

The evolution of the feather: *Sinosauropteryx*, a colourful tail

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The candy-striped dinosaur. At least Winnie the Pooh accepted he was deluded when he was found chasing his own tracks, "I am a bear of very little brain." Compliments of T. Lingham-Soliar

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Abstract A recent development in the identification of feathers in fossils by means of melanosomes was used to suggest that structures observed in an SEM of a filament in the basal theropod dinosaur, *Sinosauropteryx*, were phaeomelanosomes and that they represented conclusive evidence that the filaments were early feathers. At the most basic level, the claims of phaeomelanosomes are shown here to be founded on an optical illusion created when the SEM is reproduced at low image size—viewed at larger image size ($\sim 2\times$ original) the structures are nondescript in both size and shape and impossible to equate with phaeomelanosomes. At a higher level of investigation, the study is seriously questioned for ignoring standard scientific protocol: despite size and shape being critical to the identification of the phaeomelanosomes, no statistically viable measurements of the structures (particles) were made—the measurements, which are simply conjectured, are shown here to be incorrect in the speculated sizes, and in shapes; inferences made on vital characters from birds and advanced non-avian dinosaurs, e.g. with respect to colour banding, are without confirmation in the test animal but conjectured on circular argumentation; alternative arguments, e.g. that the particles might be bacteria or colour from the overlying skin, are peremptorily dismissed or not considered;

suggestions that the particles are embedded within the filament are without support since there is no evidence of cross-sections or tangential sections either made or occurring serendipitously—only a single section is reported, apparently of the filament's surface. False dichotomies such as, if the structures are not bacteria they must be melanosomes, are questioned given that one of the most important factors in the taphonomy of ancient (structures in question, ~ 130 MYR) fossilised filaments i.e., decomposition—that the structures might reasonably represent the degraded remains of the filaments—is not even considered. Here, from experiments on the decomposition of native collagen in fish and reptilian dermis, SEMs of their ultrastructure show that distinctive spherical, elliptical or oblate particles, even more so than those figured in *Sinosauropteryx*, typically form during degradation. This is confirmed in SEMs of degraded collagen fibres in a 225-MYR ichthyosaur fossil, virtually point by point. In addition numerous small bead-like structures in the filament of *Sinosauropteryx* bear a striking resemblance to the unique 67-nm D-bands of collagen, in both shape and size. This paper does not question the value of scientifically meritorious identifications of melanosomes, as indeed of collagen and keratin, in interpreting the integumental structures of fossil animals. However, allegations of phaeomelanosomes in *Sinosauropteryx* are shown to be without scientific merit.

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Zusammenfassung Eine kürzlich entwickelte Methode zur Bestimmung von Federn in Fossilien mit Hilfe von Melanosomen wurde zur Behauptung herangezogen, dass Strukturen, die in einer REM-Aufnahme eines Filaments

von einem basal stehenden, theropoden Dinosauriers, *Sinosauropteryx*, Phaeomelanosomen seien und sie den endgültigen Beweis erbrächten, dass diese Filamente frühe Federn darstellten. Auf einfachstem Niveau zeigen wir hier, dass der angebliche Nachweis von Phaeomelanosomen auf einer optischen Täuschung beruht, die entsteht, wenn eine REM-Aufnahme bei geringer Auflösung kopiert wird – betrachtet man die Strukturen vergrößert ($\sim 2\times$ Originalgröße) sind die Strukturen sowohl in Größe, als auch in Form undefinierbar und unmöglich mit Phaeomelanosomen in Deckung zu bringen. Auch auf höherer Ebene betrachtet, stellen wir die Studie ernsthaft in Frage, weil sie wissenschaftliches Standardprotokoll ignoriert: Obwohl für die Bestimmung der Phaeomelanosomen Größe und Form unbedingt notwendig sind, wurden von den Strukturen (Partikeln) keine statistisch verwendbaren Messungen durchgeführt, und wir zeigen hier, dass die Messungen, die schlichtweg gemutmaßt sind, weder in der Größe noch in der Form korrekt sind; Rückschlüsse, die aus Lebendmerkmalen rezenter Vögel und höher entwickelter, nicht-aviärer Dinosaurier z.B. bezüglich von Farbbänderungen gezogen werden, sind am Testorganismus nicht bestätigt, sondern spekulativ auf einem Zirkelschluss beruhend; Alternativbehauptungen, dass die Partikel beispielsweise Bakterien oder Farbe der darüber liegenden Haut sein könnten, sind mutwillig weggelassen oder nicht in Erwägung gezogen; der Vorschlag, dass die Partikel in das Filament eingebettet sind, bleibt ohne Untermauerung, da es keinerlei Hinweise auf durchgeführte oder zufällige Quer- oder Tangentialschnitte gibt – lediglich eine einzige Sektion wird vorgestellt, offensichtlich von der Oberfläche des Filaments. Wir stellen die fälschlicherweise dichotom geführte Argumentationen, wie etwa, wenn die Strukturen keine Bakterien sind, müssen es Melanosomen sein, deshalb in Frage, weil einer der wichtigsten Faktoren in der Taphonomie urzeitlicher (besagte Strukturen, ~ 130 Millionen Jahre), fossilisierter Filamente, d.h. die Degradation – dass die Strukturen begründbar die degradierten Überreste von Filamenten darstellen könnten – nicht einmal in Erwägung gezogen wird. Aus Degradierungsexperimenten von natürlichem Kollagen in Fisch- und Reptilienhaut zeigen hier REM-Aufnahmen von deren Ultrastruktur, dass charakteristische sphärische, elliptische oder abgeflachte Partikel, sogar noch deutlicher als die bei *Sinosauropteryx* festgestellten, typischerweise während der Degradation entstehen. Dies wird noch quasi Punkt für Punkt durch REM-Aufnahmen von degradierten Kollagenfasern eines 225 Millionen Jahre alten Ichthyosaurier Fossils bestätigt. Zusätzlich zeigen zahlreiche perlenartige Strukturen im Filament von *Sinosauropteryx* in Form wie in Größe eine hochgradige Ähnlichkeit zu 67 nm D-Einzelbändern von Kollagen. Diese Publikation stellt nicht den Wert wissenschaftlich fundierter Nachweise von

Melanosomen, oder die von Kollagen und Keratin für die Interpretation von Integumentstrukturen fossiler Tiere in Frage. Behauptungen zu Existenz von Phaeomelanosomen bei *Sinosauropteryx* erscheinen allerdings wissenschaftlich wertlos.

“If the difficulties [of science] are intentionally concealed, or merely removed by palliatives, then sooner or later they burst out into incurable mischiefs” (Kant 1788).

Introduction

How the feather evolved has attracted widespread attention. Zhang et al. (2010) recently investigated the presence of melanosomes in the integumental structures of certain non-avian dinosaurs and fossil birds (Vinther and Briggs 2008). Finding melanosomes deep within such integumental structures may be taken to imply that they are feather homologues. In the dromaeosaurs examined, the group that birds are generally assumed to have been derived from (considered by some to be birds), the images are interesting, albeit not unexpected in the light of a similar study in a fossil bird (Vinther and Briggs 2008). However, what is remarkable, if true, is Zhang et al.’s (2010, fig. 3c; my Fig. 1, see below) claim that melanosomes were found embedded inside the integumental structures of the basal theropod dinosaur *Sinosauropteryx*, considerably removed phylogenetically from the advanced dromaeosaurs or the troodontids (James and Pourtless 2009; Hu et al. 2009). Melanosomes found embedded inside the integumental structures of *Sinosauropteryx* would be sound evidence, not only that the integumental structures in *Sinosauropteryx*, which are not obviously feathers, are primordial feathers but that they occur in a basal coelurosaurian dinosaur. Thus, *Sinosauropteryx* was

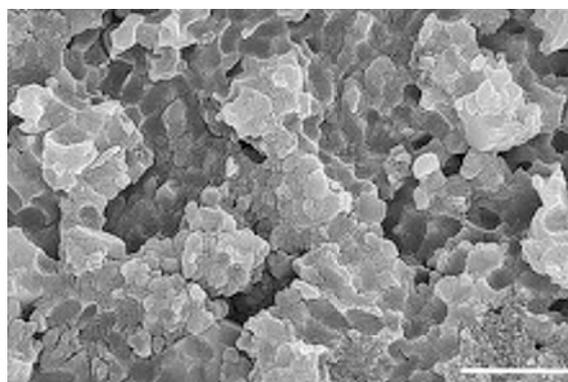


Fig. 1 SEM. Reproduced from Zhang et al.’s (2010) figure 3c. (Reprinted with permission from Zhang et al. 2010. Copyright Macmillan Publishers, Ltd.)

clearly the singular factor of the study that made it *Nature*-worthy, a fact emphasised by it alone being singled out in the paper's abstract (Zhang et al. 2010), *Nature*'s editorial (Gee 2010), and by the extensive media coverage devoted to colour in *Sinosauropteryx* (e.g. BBC's *Science in Action* and *The New York Times*). Widespread claims that the integumental structures of *Sinosauropteryx* are feather homologues are not new and had provoked a study that was highly critical of earlier alleged evidence (Lingham-Soliar et al. 2007 and references therein). The latter study, which included a new specimen of *Sinosauropteryx* and several facets of evidence, roundly concluded that the integumental structures were probably collagen structural fibres (of, e.g., display crests/fringes) and not feather homologues (Chen et al. 1998; Currie and Chen 2001). It is imperative, therefore, that new alleged evidence of melanosomes in *Sinosauropteryx* should stand on its own merits, given the basal theropod status of *Sinosauropteryx* as opposed to that of the dromaeosaurid and troodontid dinosaurs, and, consequently, the profound biological and evolutionary ramifications with respect to feather and bird origins.

The present study questions the methodology used and the analysis of the alleged phaeomelanosomes in the integumental structures in the tail of *Sinosauropteryx* (Zhang et al. (2010). Importantly, relevant stages of investigation in support of their hypothesis are often unclear or entirely absent and relevant alternative hypotheses are peremptorily dismissed or not considered. Crucially, they make major assumptions in *Sinosauropteryx* based on results on melanosomes either from the dromaeosaurid dinosaurs and fossil birds from their own results or from those of other workers (Vinther and Briggs 2008). This is scientifically unfeasible. It must be emphasised, however, that the present study's criticism of phaeomelanosomes in *Sinosauropteryx* may in no way be construed as a comment one way or the other of such structures in other non-avian dinosaurs and fossil birds nor of the hypothesis of the dinosaurian origin of birds (Lingham-Soliar et al. 2007, p. 1823). With this in mind, the new proposed evidence of melanosomes or more specifically phaeomelanosomes as irrefutable evidence of feathers in *Sinosauropteryx* is examined and alternative evidence presented.

Testing the hypothesis of fossilised melanosomes in *Sinosauropteryx*

(Material and Methods, see ESM).

Zhang et al. (2010, p. 1077) state, "occurrence of melanosomes embedded inside the filaments of Jehol non-avian dinosaurs thus confirms that these structures are unequivocally epidermal structures, not the degraded

remains of dermal collagen fibres, as has been argued recently [Lingham-Soliar 2003; Feduccia et al. 2005; Lingham-Soliar et al. 2007]." Central to their hypothesis of melanosomes in the non-avian dinosaurs and birds they examined, they state that it is essential to distinguish between melanosomes and bacteria, given that they "are generally similar in size (one micrometre or less) and shape (spherical, oblate or elongate)." They propose three criteria by which this may be achieved:

(1) Structures interpreted as melanosomes conform to size and shape, are preserved inside the feathers and like degraded keratin not a superficial coating as bacteria; (2) the melanosomes occur only in dark bands, contrary to bacteria; and (3) packing and layering of melanosomes is unlike that of bacteria.

Zhang et al.'s arguments will be considered first.

Argument 1 The authors rightly believe that, in order for the structures to be identified as melanosomes, they have to be conclusively distinguished from bacteria (albeit this is only one alternative). While it is true that bacterial presence in some fossils has been observed as a superficial coating or halo, there are no ultrastructural studies that have investigated the fossilised integumental structures of the Chinese dinosaurs with respect to microbial decomposition in deeper layers of the integument. Zhang et al. (2010) refer to the absence of the calamus and proximal part of some filaments in certain Jehol dinosaurs as evidence that they lacked melanosomes and that they were not a consequence of bacterial decay i.e., "[t]here is no reason to suppose that a film of keratinophilic bacteria would have developed elsewhere over the surface of the feather, but not on these parts, nor could their absence imply that these portions were buried in the skin and so escaped bacterial replacement." Incidentally, this is terribly flawed reasoning—the authors surely cannot question that the calamus must possess structure, especially if lacking melanosomes? Hence, if it is accepted that the calamus has structure not connected with melanosomes and it is missing in certain Jehol dinosaurs, what do Zhang et al. (2010) suggest is responsible for its absence, if not bacteria or other microorganisms? However, let us consider some viable alternative explanations in fossil birds and non-avian dinosaurs for this entire question of melanosomes versus bacteria. Bacteria, which themselves, as all other organic matter, while occasionally preserved, invariably also suffer the effects of degradation and consequently leave little or no trace of their activities (Lingham-Soliar et al. 2010, figure 1)—otherwise, the consequences would be disastrous; the earth would be engulfed by bacterial remains. Put simply, a bacterial film may give evidence of bacterial activity but bacterial activity may not give evidence of a bacterial film (as the Mad Hatter remonstrated with Alice 'You might

just as well say that “I see what I eat” is the same thing as “I eat what I see”!). Furthermore, with respect to absence of the calamus, as has frequently been mentioned in the literature by supporters of protofeathers, the reason that there are few traces of integumental structures “against the skin and other tissues” of fossil birds and non-avian dinosaurs is because they were “destroyed as the flesh decomposed” (Currie and Chen 2001, p. 1723), which would reasonably include the calamus “buried in the skin” and proximal parts of the filaments rather than the more distal parts on the sediment matrix. Nevertheless, absence of the proximal regions of the integumental structures has not been a notable feature in *Sinosauropteryx* (Currie and Chen 2001; Lingham-Soliar et al. 2007, see holotype and IVPP V12415, respectively). Purely, with respect to the dichotomy proposed by Zhang et al. (2010), i.e. if the structures in the filaments are not bacteria then they must be melanosomes, the reasoning is again flawed. Even if the filaments represent feathers, there are alternative explanations. The calamus in the first stage of one model of feather evolution proposed (Xu et al. 2001; Prum and Brush 2003), as in the modern feather, is considered to be hollow, a characteristic that should account for more ready degradation compared with distal parts of the integumental structures. The modern feather rachis, unlike the calamus, has a central medulloid pith that provides considerable biomechanical strength as well as strong resistant to biodegradation (Lingham-Soliar et al. 2010).

The filaments in *Sinosauropteryx* have consistently been regarded as factually hollow (e.g. Xu et al. 2001) ever since speculations by Chen et al. (1998). However, Zhang et al.’s (2010) SEM fails to confirm this despite their allegation that it represents an inside view of the filament and given that a hollow filament in *Sinosauropteryx* was cited as a key structure in support of stage 1 of a proposed model of feather evolution (Prum in Xu et al. 2001). There is no mention either of Xu et al.’s (2001, p. 203 and figure 6) alleged support from Chen et al. (1998) for another vital character of stage 1 of the model i.e., “UNBRANCHED [my emphasis] integumental appendages.” This is a serious mischaracterisation that was repeated subsequently (Prum and Brush 2003, p. 92). Chen et al. (1998, p. 152) described the filaments of *Sinosauropteryx* quite unambiguously as “multibranched integumentary structures” and, even in the penultimate line of the text as, “branched structures.” Such mischaracterisations of vital characters in a model, no less, of feather evolution do not engender confidence in this controversial field.

Argument 2 Identification of phaeomelanosomes? If the colour stripes in *Sinosauropteryx* are to be seriously considered then their claim (Zhang et al. 2010) that they are based on empirical evidence needs examination. First, the

authors have shown a single image of alleged melanosomes in an area we are obliged to infer is from what they refer to as the “dark-coloured stripes” in the tail filaments of “chestnut to rufous (reddish-brown)” hue. The authors’ “Methods summary” on *Sinosauropteryx* (5 lines) shows no information on how the material was tested (space constraints are no problem in this day and age with ample opportunity to include exhaustive data online). Judging from their figure 3b (inset), the filaments they depict are approximately 2 mm in length allowing potentially ~150–200 sectors of comparable dimensions to their figure 3c for investigation. Yet, besides the section represented in their figure 3c (Zhang et al. 2010; my Figs. 1 and 2), there is no evidence of even one other section being examined [e.g. with even greater constraints with respect to limited material in an ichthyosaur, data on 329 D-bands in 38 individual fibrils were collected (Lingham-Soliar and Wesley-Smith 2008)—essential in any attempt to verify any critical morphological structure]. An isolated observation based upon which there are profound evolutionary ramifications raises serious questions that will be dealt with in the course of this study. Second, because size and shape are the only physical properties that Zhang et al. (2010, p. 1077) use to define the structures as melanosomes, supporting data are imperative. Despite this, the authors merely allude to the size of the melanosomes as, “most are between 500 and 700 nm long (occasionally up to 900 nm) and 300 and 600 nm wide.” There are no vital, basic statistical data of measurements, e.g. numbers measured (n) their mean (\bar{x}) and standard deviation (SD) or how the ranges were obtained. Hence, it is clearly speculation. Certainly, there is no record of melanosomes conforming to the highly irregular and random shapes of the alleged phaeomelanosomes. Zhang et al.’s (2010) implication of some form of uniformity of particle shape and size is incorrect, which, however, would have made the particles more easily measureable than they actually are. Nevertheless, despite the obvious difficulties, measurements are made here [electronic supplementary material, ESM, Material and Methods). My results show a mean length of 302.06 nm ($n = 55$, SD = 88.18, min = 166.82, max = 571.87) and mean width of 222.6 nm ($n = 55$, SD = 74.96, min = 98.9, max = 492.55); ESM Table 1]. These are very different from Zhang et al.’s (2010) speculations. Third, Zhang et al. (2010) claim to have identified dark stripes in the integumental structures of *Sinosauropteryx* but data are strikingly absent. There are no SEM results for the lighter bands nor even any indication that they were even tested nor the precise area in the depicted filaments from which the SEM image came (by either high power optical microscopy or low power SEM such as, e.g., the use of high- and low-power SEMs to contextualise fibril structure within a collagen fibre (Lingham-Soliar and Wesley-Smith 2008; see

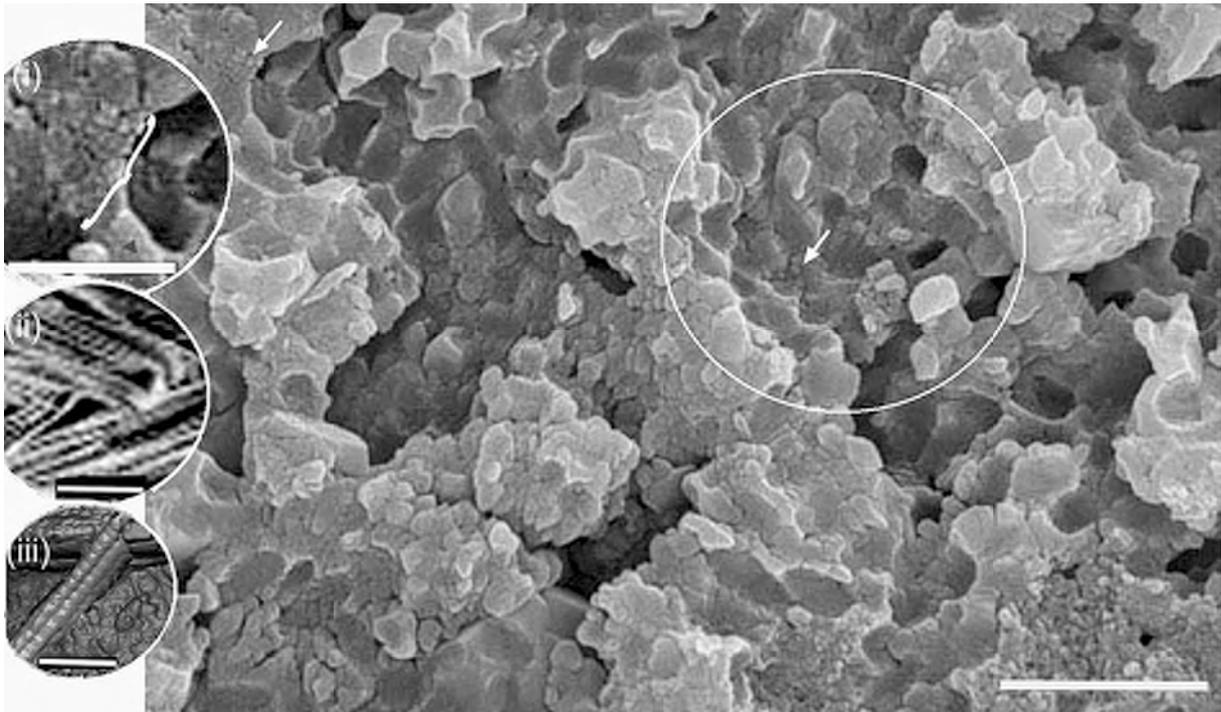


Fig. 2 SEM. Reproduced from Zhang et al.'s (2010) figure 3c at $\sim 2\times$ the size printed in their article (see Fig. 1). In the *bottom right*, biodegradation of the structures has advanced even further with the particle size 70–100 nm in diameter. In *inset i*, small bead-like

structures in parallel rows are reminiscent of 67 nm *D*-banding of collagen e.g. in an ichthyosaur, *inset ii* and rat, *inset iii* (see Fig. 5, text, and ESM Table 1). Scale bar 2 μm ; insets *i* 1 μm , *ii* and *iii* 0.5 μm

Fig. 3a, below). Their optical microscopic image of the filaments (Zhang et al. 2010, figure 3a, b, plus inset) shows minimal information and, in particular, no detail whatsoever of their external structure nor the alleged colour banding. With respect to the light part of the alleged stripes, there are no data at all. Are the stripes formed by white alternating with rufous colour in each filament or do entire rufous coloured filaments alternate with entire white filaments? Either way, since the white filaments lack melanosomes what is their structure, or are they structure-less, if so then like the calamus, what holds them up? Zhang et al. (2010, p. 1076) state it “has been shown (Vinther and Briggs 2008, figure 1a) that eumelanosomes occur only in dark bands of banded FEATHERS [my emphasis], and not in light bands” (the white bands show no structure). However, that specimen refers to black and white areas of a single fossil feather found in an entirely different formation in Brazil and cannot be used as a general rule. Nevertheless, it is a circular argument, i.e., the proposition that the filaments in *Sinosauropteryx* are feathers is being used as proof of its own conclusion i.e. it would first have to be shown that there *are* striped patterns in *Sinosauropteryx* before the comparison is made with feathers from a bird or even a dromaeosaurid or troodontid dinosaur. Furthermore, it would seem curious that only melanosomes would be preserved in the Jehol vertebrates and not the robust structural

protein of feathers i.e., keratin given that we know that dermal collagen (identified by its unmistakable multi-layered, geometrically precise architecture in typical alternating right- and left-handed weft), a less robust protein than keratin, was preserved in e.g. *Psittacosaurus* (Lingham-Soliar 2008, figure 2b, c), *Sinosauropteryx* (Lingham-Soliar et al. 2007, figure 4) and *Xianglong zhaoi*, a gliding lizard (as filaments; Li et al. 2007), all from the Early Cretaceous Jehol Group of China. Thus, crucial evidence for Zhang et al.'s (2010) proposals for stripes in *Sinosauropteryx* is not produced.

Argument 3 The idea of packing and layering of the structures identified in *Sinosauropteryx* is not apparent in Zhang et al.'s (2010) figure 3c, which simply shows a compressed mass of structures, which may or not have been systematically layered (highly unlikely given the considerable discrepancies in size of the structures as layering implies a consistency of thickness at least in each layer). Furthermore, despite suggesting layering of the melanosomes in *Sinosauropteryx*, at no point do Zhang et al. (2010) provide information on how this idea is supported because they provide no evidence that cross-sections or tangential sections of the integumental structures were either made or had occurred serendipitously during the fossil's taphonomic history. Thus, with no evidence to the contrary, it is

reasonable to conclude that the idea of layering of the alleged melanosomes is based solely on surface examination of a single section of a single filament and that there is little evidence for their claim that “the bodies occur embedded inside the feathers” and not as “a superficial coating” (Zhang et al. 2010, p. 1076, figure 3c). With respect to the SEM image, even if we accept that layering of even the largest alleged “globular” structures (900 nm long) occurred in at least 3–4 layers, this would only account for a depth of 3–4 μm in a filament $\sim 80 \mu\text{m}$ in diameter (Lingham-Soliar et al. 2007), i.e. $\sim 5\%$ thickness. This is no more than superficial and not the “inside” view proposed. Nevertheless, despite lack of evidence for systematic layering (Zhang et al. 2010, figure 3c) the concept that layering might only occur in melanosomes is faulty as shown in microbial degradation of feather keratin (Lingham-Soliar et al. 2010, figure 1; Lingham-Soliar and Glab 2010, figure 1a), which occurs through the depth of the fibre-matrix texture, a phenomenon that is clearly not unique to melanosomes. Furthermore, with respect to collagen and keratin, the fibre bundles, fibres and fibrils comprise successive layers, hence not only would bacterial degradation occur in such layers but also the degraded fibre/fibril remains (below).

Besides the above arguments, it is worth mentioning in parenthesis, Zhang et al.’s (2010) failure to consider pigment from the epidermis overlying structures below as was recently demonstrated in bone and cartilage in *Psittacosaurus* (Lingham-Soliar and Plodowski 2010). Zhang et al.’s (2010) figure 3a shows considerable occurrences of pigment associated with the specimen of *Sinosauropteryx*, including overlying of bone. However, despite little evidence to uphold Zhang et al.’s (2010) dismissal of other possibilities for the structures in *Sinosauropteryx*, besides melanosomes, the question is academic. Zhang et al.’s (2010, p. 277) arguments do not bear scrutiny at a much more basic level—the observations and conclusions of “ovoid to sub-spherical” structures of specific shape and size are based on an optical illusion. The image as presented in their figure 3c (Fig. 1) certainly gives the impression the authors suggest—but only at low image size. However, by no more than doubling the size of the image as in my Fig. 2, it becomes very clear that there is a mishmash of indefinable shapes and sizes with only a small number that could be interpreted as spherical to oblate and conforming to the sizes mentioned by the authors.

Fundamentally, Zhang et al. (2010) fail to consider one of the most important phenomena with respect to the fossilisation of soft tissue—decay and degradation (the byproducts or consequences of, not just the agents i.e., microbes), a failing that is likewise reminiscent of other interpretations of protofeathers (discussed in Lingham-Soliar 2003). Figure 2 clearly shows ongoing degradation,

i.e. larger structures being broken down further into smaller and smaller particles (the smallest the diameter of a collagen fibril *D*-band), besides numerous particles of indefinable random shapes. An appreciation of how protein structural fibres such as collagen degrade may shed further light on this.

Fossilised melanosomes or degraded structural fibres (collagen?) in *Sinosauropteryx*?

It is worth noting that the burden of proof that the fossilised particles in the integumental structures of *Sinosauropteryx* are phaeomelanosomes ultimately rests with Zhang et al. (2010) and not whether or not counterarguments can prove the particles represent something else—i.e. proof by default. Nevertheless, I shall examine viable counterarguments.

Collagen fibres are typically about 4–20 μm in diameter and are comprised of hundreds of fibrils. For more effective structural units, e.g. in the skin and in control surfaces or display organs of many animals, the fibres are usually grouped into thicker bundles frequently between 50 and 400 μm thick (the thickest recorded is $\sim 1,000 \mu\text{m}$ in the white shark, *Carcharodon carcharias* (Lingham-Soliar 2005). Fibre bundles were estimated at 80–120 μm in diameter in *Sinosauropteryx* (Lingham-Soliar et al. 2007). Fibre bundles in *C. carcharias* (some approximately the same thickness as in *Sinosauropteryx*) show multiple layers of fibres and fibrils through a serendipitous fracture (Fig. 3a). Despite degradation, a collagen fibre from the 200+ MYR ichthyosaur, *Ichthyosaurus* (Lingham-Soliar and Wesley-Smith 2008), reveals in striking detail the fibril structure in an angled partial longitudinal and transverse view (Fig. 3b).

Hence, expecting evidence with respect to Zhang et al.’s (2010) statement that the melanosomes lie embedded within the filament is not extraordinarily demanding.

Rather than simply speculate, an understanding of some of the stages of collagen decay is considered vital. In decomposing *Caretta caretta* tissue (ESM Material and Methods) fibril bundles start to break up into globular units of approximately 200–1,000 nm (Fig. 4, arrowheads) and with further decay even the *D*-bands of fibrils (Lingham-Soliar 2003, 2008) break-up into “beads,” as in a broken rosary, of $\sim 64 \text{ nm}$ diameter (identified by x-ray diffraction; Lingham-Soliar and Glab 2010, figure 4). In addition to the larger particles, numerous globular structures, identified here, of $\sim 66 \text{ nm}$ in diameter in Zhang et al.’s (2010) *Sinosauropteryx* (Fig. 2, arrows and elsewhere in the figure) emphasise the state of decay represented by the section. Presence of numerous globular structures in a single collagen fibre, e.g. that of *Caretta caretta*, indicates the potential for many more in a collagen fibre bundle

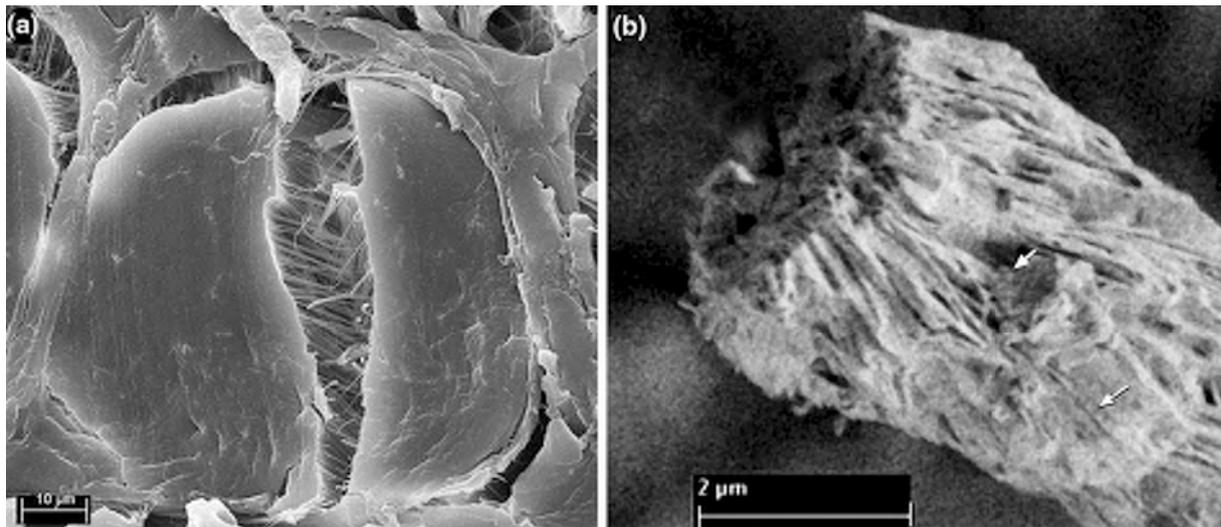


Fig. 3 SEM. Collagen. **a** transverse section of a structural collagen (type 1) fibre bundle from the dermis of *Carcharodon carcharias* show the internal fibrils through a crack. **b** Transversely sheared collagen fibre

from *Ichthyosaurus*; the angle allows a view of the fibrils in both longitudinal and transverse planes (*arrows* show beading in some fibres (see text))

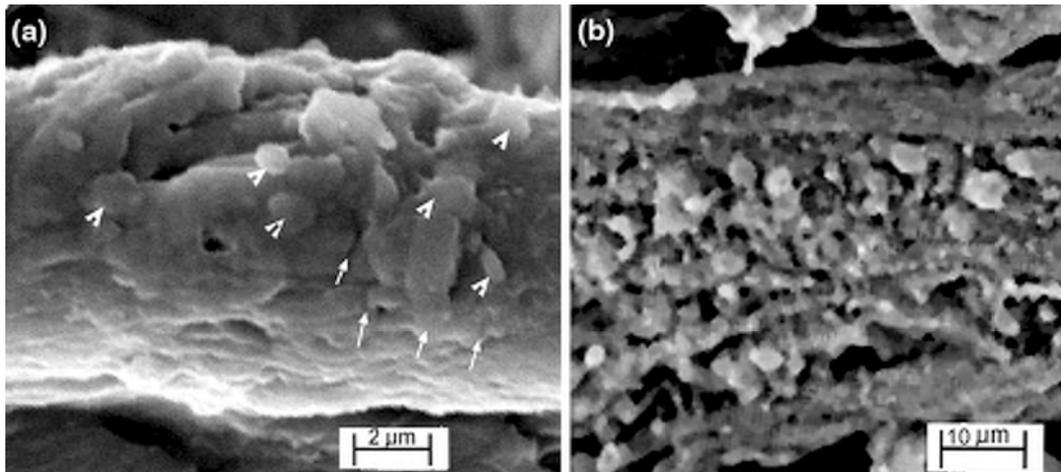


Fig. 4 SEM. **a** Part of a decomposing collagen (type 1) fibre from *Caretta caretta*, in longitudinal view. At the *bottom*, the fibres can be seen as waves (*arrows* show compacted bundles of fibrils breaking up into globular units; *arrowheads* show globular structures

~300–800 nm diameter). **b** The surface of a small collagen fibre bundle of the mako shark (*Isurus oxyrinchus*) showing widespread decay and numerous globular structures that include probable microbes

(a small fibre bundle of ~80 µm, as, e.g., in *Sinosauropteryx*, would comprise over 100 fibres; see Lingham-Soliar 2003, 2005). One reason for the formation of the globular structures (Fig. 4, arrowheads) lies in an important change that occurs in the morphology of the collagen fibre following the death of an animal, i.e. loss of muscle tension. Consequently, the collagen fibres, and frequently bundles of fibres, contract and become more bulbous giving an overall wavy appearance (Fig. 4a, bottom, also arrows; Fig. 4b shows even larger globular structures in addition to numerous small ones). With continued decay, the bulbous structures break off at the constricted points, initially into

globular structures, which, with further degradation, become less distinctive in shape and size. Thus, given a hierarchy in the filaments, of fibre bundles, fibres, down to fibrils (*D*-bands), the permutation for the formation of globular structures during decay of widely varying diameter is understandably high.

The decay and breakdown of collagen as observed in the laboratory on *Caretta caretta* (Fig. 4a) is recognised almost point by point in an incidental SEM image of a collagen fibre in a 200+ MYR specimen of *Ichthyosaurus* (Fig. 5), an emphatic endorsement of the value in attempts to reconstruct conditions of biological decomposition in the

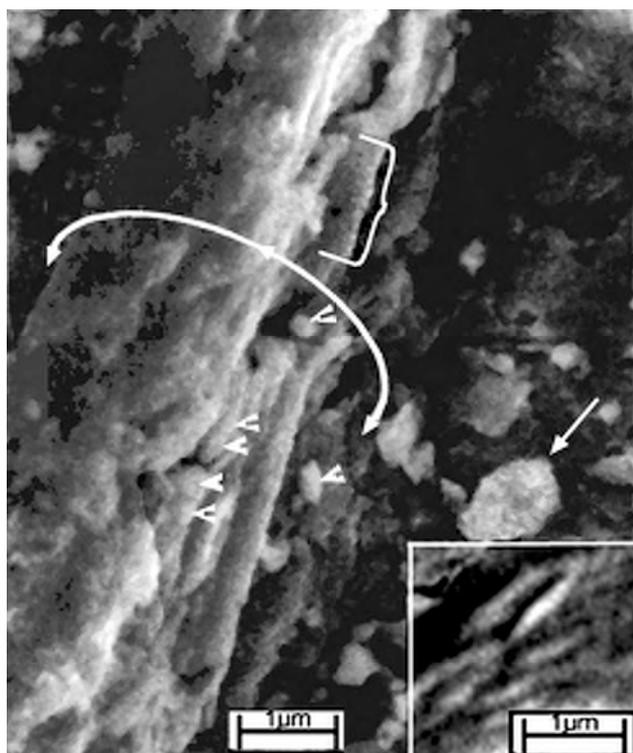


Fig. 5 SEM. Part of a collagen fibre from *Ichthyosaurus* in longitudinal view. *Arrowheads* show some sub-spherical and ovoid structures breaking away. *Bracketed area* shows 67 nm *D*-banding of fibrils giving the impression of fine beads. To the *right*, a large globular structure ($\sim 1.0\ \mu\text{m}$ long, *arrow*) has broken off the collagen fibre (comprising traces of the fibril *D*-banding). *Inset* sinuous fibrils, twisted into shorter strands are seen with traces of *D*-banding

laboratory in order to shed light on the taphonomy of fossilised soft tissue hundreds of millions of years old. Zhang et al.'s (2010) treatment of fossilised soft tissue in a similar way to that of hard or skeletal tissue, i.e. as though they would be unchanged morphologically from the native state (e.g. Currie and Chen 2001; Xu et al. 2009) exposes a serious gap in understanding, and, perhaps more worryingly, lack of concern, for the processes of decomposition (see Lingham-Soliar 2010a, b). It is through such studies that we are now able to say with reasonable confidence that the degradation of the ichthyosaur fibre, for example, is pre-fossilisation and consistent with the decay of collagen, that it occurred very soon after the animal's death, and that structures observed (Fig. 6) are not intact bodies but by-products of decay. Important, too, is the knowledge, as established here through native and fossilised soft tissue, that breakdown of filaments is usually as globular particles. Rapid mineralisation would have helped stall further decay and "freeze" the moment in time. Just as in the decomposing fibre of *C. caretta*, the long fibre bundles and fibres are being degraded into shorter ovoid and sub-circular units (Fig. 5, arrows) some as large as 1,000 nm while the

smallest components are the ultimate fibril repeat patterns, the 67 nm *D*-bands (Lingham-Soliar and Wesley-Smith 2008; here, see Fig. 2ii, iii and bracketed area in Fig. 5). Figure 5 (inset) shows another fibre of *Ichthyosaurus* in which fibrils have become twisted and attenuated before eventual break-up (traces of *D*-banding of the fibrils are apparent).

Where biological decay has proceeded much further, as seen in another collagen fibre from the same ichthyosaur specimen, all that remains is a mass of organic "debris" including many structures that are globular in shape (Fig. 6; and ESM Table 1; see also ESM Fig. 1 of the same figure with programmed minor accented edges). The globular "debris" of the collagen filaments in *Ichthyosaurus* are less compacted or flattened and somewhat more 3-dimensional in shape (preserved in a nodule; Lingham-Soliar 1999; Lingham-Soliar and Wesley-Smith 2008), compared to those of *Sinosauropteryx*. SEM-EDX analysis (Lingham-Soliar and Wesley-Smith 2008) has also shown they were mineralised (predominantly calcium) as opposed to the carbonaceous preservation in *Sinosauropteryx*. Notwithstanding, the similarities in the processes of degradation and of preservations are striking (Fig. 6; ESM Table 1).

The structures in Zhang et al.'s (2010) figure 3c, as demonstrated here by experiment and comparative studies (Figs. 4, 5 and 6) are most probably the degraded remains of soft-tissue filaments, probably collagen. However, could they be the degraded remains of melanosomes? This cannot be ruled out, but would be entirely speculative because Zhang et al. (2010) provide no evidence for it by, e.g., comparative studies on the decomposition of melanosomes (native and fossilised), and besides, they claim that the structures are preserved melanosomes not their degraded remains. Although also unlikely, we may not rule out that the filaments could represent structural keratin of a frill either solely or in combination with collagen. For example, external spines, bristles and horns in modern-day lizards are scale derivatives and comprise varieties of β -keratins (Toni et al. 2007) as do turkey bristles (Sawyer and Knapp 2003).

We are in the advantageous position of knowing that the "debris" in the integument of *Ichthyosaurus* (Figs. 5 and 6) represents the degraded remains of collagen fibres since they had been identified by the *D*-banding fibril ultra-structure (mean = 66.12, $n = 329$), the unique fingerprint of collagen (Lingham-Soliar and Wesley-Smith 2008; Figs. 3a and 2ii). Note that the 67 nm *D*-banding in hydrated rat tail collagen is frequently used as a control (Lingham-Soliar and Glab 2010; Fig. 2iii). Interestingly, in Zhang et al.'s (2010) SEM, (my Fig. 2, arrow and inset i) a parallel formation of the small beadlike structures reminiscent of *D*-banding of collagen fibrils in both native (Fig. 2iii) and fossilised collagen (Lingham-Soliar and

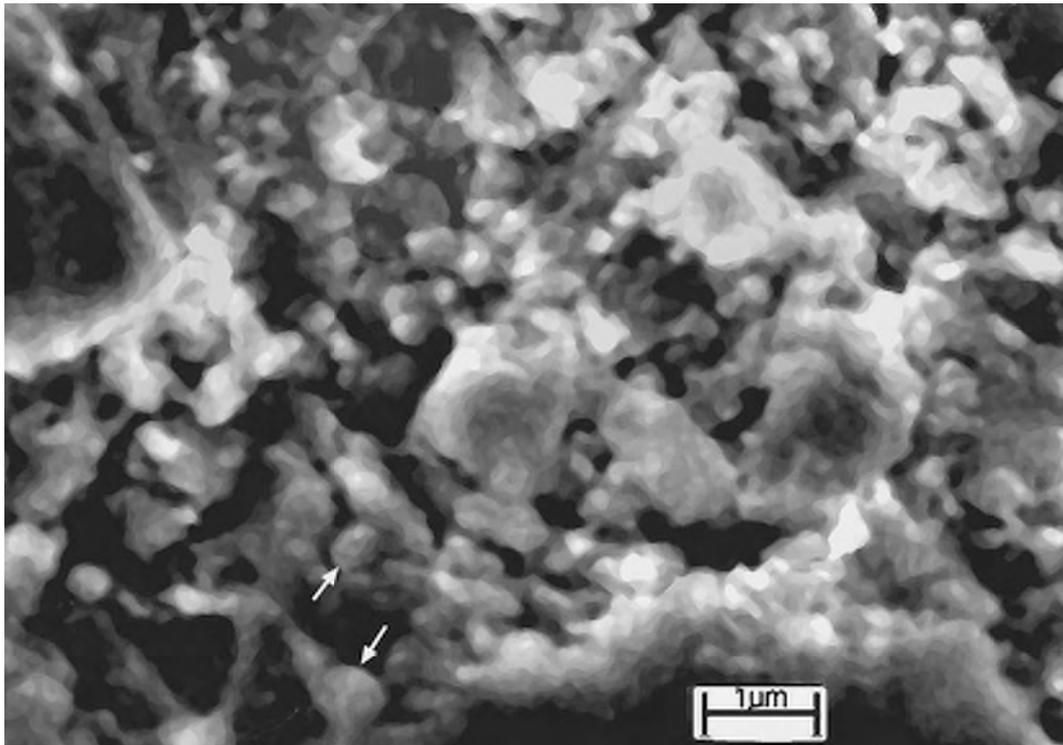


Fig. 6 SEM. Section showing advanced stage in the decomposition of collagen fibres in *Ichthyosaurus*, (longitudinal or tangential view of degraded fibre; phosphatic preservation, see text). Many globular structures show traces of the beaded *D*-bands of the basic fibrils (arrows)

Wesley-Smith 2008; here, Fig. 2ii). They are the smallest degradation by-products in the SEM (not mentioned in Zhang et al. 2010) and coincide in their average size of 66.36 nm ($n = 55$, $SD = 5.91$; ESM Table 1) with that of the *D*-bands of native and fossil collagen fibrils. The “twist in the tail” is that they might provide definitive evidence that the integumental structures of *Sinosauropteryx* were collagenous. However, in the interests of statistically viable science, study of more than just a single section of a fibre is warranted, but it opens the exciting potential for future detailed investigations. In contrast, the structures preserved in *Sinosauropteryx* as allegedly phaeomelanosomes (either preserved intact or degraded) are not supported by a reasonably expected level of evidence (Zhang et al. 2010). To accept such poorly supported allegations would foster further chaos pertaining to vital questions with respect to the evolution of the feather and of birds.

Unfortunately, Zhang et al. (2010, p. 1077) have used provocative terms such as their findings on *Sinosauropteryx* are based on “empirical” evidence “refuting recent claims [Lingham-Soliar 2003; Lingham-Soliar et al. 2007]” and “demonstrate conclusively” that “these structures are unequivocally epidermal structures”. It is regrettable therefore to summarise in strong terms a refutation of those allegations and their failure to observe scientific procedure in a number of ways that do not help their claims:

1. Fallacy of the crucial experiment. Allegations that the structures are melanosomes and a pivotal discovery in a basal theropod are not supported by empirical evidence but rather by metaphysical assertions. While the SEM image is real, the vital questions relating to it are speculative, e.g., (1) allegations of the light and dark stripes (see above); (2) allegations that the melanosomes are “embedded inside the filaments” (implying depth) are made without cross-sections or tangential sections but are based on a single SEM image of the filament’s surface or near-surface; and (3) size and shape of the structures, key to their identity, are speculative—they lack vital, basic statistical measurement data.
2. Fallacy of generalisation. Proof in one group of animals used as proof in another disparate group without testing; e.g. the authors state the evidence for stripes in *Sinosauropteryx* is that eumelanosomes only occur in dark bands in bird feathers (Vinther and Briggs 2008).
3. Tautology. The preceding comparison with respect to feathers (Vinther and Briggs 2008) is classic circular reasoning, i.e. the proposition that the filaments in *Sinosauropteryx* are feathers is being used as proof of its own conclusion.
4. False dichotomy. The only alternative is the one the authors propose, e.g. alleged globular shape/size was

used as the sole argument and bacteria were ruled out for a number of reasons. Hence, by default, the structures in the filaments, having been declared non-bacteria, have to be melanosomes. A similar dichotomy is given for the missing calamus, i.e. absence of a bacterial film equals absence of bacterial degradation and, therefore, absence of a calamus is a consequence of absence of melanosomes in the calamus—not bacterial degradation (see above).

Despite the clear dangers of making profound evolutionary assertions on inadequately tested hypotheses, e.g. in Zhang et al. (2010) on *Sinosauropteryx* (also Xu et al. 2009 on *Beipiaosaurus*) and on overt mischaracterisations (e.g. Xu et al. 2001, see above), such studies are being given attention and credibility in highly respected journals (e.g. *Nature* and *Proceedings of the National Academy of Sciences*).

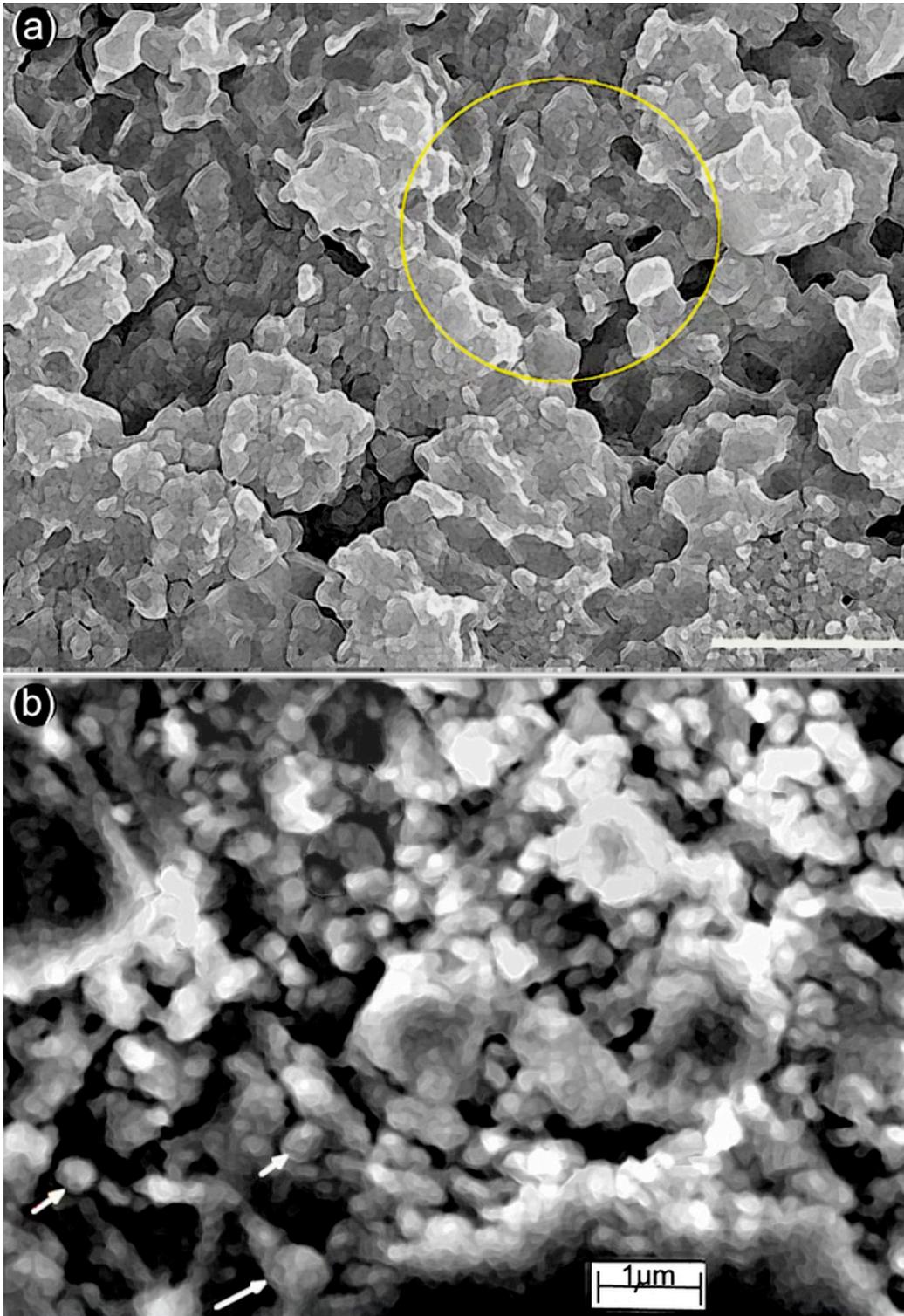
Finally, while Zhang et al.'s (2010) study was an apparently sincere attempt to investigate potential phaeo-melanosomes in the integumental structures of *Sinosauropteryx* and settle in their view the question of whether they were early feathers or collagen fibres, the methodology and results are critically flawed. SEM of fossilised melanosomes as shown earlier (Vinther and Briggs 2008) may be an extraordinarily useful tool in authenticating ambiguous fossilised integumental structures, when objectively applied, but one should be mindful that rarely if ever do we find a “magic bullet” in science as a universal solution. However, poor use of relevant technology, as shown in Zhang et al.'s SEM (2010, figure 3c), and abandonment of scientific procedure, give many reasons to be sceptical of how future ultrastructural studies of other non-avian dinosaurs, e.g. *Psittacosaurus* and *Tianyulong* (Zhang et al. 2010, p. 2) will be applied. Witmer (2009, p. 294), a supporter of the dinosaur protofeather hypothesis, recently intimated the dangers of rhetoric and concocting “complicated scenarios for feather evolution” based on what was all along thought by most workers to be a “seemingly simple question” of whether the filaments are epidermal or dermal, acknowledging that it “is surprisingly hard to answer.” Yet, there were criticisms (e.g. Ruben and Jones 2000; Lingham-Soliar 2003; Feduccia et al. 2005; Lingham-Soliar et al. 2007, and references therein) of precisely such rhetoric and more especially of a lack of scientific rigor, which were spurned in articles and dismissed as minority views (Sues 2001, p. 1036; Prum and Brush 2002, p. 4). If a lesson has been learnt, as implied by Witmer (2009), I am skeptical given the report on Witmer's comments in *The New York Times* (Zimmer 2010), specifically concerning *Sinosauropteryx* (Zhang et al. 2010), “the study decisively closes the case on whether the whiskers are feathers or collagen.” His denouncement of rhetoric is unconvincing given that

this comment appeared before any chance of potential counter-studies. The problem of this “surprisingly hard” question to answer has far from diminished as demonstrated here by Zhang et al.'s (2010) simplistic treatment of melanosomes in *Sinosauropteryx*, underscoring that, while the application of technology and interpretation of novel ideas are welcomed, they can nevertheless become self-serving to preconceived notions that continue to fuel the rhetoric of “complicated scenarios of feather evolution” and now feather colour patterns. As important as establishing the legitimacy of a hypothesis is the regard for the due processes of science (otherwise science may just as well be relegated to the casting of a die where the law of averages states that some of the time the call will be correct) and the avoidance of Machiavellian politics—i.e. the end justifies the means. A blanket view that all sinuous structures in the Chinese dinosaurs were “protofeathers,” is now being compounded by a blanket view that all micro-particles found in the same integumental structures are melanosomes. Is it possible that most workers will so soon be lulled once more into repeating the former complacency that it is a “seemingly simple question” to answer? While feathers in birds and some non-avian dinosaurs may be correctly identified by melanosomes, in others, specifically in *Sinosauropteryx*, they are almost certainly not. Zhang et al.'s (2010) study does nothing to detract from previous conclusions based on a number of lines of evidence (Lingham-Soliar et al. 2007, p. 1826) that the filamentous structures were in all probability collagenous.

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ESM Figure 1. Programmed accented (slight) edges (Adobe Photoshop). A, text figure 2 (Zhang et al. 2010 fig. 3c). The accented edges show that what appears as a single particle, clearly comprises a number of smaller, variously shaped and sized particles (the smallest, rounded "beads") compressed into one "globule". B, text figure 6, ichthyosaur degraded fibres. Scale bar: A = 2 μm ; B = 1 μm .