than partial descriptions based on individual sections (e.g. Beaver et al. 1967; Breimer 1988a, 1988b). For example, Bauer et al. (2017) created virtual models of the hydrospires of six eublastoid species from the Carboniferous–Permian of the United States and Timor by digitizing a dataset of serial acetate peels. This allowed them to redescribe the anatomy of the hydrospires in great detail, identifying differences within and between previously recognized families.

The increasing availability of non-destructive imaging techniques, such as X-ray tomography, has provided new opportunities to reexamine the internal structures of blastozoans. This proved particularly important for the Late Ordovician blastoid *Macurdablastus*, which was previously unassignable outside of class Blastoidea. Only a few well-preserved specimens are known, therefore traditional destructive techniques such as serial sectioning could not be used. The two most complete specimens (holotype and paratype) were analyzed using synchrotron tomography in order to enhance the contrast between fossil and matrix (Bauer et al. 2019). This revealed that the morphology of the reconstructed respiratory structures differs from that of the two known eublastoid orders, fissiculates and spiraculates. See Sheffield et al. forthcoming (in this series) for a more detailed overview of blastozoan water vascular systems.

3.2.2 Asterozoa

In extant asterozoans, the WVS consists of a ring canal and a set of radial canals, each of which gives rise to numerous lateral canals and tube feet. Among fossil forms, aspects of this system can be inferred indirectly from skeletal