Discussion

A densely feathered ornithomimid (Dinosauria: Theropoda) from the Upper Cretaceous Dinosaur Park Formation, Alberta, Canada: A comment

Theagarten Lingham-Soliar

Environmental Sciences, Nelson Mandela Metropolitan University, Summerstrand, Port Elizabeth 6001, South Africa

ARTICLE INFO

A R T I C L E   I N F O

Article history:
Received 25 November 2015
Accepted in revised form 4 December 2015
Available online xxx

Keywords:
Ornithomimus
Feathers
Collagen fibres
Biology
Taphonomy

A B S T R A C T

The presence of feathers in Ornithomimus is questioned on poor evidence and a failure to observe scientific process and procedure.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

van der Reest et al. (2016) describe an Ornithomimus with alleged plumaceous feathers including reconstructions/interpretations; most of latter will not be considered in any detail in this commentary for reasons that will become clear in my concluding remarks. I shall, however, examine independently, where possible, the paleontological evidence behind the authors' interpretations. However, findings discussed in this study must not be interpreted as antagonistic to the idea of feathered dinosaurs but rather, as in any hypothesis, whether or not they are circumscribed by sufficient scientific rigour.

2. Discussion

van der Reest et al. state, “[t]he most common integumentary structures are unambiguous feathers comprising filaments that range from 25 to 87 mm in length and 0.2—0.5 mm in width, preserved as dark carbonaceous imprints surrounding specific portions of the skeleton (Fig. 4).” Most are preserved as dark brown to black carbonaceous traces.” Their figure 4, in particular Fig. 4a, is indeed the only one in which one can make a reasonable and independent assessment of the alleged feathers. The dimensions they give are a very good place to start.

Measurements may be reasonably interpreted as a defining principle of science. Notwithstanding the importance of establishing rachidial widths of their alleged feathers (innumerable according to the authors reconstructions in figs. 3, 5, 6), the authors have provided no statistical measurements. This is conflated by a vague reference to the ‘feather’ width range of between 0.2 and 0.5 mm and to a solitary example “on the body of UALVP 52531 is 0.4 mm laterally (their fig. 4b and c).” This leaves no option but to trust to the scale bar on their fig. 4 and to try to establish what they mean by feathers in the context of width and structure, at least in their fig. 4a. The sections in their figure 4b, c, which is considerably eroded, will not be considered in any depth because it is based on one alleged rachis and on allegations of a “clearly branching plumage”, based on one v-shaped configuration and another in which the all-important point of origin of the alleged branch is absent (hence an assumption).

Reading between the lines, the authors’ interpretation of feathers is based on two criteria, feather rachides 0.2—0.5 mm wide and an internal system of filaments, both of which were at some point organic. It is possible to see how this interpretation came about (my Fig. 1a). However, to understand why this interpretation is fundamentally flawed, first, we need to understand, crucially, the nature of the substrate upon which the integumental structures are preserved. It is a coarse sandstone substrate that forms a craggy, highly uneven surface (troughs separated by flats or crests), riddled by cracks. Second, we need to know how and why filaments from the ornithomimid were preserved on this surface. The coarse sandstone substrate probably enabled rapid dehydration of the soft
tissue (skin) soon after the animal’s death thereby avoiding fibre swelling in different stages of decay (it is impossible to tell whether or not these are part of larger bundles). Furthermore, the coarse-grained sandstone substrate may have helped drain away enzymes of decay and suppurating fluids that may speed up the processes of decay (Schweitzer, 2003; Lingham-Soliar and Glab, 2010). We may presume that the fibres as a consequence have not suffered severe microbial decay.

van der Reest et al. are, however, only half right with respect to the integumentary structures. Here, Fig. 1b (Si Fig. 1, rectangle 1), shows a cavity that is clearly part of the topography of the substrate (also seen in a number of different parts of the section in Si Fig. 1) i.e. part of the inorganic sandstone substrate whereas they are associated with ‘internal’ filaments (~50 µm diameter) that were originally organic, clearly falsifying the hypothesis of rachides. The extreme filament contortions and ready ability to separate into individual strands are a clue to their chemistry.

Unlike in some preservations, the filaments in the authors’ fig. 4a are fairly uniform in diameter (~50 µm). The fibres also show a typical collagenous feature of beading, which is a feature of dehydration and crumbling or slight contractions along the fibre length when muscle tone in the tissue is lost (Lingham-Soliar, 2003; Lingham-Soliar and Glab, 2010). The troughs (marked frequently by being a sinkhole for pigment/carbon/iron-oxide; see Lingham-Soliar and Glab, 2010) and ridges have acted to redirect the collagen fibres. The latter are highly malleable when muscle tone is lost and capable of following the shape of underlying structures as the fibres are pressed down. It is possible to see how readily collagen fibres are separated and deflected from their original geometrical cross-fibre patterns in the skin of a decaying dolphin, where they follow the course of the ribs and in histological preparations of shark skin (Lingham-Soliar, 2003, 2005; Fig. 1c, d resp.). Similar deflections or diversions are frequently seen in van der Reest’s fig. 4a, initiated by even the slightest obstacles (see Si for details e.g. rectangular boxes) frequently with the filaments separating into single strands emphasizing that whatever bonds that exist between adjacent fibres, they are weak as e.g. in collagen (Fig. 1d). They are in striking contrast to β-keratin, the toughest, natural fibre known with a limited degree of elasticity and constructed of internal fibres that are ‘glued’ by a tough amorphous α-keratin matrix (we have shown that it takes years of microbial degradation under ideal conditions to degrade and even then the filaments are arrow-straight (Lingham-Soliar et al., 2010; Fig. 1e, F)). On the other hand where the substrate surface may be fairly flat (Si Fig. 1, curved bracket adjacent to arrow 1) a wide band of fibres (~1.5 mm) can be seen without the ‘channeling’ and deflections seen elsewhere. The filament characteristics and biomechanics have little factual resemblance to avian β-keratin fibres (Lingham-Soliar et al., 2010; Lingham-Soliar and Murugan, 2013; Lingham-Soliar, 2014, 2015).

Next, the authors state, “[t]he other and more complete adult [of Ornithomimus] has oblique carbonaceous markings on the ulna and radius that are interpreted by Zelenitsky et al. (2012) as attachment traces for the calami of pennaceous feathers” with which they concur, despite the absence of quill knobs. Given, that these are no more than stains without even a trace of depressions, both sets of authors’ conclusions are untested and suffer from confirmatory bias i.e. the authors’ make no effort to provide alternative scenarios that might falsify their conclusions. The functional significance of stains as opposed to quill knobs (as in modern birds and Velociraptor) has not been explained. The parsimonious explanation is that these are pigment impressions from the decaying epidermis as seen in a dolphin and Psittacosaurus (Lingham-Soliar and Plodowski 2010; Fig. 1c and 1g–k resp.). In Psittacosaurus the pigment stains (including on the ulna) take on a variety of shapes dependent on the structures/partial structures in the overlying epidermis but if one was to interpret them as marking the sites of the calami, they are resoundingly more impressive than the muted stains in Ornithomimus, perhaps facilitated by the high quality of soft tissue preservation and highly pigmented nature of the Jehol’s Psittacosaurus. However, they are also preserved on the ribs, scapula, etc. of Psittacosaurus, precluding in this case any possible misinterpretation of calami insertions points.

Figures 3, 5 and 6 (van der Reest et al., 2016) are extremely poor quality images from which the authors purport to distinguish feathers. While it is barely possible to discern some kind of filamentous structures in these figures, the poor quality makes no meaningful independent analysis possible. Thus it is impossible to falsify the interpretive drawings by van der Reest et al. (2016). The only area that I have been able to enlarge meaningfully from these figures is in the authors’ fig. 3, an area not included in their interpretative drawing. It shows what appears to be a cross-fibre architecture (Si fig. 2) of regularly oriented filaments that is reminiscent of the dermis in many vertebrates including Sinosauropteryx (Lingham-Soliar, 2011*, 2012). These closely adjoin the authors depicted more variably oriented ‘feathers’ from considerably more blurry parts of the photo. However, more general remarks will be made in my conclusions below.

“It was shown that highly irregularly-shaped degraded material in Sinosauropteryx had no resemblance to phaeomelanosomes (once thought to be irrefutable evidence of feathers in the animal) whatsoever and subsequently questioned by other workers (Manning et al., 2013; Lindgren et al., 2015).

3. Conclusions

The hypothesis of feathers in Ornithomimus lacks sufficient scientific rigour and depends wholly on confirmation. Mahoney (1977) drew attention to the fundamentally illogical nature of confirmatory bias, which we see amply demonstrated in van der Reest et al. (2016), i.e. the tendency to emphasize and believe experiences that support one’s views and to ignore or discredit those that do not. The definition of science is that it should be testable and capable of being falsified (Popper, 1959) as opposed to verificationist systems. Let us look at the authors findings in this context: i) the authors state that the integumentary structures are “unambiguous feathers” without feeling the need to consider why they might not be or consider an alternative, collagen for example; ii) the authors give ‘feather’ widths of 0.2–0.5 mm without basic statistics from any of the figures, thus lacking any significance, given the fundamental importance of such measurements; iii) despite the substrate surface being highly cragggy and cracked (fig. 4a) the authors see no other explanation for the troughs than that they are feather rachides (0.2–0.5 mm wide); iv) their
interpretation of a solitary v-shaped structure as a branched feather considers no other explanation e.g. taphonomy (see v-shaped filaments in ichthyosaur skin, Fig. 1); v) the authors see no contradiction to their unambiguous feather hypothesis namely in the highly malleable nature of the filaments that mould themselves to extreme contours and curves, which is consistent with collagen but inconsistent with β-keratin, even during decay; vi) the authors see no reason to explain how keratin filaments separate into single strands with such ease (see above and SI Fig. 3); vii) the authors fail to explain the crumbling of the filaments to produce ‘beads’ when tension is lost, which has consistently been associated with collagen fibres.

There is also, perhaps requiring the strongest criticism of all, a general breakdown in scientific protocol in the paper by van der Reest et al. (2016). With respect to their figures 3, 5 and 6 the authors produce interpretive drawings. On the other hand their respective photos are of such poor quality (lacking even a single detailed image), that it generally precludes an independent analysis. The Council of Biology Editors (CBE), an authoritative professional organization (in biology, at least) defined a primary scientific publication as the first disclosure “containing sufficient [my italization] information to enable peers (1) to assess observations, (2) to repeat experiments, and (3) to evaluate intellectual processes. There is no proviso for “the appeal to authority” that precludes scientific assessment by peers. A paper is under review not an authors’ knowledge or experience (even if greater) etc. Scientific papers are the way we communicate science and its persuasiveness must lie entirely in what is contained within the paper’s pages (figures and data). An independent assessment cannot be made of van der Reest et al.’s interpretive drawings without adequate photos or it becomes an “appeal to authority.” This kind of major scientific failing goes back at least 15 years in this field e.g. when Currie and Chen (2001) produced subjective inky outlines of integumental structures that are meant to represent no less important structures than branched feathers. Most deserving of censure, however, in that same study was, “[u]nder magnification, the margins of the larger structures are darker along the edges but light medially, which suggests that they may have been hollow.” They did not produce the evidence though and yet their untested comments stand to this day for one of the most crucial features of real feathers, hollowness. What was clearly needed was close-up microphotographic evidence so that an independent analysis of what the authors’ claimed to see in their paper could be made by peers. They are hearsay evidence and an “appeal to authority,” which is recorded here to emphasize how little has changed to the present day.

van der Reest et al. (2016) demonstrate a spectacular failure to appreciate both the biological predictabilities of organic structures and the idiosyncrasies of taphonomy, confounded by a clear lack of attention to scientific procedure. Their study does not bode well for similar claims on Ornithomimus by Zelenitsky et al. (2012).

Acknowledgements
The study is supported by National Research Foundation, South Africa.

References

Appendix A. Supplementary data
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cretres.2015.12.001.
Discussion

Reply to comment on: “A densely feathered ornithomimid (Dinosauria: Theropoda) from the Upper Cretaceous Dinosaur Park Formation, Alberta, Canada”

Aaron J. van der Reest*, Alexander P. Wolfe, Philip J. Currie

Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada

Abstract

We confirm the presence of pigmented keratinized integumentary structures attributable to feathers in the Late Cretaceous Ornithomimus specimen UALVP 52531. We falsify the hypothesis that these features represent collagen fibers and address additional criticisms of our paper made by Lingham-Soliar (2016).

1. On measurement and scale

We welcome the opportunity to address comments made by Lingham-Soliar (2016) on the integumentary structures associated with UALVP 52531, a recently described Ornithomimus from the Late Cretaceous of Alberta, Canada (van der Reest et al., 2016). We first address the issues of measurement and scale, stated bluntly by Lingham-Soliar (2016) as “a vague reference to the ‘feather’ width range of between 0.2–0.5 mm and to a solitary example on the body of UALVP 52531 that is 0.4 mm” followed shortly thereafter by “This leaves no option but to trust the scale bar on their Fig. 4 and try to establish what they mean by feathers in the context of width and structure.” In no manner are our measurements or illustrated scale bars lacking in accuracy or precision. To make this point clear, in Fig. 1 of this rebuttal Figs. 1A–C are reproduced from the original alongside the unedited photograph of the specimen in question with ruler in place (Fig. 1D). Simply stated, there is nothing remotely disingenuous or deceptive about this scale bar, or any of the others presented by van der Reest et al. (2016). Indeed, these illustrations were deemed satisfactory, as initially submitted, to both anonymous reviewers and the editorial office of Cretaceous Research. Moreover, this scale bar is consistent with, and enables confirmation of, the filament dimensions reported in the text of van der Reest et al. (2016). As can be seen in Figs. 1B and C, there are many more than a “solitary example” of the two filament populations observed. There are numerous fine elements branching from more robust structures that are interpreted as feather rami and rachises, respectively. There is no evidence of feather barbule preservation in UALVP 52531. As stated, the detailed analyses of the microstructure and chemistry of preserved integumentary features are forthcoming, but we can specify that these two filament populations have dimensions of 49 ± 9 μm (range: 30–60 μm, n = 79) and 253 ± 66 μm (range: 110–450 μm, n = 81). We thank Lingham-Soliar (2016) for requesting this information and acknowledge our omission, but we emphasize that the data and illustrations presented by van der Reest et al. (2016) are correct, and that our interpretation of UALVP 52531 remains unchanged.

2. Feathers adorning UALVP 52531

On page 109 of van der Reest et al. (2016) we wrote: “The most common integumentary structures are unambiguous feathers comprising filaments that range from 25–87 mm in length and 0.2–0.5 mm in width, preserved as dark carbonaceous imprints
Fig. 1. Keratinous integument and collagen fibers. (A–C) Direct reproduction of Fig. 4 from van der Reest et al. (2016), with the following additions: measurements of coarse (rachises) and fine (rami) feather elements in (B) and (C), and shading of the fractured vertical margin of the specimen that was edited from the original (D) in order to restrict (A) to surfaces in focus. (D) The original unedited photograph that became Fig. 4A, where the ruler on left is the basis for the digital scale, identical as that in (A). The digital scale is slightly longer that the photographed ruler in order to compensate for specimen height. (E) Surface and fracture margin of one of the “dark carbonaceous imprints” from the ilium of UALVP 52531 observed with field-emission scanning electron microscopy (FE-SEM). The μm-scale bacilliod shapes are melanosomes. (F) Comparable features, at the same scale as (E), from a rufous tail feather of Buteo jamaicensis (red-tailed hawk) digested briefly in 1 M Na2S. In (G) and (H) are FE-SEM images of collagen fibers extracted from a Middle Pleistocene Mammuthus primigenius pelvis, ~600 ka in age, from the Yukon Territory and buried in frozen ground (Dominion Creek locality, Klondike gold fields, 63°46′N, 138°32′W). Each fiber has a diameter ≤15 μm, and is comprised of much finer (<1 μm) fibrils. The finest carbonaceous elements in UALVP 52531 measure 30 μm in diameter, but average 49 ± 9 μm, much wider than the coarsest collagen fibers, as shown by comparison of (B) and (C) to (G) and (H). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
surrounding specific portions of the skeleton.” What we failed to convey in this sentence, perhaps leading to some of the objections of Lingham-Soliar (2016), is that our claims are neither flippant nor unsubstantiated. Rather, this sentence and the associated images distil a large amount of hands-on research addressing UALVP 52531 (mainly by AvdR and APW), while simultaneously benefitting from one of us (PJC) having almost two decades of experience interpreting theropod integumentary structures from localities with exceptional preservation (e.g., Ji et al., 1998). When we began writing the manuscript (summer-autumn 2015), we had preliminary inklings from field-emission scanning electron microscopy (FE-SEM) that the integument of UALVP 52531 was consistent with pigmented keratin. The results were deemed insufficient for inclusion in the paper at that time, although subsequent work supports the interpretation of feathers, including extensive comparisons with modern avian material as well as ongoing chemical analyses using Fourier transform infrared spectroscopy (FTIR) and time of flight-secondary ion mass spectrometry (ToF-SIMS). Much of the “dark carbonaceous material” surrounding the dorsal margin of UALVP 52531 has densely packed melanosomes (melanin containing organelles) that are homologous to those observed in modern bird feathers (Figs. 1E, F). In both cases, the melanosomes are intimately associated with sheets of keratin. While melanosomes from Corvus feather (American crow) retain a distinct fabric with preferential long-axis orientation, those of UALVP 52531 appear randomly oriented, and are furthermore more variable with respect to size and shape. Importantly, in neither case is there any evidence of cellular division, which would be expressed as medial constrictions and would signify a bacterial origin for these structures.

Composititionally, provisional FTIR spectra (Fig. 2) reveal that the integumentary structures in UALVP 52531 are highly overprinted with mineral phases, as expected for any geological sample. However, the material retains moderately strong expressions of the alkane CH doublet (2850 cm$^{-1}$ and 2930 cm$^{-1}$), which denotes hydrocarbon side chains on the protein backbone, and thus confirms the survival of parts of the material’s original organic chemistry. The other organic bands that are strongly expressed in the UALVP 52531 samples are those associated with carbonyl functional groups (C=O) at 1400 cm$^{-1}$ and 1580 cm$^{-1}$, which confirm the presence of carboxylic acids. These peaks are also prominent in the FTIR spectra of black crow feathers and cuttlefish ink (Fig. 2C–D), and most likely relate to the presence of dihydroxyindole carboxylic acid (DHICA), an essential building block of the black pigment eumelanin.

Through the combination of microscopic and spectroscopic analyses, the integumentary vestiges of UALVP 52531 can be confidently identified as melanosomes within a keratin matrix, which bolsters the original interpretation of feather preservation on this specimen. Even in light of the stringent criteria presented by Moyer et al. (2014), we find no evidence that these structures represent vestiges of extracellular bacterial biofilms. We surmise, from the combination of what we knew at the time of writing and what we know now, that the admittedly strong wording of “unambiguous feathers” in van der Reest et al. (2016) remains entirely appropriate with respect to the observations.

3. Collagen matters

Collagen fibers do not represent an adequate interpretation for the integument of UALVP 52531. We extracted collagen fibers from Pleistocene mammoth pelvic bone by sequential decalcification, gelatinization, and ultrafiltration, following the protocol of Beaumont et al. (2010). While mammoths and dinosaurs are clearly not the same, collagens are remarkably conserved across and between clades, in addition to representing the most abundant proteins in bone (Asara et al., 2007). This level of conserveness has been exploited before in drawing inferences from mammalian, reptilian, and elasmostegranic collagen that potentially bear on integumentary structures of dinosaurs (Lingham-Soliar, 2003; Feduccia et al., 2005). Field-emission SEM of collagen fibers from mammoth (Figs. 1G, F) reveal structures of much smaller diameter (<25 μm) than even the thinnest filaments preserved as carbonaceous structures on UALVP 52531. These dimensions overlap collagen fibers from various extant reptiles, whereas homologous structures extracted from hadrosaur bone are even finer (Schweitzer et al., 2009). On the other hand, the diameter of collagen fibers does overlap with those of barbules from Late Cretaceous bird and dinosaur feathers preserved in amber (McKellar et al., 2011). However, barbules possess distinctive nodes and internodes that produce segmented structures, whereas collagen fibers comprise bundles of slightly whorled fibrils that are considerably more flexible (Fig. 1G). In FTIR spectroscopy, the mammoth collagen fibers resemble neither UALVP 52531 integument nor avian feather or cuttlefish ink, most notably by the absence of carbohydrate absorbance bands (Fig. 2). Summarily, we remain unable to falsify the hypothesis that the integumentary structures in UALVP 52531 represent feathers, while effectively dismissing collagen fibers as a candidate for any of the observations made by van der Reest et al. (2016). We also note that in one of our images reproduced by Lingham-Soliar (2016, Supplemental Fig. 2), marks created during preparation using pneumatic scribes have been mistaken for primary structures. Had we been contacted with a request for detailed images of the region, it would have been evident as to what these marks truly represented. Furthermore, a simple comparison to the line drawing associated with the original figure clearly illustrates what is matrix and is not matrix. Had the line drawing been consulted, it would have been clear that other structures suggested as possible collagen are in fact the dorsal margin of both ilia and the neural spine of the posterior sacral vertebrae. These facts clearly illustrate the importance of requesting high resolution images (which may not always be published due to file size, or page limitation) or to personally view specimens for confirmation of reported characters and structures.

4. Conclusion

While we respect the spirit of exploration for alternative interpretations (Lingham-Soliar 2016), collagen preservation is extremely rare in the Mesozoic fossil record (Schweitzer et al., 2007; Schweitzer, 2011), and almost certainly less common than the preservation of feather keratin (Norell and Xu, 2005), which itself is restricted to relatively few localities. In a general sense, the preservation potential of keratin is higher than that of integumentary non-apatitic collagen because of the more hydrophobic character of cross-linkages involving non-polar amino acids in keratin, which effectively exclude water at the cellular loci of keratin synthesis and maturation (Schweitzer, 2011). A similar conclusion can be reached by comparing keratins and collagens from a materials science perspective (Meyers et al., 2008). However, in the exceptional instances where intact dinosaur collagen has been recovered from multiple specimens, entombed in rapidly-deposited sandstone, the resulting sequences for collagens $\alpha$1 type I and $\alpha$2 type I obtained by mass spectrometry appear to confirm the monophyly of dinosaurs and birds (Schweitzer et al., 2009). The exact same conclusion can be drawn for non-fossil feathers and feather-like integumentary structures, bolstered by an additional suite of anatomical synapomorphies discussed by van der Reest et al. (2016) among many other authors (e.g., Ostrom, 1976; Wagner and Gauthier, 1999; Vargas and Fallon, 2005). While we

Please cite this article in press as: van der Reest, A.J., et al., Reply to comment on: “A densely feathered ornithomimid (Dinosauria: Theropoda) from the Upper Cretaceous Dinosaur Park Formation, Alberta, Canada”, Cretaceous Research (2016), http://dx.doi.org/10.1016/j.cretres.2016.01.005
are sensitive to alternate hypotheses that postulate more distant phylogenetic relationships between dinosaurs and birds (Feduccia and Wild, 1993), and to the crucial role played by the diagnosis of integumentary structures in this debate (Jones et al., 2000; Feduccia et al., 2005), the observations from UALVP 52531 remain inconsistent with such interpretations. Moreover, the extension of Lingham-Soliar’s (2016) critique of our work towards that of Zelenitsky et al. (2012) is neither scientifically nor professionally justified. UALVP 52531 supports wholly the earlier publication of feathered ornithomimids from the Dinosaur Park Formation, while
adding new dimensions to the quality of preservation that exists within these remarkable sediments.

Acknowledgments

Research was funded by the Natural Sciences and Engineering Research Council of Canada (Discovery Awards to APW and PJC). Wayne Moffat (Department of Chemistry, University of Alberta) is thanked for assistance with FTIR spectroscopy.

References


