

ON THE PURPORTED PRESENCE OF FOSSILIZED
COLLAGEN FIBRES IN AN ICHTHYOSAUR AND A
THEROPOD DINOSAURby FIANN M. SMITHWICK¹ , GERALD MAYR² , EVAN T. SAITTA¹ ,
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Abstract: Since the discovery of exceptionally preserved theropod dinosaurs with soft tissues in China in the 1990s, there has been much debate about the nature of filamentous structures observed in some specimens. *Sinosauropteryx* was the first non-avian theropod to be described with these structures, and remains one of the most studied examples. Despite a general consensus that the structures represent feathers or feather homologues, a few identify them as degraded collagen fibres derived from the skin. This latter view has been based on observations of low-quality images of *Sinosauropteryx*, as well as the suggestion that because superficially similar structures are seen in Jurassic ichthyosaurs they cannot represent feathers. Here, we highlight issues with the evidence put forward in support of this

view, showing that integumentary structures have been misinterpreted based on sedimentary features and preparation marks, and that these errors have led to incorrect conclusions being drawn about the existence of collagen in *Sinosauropteryx* and the ichthyosaur *Stenopterygius*. We find that there is no evidence to support the idea that the integumentary structures seen in the two taxa are collagen fibres, and confirm that the most parsimonious interpretation of fossilized structures that look like feather homologues in *Sinosauropteryx* is that they are indeed the remains of feather homologues.

Key words: theropod, *Sinosauropteryx*, ichthyosaur, Jehol, feathered dinosaur, collagen.

THE debate on the origins of birds is one of the oldest in palaeontology (Huxley 1870; Heilmann 1926), and there has been a substantial increase in research on this question in the past two decades, thanks to the discovery of numerous exceptionally preserved fossils in China (reviewed in Zhou *et al.* 2003; Xu 2006). These discoveries included extraordinarily well preserved stem birds and theropod dinosaurs complete with integumentary preservation (Ji & Ji 1996; Zhou *et al.* 2003; Xu 2006; Zhang *et al.* 2006). The first of these theropods to be described with features attributed to filamentous integumentary structures was the compsognathid *Sinosauropteryx* (Ji & Ji 1996; Fig. 1). These authors noted the unique integumentary structures found mostly across the dorsum and tail of the animal, which have subsequently been observed in all known specimens (Ji & Ji 1996; Currie & Chen 2001; Ji *et al.* 2007). Through the original descriptions and a series of subsequent studies, a general consensus has arisen that the structures represent feather homologues or ‘protofeathers’ (see Prum (1999) and Xu (2006) for

discussions on feather evolution and nomenclature). This consensus conforms to previous phylogenetic hypotheses about the origins of birds and their nesting within Theropoda, established long before any non-avian theropod with proposed feathers had been discovered (Ostrom 1976; Prum 2002, 2003; Norell & Xu 2005; Smith *et al.* 2015).

Evidence supporting a feather affinity of integumentary structures in *Sinosauropteryx* includes morphological analysis of the structures, their distribution across the body which is conserved in multiple specimens (Fig. 1), SEM data showing the presence of melanosomes within the structures, and both morphological and molecular phylogenetics (Currie & Chen 2001; Prum 2002, 2003; Norell & Xu 2005; Li *et al.* 2010; Zhang *et al.* 2010; Smith *et al.* 2015). However, alternative explanations have been put forward for the structures, suggesting that they represent partially degraded collagen fibres from the skin rather than feathers (Ruben & Jones 2000; Lingham-Soliar 2003a; Feduccia *et al.* 2005; Lingham-Soliar *et al.* 2007).

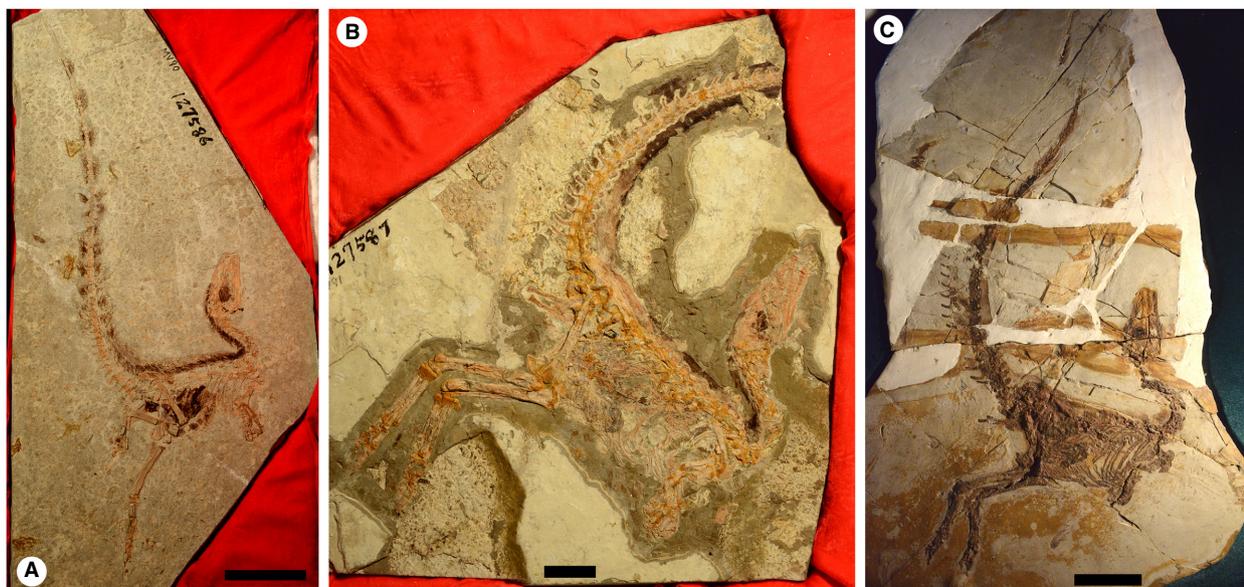


FIG. 1. The three best preserved described specimens of *Sinosauropteryx* from the Early Cretaceous Jehol Biota of Liaoning Province, China. All three specimens show the preservation of integumentary structures identified by some as feathers, and others as degraded collagen fibres. The integument can be seen as the dark brown patches across the dorsum and tail. A, NIGP 127586; counterpart of the holotype. B, NIGP 127587. C, IVPP V12415; orange staining present across the matrix is probably due to the presence of iron oxides, and is different from the clearly darker colour of the integument. Scale bars represent: 50 mm in (A, B); and 100 mm (C). Colour online.

The idea that the integumentary structures first identified as feathers in *Sinosauropteryx* could actually represent degraded collagen fibres originated soon after the original description of the theropod, based on similarities with modern snake and lizard collagen (Gibbons 1997; Ruben & Jones 2000). The claim was then further fuelled by the observations of similar structures in ichthyosaur fossils (Feduccia *et al.* 2005; Lingham-Soliar 2003a). A description of the integumentary structures on a *Sinosauropteryx* specimen (IVPP V12415; Fig. 1C) using light microscopy, was used to suggest that they were collagen fibres (Lingham-Soliar *et al.* 2007). This work has formed the basis of most criticisms and rebuttals of the feathered theropod hypothesis (Lingham-Soliar *et al.* 2007; Lingham-Soliar 2010a, 2012; Feduccia 2013). One of the key observations made about the structures of IVPP V12415 as evidence of a collagen affinity is the beaded appearance of the fibres (Fig. 2; Lingham-Soliar *et al.* 2007, fig. 2). Beading is said to occur in modern collagen due to contraction caused by dehydration (Lingham-Soliar *et al.* 2007; Lingham-Soliar & Wesley-Smith 2008). Further evidence for a collagen affinity came from the pattern and orientation of their arrangement. In certain regions, an overlap of 'geometrically precise bands of parallel fibres' is said to be consistent with structural reinforcement of the skin when compared to modern animals (Lingham-Soliar *et al.* 2007). Observations of decaying dolphin tissues were also used to demonstrate how collagen can show a similar

morphological appearance to feathers (Lingham-Soliar 2003b).

The integumentary structures in *Sinosauropteryx* were interpreted by Lingham-Soliar *et al.* (2007) as remnants of a frill along the dorsum, reinforced with structural collagen fibres, thus accounting for the way in which the integument extends dorsally from the vertebrae. This was suggested to add stiffening support to the long tail, to protect against injury, as well as to provide a decorative display organ (Lingham-Soliar *et al.* 2007). The banded pattern of the integument was interpreted as 'scallopings', analogous to features in modern basilisk lizards such as *Basiliscus basiliscus* and *B. plumifrons* (Lingham-Soliar *et al.* 2007; Lingham-Soliar 2012, 2013). It has been further suggested that the purported frill could be used to aid swimming in *Sinosauropteryx* as the dinosaur was found in a lacustrine environment (Lingham-Soliar 2012, 2013).

In addition to the hypothesis that the structures on *Sinosauropteryx* are collagen fibres rather than feathers, it has been claimed that scales are present overlying the fibres making them unlikely to represent feathers. Martin & Czerkas (2000) were the first to suggest that scales were present in *Sinosauropteryx* four years after its original description, when it was stated that a slab with scale impressions was originally removed from a specimen. No evidence to support this claim was ever provided, however, nor any images (Martin & Czerkas 2000, p. 688;

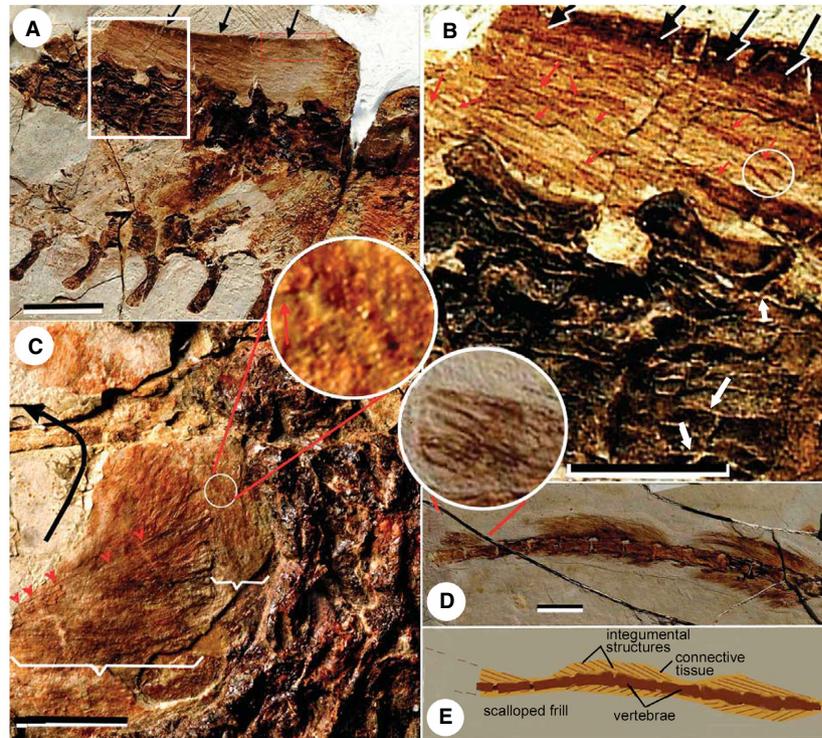


FIG. 2. Original figure published in Lingham-Soliar *et al.* (2007) of the integument of *Sinosauropteryx* specimen IVPP V12415. A, integument preserved next to the skeleton in the proximal tail region. B, close-up of the area denoted by a rectangle in A purportedly showing an aberrant association of fibres (highlighted by the white circle); white arrows indicate areas of the internal structure of the vertebrae misidentified as collagen fibres (Fig. 5E). C, purported collagen fibres of the dorsal cervical region, apparently showing beading indicative of degraded collagen (highlighted in the expanded circle). D, mid-tail region showing the integument with an expanded area (inset circle) purporting more beading of individual fibres. E, schematic illustration of the mid tail region depicting the fibres as running straight and parallel posteriorly outwards from the vertebrae from the axial skeleton, contrary to the genuine pattern of orientation where they recurve back towards the vertebrae (D) of the integumentary structures seen in the specimen (Fig. 4A). Scale bars represent: 20 mm (A, D); and 10 mm (B, C). Reproduced with original labelling from Lingham-Soliar *et al.* (2007) with permission from the Royal Society. Colour online.

Lingham-Soliar 2013, p. 460). More recently, scales have been purportedly identified on another specimen and used as evidence of a frill as well as to refute the presence of feathers (Lingham-Soliar 2013).

Many flaws in the arguments given by proponents of the collagen hypothesis have been noted (e.g. Mayr 2010; Smith *et al.* 2015; van der Reest *et al.* 2016), but claims of a likely collagen affinity have propagated through the literature. Often, when avian origins and theropod integuments are discussed, both feather and collagen hypotheses are treated as equally parsimonious and credible (Perrichot *et al.* 2008; Dhouailly 2009; Geist 2009; Ruben 2010; Dove & Straker 2012). The debate is clearly an important one, with major implications for our understanding of the early evolution of birds, feathers and flight, and thus scrutiny of the proposed evidence is crucial. As *Sinosauropteryx* has been the focus of the majority of the debate on theropod integumentary structures, a clearer understanding of the known specimens is essential.

Here, we review the evidence given for the collagen model of theropod integumentary structures in *Sinosauropteryx* as well as similar structures observed in ichthyosaurs. We highlight a shortcoming in this model, in that nearly all the evidence comes from low-resolution photographic images. We present our results, based on close study of the three best preserved *Sinosauropteryx* specimens (Fig. 1). From our observations and photographs, previous work using lower resolution images is reconsidered, and errors arising in earlier interpretations discussed. The aim of this paper is to clarify a number of misconceptions surrounding soft tissue preservation in ichthyosaurs and the theropod *Sinosauropteryx* as well as to highlight errors used in many of the arguments surrounding theropod integumentary structures.

Institutional abbreviations. GMV, Vertebrate Collections of the Geological Museum of China, Beijing; NIGP, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences,

Nanjing, Jiangsu Province; IVPP, Institute of Vertebrate Palaeontology and Palaeoanthropology, Chinese Academy of Sciences, Beijing; SMF, Senckenberg Research Institute, Frankfurt am Main, Germany.

EVIDENCE FOR COLLAGEN FIBRES IN ICHTHYOSAURS AND *SINOSAUROPTERYX* REAPPRAISED

While the evidence for a feather affinity of the integumentary structures in *Sinosauropteryx* has been criticized heavily since the hypothesis was first put forward (Gibbons 1997; Feduccia 1999; Lingham-Soliar *et al.* 2007; Feduccia 2013) the evidence for the collagen model has

never been rigorously tested. The statements provided by proponents of the collagen hypothesis will be evaluated in turn, highlighting errors that include misidentification of sedimentary structures, surface topography and marks made during the fossil preparation process. New, high resolution images (7360×4912 pixels) of three *Sinosauropteryx* specimens (IVPP V12415, NIGP 127586 and NIGP 127587; Fig. 1) were taken using a Nikon D800 camera with a Micro Nikkor 60 mm lens mounted on a tripod with a 10 s delayed timer to maximize image sharpness. Specimens were illuminated with a mounted tungsten light source with a polarizing filter attached. Images were taken under both normal lighting conditions and using a polarizing filter on the camera to allow cross-polarization to reduce glare. These methods provided very accurate and detailed images. Images of the ichthyosaur *Stenopterygius quadriscissus* (SMF R 457) were taken by Sven Tränkner (SMF).

Fibres in ichthyosaurs

Further study of purported ichthyosaur collagen fibres used in comparisons to the integumentary structures of *Sinosauropteryx* has revealed a number of misinterpretations in the original descriptions. Identified fibres on *Stenopterygius quadriscissus* (SMF R 457) said to show three-dimensional preservation and resemblances to theropod fossil fibres are, in many cases, actually preparation marks made when the matrix of the ichthyosaur was being smoothed around the genuine preserved soft tissue (Fig. 3). The new images of the same specimen clearly

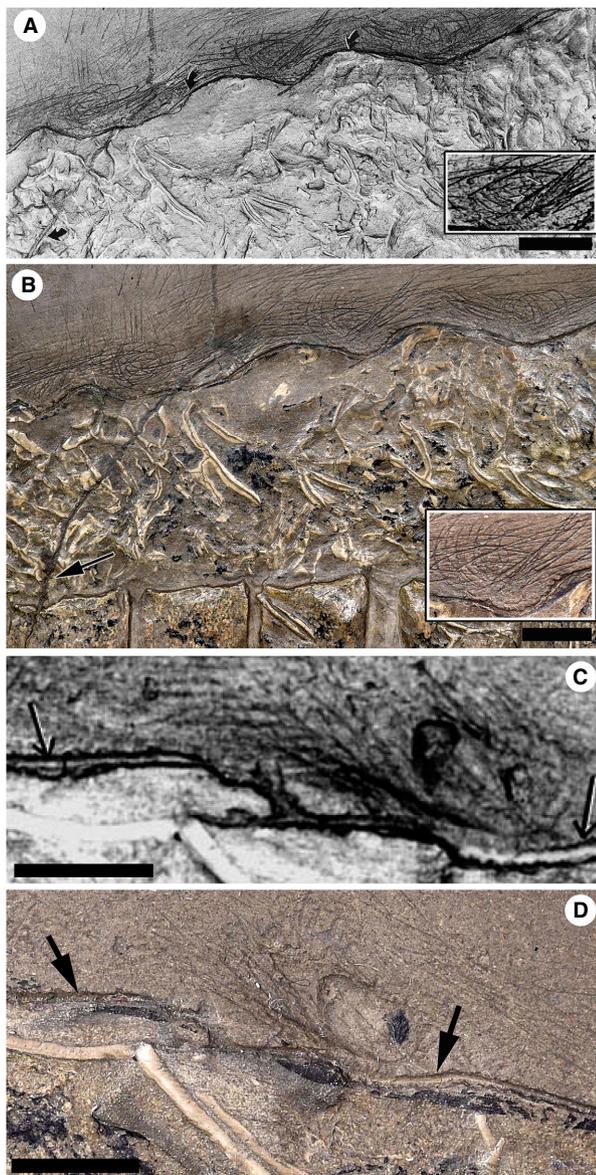


FIG. 3. Soft tissue preservation in the ichthyosaur *Stenopterygius quadriscissus* (SMF R 457). A, the original image provided by Lingham-Soliar (2001, fig. 5a) showing purported preserved collagen fibres; black arrows mark suggested fibre types including a dorsal longitudinal fibre (DLF); inset shows an area above the DLF of purported fibres resembling feather rachis and barbs (from Lingham-Soliar 2003a, fig. 1d). B, new image of the same area depicted in A showing that a previously identified fibre can be seen to be a crack in the matrix (black arrow) and the purported collagen fibres said to resemble feathers in *Sinosauropteryx* appear to be deep scratches in the matrix made during preparation of the fossil (inset). C, close-up of the DLF (indicated by arrows) allegedly showing mineralization patterns similar to those seen in *Sinosauropteryx* (from Lingham-Soliar 2003a, fig. 1f). D, new image showing the DLF to actually be a deep trench in the matrix (indicated by arrows) which casts a strong shadow, thus creating an illusion of differential mineralization. Scale bars represent: 10 mm (A, B); and 5 mm (C, D). A reproduced with original labelling from Lingham-Soliar (2001) with permission from Wiley; C reproduced with original labelling from Lingham-Soliar (2003a) with permission from Springer. Colour online.

show that these marks are deep scratches within the matrix, which is not so obvious from the original black and white images (Fig. 3A). These scratch marks, frequently described as ‘class 2’ or ‘radial’ fibres were said to show superficial resemblance to a feather rachis (referred to as pseudo-rachis), an argument against genuine feather preservation in theropods (Lingham-Soliar 2001, 2003a, fig. 1d). These fibres are noted as only being present on SMF R 457, which is presumably because the other fossils studied do not have these preparation marks still on the matrix or fossil. Further purported ‘radial’ fibres can now also be shown to be misidentified cracks running through the matrix which had been repaired when the multiple blocks of the fossil were put back together (Fig. 3A, B).

It was claimed that mineralization patterns of a ‘dorsal longitudinal fibre’ (DLF) in SMF R 457 also resembled that of filaments in *Sinosauropteryx* (Fig. 3A, C; Lingham-Soliar 2001, 2003a). This feature can be seen to run parallel to the body, separating the soft tissue from the preparation marks misidentified as ‘radial fibres’ in the ichthyosaur (Fig. 3A; Lingham-Soliar 2001, figs 5, 9). The ‘DLF’ runs continually, marking the boundary between soft tissue and matrix (Lingham-Soliar 2001). Re-examination of the specimen indicates that rather than a single primary feature, this is in fact a deep trench within the matrix, with the three-dimensional structure creating a light centre with darker ridges due to shadowing (Fig. 3B, D). This is therefore not a pattern created by mineralization, but is probably also a preparation mark, and is therefore not an appropriate analogue for any structure seen on *Sinosauropteryx*.

While many of the soft tissue features preserved within the halo of the body of SMF R 457 do appear to be remnants of organic structures, none that resemble the filaments found on *Sinosauropteryx* stands up to scrutiny. Therefore, references to the similarities of ichthyosaur fibres and theropod integumentary structures can be shown to be baseless. Published SEM images of what is probably genuine ichthyosaur soft tissue (Lingham-Soliar & Wesley-Smith 2008; Lingham-Soliar 2011) and that of *Sinosauropteryx* (Zhang *et al.* 2010) show no microstructural resemblance, with ichthyosaur tissue forming a rope-like structure of fine filaments and the integumentary structures of *Sinosauropteryx* showing preserved melanosomes with no fibre-like features.

Evidence of beading in Sinosauropteryx

The purported evidence that the structures found on *Sinosauropteryx* are beaded, conforming to collagen, falls short for a number of reasons. Beading in collagen is not a commonly observed phenomenon in modern vertebrate tissue. It has only been reported in decaying collagen

from marine vertebrates (Lingham-Soliar 2003b; Lingham-Soliar & Wesley-Smith 2008). The images of beading in this modern collagen (Lingham-Soliar 2003b; Lingham-Soliar *et al.* 2007) are of insufficient quality to determine any genuine resemblance to structures seen in the fossils. References to observations of beading in modern mammal collagen are scant, and in the cited publications the authors note only sub-micrometre scale granular textures sometimes induced by experimental procedures. Such textures would not be visible without high magnification, and this was not used in the description of the *Sinosauropteryx* integument (Lewis & Johnson 2001; Young 2003; Reichlin *et al.* 2005; Lingham-Soliar *et al.* 2007). In other words, beading in collagen is not ubiquitous, and in any case the scale differs from that supposedly seen in *Sinosauropteryx*.

There are also problems with the identification of a purported beaded structure in the *Sinosauropteryx* integument. The high-resolution images in the same areas as those depicted by Lingham-Soliar *et al.* (2007) show that the structures present no evidence of beading (Figs 4, 5). When well preserved, the structures instead appear to be long, smooth filaments tapering distally (Fig. 4). In figure 3b of Lingham-Soliar *et al.* (2007) isolated fibre structures are shown suggesting beading, which have been digitally cut and reorientated alongside one another on a background mimicking matrix (Fig. 5C). For most of these structures, it is not clear where they have been cut from and so comparison to the original cannot be made. One of the structures, however, is noted on a larger figure (Fig. 2B), and is said to show aberrant associations between multiple beaded fibres, forming a ‘Y’ shape (Lingham-Soliar *et al.* 2007, fig. 2b). Our specimen photograph, however, shows no such structure other than a slight undulation in the sediment of the matrix, with no clear association between the integumentary structures (Fig. 5B, D). The digitally cut and reproduced fibres also show large discrepancies in scale, with the Y-shaped fibre depicted as around 633 μm (Fig. 5C) while in larger figures it can be seen to measure around 3000 μm (Figs 2B, 5D; measured using ImageJ; <https://imagej.nih.gov/>). Beaded integumentary structures are also identified as overlying the vertebrae, showing apparent branching (Fig. 2B; Lingham-Soliar *et al.* 2007, fig. 2b). Close inspection of the new images however, shows that these are three-dimensionally preserved parts of the bones themselves, and are probably part of the internal structure as they appear to be broken when compared to adjacent complete bones. These structures readily cast shadows that appear to have been misidentified as integument (Fig. 5B, D). One of these skeletal structures was digitally cut from the bone and again presented as an isolated collagen fibre (Fig. 5C; Lingham-Soliar *et al.* 2007, fig. 3b). It cannot be assumed that any of these cut fibres are

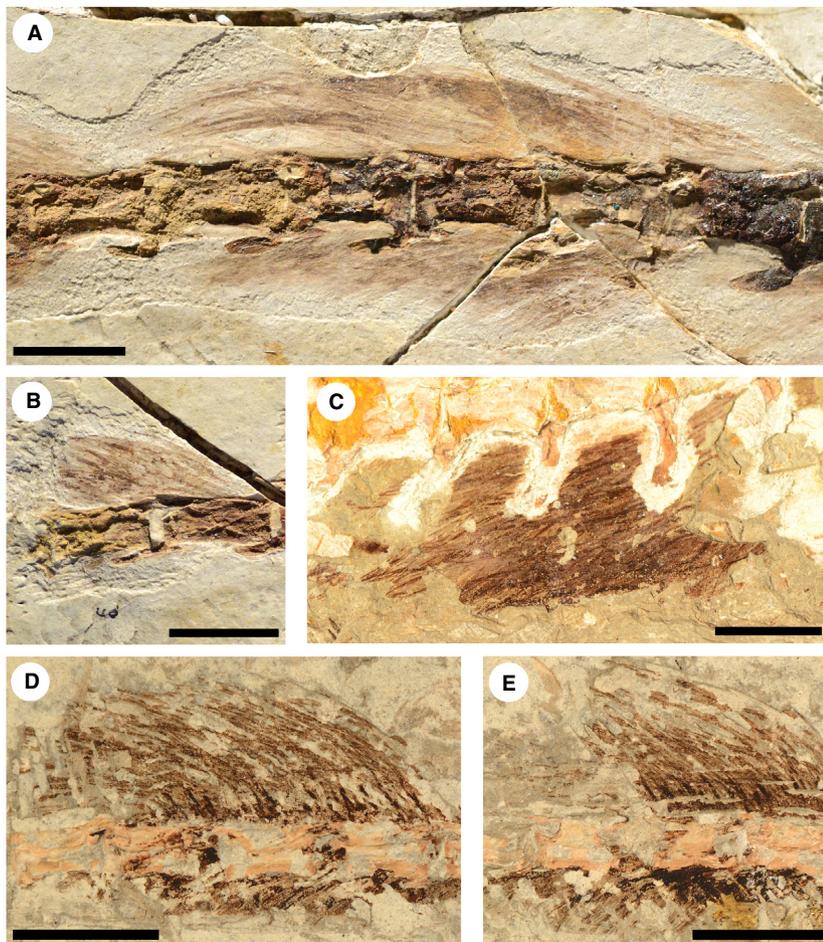


FIG. 4. Areas of exceptionally well preserved integumentary structures on the tail of *Sinosauropteryx*. All regions show smooth filament-like structures which taper towards their tips, with no evidence of beading, as is suggested for degraded collagen. A, mid-tail region of IVPP V12415, showing long smooth filaments protruding from the tail axis before recurving back in towards it. B, region of integument at the break of the tail in IVPP V12415. C, ventral integumentary structures in the mid-tail region of NIGP 127587, again showing long smooth filaments with a clearly flexible structure allowing curvature and overlap. D, E, posterior regions of the tail in NIGP 127586 showing further flexible filaments recurving posteriorly after protruding from the tail. All scale bars represent 10 mm. Colour online.

integumentary features showing beading, as the only two that have their original location shown are clearly not fibres, as noted above. In conclusion, it seems that the illustrations of supposedly beaded collagen fibres from the *Sinosauropteryx* specimens are based on undulations of the matrix which create differential lighting and shadowing. Furthermore, we could find no examples of these beaded structures on close inspection of the actual specimens.

Orientation of the filaments in Sinosauropteryx: parallel and cross-fibre patterning

Cross-fibre patterning of the dermis reported from NIGP 127587 and IVPP V12415, used to support the collagen hypothesis, also fails to stand up to scrutiny (Lingham-Soliar *et al.* 2007; Lingham-Soliar 2012, 2013). In Lingham-Soliar (2013), figure 4 shows a section of the tail of IVPP V12415 which has broken and separated, with arrows indicating the proposed opposing direction of fibre orientation suggested to represent the original

collagen structural arrangement (Fig. 6A; Lingham-Soliar 2013, p. 457). Our photographs, however, show that the so-called 'fibres' are in fact deep scratches made during preparation of the specimen, probably by a pneumatic air scribe, and so with no biological significance (Fig. 6C). Similar preparatory scratch marks can be seen across the matrix around the entire animal (Fig. 7). The same marks are found in NIGP 127586, with some even cutting through the genuine integumentary structures (Fig. 7C). These marks cast shadows, which appears to have led to their misidentification. Interestingly, these preparation marks are clear and obvious in the region of IVPP V12415 in which the tail has been displaced (Fig. 6D), a feature blocked out on the original image by a black square with no explanation by Lingham-Soliar (2013, fig. 1).

Further evidence for geometrically precise bands of parallel collagen fibres comes from observations made on an image of NIGP 127587 (Lingham-Soliar *et al.* 2007, fig. 4) first published by Chen *et al.* (1998, fig. 6). The preservation of the structures in this area appears markedly different from those in other areas, with a more

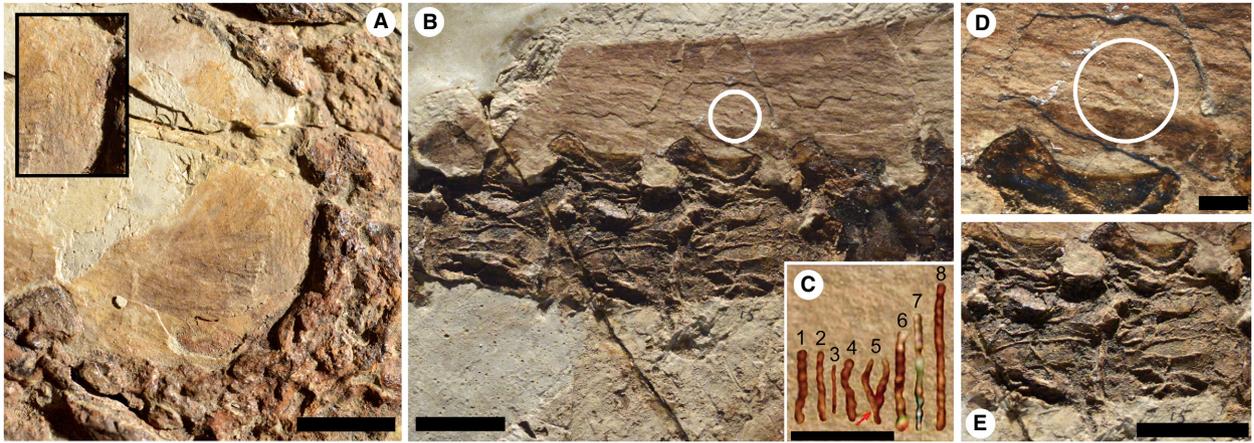


FIG. 5. Areas of well-preserved integument in *Sinosauropteryx* specimen IVPP V12415. A, integument dorsal to the cervical vertebrae previously depicted in Lingham-Soliar *et al.* (2007; Fig. 2C); the structures appear as long, smooth filaments, with no evidence of beading even when viewed close-up (inset); despite the extreme arching of the neck, the filaments remain straight and parallel. B, dorsal integumentary structures of the anterior tail region with purported associated collagen fibres (circle). C, original figure from Lingham-Soliar *et al.* (2007, fig. 3b) showing digitally cut, and reorientated fibres including an aberrant association forming a Y-shape (fibre 5) cut from the integument shown in B. D, a close-up of the purported aberrant association of fibres circled in C; from this image it is unclear whether the structures preserved are associated, or if undulations in the matrix have caused the apparent shape of the structures; a large discrepancy in size can be seen between the cut fibre (633 μm) and the original area of interest (3000 μm). E, vertebrae of the same region imaged under bright conditions to highlight the shadows cast by the three-dimensional structure of the bones, which have previously been misidentified as part of the integument (Fig. 2D; Lingham-Soliar *et al.* 2007); one of these ridges running across the bone was digitally cut and presented as collagen fibre 7 in Lingham-Soliar *et al.* (2007) (reproduced in C). Scale bars represent: 10 mm (A, B, E); 1 mm (C); and 2 mm (D). C, reproduced with original labelling from Lingham-Soliar *et al.* (2007) with permission from the Royal Society. Colour online.

three-dimensional and organized appearance, which is explained by Lingham-Soliar *et al.* (2007, fig. 4) as showing different stages of collagen preservation (Fig. 8A). The layer containing the purported fibres clearly sits underneath the main integumentary layer, as can be seen from the shadow cast by the overlying layer. Our specimen photographs show that subsequent preparation of the specimen has removed most of the overlying layer of integumentary structures, and the matrix now sits entirely at the level of the aforementioned purported parallel fibres (Fig. 8). This new preparation work was presumably done in order to reveal more depth around the bones themselves, as can be seen in the more exposed ventral side of the vertebrae in the new image (Fig. 8) in comparison to the pre-preparation image. Due to this preparation, information from the integument in the region has been lost. However, it reveals that the identified purported fibres are actually sedimentary layers of the matrix (Fig. 8). The area where the suggested fibres were in the original figure is still present after the preparation, but all that can be seen are contrasting coloured sediment layers exactly conforming to the outer margin of the 'fibres' described in the original image. The same appearance can be seen on the dorsal side of the same area, with another clear boundary created by sedimentary changes (Figs 8B, 9). From close observation of these and

other areas of the specimen, it is clear that a superficial halo is present where the sedimentary layers are visible around the animal's body, creating a transition from dark to light matrix (Figs 8B, 9). The preserved integument is markedly different in colour, being a deep brown in contrast to the lighter grey and buff sediment, a feature that is highlighted when the whole specimen is observed under strong light (Fig. 10A). In conclusion, the purported 'fibres' are in fact shadow effects caused by surface undulations in the rock and overlying layers prior to preparation (compare the shadows in Fig. 8A compared to Fig. 8B). The same error occurs in a more recent paper (Lingham-Soliar 2012, fig. 3), in which even lower-quality images of the same area are used to identify 'dermal tissues' showing 'fine horizontal geometric fibres' 'unique to dermal tissue' which are the sedimentary layering features already noted. In this paper, images of the whole specimen prior to the recent preparation are presented where the region of interest can clearly be seen to form part of the sedimentary halo shown here (Lingham-Soliar 2012, fig. 1). From a reconstruction in the same paper (Lingham-Soliar 2012, fig. 6) it appears that this halo has been misinterpreted as the remnants of the outline of the skin of the animal in the form of a frill. This has been used as evidence in support of a collagen affinity (Lingham-Soliar *et al.* 2007; Feduccia 2013). The halo present on NIGP

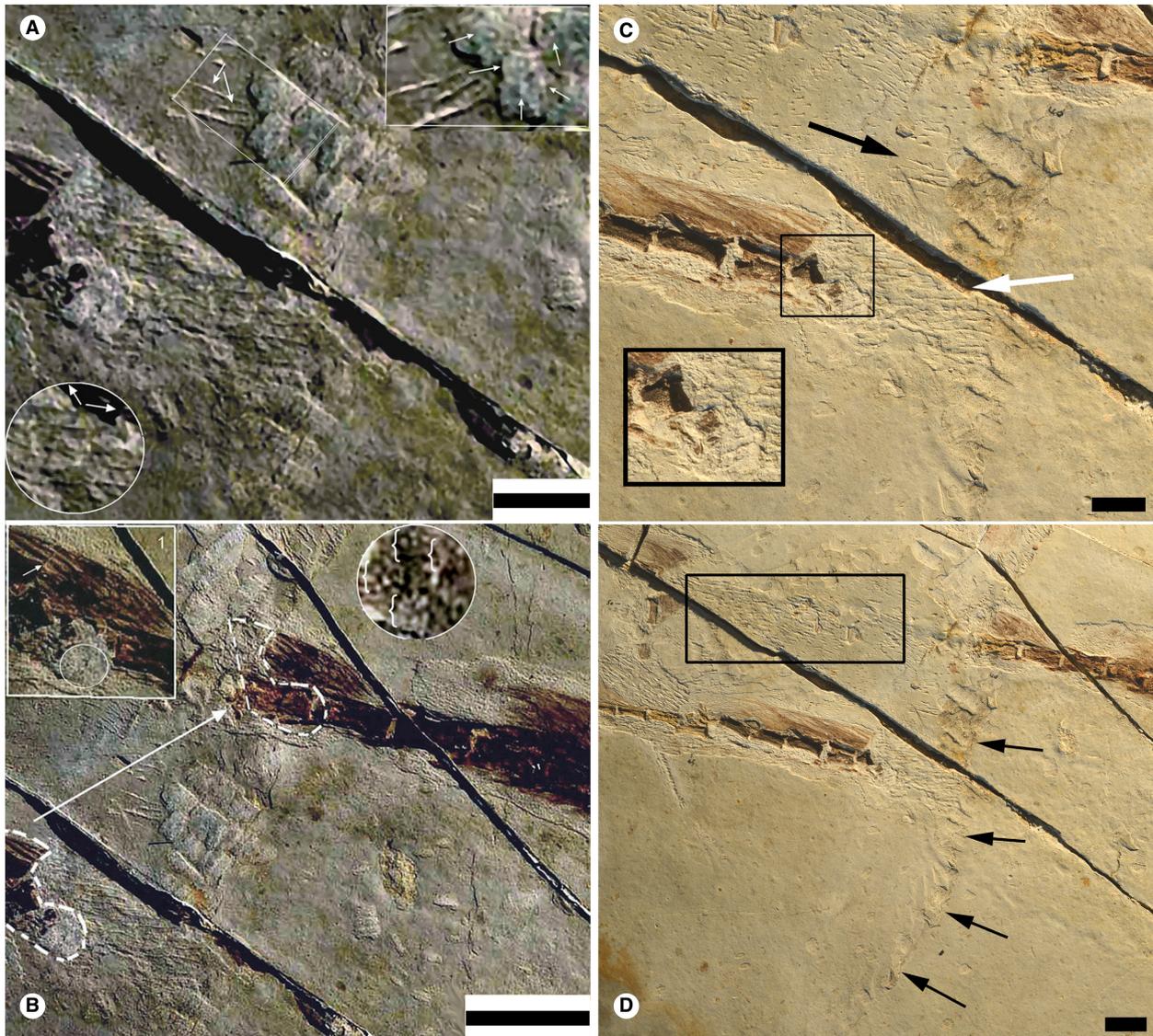


FIG. 6. Images of a break in the mid-tail of *Sinosauropteryx* specimen IVPP V12415 reported in Lingham-Soliar (2012, figs 3, 4) alongside new high-resolution images of the same area. A, region between the broken tail sections purportedly showing cross-oriented collagen fibres (inset circle) and scales with attachment fibres (inset rectangle). B, the same region showing purported organic scale preservation shown in the inset circle; despite attempts to highlight the scales and a papulose pattern using brackets, no clear structures can be made out due to the low resolution of the image. C, new high-resolution image of the same region, showing that the structures identified as cross fibres (white arrow) and scale attachment fibres (black arrow) in A are in fact preparation marks which appear throughout the matrix, probably made by a pneumatic air scribe; inset shows the area of purported scales in B with no clear structure being present. D, expanded view of the same area, showing potential sedimentary structures and undulations in the matrix misidentified as scales in A, running across the matrix far from the animal itself (black arrows); the highlighted rectangle shows extensive preparation scratches resembling fibres identified in A, which were blocked out of an original image in Lingham-Soliar (2013). Scale bars represent 10 mm in (A, C, D); 20 mm (B). A–B, reproduced with original labelling from Lingham-Soliar (2013) with permission from Springer. Colour online.

127587 is not seen in the other two fossils (Fig. 1), which both sit on a more uniform flat surface layer, highlighting the fact that only where the sediment layers are cut through in NIGP 127587 can the lines suggested as ‘fibres’ be seen. Shadowing effects on fossil specimens from

images in previous work have also been misinterpreted as genuine biological structures in a similar fashion, as noted by Mayr (2010).

The orientation of the genuine integumentary filaments (outwards and posteriorly along the body axis), conforms



FIG. 7. Evidence of preparation scratches likely to have been made by pneumatic air scribes in multiple specimens of *Sinosauropteryx* which exactly conform to structures previously identified as primary collagen fibres (Fig. 6). Black arrows show the orientation of the preparation scratches. A, anterior tail region of IVPP V12415 with long preparation marks running at the same angle as the integumentary structures. B, mid-tail region of IVPP V12415 showing further preparation scratches running parallel to the genuine integument. C, NIGP 127586 mid-tail region showing preparation scratches cross-cutting the genuine integumentary structures. All scale bars represent 10 mm. Colour online.

to pliable integumentary appendages such as feather homologues rather than collagen fibres, especially as they often curve back in towards the body distally, showing apparent flexibility (Currie & Chen 2001). This is most obvious in the tail region of IVPP V12415 (Fig. 4A, B), and is also clear in the tails of NIGP 127586 (Fig. 4D, E) and NIGP 127587 (Fig. 4C). A schematic figure in Lingham-Soliar *et al.* (2007, fig. 2f) ignored the actual pattern of orientation of the filaments and depicts them as being aligned straight and parallel, running posteriorly and outwards from the skeletal axis, in support of a structural collagen fibre affinity. This is in spite of the fact that the genuine orientation of the fibres is presented in a panel in the same figure (Fig. 2D, E). Our high-quality images of the filaments (Fig. 4) also clearly refute the suggestion that they become progressively more degraded towards their tips, a feature suggested to support identification as collagen (Lingham-Soliar *et al.* 2007).

Further misidentification of non-organic structures occurs through the use of low resolution images of NIGP 127587. Holes in the matrix which cut through the genuine integumentary structures were identified as neural spines with geometrically parallel fibres impressed into them (Lingham-Soliar 2012, fig. 3a). The new images show that these holes are not part of the theropod fossil nor do they show any evidence of fibre association (Fig. 10B). It is also claimed that a cartilaginous trachea is preserved in NIGP 127587 (Lingham-Soliar 2012, fig. 1). In fact, the high-resolution images show that no tracheal structure is present (Fig. 10C). In the original figure, a vague bend has been highlighted to create the impression of a structure, which the new images reveal as a joining of unrelated features of the matrix. It is also possible that the conchostracans that appear throughout the matrix were interpreted as tracheal rings (Li *et al.*

2007). Genuine tracheal preservation is rare, but has been found in some exceptional fossils including a mosasaur where tracheal rings were present in the throat region of the animal (Lindgren *et al.* 2010, fig. 3) and the theropod *Scipionyx samniticus* (Dal Sasso & Signore 1998). When present in fossils however, these tracheal features bear no resemblance to any structure seen in *Sinosauropteryx* (Dal Sasso & Signore 1998; Lindgren *et al.* 2010).

Evidence of scales in Sinosauropteryx

In IVPP V12415, a dark patch found at a break in the tail was identified as comprising four distinct scales with papulose surface patterns (Fig. 6B; Lingham-Soliar 2013, fig. 3). The original figure is of such low resolution however, that no features can be seen. Even using the highest resolution of the new images obtained, identifiable structures cannot be made out within the dark patch (Fig. 6C).

A further area is also claimed to represent scales, this time with 'attachment fibres' as further evidence of their affinity (Fig. 6A; Lingham-Soliar 2013, fig. 4). Superficially these appear more convincing, and scale-like shapes can be made out along with apparent fibres protruding from them (Fig. 6A). However, examination of the high resolution images reveals that these scales are sedimentary features of the matrix which continue not only across the tail break, as in the original figure, but right across the matrix far from the animal in a distinct linear arrangement; those furthest from the fossil show no scale-like structures (Fig. 6D). The claim that these structures also show a papulose pattern is incorrect, and no evidence of this is seen in the specimen or the new images (Fig. 6). Closer inspection of the high quality images also reveals that the proposed 'attachment fibres' are misidentified; these are clearly scratch marks,

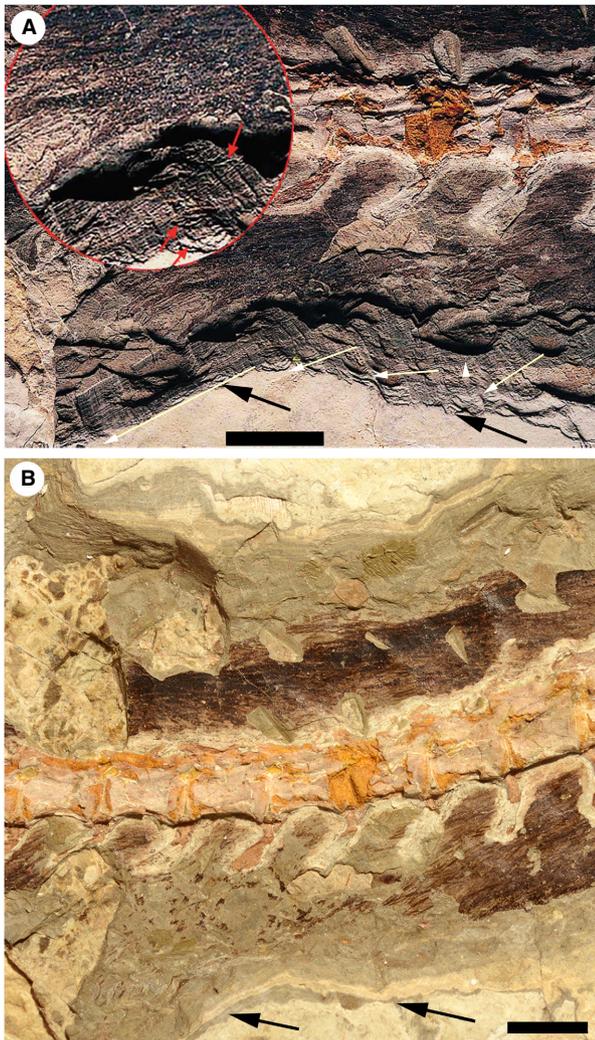


FIG. 8. The mid-tail region of NIGP 127587 before (A) and after (B) preparation. In the description of the integument in this region by Lingham-Soliar *et al.* (2007), large areas of sediment underlying the integumentary layer were misidentified as primary structures. Small arrows (A) were placed by Lingham-Soliar *et al.* (2007) to show apparent orientations and positions of fibres. From the new image (B) it can be seen that these are most likely to be shadow effects of the change in sediment layer height, the outline of which exactly conforms to the original image prior to preparation (large black arrows indicate the same area in both images). The transition from dark to light matrix is clear, and is also present on the dorsal side of the tail, forming a superficial halo around the animal. The genuine integument is markedly different, with a dark brown colour contrasting with the lighter matrix. Both scale bars represent 10 mm. A was originally published in Currie & Chen (2001) and is reproduced with original labelling from Lingham-Soliar *et al.* (2007) with permission from the Royal Society. Colour online.

probably made by an air scribe during the preparation of the fossil, and match those made throughout the matrix of the specimen (Figs 6, 7).

Along with the misidentification of preparation marks as collagen fibres in both *Sinosauropteryx* and *Stenopterygius*, similar features have been misinterpreted as biological structures in other important fossils, as in the critique of work showing feathers in an ornithomimid dinosaur from Canada (Van der Reest *et al.* 2016). Furthermore, preparation marks were misinterpreted in support of the view that integumentary structures on the ornithischian *Psittacosaurus* (Mayr *et al.* 2002) were collagen, in a critique by Lingham-Soliar (2010a, b) who misidentified a sand-blasted bristle as primarily ‘degraded’ (Mayr 2010).

CONTEMPORANEOUS JEHOLO FOSSILS REFUTE COLLAGEN PRESERVATION

Following these discussions, there is no evidence of scales on the known specimens of *Sinosauropteryx*. On the other hand, scaled dinosaurs have been found in the Jehol Biota, and so there is evidence available of what preserved scales look like in these lithologies. The ornithischian dinosaur *Psittacosaurus* shows a covering of well-preserved scales complete with pigment preservation (Mayr *et al.* 2002, 2016; Lingham-Soliar & Plodowski 2010; Smith *et al.* 2015; Vinther *et al.* 2016). Despite this, there is no evidence that any structures similar in appearance to the integumentary structures of *Sinosauropteryx* are present, other than long bristles on the dorsal side of the tail of *Psittacosaurus* (Mayr *et al.* 2016). It seems implausible that one scaled dinosaur would be preserved with no scales but with the underlying collagen fibre structure *in situ*, when another in the same deposit would have a covering of scales but no evidence of collagen preservation. Furthermore, the scales of *Psittacosaurus* are probably preserved because of the refractory properties of melanin (in melanosomes) and calcium phosphate, residues of which are embedded in mineral salts commonly found in the vertebrate epidermis, rather than through mineralization or organic preservation of collagen or keratin (Pautard 1964; Mayr *et al.* 2016; Vinther *et al.* 2016).

The exceptionally preserved feathers of stem birds in the same deposits show similarities with the integument of *Sinosauropteryx*, including pigment preservation, but no evidence of preserved collagen fibres (Clarke *et al.* 2006; Zhang *et al.* 2006, 2010; Wogelius *et al.* 2011). Furthermore, pterosaurs from the Jehol Biota show both dermal soft tissue preservation (actinofibrils) and integumentary appendages (pynofibres), which are preserved in different manners within individual specimens (Kellner *et al.* 2010). The actinofibrils are lightly coloured, geometrically arranged structures (herein shown not to exist in *Sinosauropteryx*) while the pynofibres are darkly coloured filaments similar to those of *Sinosauropteryx*, which are most likely to have been preserved by their

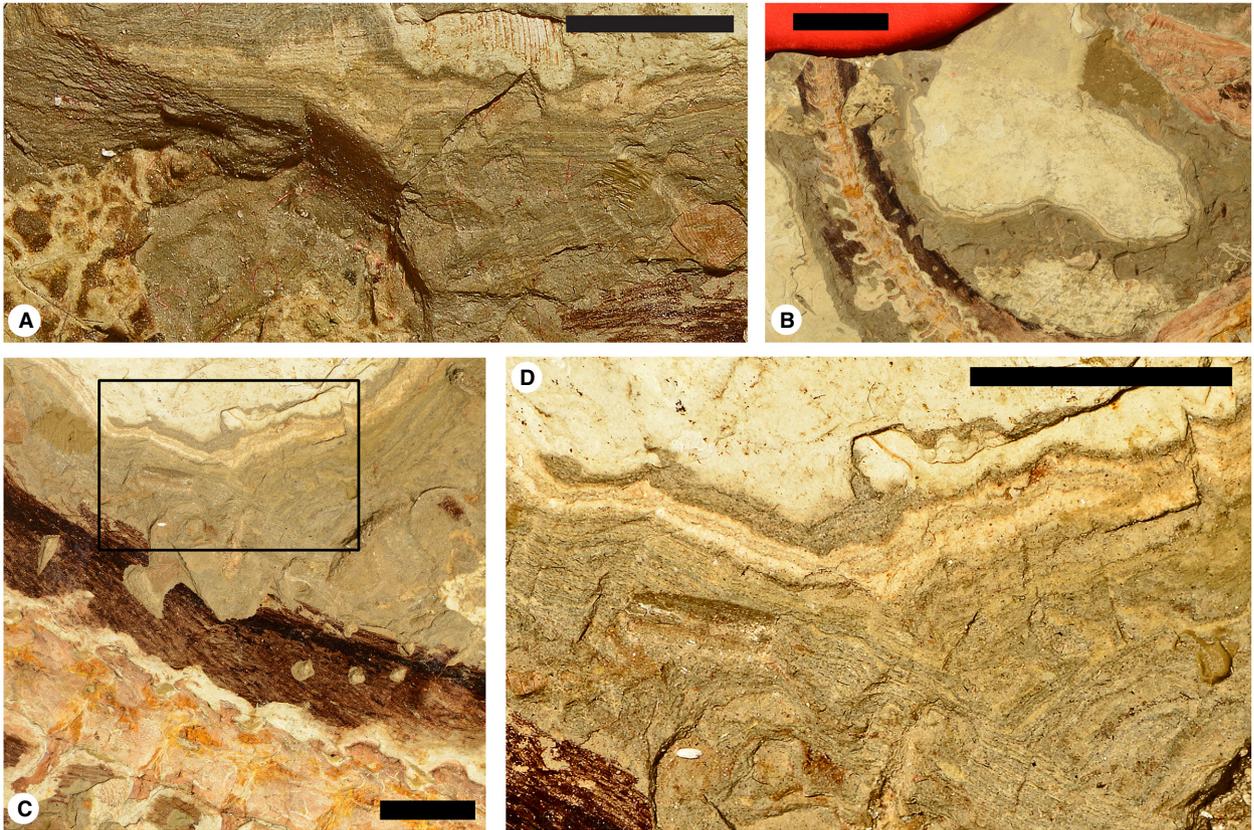


FIG. 9. The sedimentary halo surrounding NIGP 127587 which has previously been misinterpreted as a corona of skin around the animal. A, close-up of the area dorsal to the tail shown in Fig. 8; the distinct layers of matrix can be seen to form parallel rows, previously identified as collagen fibres from low-resolution images. B, the change in sediment colour from dark to light forming a halo around the entire animal previously misidentified as skin in Lingham-Soliar *et al.* (2007) and Lingham-Soliar (2012); areas of genuine integumentary preservation are clear from the dark brown patches close to the skeleton, the colour of which is most likely due to the presence of preserved melanosomes. C, region dorsal to the anterior tail, showing clear dark integumentary structures above the vertebrae, followed by the dark grey matrix which transitions to light grey/buff creating a series of parallel lines. D, close-up of the area highlighted in C, showing the parallel lines of sediment layers in detail, which conform to previous images in Lingham-Soliar *et al.* (2007) where they were identified as collagen fibres (Fig. 8); the clear dark integument can be seen in the bottom left-hand corner. Scale bars represent: 10 mm (A, C, D); and 50 mm (B). Colour online.

pigment content (Kellner *et al.* 2010). Original colour patterns have been found in pterosaur integument, indicating the high likelihood of pigment preservation (Vinther 2015a). The presence of pigment (via melanosomes) is a key piece of evidence uniting the integumentary appendages of vertebrates including theropods, ornithischians, pterosaurs and early representatives of Aves in the Jehol Biota. Thus far, no satisfactory alternative explanation for the dark colour of these structures has been provided (Vinther 2015a, b). Pan *et al.* (2016) conducted antibody immunization experiments which suggested that keratin could be preserved; however, this requires chemical testing to be confirmed. Melanosomes are characteristic for harbouring melanin in vertebrates and are often present in high abundance in hair and feathers (Vinther 2015a). They are now known to be the predominant reason that hair and feather outlines are

present in exceptional fossils, and are responsible for their preservation as organic (dark coloured) residues (Colleary *et al.* 2015; Vinther 2015a, b). Alongside morphological evidence of similarity between fossil and modern melanosomes, a number of recent papers have confirmed the presence of melanin in fossil integumentary structures chemically (Colleary *et al.* 2015; Gren *et al.* 2017; Pan *et al.* 2016). The melanosomes found in *Sinosauropteryx* conform to the morphology of modern phaeomelanosomes, which are known to impart rufous or chestnut hues to feathers (Zhang *et al.* 2010; Vinther 2015a). Criticisms of the presence of melanosomes in the integument of *Sinosauropteryx* (Lingham-Soliar 2011) are poorly supported, and include observations that a chestnut colour appears throughout the matrix, which does not conform to our understanding of the nature of pigment preservation and the taphonomy of melanin (Colleary *et al.* 2015;

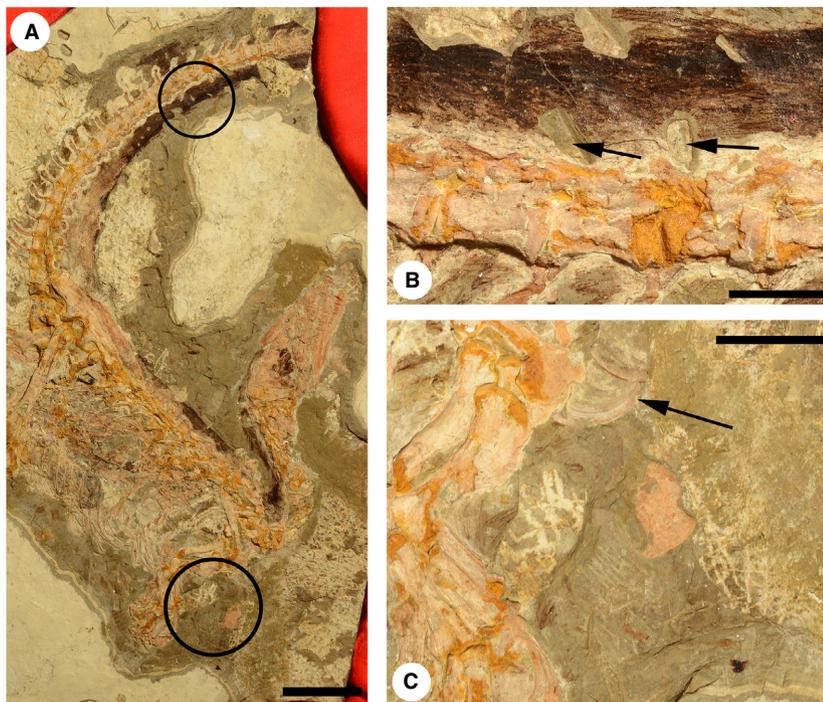


FIG. 10. Examples of misidentifications of non-biological structures in previous work using low-resolution images. A, NIGP 127587 showing the two areas where erroneous identifications have been made; note the sedimentary halo previously identified as the outline of the animal's integument and the starkly darker genuine integument. B, clear holes in the matrix, devoid of any primary structures (highlighted by black arrows) which were identified as neural spines by Lingham-Soliar (2012; fig. 3). C, area ventral to the cervical region of the animal, identified as a preserved trachea by Lingham-Soliar (2012; fig. 1) with only conchostracans potentially resembling tracheal rings at the centre top of the image (black arrows). Scale bars represent: 100 mm (A); and 10 mm (B, C). Colour online.

Vinther 2015a). Preserved pigments rarely retain their original colour through diagenesis, and no case is known where phaeomelanosomes provide rufous or chestnut colours in a fossil (Li *et al.* 2010; Colleary *et al.* 2015; Vinther 2015a). This can be seen in the integument of all specimens of *Sinosauropteryx*, which is preserved as dark brown-black pigment, differing markedly from any other features of the matrix except for the eyes and abdominal soft tissues (Fig. 1), which also contain abundant melanin in modern animals (Lindgren *et al.* 2012; Vinther 2015a). In *Sinosauropteryx*, the preserved dark integumentary pigment is confined to the filaments themselves, marking out their structure, and is not found outside the filaments as would probably be the case if claims that leaching from overlying skin were true (Lingham-Soliar & Plodowski 2010). Orange colours in the matrix are probably produced by iron oxides and are not related to biological pigments. Claims that SEM images of melanosomes in *Sinosauropteryx* resemble collagen 67 nm D-banding are based on low resolution expanded images of the originals, which show little more than groups of pixels highlighted to infer a desired pattern (Zhang *et al.* 2010; Lingham-Soliar 2011, fig. 2).

Recently, an ornithischian dinosaur (*Kulindadromeus*) from Siberia was described with both scales and feather-like structures preserved together, with extensive comparison made between the preservation and morphology of each structure type (Godefroit *et al.* 2014a). This is further evidence that scales and feathers have a similar propensity for preservation within specific deposits, and the specimens show that when scales and feathers are

preserved in the same specimen they can readily be distinguished. Reinterpretations of the feathers in *Kulindadromeus* as collagen fibres by Lingham-Soliar (2014) have been rebutted and cannot be considered likely in the absence of evidence (Godefroit *et al.* 2014b).

CONCLUSIONS

The debate about avian origins has generated a great deal of controversy in the past few decades. Despite an accumulation of substantial evidence that birds are dinosaurs, some voices continue to challenge this evidence. Our focus here has been on the papers that criticize the evidence for feathers in *Sinosauropteryx*, and we show that these studies have largely misinterpreted sedimentary and preparation structures as primary anatomical features. We believe that the use of low quality images instead of first-hand study of the specimens has made it difficult to determine which traces in the fossils might be bone, feathers, sedimentary features or preparation marks. Our examination of the specimens and use of high quality images has shown how these features have been misidentified. Our re-analysis shows that the studies arguing for collagen preservation in the integument of *Sinosauropteryx* and *Stenopterygius* are thus refuted.

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