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## VISUAL DISPLAY

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pterosaurs used  
coloured feathers for  
visual communication

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malnutrition crisis

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# Pterosaur melanosomes support signalling functions for early feathers

<https://doi.org/10.1038/s41586-022-04622-3>

Received: 22 October 2021

Accepted: 7 March 2022

Published online: 20 April 2022

Open access

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Remarkably well-preserved soft tissues in Mesozoic fossils have yielded substantial insights into the evolution of feathers<sup>1</sup>. New evidence of branched feathers in pterosaurs suggests that feathers originated in the avemetatarsalian ancestor of pterosaurs and dinosaurs in the Early Triassic<sup>2</sup>, but the homology of these pterosaur structures with feathers is controversial<sup>3,4</sup>. Reports of pterosaur feathers with homogeneous ovoid melanosome geometries<sup>2,5</sup> suggest that they exhibited limited variation in colour, supporting hypotheses that early feathers functioned primarily in thermoregulation<sup>6</sup>. Here we report the presence of diverse melanosome geometries in the skin and simple and branched feathers of a tapejarid pterosaur from the Early Cretaceous found in Brazil. The melanosomes form distinct populations in different feather types and the skin, a feature previously known only in theropod dinosaurs, including birds. These tissue-specific melanosome geometries in pterosaurs indicate that manipulation of feather colour—and thus functions of feathers in visual communication—has deep evolutionary origins. These features show that genetic regulation of melanosome chemistry and shape<sup>7–9</sup> was active early in feather evolution.

Feathers are remarkable integumentary innovations that are intimately linked to the evolutionary success of birds<sup>10</sup> and occur in diverse non-avian dinosaurs from the Middle Jurassic onwards<sup>1</sup>. The early evolutionary history of feathers, however, remains controversial as relevant fossils are rare<sup>3,11</sup>. Integumentary appendages in pterosaurs, traditionally termed pycnofibres, were recently reinterpreted as feathers on the basis of preserved branching<sup>3</sup> but their homology with feathers is debated<sup>3,11</sup> and their functions remain unclear<sup>4</sup>. The small size and lack of secondary branching in pterosaur feathers precludes functions in active flight, but their dense packing and distribution over the body are consistent with thermoregulation<sup>12</sup>. This in turn is consonant with functional hypotheses for small, simple feathers in theropod dinosaurs<sup>1,4</sup>. Even simple unbranched feathers in theropods, however, functioned in visual signalling, as evidenced by melanosome-based colour patterning<sup>13,14</sup>. Whether feathers in earlier-diverging taxa also functioned in patterning is unclear: feathers and filamentous integumentary structures in non-coelurosaurian dinosaurs and pterosaurs are rare and their taphonomy is difficult to interpret. As a result, the timing and phylogenetic and ecological context of the evolution of melanin-based colour patterning in feathers is unknown.

Resolution of this issue requires evidence of colour patterning, including spatial zonation of melanosomes<sup>15</sup>, but this could be a taphonomic artefact. More definitive evidence includes variation in the morphology of melanosomes, as this is linked to feather colour in extant birds<sup>16</sup>. Previous observations of feather melanosomes in pterosaurs have revealed indiscriminate ovoid geometries<sup>2</sup>. These

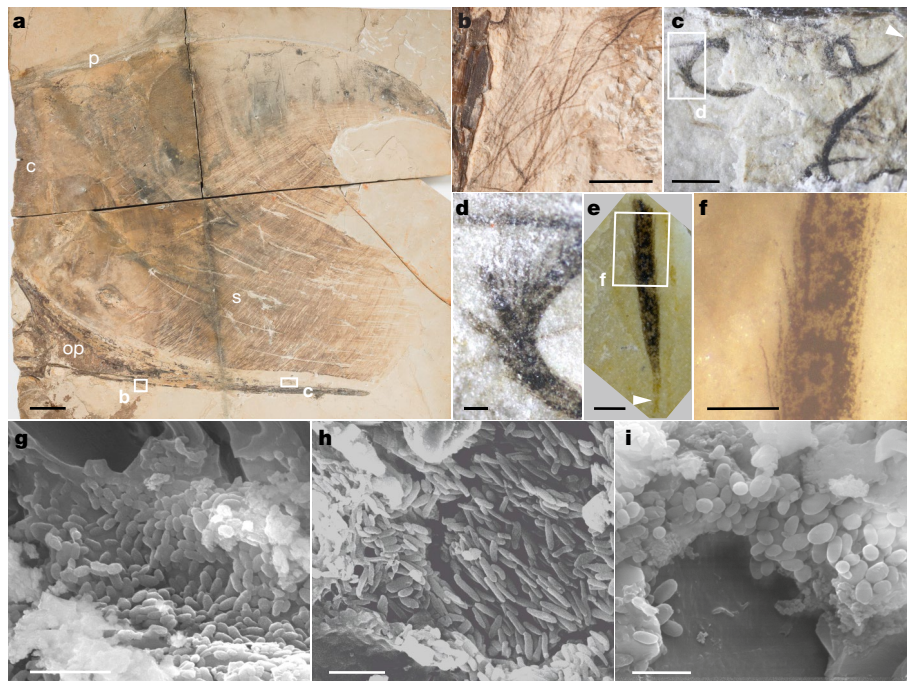
resemble melanosome geometries in the skin of extant reptiles (where visible colour is independent of melanosome geometry<sup>6</sup>) and preserved melanosomes in the skin of fossil non-dinosaurian reptiles. These data indicate that within Avemetatarsalia, the ability to vary melanosome geometry (and control the colour of integumentary appendages) is unique to theropods. Variable melanosome geometries in extant mammals, however, suggest earlier origins for this feature in a common amniote ancestor and a secondary loss in pterosaurs.

Here we resolve this issue using a new specimen of an adult tapejarid pterosaur from the Lower Cretaceous Crato Formation<sup>17</sup> (Araripe Basin, Brazil; Fig. 1, Extended Data Fig. 1, Supplementary Information). The specimen comprises an incomplete cranium associated with preserved skin, monofilaments and branched integumentary structures. These integumentary tissues preserve melanosomes that show tissue-specific geometries, a feature previously known only from theropod dinosaurs, including extant birds<sup>18</sup>. Collectively, these results confirm that branched integumentary structures in pterosaurs are feathers and provide evidence that tissue-specific partitioning of melanosome geometry—critical for melanin-based plumage patterning—has deep evolutionary origins in ancestral avemetatarsalians in the Early to Middle Triassic.

## Preserved pterosaur feathers

The cranium of a new specimen of *Tupandactylus cf. imperator* (MCT.R.1884; Pterosauria: Tapejaridae) (Supplementary Information) is preserved on five limestone slabs from the Lower Cretaceous Crato

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**Fig. 1 | Details of the cranial crest of MCT.R.1884, a new specimen of *Tupandactylus cf. imperator* (Pterosauria: Tapejaridae) from the Lower Cretaceous Crato Formation, Brazil. a**, Incomplete cranium showing preserved soft tissue crest. **b–f**, Detail of the integumentary structures associated with the posterior part of the skull. **b**, Monofilaments. **c**, Branched feathers. **d**, Detail of curved branched feather in **c**. **e, f**, Straight branched

feather (**e**) with detail (**f**). White arrowhead in **e** indicates the basal calamus. **g–i**, SEM of melanosomes in the soft tissues of MCT.R.1884. **g**, Ovoid melanosomes from the elongate fibres of the soft tissue crest. **h**, Elongate melanosomes from a monofilament. **i**, Ovoid melanosomes from a branched feather. **c**, Cristae; **p**, postmaxillary process; **op**, occipital process; **s**, skin. Scale bars, 50 mm (**a**); 5 mm (**b**); 2 mm (**c**); 250  $\mu$ m (**d–f**); 2  $\mu$ m (**g–i**).

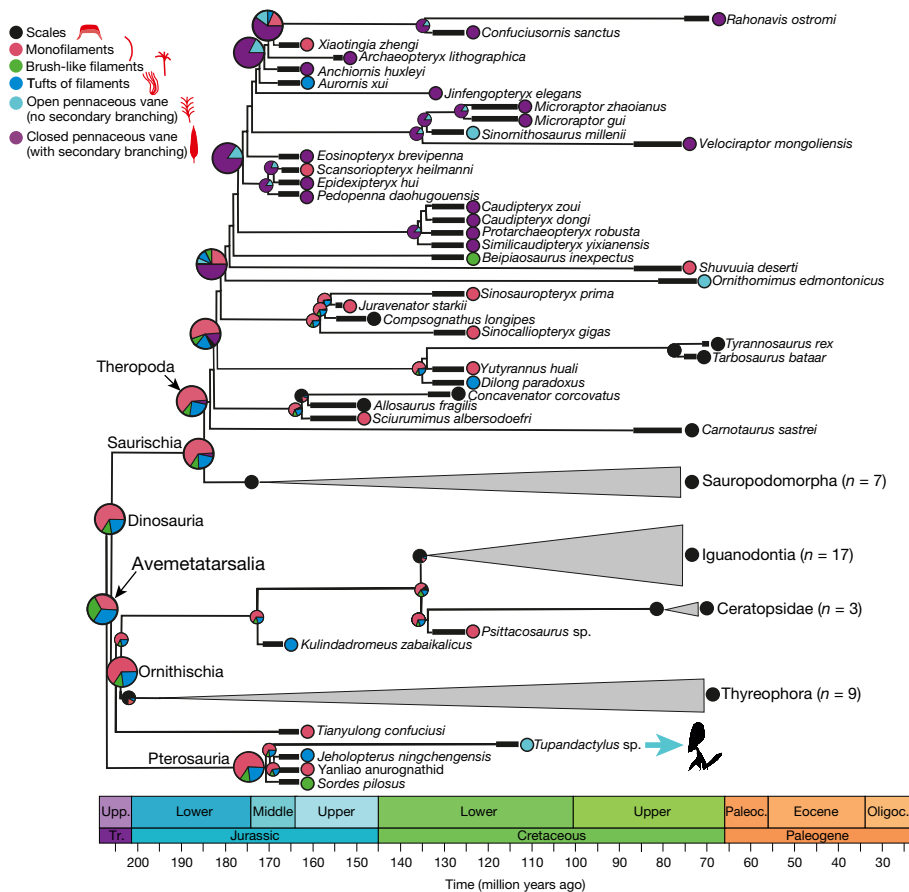
Formation in Brazil. Only the posterior portion of the cranium is present, comprising part of the left orbit, left nasoantorbital fenestra, fibrous crista and occipital process. The preserved soft tissue cranial crest extends between the postpremaxillary and occipital processes (Fig. 1a, Supplementary Information). Two types of filamentous integumentary structure occur close to (within 15 mm of) the occipital process (Fig. 1b–f). The proximal portion of the occipital process is mostly associated with monofilaments (approximately 30 mm long and 60–90  $\mu$ m wide; Fig. 1b, Extended Data Figs. 1, 2). These resemble stage I feathers<sup>19,20</sup> and monofilaments in the anurognathid *Jeholopterus ningchengensis*<sup>21,22</sup>, *Sordes pilosus*<sup>23,24</sup> juvenile anurognathids<sup>2</sup>, the ornithischian dinosaur *Tianyulong*<sup>25</sup> and the theropod *Beipiaosaurus*<sup>26</sup>.

The distal part of the occipital process is associated with short (2–5 mm long) branched integumentary structures (Fig. 1c–f, Extended Data Fig. 2). Each shows a poorly defined central shaft (approximately 60  $\mu$ m wide; Extended Data Fig. 3) that thins close to the proximal tip (Fig. 1c, e). This narrow, light-toned proximal portion of the shaft resembles a basal calamus (Fig. 1e). Short (approximately 100–200  $\mu$ m long), straight and closely spaced secondary fibres extend from the shaft along almost its entire length, forming a branched structure (Fig. 1d–f). These branched structures can be straight but are often curved; when curved, the branches are characteristically splayed (Fig. 1c, d). Such splaying can be generated only where a central shaft and lateral branches are stiff and where the branches diverge along the length of the shaft, rather than diverging from a single point or limited region of the shaft (Extended Data Fig. 3). This mode of branching is directly comparable to that in stage IIIa feathers<sup>19,20</sup> of extant birds, that is, with barbs branching from a central rachis. This is strong evidence that the fossil branched structures are feathers comprising a rachis and barbs. This is consistent with and supports recent claims of branched feathers in other pterosaurs<sup>1</sup>. The monofilaments are thus most plausibly interpreted as stage I feathers.

To our knowledge, stage IIIa feathers have not previously been reported in pterosaurs. The *Tupandactylus* branched structures resemble those in the dromaeosaurid dinosaur *Sinornithosaurus millenii*<sup>27</sup>, which are considered homologous to avian feathers<sup>28</sup>, and differ from the three types of branched feathers described in anurognathid pterosaurs<sup>2</sup>. Branching in the anurognathid feathers can be distal (brush-like ‘type 2’ feathers<sup>2</sup>), near the midpoint (brush-like ‘type 3’ feathers<sup>2</sup>) or proximal (tuft-like ‘type 4’ feathers<sup>2</sup>; see Extended Data Table 1 for comparison of fossil feather nomenclature systems). Unlike these three anurognathid feather types, all of which branch in a narrow zone along the feather shaft, the branched feathers in *Tupandactylus* branch along almost the entire length of the rachis. Further, the consistent length of the *Tupandactylus* secondary fibres (barbs) differs from the varying length of those in anurognathid feathers<sup>2</sup>.

The *Tupandactylus* feathers are not taphonomic artefacts. Both monofilaments and branched feathers occur in the specimen, which is consistent with the presence of multiple feather types in anurognathids<sup>2</sup>, feathered dinosaurs<sup>29–31</sup> and fossil<sup>32,33</sup> and extant birds<sup>34</sup>. Critically, *Tupandactylus* includes many isolated (non-superimposed) feathers where branching is obvious (Fig. 1c–f) and thus cannot be explained by superposition of monofilaments<sup>35</sup>. Nor does branching reflect degradation of monofilaments<sup>35</sup>—branched feathers show a consistent morphology, unlike the random pattern of fragmentation expected from decay. Further, the branched feathers do not represent structural fibres of the skin that have decayed, as the feathers are restricted to a portion of the skull (occipital process) that should be devoid of such fibres. Moreover, the cranial crest lacks feathers despite the preservation of long straight fibres (100–150  $\mu$ m wide; up to approximately 300 mm long) that presumably represent preserved structural skin fibres (Supplementary Information and Extended Data Figs. 1, 4).

Our phylogenetic reconstruction used a recently published phylogeny for pterosaurs, birds and non-avian dinosaurs<sup>2</sup> that preserve



**Fig. 2 | Time-tree phylogeny of Avemetatarsalia.** The phylogeny shows the results of ancestral-state estimations for the origin of feathers with the highest likelihood (−72.52), in addition to the lowest AICc (168.32) and the highest AICc weighting (64.56). Only the most complex integumentary structure present is shown for each taxon. Feathers are reconstructed as ancestral to the common avemetatarsalian ancestor of dinosaurs and pterosaurs. Branch lengths are estimated using the mbl branch length estimation and reconstructed

according to the best model (that is, with the highest likelihood, lowest AICc and highest AICc weighing), which estimates trait transition rates following ordered evolution. The pie charts at the nodes show the scaled likelihoods of different integumentary structures. The likelihood values for model parameters are shown in Extended Data Table 2. The *Tupandactylus* silhouette is drawn by E. Boucher from www.phylopic.org. Silhouettes of integumentary appendages are reproduced from ref. <sup>2</sup>, Springer Nature Limited.

integumentary structures. Given their lack of secondary branching (that is, barbules), branched feathers in *Tupandactylus* correspond to an open pennaceous vane. Ancestral-state estimations indicate that the statistically most likely result (corrected Akaike information criterion (AICc) weight = 84%) is that the avemetatarsalian ancestor of pterosaurs and dinosaurs possessed integumentary filaments, with approximately equal likelihood of possessing monofilaments, tufted feathers and brush-like feathers (Fig. 2, Extended Data Figs. 5–7, Extended Data Table 2). This is not inconsistent with the hypothesis that filamentous integumentary structures originated independently in both groups<sup>36</sup>. The more parsimonious interpretation, however, is that monofilaments and branched feather morphologies have a single origin in Avemetatarsalia. Our model predicts that progressively more complex integumentary structures arose within both Pterosauria and Theropoda (Fig. 2, Extended Data Figs. 5–7, Extended Data Table 2). This does not imply that identical feather types evolved in each group. Some feather morphologies are shared (that is, monofilaments, brush-like and tufted feathers and feathers with along-rachis branching), but others are not—for example, feathers with midpoint branching in pterosaurs and all feathers with barbules in theropods. Barbules are thus a unique innovation of theropod feathers. Progressive evolution of feather complexity is consistent with the younger age of *Tupandactylus* (with open vane branched feathers) relative to the previously studied anurognathids (with branching restricted to a narrow zone on the shaft).

### Tissue-specific melanosome geometries

We analysed samples of soft tissue from the fossil monofilaments, branched feathers and fibrous soft tissues from the cranial crest (Extended Data Fig. 8). Scanning electron microscopy shows that all soft tissue samples contain abundant ovoid or elongate microbodies approximately 0.5–1 μm in length (Extended Data Table 3). These microbodies are often embedded in an amorphous matrix similar to that preserved in feathers of other pterosaurs<sup>2,6</sup> and some non-avian dinosaurs and early-diverging birds<sup>13,36,37</sup> and interpreted as the degraded remains of the feather keratin matrix<sup>2,37,38</sup>. Samples of sedimentary matrix adjacent to the cranial crest lack microbodies (Extended Data Fig. 1, samples 1 and 9), confirming that the latter are restricted to the soft tissues. Microbodies with relatively homogeneous ovoid geometries were previously reported in fibrous soft tissues of the crest of another *Tupandactylus* specimen from the Crato Formation<sup>5</sup> and in filamentous structures from a pterosaur from the Jehol Group<sup>6</sup>. In each case, the microbodies were interpreted as preserved melanosomes<sup>5,6</sup>. This is consistent with the broad consensus (based on extensive morphological, ultrastructural, chemical and contextual evidence) that similar microbodies, preserved in dark carbonaceous soft tissue films associated with other fossil vertebrates, represent fossil melanosomes<sup>39,40</sup>.

In *Tupandactylus*, melanosomes from the skin fibres in the crest, monofilaments and branched feathers differ significantly in geometry

(analysis of variance (ANOVA):  $F(4, 2,989) = 449.3, P < 0.0001, n = 2,994$ ). Elongate melanosomes are restricted to the monofilaments (Fig. 1h, Extended Data Fig. 8) ( $848 \pm 172$  nm long and  $255 \pm 62$  nm wide;  $n = 406$ ). Melanosomes in the branched feathers are ovoid ( $794 \pm 127$  nm long and  $303 \pm 50$  nm wide;  $n = 878$ ; Fig. 1i, Extended Data Fig. 8). Melanosomes are ovoid in skin fibres located between the base of the cranial crest and the occipital process (Fig. 1g, Extended Data Fig. 8; area 1, Extended Data Table 3;  $835 \pm 145$  nm long and  $371 \pm 92$  nm wide;  $n = 786$ ) and in the posterior part of the cranial crest (Extended Data Fig. 8; area 2, Extended Data Table 3;  $702 \pm 153$  nm long and  $344 \pm 92$  nm wide;  $n = 693$ ). In the dorsal part of the crest (area 3, Extended Data Table 3), melanosomes are spheroidal ( $649 \pm 156$  nm long and  $400 \pm 120$  nm wide;  $n = 231$ ). Similar tissue-specific partitioning of melanosome geometry has been reported in diverse other fossil and extant vertebrates<sup>40–42</sup>. The absence of multiple distinct melanosome populations in the other studied specimen<sup>5</sup> of *Tupandactylus* may reflect limited sampling.

The diversity of melanosome morphologies reported here expands the known range<sup>2,6</sup> of geometries of pterosaur melanosomes (Extended Data Fig. 9c): rods and spheres had previously been reported only from mammalian hair and dinosaur (non-avian and avian) feathers. The geometry of the melanosomes in *Tupandactylus* overlaps with that of extant animals (Extended Data Fig. 9a–d). This further supports the hypothesis that the branched integumentary structures in pterosaurs are feathers. It does not, however, completely exclude the alternative (albeit unlikely) hypothesis that pterosaur filamentous integumentary structures represent a third type of vertebrate integumentary outgrowth (in addition to hair and feathers) that is capable of imparting, and varying, melanin-based coloration.

The different geometries of the preserved melanosomes in the monofilaments and branched feathers are suggestive of different visible colours. Irrespective of the actual colour produced, the data confirm tissue-specific melanosome populations in MCT.R.1884. In turn, this strongly suggests that the genomic and developmental mechanisms required for tuning melanosome geometry were already in place in the avemetatarsalian ancestor of pterosaurs.

## Origins for visual signalling in feathers

Our study has important implications for understanding the evolution of melanin-based colouration. Melanosomes in other pterosaur fossils have ovoid to spheroidal shapes, even in integumentary filaments or feathers<sup>2,5,6</sup>. This low melanosome diversity resembles that in the skin of extant reptiles, where many colours are generated by non-melanin pigments housed in iridophores and xanthophores<sup>41–43</sup>. Preservation of ovoid and spheroidal melanosomes in pterosaur feathers and skin was therefore previously interpreted as evidence for retention of the ancestral state in pterosaurs<sup>40</sup>. Unlike those fossils, however, MCT.R.1884 shows important differences in melanosome geometry between the skin and feathers, with evidence for expanded diversity of melanosome geometry (that is, elongate melanosomes) in the feathers. This tissue-specific partitioning of melanosome geometry—and, in particular, the greater morphological diversity of melanosomes in integumentary appendages (feathers and hair) than in skin—also characterizes extant birds and mammals<sup>6</sup>. This feature may reflect preferential selection of more extreme, oblate melanosome geometries in order to expand melanin-based colour space<sup>40</sup> into regions associated with eumelanin-dominated darker and iridescent hues. In turn, this may be a response to the loss of non-melanin-containing chromatophores during the evolution of integumentary appendages<sup>44</sup>. Alternatively, these fundamental changes in skin structure may derive from changes in metabolism<sup>6</sup> and immunity<sup>40</sup> during the evolution of endothermy. At a genomic and developmental level, the production of elongate, eumelanin-rich melanosomes reflects earlier activation of  $\alpha$ -melanocyte-stimulating hormone<sup>7</sup> ( $\alpha$ -MSH) and/or enhanced production of premelanosome proteins<sup>8,45</sup> that form a scaffold for

eumelanin deposition during melanosome development<sup>8</sup>. The discovery of elongate melanosomes in the feathers, but not skin, of the specimen of *Tupandactylus* described here expands the known range of feather melanosome geometries in pterosaurs and confirms that pterosaurs show similar tissue-specific trends in melanosome geometry to fossil and extant birds and other theropods<sup>46,47</sup>. This could reflect one of three evolutionary scenarios related to the timing of origin of the genomic regulatory networks governing melanogenesis (especially linked to  $\alpha$ -MSH, agouti signaling protein, SRY-box transcription factor 10 (Sox10) and melanocortin-1-receptor)<sup>45</sup> and their phenotypic expression. The genotypic and phenotypic characters could both be ancestral to avemetatarsalians; alternatively, both evolved independently in theropods and pterosaurs, or the genes are ancestral and the phenotypic expression occurred independently in the two groups. Our ancestral-state estimations (Extended Data Fig. 9e) reveal that the most parsimonious scenario is that feathers in the avemetatarsalian ancestor had melanosomes with different geometries. This is consistent with a single, deep evolutionary origin for this feature, whereby critical shifts in the genetic machinery facilitating plasticity in melanosome shape occurred in the common ancestor of pterosaurs and birds. Key genomic controls on melanin-based colouration that define the plumage colours of theropods and fossil and extant birds were therefore already in place in early-diverging avemetatarsalians in the Middle to Late Triassic.

## Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-022-04622-3>.

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## Methods

### Fossil material

Twenty-two soft tissue samples were collected using sterile tools from MCT.R.1884. These samples represent: (1) six distinct integumentary appendages located close to the posterior part of the occipital process (Extended Data Fig. 1, samples 3, 4, 6, 7, 23 and 24); (2) three skin fibres projecting from the crest towards the occipital process (Extended Data Fig. 1, samples 2, 5 and 8); (3) four skin fibres from the posterior part of the crest (Extended Data Fig. 1, samples 10, 11, 15 and 18); (3) nine skin fibres situated on the anterior portion of the crest (Extended Data Fig. 1, samples 12–14, 16, 17, 19–22). We also collected two samples of the sedimentary matrix (Extended Data Fig. 1, samples 1 and 9) in the region located between the cranial crest and the posterior extension of the skull.

### Scanning electron microscopy

Samples of soft tissue were mounted on double-sided carbon tape and sputter-coated with gold. Scanning electron microscopy (SEM) was performed with an environmental FEI Quanta 200 SEM and a FEI Quanta 650 FEG-SEM, using a working distance of 8.6–13 mm, accelerating voltage of 10–30 kV and a probe current of 1.5–3.0.

### Measurements of melanosome geometry

Long and short axis were measured for a total of 2,994 melanosomes using ImageJ<sup>48</sup> (version 64-bit Java 1.8.0\_172; <http://imagej.nih.gov/ij/>). Orientation was measured for selected samples. For melanosomes in each sample, values for the mean, standard deviation, skew and coefficient of variance were calculated for melanosome length, width and aspect ratio. The significance of variation in the data was tested statistically using the ANOVA test in the freeware PAST<sup>49</sup> (version 4.09; palaeontological statistics: <https://www.nhm.uio.no/english/research/infrastructure/past/>).

### Ancestral-state estimations

Data on melanosome geometry were analysed using quadratic discriminant analysis and multinomial logistic regression using the MASS package<sup>50</sup> and the Nnet-package, both implemented in R using a published melanosome dataset<sup>51</sup>.

Ancestral-state estimations for integumentary appendages in *Avemotarsalia* were performed using the methodology and data in ref. <sup>2</sup>. In short, the integumentary appendages were assigned to one of six possible categories: scales, monofilaments, brush-like filaments, tufts of filaments joined basally, open pennaceous vane lacking secondary branching and closed pennaceous feathers comprising a rachis and barbs. We extended the above-mentioned database<sup>2</sup> via the inclusion of data on feathers from MCT.R.1884 as an open-vaned structure. We used maximum-likelihood estimations implemented in the ‘ace’ function of the ape 4 package<sup>52</sup>. Tree branch lengths were estimated using two methods: ‘equal branch’ length and ‘minimum branch’ length (mb1); using the ‘DatePhylo’ function in the strap R package<sup>53</sup>. For more details, see ref. <sup>2</sup>.

We ran our analyses using four evolutionary models with different state transition rates: an equal-rates model, a symmetrical rates model, an all-rates-different and an ordered-rates model. In the last example,

transition can occur only to and from successive states; that is, feathers with a closed vane can evolve only if open-vaned feathers have already evolved. We compared models by calculating log-likelihood, Akaike information criterion (AIC) and AICc; the latter model corrects for sample size and is summarized as weighed AICc values (Extended Data Table 2). Because of the large parameter space, ‘ace’ was not able to estimate ancestral states for the mb1-ARD model. As such, we used the ‘make.simmap’ function of the phytools package<sup>54</sup>.

### Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

### Data availability

Additional data on melanosome geometry and the character matrix used in the phylogenetic analyses are available in the Zenodo.org data repository at <https://doi.org/10.5281/zenodo.6122213>. SEM images and samples are available from the corresponding authors on request.

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**Acknowledgements** This work was funded by a Fonds National pour la Recherche Scientifique (F.R.S.-FNRS) FRIA grant (F3/5/5-MCF/ROI/BC-2319784), an Irish Research Council Government of Ireland Postdoctoral Fellowship (GOIPD/2018/768) awarded to A.C. and an ERC Starting Grant H2020-2014-StG-637691-ANICOLEVO and an ERC Consolidator Grant H2020-2020-CoG-101003293-PALAEOCHEM awarded to M.N. We thank M. Benton for providing the original data and code used in the phylogenetic reconstruction<sup>2</sup>, Z. Yang for providing raw melanosome measurements used to compare melanosome geometry in pterosaurs and J. Cillis for assistance with SEM. MCT.R.1884 was photographed by T. Hubin (RBINS).

**Author contributions** A.C. conceived the study, designed and performed analyses (SEM, melanosome measurements, taphonomy and ANOVA), interpreted data, prepared figures and tables and co-wrote the paper with M.M. M.N. performed analyses (ancestral-state estimations), described the specimen, interpreted data, prepared figures and tables and revised drafts of the paper. H.B.N.C. described the specimen; M.M. performed analyses (SEM), interpreted data, prepared figures and co-wrote the paper with A.C. L.D. supervised the study, prepared figures and provided data. M.D.S. supervised the study and provided data. E.-E.K. provided data. J.Y. supervised the study and revised drafts of the paper. R.C. performed analyses and interpreted data. F.E. provided data. P.G. conceived, designed and supervised the study, described the specimen and revised drafts of the paper. All authors discussed the manuscript and approved the submitted version.

**Competing interests** The authors declare no competing interests.

### Additional information

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41586-022-04622-3>.

**Correspondence and requests for materials** should be addressed to Aude Cincotta or Maria McNamara.

**Peer review information** Nature thanks Michael Benton, Stephen Brusatte and Xing Xu for their contribution to the peer review of this work. Peer reviewer reports are available.

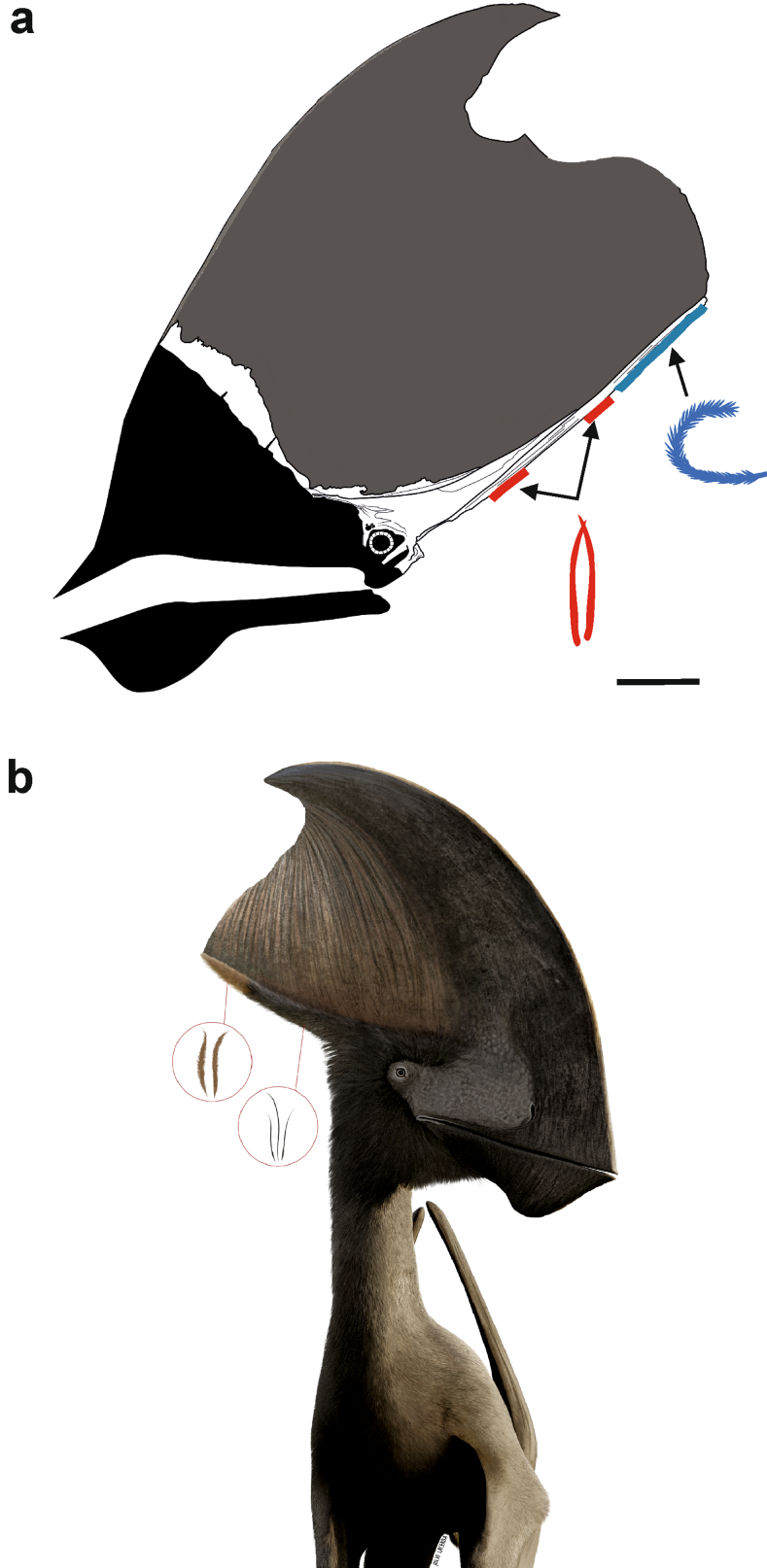
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**Extended Data Fig. 1 | Location of the samples collected from the soft tissue cranial crest, monofilaments and branched feathers and sedimentary matrix.** The soft tissue crest is characterized by elongate brown fibres.

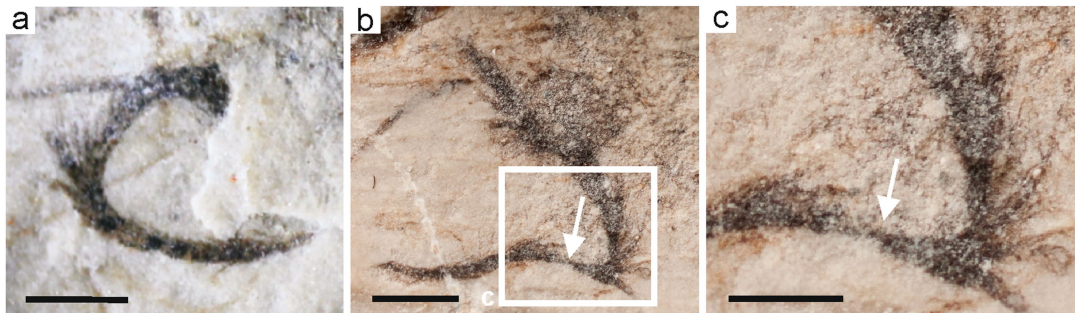
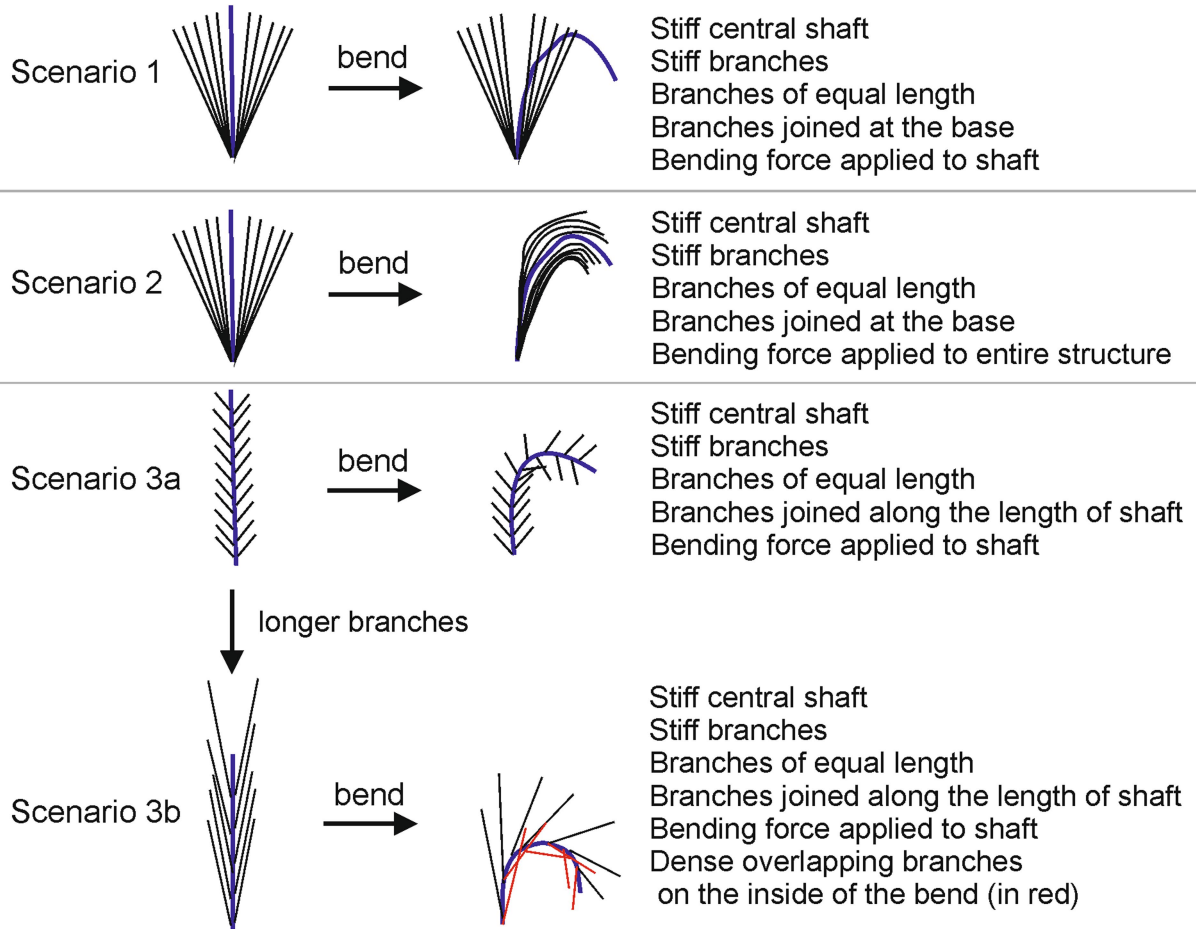
The posterodorsal part of the crest is darker than the rest of the crest and the brown fibres are faint or not evident in that area. Scale bar, 100 mm.





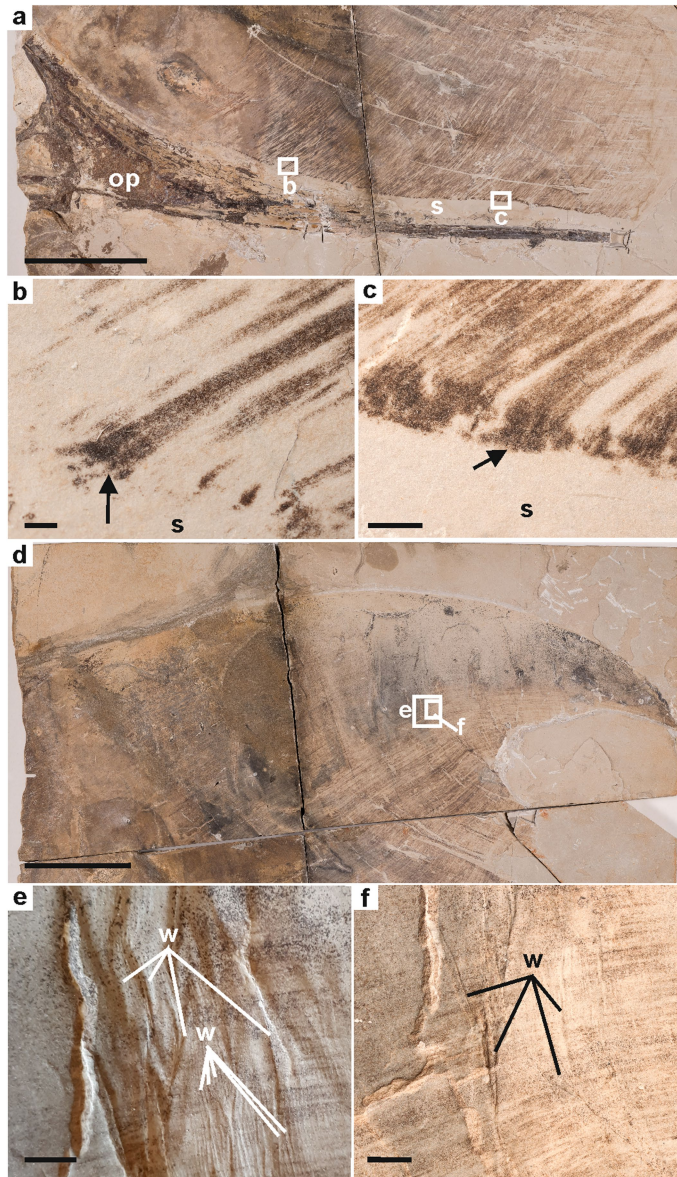
**Extended Data Fig. 2 | Distribution of feather types in the tapejarid pterosaur *Tupandactylus cf. imperator* (MCT.R.1884).** **a**, Schematic illustration of MCT.R.1884. Monofilaments (red) are restricted to the region immediately adjacent to the proximal part of the occipital process and the branched feathers (blue) to the region adjacent to the distal part of the

occipital process. The cranial soft tissue crest is shown in dark grey and the preserved bones are shown in white. The proximal part of the skull (in black) is not present on the slab. **b**, Reconstruction of MCT.R.1884 showing the distribution of feathers along the occipital process (colours are not reconstructed here). Image credit, Julio Lacerda. Scale bar in (a), 100 mm.

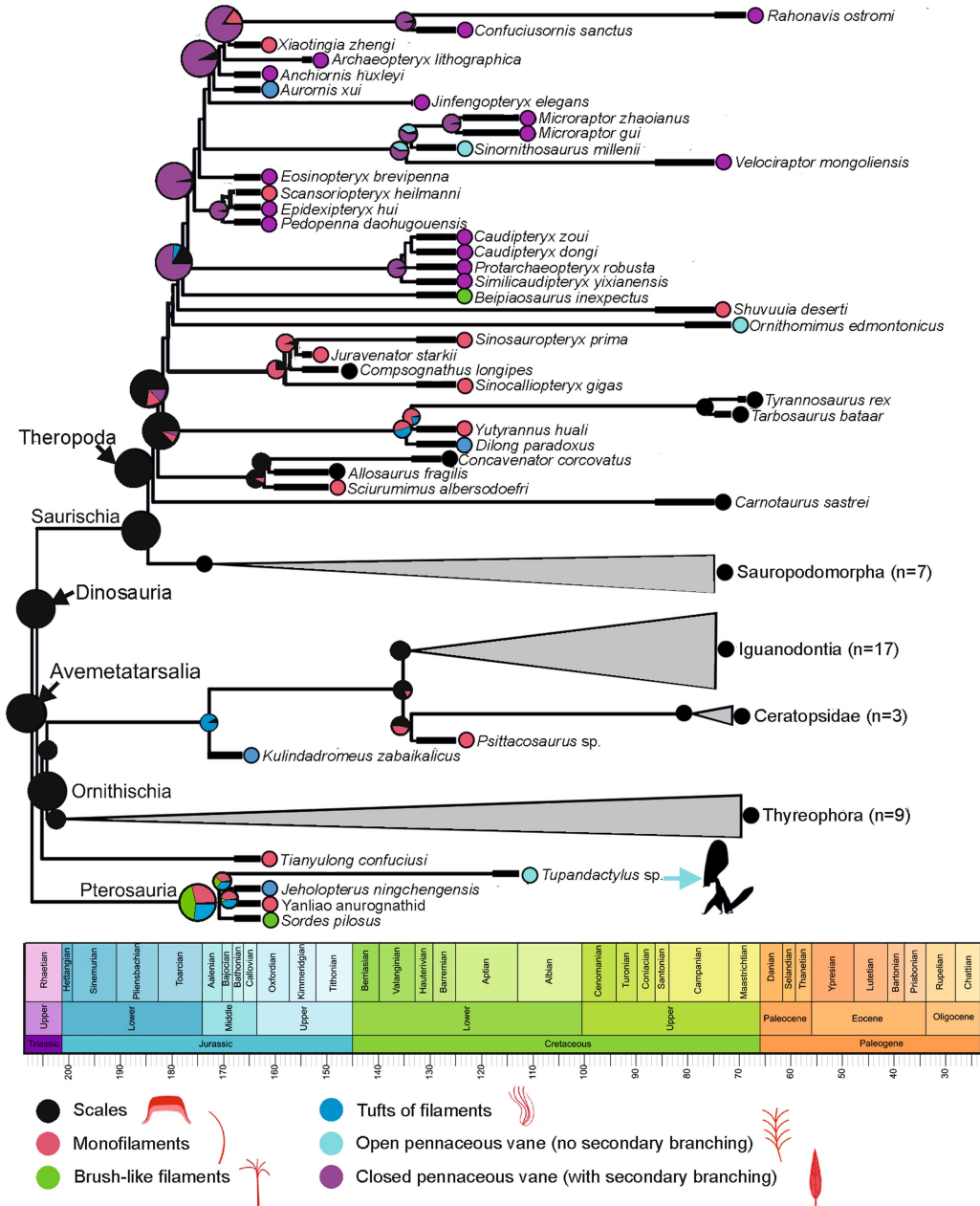


**Extended Data Fig. 3 | Taphonomic scenarios to explain the origin of the splayed appearance of the branched feathers, based on different styles of feather branching and stiffness.** Only scenario 3, in particular scenario 3b, with a stiff central shaft and stiff barbs of equal length, can explain the

particular structures observed in *Tupandactylus* feathers. **a–c**, Branched feathers from MCT.R.1884. **c**, Close-up of the splayed structure in **(b)** showing branching and a thin shaft at the point of flexure of the barbs (arrow). Scale bars, 1 mm (**a**, **b**), 250  $\mu$ m (**c**).

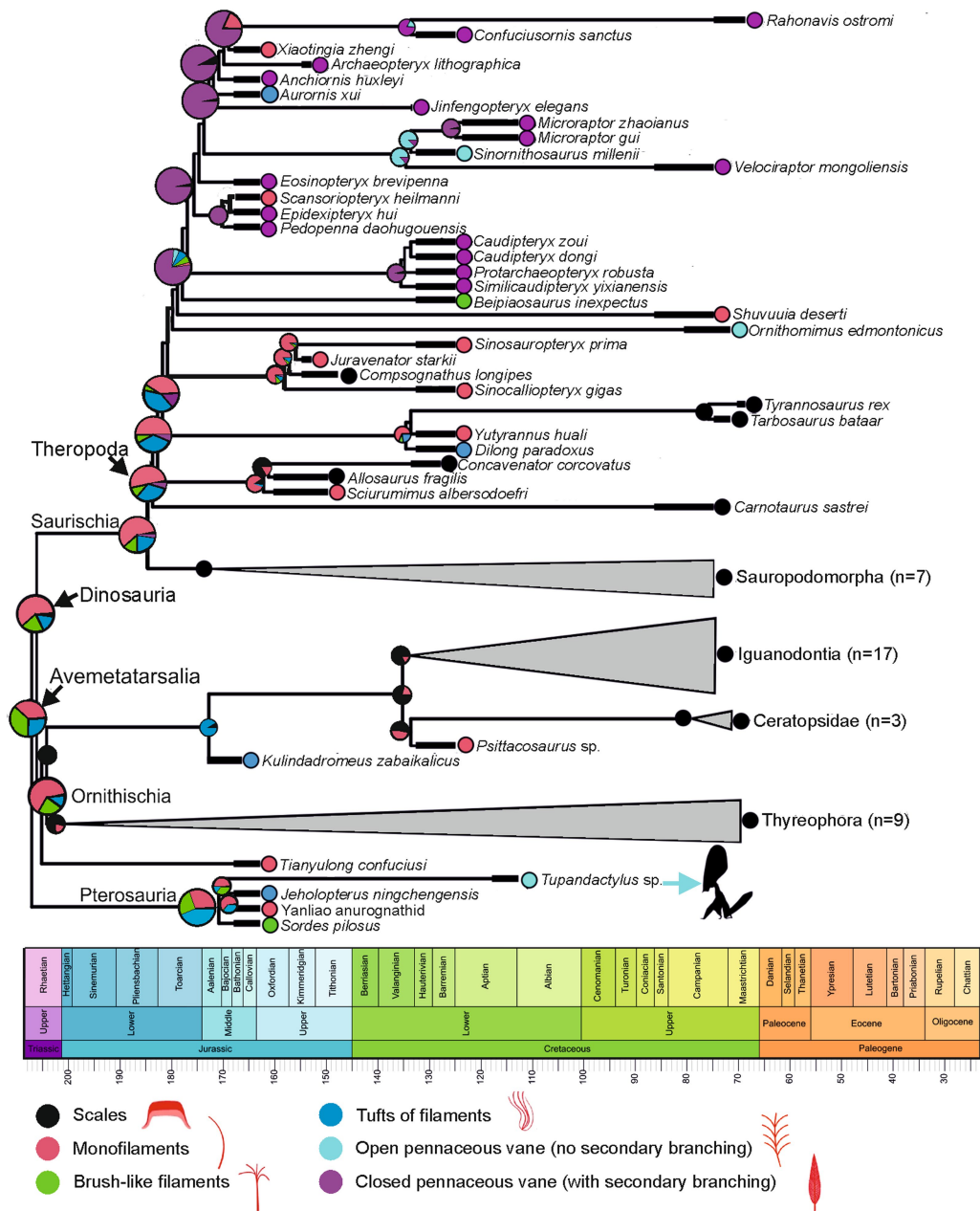


**Extended Data Fig. 4 | Integumentary structures of the cranial crest of MCT.R.1884.** **a**, Ventral part of the soft tissue crest separated from the occipital process (**op**) by a zone lacking soft tissue and showing only sediment (**s**). **b, c**, Detail of the basal part of the cranial crest showing dark brown structures at the base of the fibres (see arrows). **d**, Posterodorsal part of the cranial crest. **e, f**, Details of regions indicated in (**d**). The brown fibres of the soft tissue crest are oriented perpendicular to prominent wrinkles, expressed as variation in the topography of the specimen. Scale bars, 10 mm (**a, d**); 2 mm (**b, c**); 5 mm (**e, f**). **op**, occipital process; **s**, sediment; **w**, wrinkle.



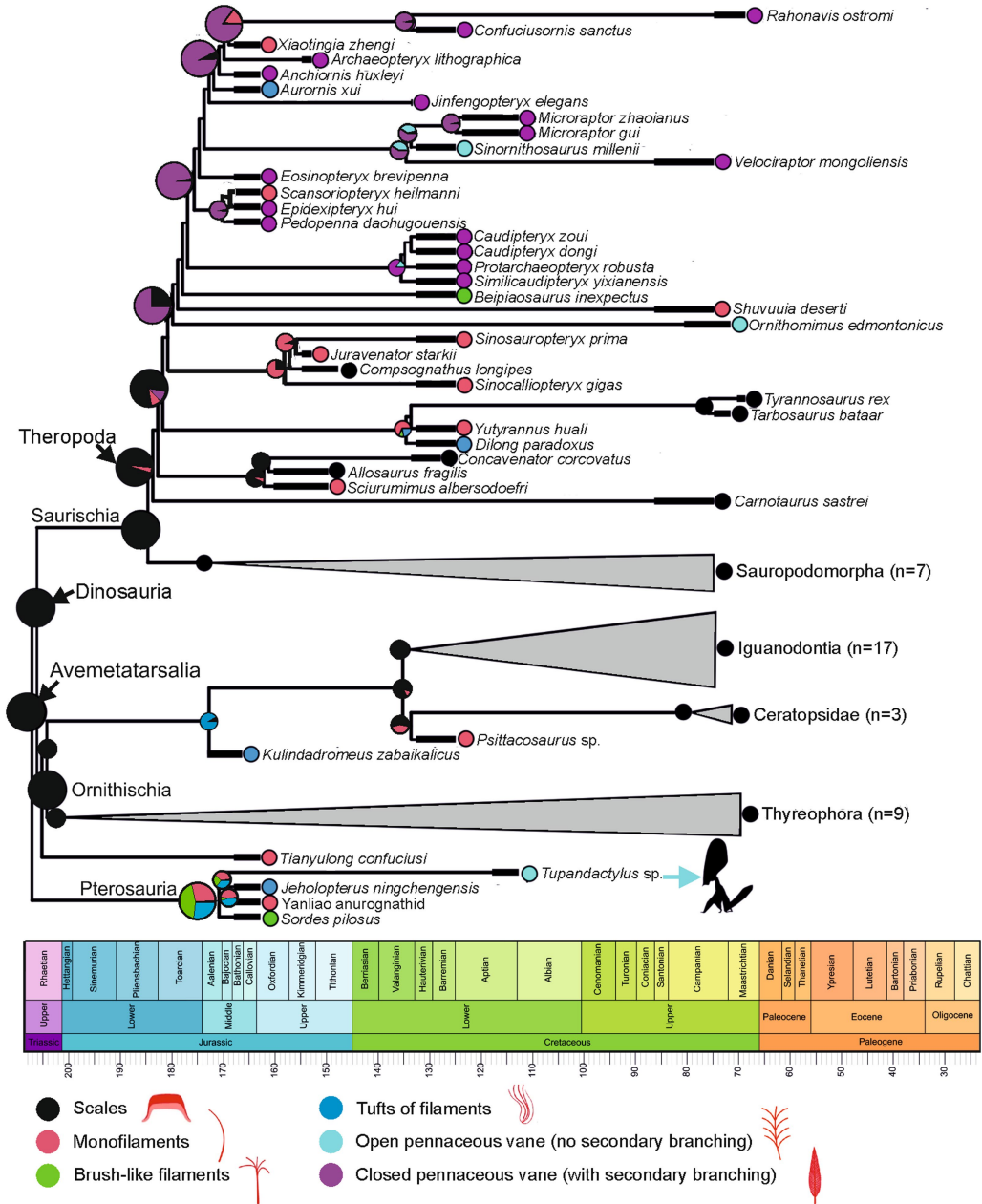
**Extended Data Fig. 5 | Time-tree phylogeny of Avemetatarsalia, estimated using the 'mbl' branch-length estimation and reconstructed according to the 'equal rates' evolutionary model.** The likelihood values for model parameters are shown in Extended Data Table 2. The different categories of integumentary structures represent: scales, monofilaments, brush-like filaments, tufts of filaments joined basally, open pennaceous vane lacking

secondary branching and closed pennaceous feathers comprising a rachis-like structure associated with lateral branches (see material and methods in the main text for more details). *Tupandactylus* silhouette by Evan Boucher from www.phylopic.org. Silhouettes of integumentary appendages are reproduced from ref. <sup>2</sup>. (Fig. 3).



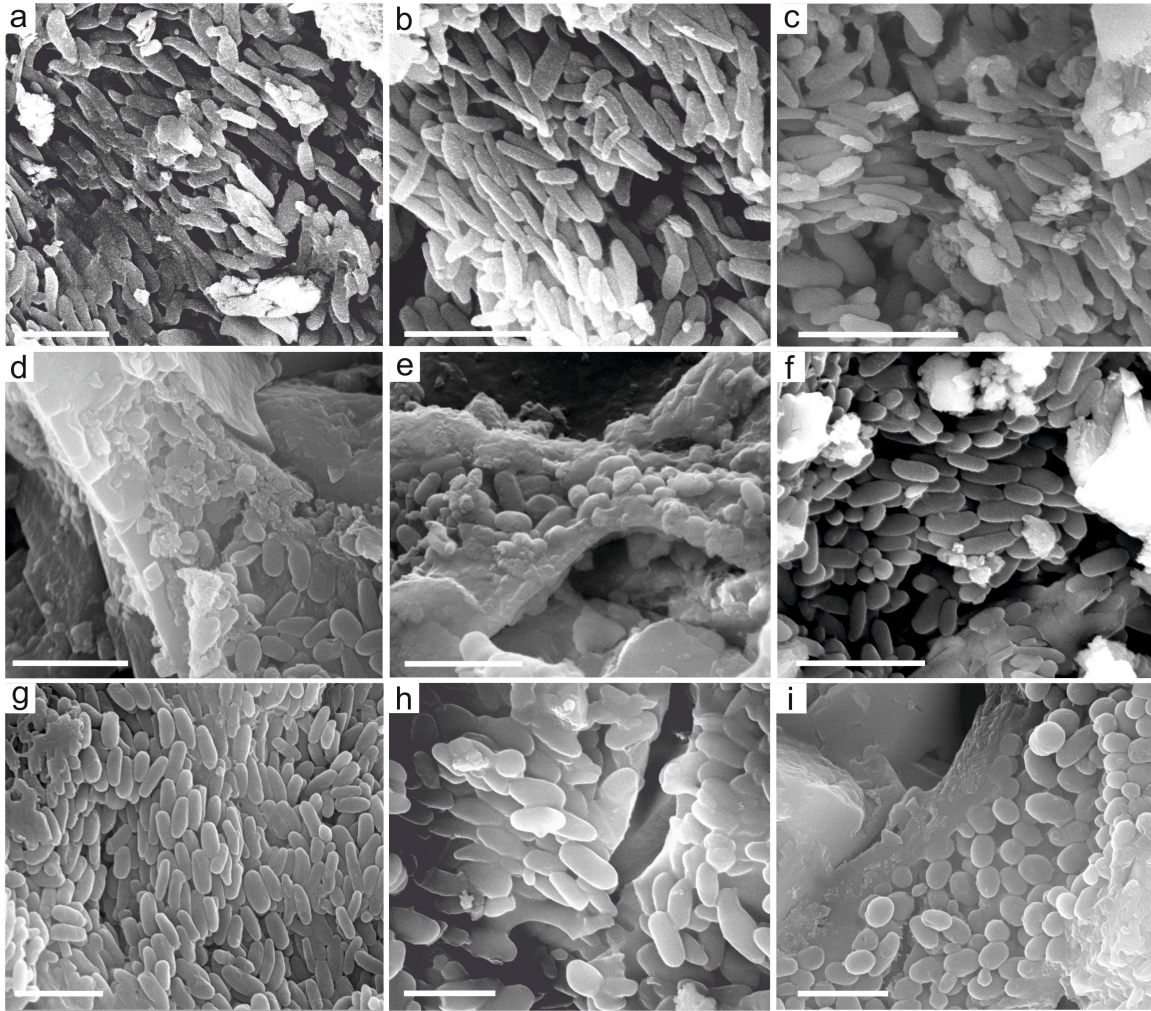
**Extended Data Fig. 6 | Time-tree phylogeny of Avemetatarsalia, estimated using the 'mbl' branch-length estimation and reconstructed according to the 'SYM' evolutionary model.** The likelihood values for model parameters are shown in Extended Data Table 2. The different categories of integumentary structures represent: scales, monofilaments, brush-like filaments, tufts of

filaments joined basally, open pennaceous vane lacking secondary branching and closed pennaceous feathers comprising a rachis-like structure associated with lateral branches (see material and methods in the main text for more details). *Tupandactylus* silhouette by Evan Boucher from www.phylopic.org. Silhouettes of integumentary appendages are reproduced from ref. <sup>2</sup>. (Fig. 3).

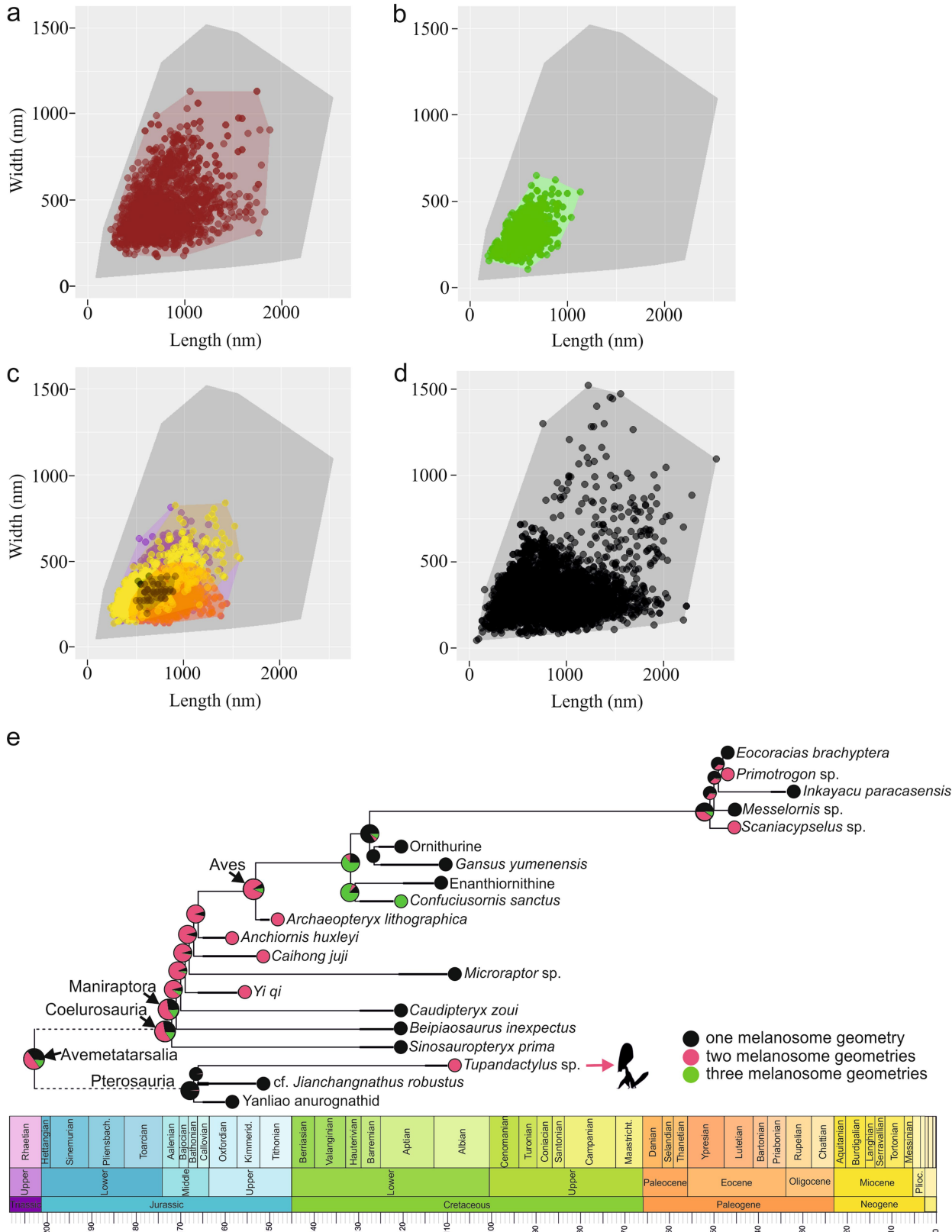


**Extended Data Fig. 7 | Time-tree phylogeny of Avemetatarsalia, estimated using the ‘mbl’ branch-length estimation and reconstructed according to the ‘all rates different’ (ARD) evolutionary model.** The likelihood values for model parameters are shown in Extended Data Table 2. The different categories of integumentary structures represent: scales, monofilaments, brush-like filaments, tufts of filaments joined basally, open pennaceous vane

lacking secondary branching and closed pennaceous feathers comprising a rachis-like structure associated with lateral branches (see material and methods in the main text for more details). *Tupandactylus* silhouette by Evan Boucher from [www.phylopic.org](http://www.phylopic.org). Silhouettes of integumentary appendages are reproduced from ref. <sup>2</sup>. (Fig. 3).



**Extended Data Fig. 8 | Scanning electron micrographs of melanosomes in the soft tissues of MCT.R.1884. a–c, Elongate melanosomes from monofilaments. d–f, Ovoid melanosomes from the branched feathers. g–i, Ovoid melanosomes from the soft tissue crest (area 1, Extended Data Table 2). Scale bars, 2  $\mu$ m.**








Extended Data Fig. 9 | See next page for caption.



**Extended Data Fig. 9 | Scatterplots of melanosome geometry in amniotes and ancestral-state estimation of the diversity of melanosome geometries within Avemetatarsalia. a–d**, Melanosome geometry in amniotes; data from refs.<sup>2,6</sup>, and this study. **a**, Mammal hair<sup>6</sup> (n = 1984). **b**, Squamate skin<sup>6</sup> (n = 734). **c**, Pterosaur skin (this study, n = 2115; melanosomes imaged from ten independent samples; purple datapoints) and pterosaur feathers (n = 2173; orange datapoints, this study (n = 1284; melanosomes imaged from four independent samples); black and yellow datapoints, previous studies<sup>2,6</sup>). **d**, extinct and extant bird feathers<sup>6</sup> (n = 3643). Data from non-avian dinosaurs are not shown here. Polygon with dark grey shading in **(a–d)** shows the range of

melanosome geometries known for extant and extinct bird feathers. Darker shades in **(a)** and **(d)** indicate more than one data point with similar measurements. **e**, Simplified time-tree phylogeny estimated using the ‘mbf’ branch-length estimation and reconstructed according to the best evolutionary model, i.e. ‘equal rates’ (ER) model. The different categories (or ‘states’) of melanosome geometry are: one geometry (in black), two geometries (in red) and three geometries (in green). Only taxa for which melanosome length and aspect ratio was known have been included in our dataset (n = 20). \*taxa showing spheroidal melanosomes in addition to any other category. *Tupandactylus* silhouette (in **e**) by Evan Boucher from [www.phylopic.org](http://www.phylopic.org).

**Extended Data Table 1 | Classification of pterosaur feathers**

Pterosaur feather morphology	Taxon	Branching	Feather type (sensu Yang et al., 2019)	Evo-devo Stage (sensu Prum (1999); Prum & Brush, 2002)	Feather morphotype (sensu Xu et al., 2010; Xu, 2020)	Assignment to evo-devo Stage
	anurognathids & <i>Tupandactylus</i>	none	type 1	Stage I	morphotype 1 ("SMFI")	Stage I
	anurognathids	terminal	type 2	n/a	morphotype 4 ("BJSFF")	Stage II+
	anurognathids	mid-point	type 3	n/a	n/a	Stage II+
	anurognathids	basal	type 4	Stage II	morphotype 3 ("BJFF")	Stage II
	<i>Tupandactylus</i>	along-rachis	n/a	Stage IIIa	morphotype 5 ("RBSFF")	Stage IIIa

Assignment of pterosaur feathers, including those reported in *Tupandactylus* cf. *imperator* (this manuscript) and two anurognathid pterosaurs<sup>2</sup>, to existing classification systems; i.e. feather type (sensu Yang et al., 2019), evo-devo stage (sensu Prum et al. 1999 and Prum & Brush, 2002) and feather morphotype (sensu Xu et al., 2010 and Xu, 2020). SMFI: slender monofilamentous integument, BJFF: basally joining filamentous feather, BJSFF: basally joining shafted filamentous feather, RBSFF: radially branched shafted filamentous feather.



**Extended Data Table 2 | Model performance of the phylogenetic reconstructions using different methods for branch length reconstruction and different transition rates**

<i>Model</i>	<i>nvar</i>	<i>LnL</i>	<i>Scales</i>	<i>Filaments</i>	<i>AIC</i>	<i>AICc</i>	<i>AICc_wt</i>
<i>equal – ER</i>	1	-92.35	98.30%	1.70%	186.70	186.75	1.28E-05
<i>equal – SYM</i>	15	-81.36	96.60%	3.40%	192.72	200.46	6.30E-07
<i>equal – ARD</i>	30	-74.35	0%	100%	208.70	248.27	2.13E-10
<i>equal – ordered</i>	10	-73.12	0%	100%	166.24	169.52	0.35
<i>mbl – ER</i>	1	-93.17	91.50%	8.50%	188.34	188.39	5.63E-06
<i>mbl – SYM</i>	15	-76.85	92.40%	7.60%	183.70	191.44	5.73E-05
<i>mbl – ARD*</i>	30	-72.74	0%	100%	205.48	245.05	N/A
<i>mbl – ordered</i>	10	-72.52	0%	100%	165.04	168.32	0.64

Parameters shown are the number of variables (*nvar*), log-likelihood (*LnL*), probability of scales being ancestral (*scales*), probability of feather-like structures being present (*filaments*), Akaike Information Criterion (*AIC*), second order bias correction of *AIC* (*AICc*) and relative weight of the corrected *AIC* (*AICc\_wt*). \* *mbl-ARD* was calculated using a different method (*make.simmap*) and was not used in the weighted *AICc* calculations.

# Article

**Extended Data Table 3 | Geometry of melanosomes (mean plus standard deviation) from various soft tissues in *Tupandactylus imperator* (MCT.R.1884)**

<i>Tissue type</i>	<i>n</i>	<i>Long axis (nm)</i>	<i>Short axis (nm)</i>	<i>Aspect ratio</i>	<i>Geometry</i>
<i>Crest fibres (area 1) (Extended Data Fig. 8g)</i>	786	835 ± 145	371 ± 92	2.37 ± 0.66	
<i>Crest fibres (area 2) (Extended Data Fig. 8i)</i>	693	702 ± 153	344 ± 92	1.97 ± 0.39	
<i>Crest fibres (dark regions of the crest; area 3)</i>	231	649 ± 156	400 ± 120	1.60 ± 0.28	
<i>Monofilaments (Fig. 1h and Extended Data Fig. 8a-c)</i>	406	848 ± 172	255 ± 62	3.57 ± 1.04	
<i>Branched feathers (Fig. 1i and Extended Data Fig. 8d-f)</i>	878	794 ± 127	303 ± 50	2.68 ± 0.56	

Schematic melanosome morphology is shown for each tissue analyzed. n, number of individual melanosomes measured for each tissue type.

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### Software and code

Policy information about [availability of computer code](#)

Data collection No software was used to collect data

Data analysis Melanosomes were measured using ImageJ freeware (version 64-bit Java 1.8.0\_172; <https://imagej.nih.gov/ij/>); The significance of variation in the data was tested statistically using the ANOVA test of the freeware PAST (Palaeontological Statistics, version 4.09; <https://www.nhm.uio.no/english/research/infrastructure/past/downloads/>); Normality tests were done using RStudio freeware (version 1.1.463); Data on melanosome geometry was analysed using quadratic discriminant analysis (QDA) and multinomial logistic regression (MLR) using the MASS-package (Venables & Ripley, 2002) and the Nnet-package, both implemented in R using a published melanosome dataset (Babarović et al., 2019); Ancestral state estimations were performed using the methodology and data presented in Yang et al., (2019). We used maximum-likelihood estimations implemented in the 'ace' function of the ape 4 package (Paradis, 2011). Tree branch lengths were estimated using two methods: 'equal branch' length and 'minimum branch' length (mbL) using the 'DatePhylo' function in the strap R package (Bell & Loyd, 2015); The evolutionary models were run using the 'make.simmap' function of the phytools' package (Revell, 2012).

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Additional data, including dimension of melanosomes and the character matrix used in the phylogenetic analyses have been deposited in a data repository at Zenodo.org (DOI: 10.5281/zenodo.6122213). SEM images and samples are available from the corresponding authors on request.

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## Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We report diverse melanosome geometries in the skin and simple and branched feathers associated with the cranial crest of a tapejarid pterosaur from the Early Cretaceous of Brazil (Crato Formation). We collected fossil soft tissue samples from the cranial crest itself and from both feather types. We imaged the samples using scanning electron microscopy (SEM) and measured the length and width of melanosomes from the SEM images. We imaged 22 samples in total.
Research sample	The research sample is a pterosaur cranial crest ( <i>Tupandactylus cf. imperator</i> ; MCT.R.1884). The specimen provides an almost complete cranial crest and two types of integumentary appendages. We targeted the soft tissue part of the crest (skin) and the feathers to study (1) their structure and (2) their taphonomy.
Sampling strategy	No statistical method was used to predetermine sample size. Small samples (a few mm wide) were collected in order to maintain the integrity of the fossil as much as possible. The size of the sample was sufficient to observe abundant melanosomes and have statistically significant data points.
Data collection	Twenty-two soft tissue samples were collected by A. Cincotta using sterile tools (tweezers). These samples are: (1) six independent monofilaments and branched feathers located around the posterior extension of the occipital process, (2) three fibres from the crest projecting from the base of the crest towards the occipital process, (3) four fibres collected on the posterior part of the crest, and (4) nine skin fibres located on the anterior part of the crest. Samples from the sedimentary matrix (from the region located between the base of the crest and the occipital process) were also collected. Samples were stored in SEM storage boxes before imaging.
Timing and spatial scale	Sample collection started in 2017 and finished in 2021. The timing for sample collection is related to the advance of our study. Timing of data collection has no importance in our study (fossil samples). Millimeter-sized samples were collected.
Data exclusions	No data were excluded from the analyses.
Reproducibility	Melanosome measurements, data for ancestral state estimations and other supplementary data are deposited in a data repository, available on:
Randomization	Samples were differentiated after their location on the fossil and their morphology: (1) cranial crest, (2) monofilaments and (3) branched feathers.
Blinding	Blinding is not relevant to our study because it does not involve randomised control trials.
Did the study involve field work?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

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- Antibodies
- Eukaryotic cell lines
- Palaeontology and archaeology
- Animals and other organisms
- Human research participants
- Clinical data
- Dual use research of concern

## Methods

- n/a Involved in the study
- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

## Palaeontology and Archaeology

- Specimen provenance The fossil was originally collected from the Crato Formation at an unknown locality. The specimen resided in private collections for an unknown period of time before being deposited at the Royal Belgian Institute of Natural Sciences (RBINS). A cooperation agreement was signed on 11 October 2021 between RBINS and the embassy of Brazil in Belgium, which led to the official repatriation of the specimen to the Museum of Earth Sciences at Rio de Janeiro, Brazil, in early February 2022.
- Specimen deposition The specimen has been deposited at the Museum of Earth Sciences, Rio de Janeiro, Brazil. Collection number: MCT.R.1884.
- Dating methods No new dates are provided in our study.
- Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.
- Ethics oversight No ethical approval was required as the specimen studied is a fossil. The specimen was repatriated to its country of origin as part of a joint collaboration between Brazil and Belgium, and in agreement with the 1972 UNESCO convention concerning the protection of the World cultural and natural heritage.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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**Supplementary information**

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**Pterosaur melanosomes support signalling functions for early feathers**

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In the format provided by the authors and unedited



Supplementary information for:

## **Pterosaur melanosomes support signalling functions for early feathers**

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## **Contents**

<b>1. SUPPORTING TEXT.</b>	<b>3</b>
<b>1.1. Origin of the specimen.</b>	<b>3</b>
<b>1.2. Geological background.</b>	<b>3</b>
<b>1.3. Description of the specimen.</b>	<b>3</b>
<b>1.4. Soft tissue anatomy.</b>	<b>5</b>
<b>SUPPLEMENTARY REFERENCES.</b>	<b>7</b>

## **1. Supporting text.**

### **1.1. Origin of the specimen.**

The specimen is believed to have been originally poached from an undetermined outcrop of the Early Cretaceous Crato Formation (Araripe Basin, north-eastern Brazil) and then resided in private collections in Europe for an unknown period of time. The specimen was deposited by its private owner at the Royal Belgian Institute of Natural Sciences (RBINS) through the French fossil preparation company Eldonia in 2020. The Brazilian authorities were contacted immediately and the specimen is now part of the collections of the Museum of Earth Sciences, Rio de Janeiro (collection number: MCT.R.1884). Negotiations between the RBINS, Eldonia and Brazilian authorities led to the official physical repatriation of the specimen to Brazil in early February 2022. The specimen was incompletely prepared when it arrived at Eldonia. Preliminary preparation under the supervision of FE allows us to certify that this specimen is not a composite and that restorations prior to its arrival at RBINS are limited to standard consolidation procedures.

### **1.2. Geological background.**

The specimen is hosted within grey laminated limestones and was probably excavated from the Nova Olinda Member of the Crato Formation (Fm). This member is located at the base of the formation; all specimens of *Tupandactylus imperator* described thus far have been recovered from here<sup>1-4</sup>. The vertebrate assemblage in the Nova Olinda Member includes well preserved fish<sup>5-8</sup>, bird feathers<sup>9-15</sup>, lizards<sup>16-19</sup>, anurans<sup>20-23</sup>, turtles<sup>24-28</sup> and rare crocodylians<sup>29-32</sup>. The member also preserves abundant arthropods<sup>33, 34</sup>, arachnids<sup>35</sup>, crustaceans<sup>36</sup> and plants, including early angiosperms<sup>37, 38</sup>.

The deposits of the Crato Fm consist of a mixture of siliciclastic deposits and laminated limestones<sup>39</sup>. The Nova Olinda Member is a laminated unit that for most of its vertical thickness (ca. 13 m) comprises pale carbonate-rich and dark carbonate-poor laminae<sup>3</sup>. This composition indicates deposition in a stratified water body characterized by a persistent thermocline or chemocline. The preservation of fossil soft tissues probably reflects (at least in part) low oxygen conditions in bottom waters. The geochemistry, sedimentology and palaeontology of the sediments indicate deposition in a brackish, restricted environment (not open marine) close to shore, probably a lagoon<sup>39-40</sup>.

### **1.3. Description of the specimen.**

#### **Systematic Palaeontology.**

Pterosauria Kaup, 1834

Pterodactyloidea Plieninger, 1901

Tapejaridae Kellner, 1989

Tapejarinae Kellner, 1989

*Tupandactylus* Kellner & Campos, 2007

*Tupandactylus* cf. *imperator* (Campos & Kellner, 1997)

### **Description and identification.**

Specimen MCT.R.1884 comprises the posterior portion of the cranium and the remains of a soft tissue cranial crest, spanning five separate slabs. The specimen is in left lateral aspect, with most bones compacted. The absence of the anterior portion of the cranium is unlikely to represent an artefact of preservation. Instead, it was probably lost during excavation by quarry workers, as indicated by clean cuts (presumably saw cuts) through the bones in the anterior part of the cranium and by no obvious disarticulation around articulation sutures, which would be expected if the cranium disarticulated naturally from the rest of the carcass.

Of the cranium, parts of the orbit and nasoantorbital fenestra are still visible (Extended Data Figs. 1, 4a). Although only the dorsal part is preserved, the orbit appears rather small with an inverted pear geometry. The dorsal margin of the nasoantorbital fenestra is set more dorsally than the dorsal margin of the orbit. Orbit and nasoantorbital fenestra are separated by a thin lacrimal process. Two long bony processes extend posteriorly, supporting a soft tissue cranial crest that spans ca. 0.3 m<sup>2</sup> (465 mm high x 588 mm long). The occipital process is robust and extends posterodorsally from the back of the skull. Its ventral margin is straight, forming an angle of ca. 130° with the dorsal margin of the nasoantorbital fenestra; its dorsal margin is markedly concave. Only the posterior portion of the premaxillary bony crest is preserved as small rods, which seem to form a transition between the ossified base of the premaxillary crest and the soft tissue crest. These bony rods have been reported in the holotypes of *T. imperator*<sup>41</sup> and *T. navigans*<sup>42</sup>. The premaxillary bony crest extends as a postpremaxillary process that is about 20% longer (but more slender) than the parietal crest and is poorly preserved as a trabecular meshwork. The postpremaxillary process forms an angle of ca. 80° with the dorsal margin of the nasoantorbital fenestra and its posterior part has a distinct ventral arch.

The soft tissue cranial crest is the most complete known for *Tupandactylus*. The crest of MCT.R.1884 is similar to another described specimen of *Tupandactylus*<sup>4</sup>, with a convex dorsoposterior margin. It comprises parallel fibres that run oblique to the occipital crest between the dorsal margin of the cranium and occipital crest. These parallel fibres are similar to the fibres described in other *Tupandactylus* specimens<sup>1, 4</sup>. Along the occipital process, the crest locally shows monofilaments and branched

integumentary structures, here identified as feathers (see Main Text). Similar monofilaments are known from the mandibular ramus of *Tupandactylus*<sup>4</sup> (reported as ‘probable pycnofibres’), but have not been reported in association with a cranial crest previously. Overall, the cranial crest of this specimen closely resembles that of the tapejarid pterodactyloid *Tupandactylus* from the Crato Fm of the Araripe Basin<sup>1</sup>. Synapomorphic characters include the presence of a spine-like and slightly caudally-inclined postpremaxillary process and of a long (only slightly shorter than the postpremaxillary process) and caudally-oriented occipital crest<sup>1, 4, 43</sup>. As in previous specimens of *Tupandactylus*<sup>1, 4, 42</sup>, no obvious sutures are present, indicating that the specimen was probably an adult.

The maximum extension of the occipital process of MCT.R.1884 cannot be inferred from the known specimens of *Tapejara wellnhoferi*<sup>44</sup>, from the Santana Fm of the Araripe Basin, but it was obviously much shorter than in *T. imperator*. Further, our specimen differs from *T. wellnhoferi* whereby the orbit of the latter lies slightly below the dorsal margin of the nasoantorbital fenestra<sup>43</sup>. The occipital process of the tapejarid *Sinopterus dongi* differs from that of *T. imperator* in morphology and size<sup>4, 45</sup>. *Tupandactylus navigans*, also from the Crato Formation, lacks an occipital process and has a more vertically-inclined postpremaxillary process<sup>42</sup>. The specimen described in the present paper differs from the four other *T. imperator* specimens described from the Crato Formation whereby its occipital crest is more dorsally inclined and by the concave dorsal margin of the occipital crest<sup>4</sup>; the soft-tissue supracranial crest is narrower antero-posteriorly than that of the other *T. imperator* specimens. Such morphological variation is not unexpected and is implied in sexual selection<sup>46</sup>. We therefore tentatively refer this specimen to *Tupandactylus* cf. *imperator*, pending further evidence on the intraspecific variability of the cranial crest in this taxon.

#### **1.4. Soft tissue anatomy.**

In addition to the monofilaments and branched feathers associated with the occipital process (and that are described in detail in the main text), the cranial crest exhibits two types of fibrous integumentary structures. First, numerous sub-parallel, light to dark brown fibres (100–150 µm wide and up to ca. 300 mm long (Fig. 1a, Extended data Figs. 1, 4)) are widespread across the cranial crest. Those elongate fibres are well defined in the posteroventral part of the crest. They are faint or not evident in the anterodorsal part of the crest, where only a thick, black layer, possibly representing decayed organic matter, is present. Most of these fibres are mutually parallel, aligned with the sagittal axis ventrally and curved dorsally. Ventrally, a series of ca. 100 striking dark brown structures, each 600–900 µm wide, are aligned close to, but isolated from, the occipital process by a 20 mm to 30 mm-wide zone that lacks soft tissues (save faint fibre impressions). Each dark brown structure gives rise to a series of these sub-parallel dark brown fibres

(usually ca. five) (Extended Data Fig. 4a–c). The crest of *T. imperator* described in Pinheiro et al. (2011) also exhibits similar fibres, but those in MCT.R.1884 show a striking curvature.

Locally, the dorsal part of the crest displays wrinkles. Those are thin (ca. 0.3 mm wide), mutually parallel and tightly packed. In the posterior part of the crest, those structures are preserved as external moulds (Extended Data Fig. 4e, f) and run perpendicular to the main set of brown fibres that emerge from the ventral part of the crest. Those wrinkles are especially visible in the posterodorsal part of the crest (Extended Data Fig. 4e, f).

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## Peer Review File

**Manuscript Title: Pterosaur melanosomes support signalling functions for early feathers**

### Reviewer Comments & Author Rebuttals

#### Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

The paper presents evidence of different melanosomes, and hence different colours expressed as patterns in the crest of a Cretaceous pterosaur. The novelty of the find is that this is the first detailed report on melanosomes and inferred colours and patterns in a pterosaur crest, and it has implications for pterosaur behaviour in providing the first solid evidence of colour patterns that indicate visual signalling. Earlier reports of pterosaur feathers and melanosomes implied rather uniform, brownish colours, and no direct evidence of adaptations for signalling. The paper suggests that tissue-specific distribution of melanosome types has deep origins among amniotes based on its occurrence in modern birds and mammals, as well as in dinosaurs and, now, in pterosaurs.

The materials, methods, and presentation are all excellent. I spotted a few typos and a couple of areas where more explanation would help.

The authors present careful descriptions of the feather types, and clarify they are really branching and not overlapping monofilaments, and that they are dermal structures, not shredded skin (lines 89–121), necessary, and repeating previous observations and arguments, but there are still a few hold-outs who are reluctant to accept the parsimonious observation that what look like feathers in dinosaurs and pterosaurs likely are feathers, or at least dermal follicle-derived structures homologous with feathers and hairs.

There is then a phylogenetic/ ancestral-states analysis (lines 131-141), demonstrating the likelihood of simple feathers as ancestral within Avemetatarsalia (itself, a debated issue; an alternative view suggesting feathers arose multiple times in Theropoda and Ornithischia for two, and presumably Pterosauria as a third independent origin). The parsimonious assumption here that identical feather types in disparate clades implies common ancestry seems most sensible for the moment. The authors need to clarify their statement (lines 135–136): “Our model predicts that progressively more complex integumentary structures arose within Avemetatarsalia...” – do you mean that identical-looking branched feathers (you’re your types 5 and 6) arose independently in Theropoda and Pterosauria, or were there different types of branched feathers in each clade.

The defence of melanosome identity vs. bacterial identity for the microbodies (lines 150–155) is crystal-clear, and needed less and less, as the small group of doubters accept that they are what they are claimed to be, namely melanosomes!

99 differ to = differ from

Fig. 2. Need to explain feather types 1–6; these should be indicated with descriptive terms (monofilaments, tufted filaments) as well as small sketches in the figure, so we don't lose track of what is what. The equal likelihoods of types 2, 3 and 4 in the avemetatarsalian ancestor makes it crucial we know which is which. Also, the enlarged pies to the left look strangely pixelated and hard to read.

Referee #2 (Remarks to the Author):

I enjoyed reading this paper. The new fossil described is exquisite, and has important bearing on our understanding of feather evolution. It stands apart from many recently described feathered fossils in two ways: it is a pterosaur (not a dinosaur) and it is from Brazil (not Liaoning, China). It deserves a high profile publication.

The key importance of this specimen, to me, is that it is the strongest evidence yet that pterosaurs had feathers homologous with those of dinosaurs. This is an active debate. A few years ago some of the current authors proposed that pterosaurs had feathers based on branching structures identified in Chinese specimens—in a paper published in *Nature E&E* that I was surprised was not published in *Nature*! Since then, other researchers have vociferously disagreed, arguing that these structures in pterosaurs are not homologous to feathers. The debate hinges in part on which statistical and phylogenetic methods are used to infer ancestral morphologies, but more than anything, on the morphologies and chemical properties of the fuzzy structures in pterosaurs that may or may not be feathers.

I found the authors' previous study on pterosaurs convincing—to me, these structures seemed morphologically similar to (and shared derived characters with) fossil dinosaur feathers I have studied, and they had the numerical analyses to back it up. But I could see how some workers might disagree. These first-described pterosaur feathers were quite simple (even though they were branching), and all previous work on pterosaur integument melanosomes indicated that these structures had simple color patterns, unlike many dinosaurs.

To me, this new discovery puts these doubts to rest, for two reasons: the morphology of the integumentary structures and their melanosomes. Morphology: some of these pterosaur structures really resemble 'type II' feathers of dinosaurs (= 'type V' in the authors' scheme). They branch along their lengths—a quite derived condition. If such structures in dinosaurs are considered feather homologues, then they should be in pterosaurs, too. Melanosomes: these epidermal structures have a great range of melanosome geometries, including rods and spheres, previously unknown in pterosaurs, but known in dinosaur/bird feathers. True, mammal hair has such a diversity too, so it's not a slam-dunk indicator of feather homology, but along with the morphological evidence it packs a strong 1-2 punch. While I can imagine that this paper might not settle the debate for all workers, to me the evidence is now overwhelming that pterosaurs had structures homologous on some fundamental level to dinosaur feathers.

Thus—I think that is the main, groundbreaking, novel result of this study. I think it should be highlighted more in the title, abstract, intro, and discussion. The authors frame the opening of their paper as if pterosaurs unequivocally had feathers, and this is accepted fact. But it's not. I think they're missing a tact here: by framing their results as the most definitive evidence yet for pterosaur feathers, this paper would have even more novelty and impact for a wider audience of scientists.

A few other points to consider for revision:

The debate over pterosaur 'feathers' needs to be acknowledged more. Yes, I do think these things are feathers, but it does come down in a sense to terminology and homology, and alternative ideas of independent origins of strand-like integuments could be explored in this paper. What would the interpretations of the new data on color patterning be if these were not feathers in the avian homology sense?

The discovery of greater melanosome diversity in these structures, relative to the simple melanosomes of the few previously studied pterosaurs, is really interesting. It is and should be a main thrust of the paper. To me, that is a huge line of evidence supporting homology with avian feathers. But the authors spend more time interpreting this finding to address a debate over whether 'the ability to vary melanosome geometry and thus control the color of integumentary appendages arose independently in birds and mammals, or is an ancestral feature that originated in a common amniote ancestor'. They come down heavily on the latter interpretation. However, I don't see how the new fossil adds anything new here. Diverse melanosome geometries were already known in the dinosaur/bird and mammal lineages, and this new finding just moves their origin slightly further down the bird line. Parsimony optimizations would not change, although perhaps Bayesian probability optimizations would, with the addition of a pterosaur (=earlier or more 'basal' member of the bird lineage) with diverse melanosomes. Which brings me to my main point: the authors should underpin this discussion of ancestral amniote melanosomes with the sort of character optimization and modelling analysis that they use to support the homology between dinosaur and pterosaur feathers (figure 2 and supplementary figures 4-6). Provide an analysis that actually demonstrates that this new discovery adds evidence that diverse melanosomes go back to the amniote common ancestor. If such an analysis cannot show this, then I suggest losing this line of framing altogether, and focusing on the pterosaur/bird feather homology theme.

Some of these feathers are associated with the cranial crest. This strikes me as interesting. In life, were they attached to the crest? Or was there another association? Have integumentary filaments been found previously in close association with a pterosaur cranial crest? Does this change our understanding of the structure and function of the crests, or what they would have looked like in life?

It is wonderful to see this Brazilian fossil rescued from the illegal fossil trade, repatriated to Brazil, and described with the glory it deserves. I just note that it is not in Brazil yet. The repatriation process is still taking place. I'll leave it to the editors to determine whether this may be an issue with the timing of publishing this paper. Just make sure this fossil gets to Brazil.

Supplementary figures 4-6 seem to have the new fossil with a dark blue color—corresponding to

feather stage 4. Whereas figure 2 in the main text has the new fossil with a light blue color—corresponding to feather stage 5, which is what is described in the text. Check these figures to make sure they are accurate.

Cool fossil, solid and convincing methods (feather descriptions, melanosome identifications, etc.), well written and structured paper. Nice job. I look forward to seeing this published.

Steve Brusatte, Univ of Edinburgh, November 4, 2021

Referee #3 (Remarks to the Author):

Cincotta and coauthors report the discoveries of feather-like structures and a diverse melanosomes from these structures and also from skin preserved in an Early Cretaceous pterosaur fossil, which have not previously been reported. The new melanosome data is unexpected, providing significant new information on our understanding of feather evolution, both morphologically and functionally. I believe this is an important contribution to the field, and it will not only interest paleontologists focusing on Mesozoic ecosystem, but also experts on integumentary development and evolution. For these reasons, I recommend the publication of this ms in Nature, pending on addressing some minor issues detailed below.

Detailed comments:

Lines 29-30: The genes  $\alpha$ -MSH, ASIP and MC1R should be fully spelled out here (also should be italic).

Lines 31-32: Is the evidence enough to infer the presence of the melanin-based coloration genomic regulatory system in the most recent common ancestor of birds and mammals? If the authors want to make such an inference, they need make an ancestral state reconstruction (see below)

Line 32: Should here be “the most recent common ancestor” rather than “the common ancestor”

Line 39: “feathers evolved not in dinosaurs but in the avemetatarsalian ancestor of pterosaurs and dinosaurs in the Early Triassic’ is confusing and need be rephrased

Line 45: change “more basal taxa” to “earlier-diverging taxa”

Line 46 change “non-maniraptoran dinosaurs” to “non-coelurosaurian dinosaurs” given relatively good integumentary data from several non-maniraptoran coelurosaurian groups such as compsognathids and tyrannosaurs

Lines 57-63: Here it is not clear what exactly the authors want to express. Ref. 5 suggests two shifts of melanosome diversity at the base of pennaraptoran theropods and mammals, respectively, and all other amniotes including other dinosaurian groups such as ornithischians and several non-pennaraptoran groups display low melanosome diversity. If this pattern is true, the authors’

discovery of an increase in melanosome diversity in pterosaurs will add a third independent shift, rather than provide evidence for a single origin.

Line 65: Change “the Early Cretaceous Crato Formation” to “the Lower Cretaceous Crato Formation”

Lines 71-72: the tissue-specific partitioning (particularly between skin and feathers) of melanosome geometry in this fossil seems to represent the first fossil evidence, and thus deserves more discussions and should be highlighted in the abstract.

Line 77: "cf" should not be italicized in “*Tupandactylus cf. imperator*”

Lines 90-97: I am a little confused by the description and the discussion, and the authors need provide some clarifications. Seems to me, figure 1 shows some morphologies different from what the authors describe. First, the central shaft seems not to be a basal calamus, but a rachis (only rachis is known to have barbs along its basal-apical length). Second, the authors suggest that Type 2 feathers in this specimen represent Stage II feathers in Prum (1999) Model, but stage II feathers display a morphology radically different from Type 2 feathers described here. Stage II feathers are basically a radially radiated structure (i.e., barbs radiating from the distal edge of the calamus). However, Type 2 feathers in this pterosaur seems to be a bilaterally symmetrical structure with barbs branching from both sides of the central shaft along the whole length, or less likely but possible, to be a radially radiated structure with barbs branching along the whole length of the central shaft rather than along the distal edge of the calamus if the compression leads to the bilateral symmetry in this fossil. If the former is true, Type 2 feathers in this pterosaur resemble Stage IIIa feathers; if the latter is true, Type 2 feathers in this pterosaur represent a morphotype that is not predicted by Prum Model. Nevertheless, the authors need clarify what exactly Type 2 feathers in this fossil look like (maybe providing an illustrative drawing to show the morphology). Also unusual is that the central shaft is extremely thick comparing to the barbs, and any interpretation?

Lines 125-126: this is confusing: the authors refer the Type 2 feathers to Stage II feathers in Prum Model in earlier paragraph, but here refer them to open pennaceous vane lacking secondary branching (i.e., Stage III or other more advanced stage)?

Line 128: Rephrase “a basal feature of pterosaur”: ‘basal’ is not normally used for describing a feature.

Lines 135-139: Need clarifications. If the authors mean to discuss the trend of increasing complexity in avemetatarsalian integumentary evolution, the *Tupandactylus* discovery is not directly relevant (it shows only the increasing complexity in pterosaurian integumentary evolution)

Line 149: change “some dinosaurs and basal birds” to “some non-avian dinosaurs and early-diverging/stem birds”

Lines 152-153: the authors mentioned Pinheiro et al (2019)’s discovery of melanosomes in another *Tupandactylus* specimen, and it will be nice for the authors to provide information on what is new from this study compared to the earlier study (maybe put it in Supp.)

Lines 171-172: “rods and spheres were reported previously only from mammalian hair and avian feathers”? There are reports of rod-shaped and sphere-shaped melanosomes in non-avian dinosaurs such as Microraptor and Caihong, and do the authors mean elongate melanosomes with a specific range of aspect ratio?

Lines 177-179: see above.

Line 192: change “integumentary appendages (feathers)” to “integumentary appendages (feathers or hairs)”

Lines 205-210: I doubt that a parsimonious analysis will produce results showing a single origin of this feature (see above). Instead, it does suggest the more complex integumentary structures, associated melanosomes, and the underlying genetic machinery have independently evolved in some pterosaurs as represented by *Tupandactylus*, birds, and mammals. Particularly, the genetic mechanisms responsible for producing the tissue-specific partitioning of melanosome morphology and for melanin-based visual communication represent a deep homology, and it is something like the Pax6 gene for the eye development: eyes are independently evolved in multiple lineages, but genetic mechanisms have a deep homology across different groups

Line 210: change “basal amniotes” to “early-diverging amniotes”.



## Author Rebuttals to Initial Comments:

*Response: We appreciate the useful and instructive reviews and have modified the text extensively in line with the reviewers' comments.*

### Referee #1

Comment 1: The paper presents evidence of different melanosomes, and hence different colours expressed as patterns in the crest of a Cretaceous pterosaur. The novelty of the find is that this is the first detailed report on melanosomes and inferred colours and patterns in a pterosaur crest, and it has implications for pterosaur behaviour in providing the first solid evidence of colour patterns that indicate visual signalling. Earlier reports of pterosaur feathers and melanosomes implied rather uniform, brownish colours, and no direct evidence of adaptations for signalling. The paper suggests that tissue-specific distribution of melanosome types has deep origins among amniotes based on its occurrence in modern birds and mammals, as well as in dinosaurs and, now, in pterosaurs.

The materials, methods, and presentation are all excellent. I spotted a few typos and a couple of areas where more explanation would help.

*Response: We note these issues and have responded to the specific comments below.*






Comment 2: The authors present careful descriptions of the feather types, and clarify they are really branching and not overlapping monofilaments, and that they are dermal structures, not shredded skin (lines 89–121), necessary, and repeating previous observations and arguments, but there are still a few hold-outs who are reluctant to accept the parsimonious observation that what look like feathers in dinosaurs and pterosaurs likely are feathers, or at least dermal follicle-derived structures homologous with feathers and hairs.

*Response: We appreciate the reviewer's understanding of our rationale for reiterating (briefly) the justification for our interpretations.*

Comment 3: There is then a phylogenetic/ ancestral-states analysis (lines 131-141), demonstrating the likelihood of simple feathers as ancestral within Avemetatarsalia (itself, a debated issue; an alternative view suggesting feathers arose multiple times in Theropoda and Ornithischia for two, and presumably Pterosauria as a third independent origin). The parsimonious assumption here that identical feather types in disparate clades implies common ancestry seems most sensible for the moment. The authors need to clarify their statement (lines 135–136): “Our model predicts that progressively more complex integumentary structures arose within Avemetatarsalia...” – do you mean that identical-looking branched feathers (you're your types 5 and 6) arose independently in Theropoda and Pterosauria, or were there different types of branched feathers in each clade.

*Response: This is a really interesting comment that belies several issues. (1) There seems to be some confusion here, which may derive in part from a lack of clarity in the text, which we acknowledge and have now fixed. We recognise that the literature now includes three separate nomenclature systems for describing the morphology of fossil feathers (Yang et al., 2019; Xu et al., 2010; Prum, 1999). This is confusing. In the current manuscript, we have produced a new supplementary table that attempts to provide a comparative basis for interpreting pterosaur feathers (including those reported in our manuscript and in Yang et al. 2019) using each of these systems. In our manuscript, we prefer to use the Prum system for describing feather morphology because of its evo-devo basis and because the feather morphologies reported thus far for pterosaurs can most readily be assigned to this system. We have therefore streamlined the text so that it now refers to this nomenclature system for*

integumentary structures and feathers. (2) In their comment, the reviewer refers to “type 5” (= open vane) and “type 6” (= closed vane) integumentary structures (note that this is the nomenclature system used in Fig. 2 of the original text, but is no longer used in the revised manuscript). Both types of integumentary structures have been reported in various theropods, but of these, only type 5 (= open vane) has been reported in pterosaurs (note that pterosaurs also possessed monofilaments (= type 2 in Fig. 2 of the original text)). (3) Our ancestral states reconstruction shows that for both pterosaurs and theropods, early-diverging taxa have a higher likelihood of possessing simple integumentary structures (e.g. scales and/or monofilaments). In theropods, almost all later-diverging taxa are most likely to have possessed complex closed-vane feathers. Pterosaurs show a similar signal (albeit the data are limited). Both groups, therefore, show a progressive increase in feather complexity during their evolution. (4) The final issue is whether the feather morphologies in the two groups arose independently or not. The feather morphologies reported thus far for pterosaurs correspond to feather Stages I, II, IIIa (as per the Prum evo-devo model), and two additional morphologies that appear to be transitional between stages II and IIIa (“Stage II+”). Although there are some morphological differences between the pterosaur feathers and feathers in theropods, in terms of their structural complexity and organisation, these four stages are also represented by fossil theropod feathers. Our ancestral state estimation shows that stages I, II and “II+” were present in the pterosaur ancestor, and that stages I, II, “II+” and IIIa were present in the common ancestor of pterosaurs and dinosaurs. Thus, this implies that feather morphologies corresponding to stages I, II, II+ and IIIa in pterosaurs and dinosaurs have a single origin. Only dinosaurs, in particular, theropods, evolved more complex feather morphotypes, i.e. feathers at stages IIIb, IIIa+b, IV and V. *New Extended Data Figure provided below.*

Pterosaur feather morphology	Taxon	Branching	Feather type (sensu Yang et al., 2019)	Evo-devo Stage (sensu Prum (1999); Prum & Brush, 2002)	Feather morphotype (sensu Xu et al., 2010; Xu, 2020)	Assignment to evo-devo Stage
	anurognathids & <i>Tupandactylus</i>	none	type 1	Stage I	morphotype 1 (“SMFI”)	Stage I
	anurognathids	terminal	type 2	n/a	morphotype 4 (“BJSFF”)	Stage II+
	anurognathids	mid-point	type 3	n/a	n/a	Stage II+
	anurognathids	basal	type 4	Stage II	morphotype 3 (“BJFF”)	Stage II
	<i>Tupandactylus</i>	along-rachis	n/a	Stage IIIa	morphotype 5 (“RBSFF”)	Stage IIIa

Comment 4: The defence of melanosome identity vs. bacterial identity for the microbodies (lines 150–155) is crystal-clear, and needed less and less, as the small group of doubters accept that they are what they are claimed to be, namely melanosomes!

**Response:** *We appreciate the reviewer’s perceptive comment here and understanding of the issues surrounding the melanosome vs. bacteria debate.*

Comment 5: 99 differ to = differ from. 194 color = colour

**Response:** *Agreed – text has been modified (lines 96 and 192).*

Comment 6: Fig. 2. Need to explain feather types 1–6; these should be indicated with descriptive terms (monofilaments, tufted filaments) as well as small sketches in the figure, so we don’t lose track of what is what. The equal likelihoods of types 2, 3 and 4 in the avemetatarsalian ancestor

makes it crucial we know which is which. Also, the enlarged pies to the left look strangely pixelated and hard to read.

*Answer: We apologise for the issues with this figure. We have fixed the pie chart and provided a more useful legend and schematic illustrations of the integumentary structures.*

#### Referee #2

Comment 1: To me, this new discovery puts these doubts to rest, for two reasons: the morphology of the integumentary structures and their melanosomes. Morphology: some of these pterosaur structures really resemble ‘type II’ feathers of dinosaurs (= ‘type V’ in the authors’ scheme).

*Response: We presume that by “type II” the referee is referring to Stage II of the Prum evo-devo model, which was referred to in line 98 of the original version of our manuscript. Note that the reference to this Stage in the original text was in fact a typo – should have read Stage IIIa. Text has been amended (Line 89).*

Comment 2: They branch along their lengths—a quite derived condition. If such structures in dinosaurs are considered feather homologues, then they should be in pterosaurs, too.

Melanosomes: these epidermal structures have a great range of melanosome geometries, including rods and spheres, previously unknown in pterosaurs, but known in dinosaur/bird feathers. True, mammal hair has such a diversity too, so it’s not a slam-dunk indicator of feather homology, but along with the morphological evidence it packs a strong 1-2 punch. While I can imagine that this paper might not settle the debate for all workers, to me the evidence is now overwhelming that pterosaurs had structures homologous on some fundamental level to dinosaur feathers.

*Response: We fully agree with the reviewer here. As he correctly points out, the expanded melanosome diversity in the pterosaur integumentary structures (monofilaments and branched structures) relative to skin is not unique to feathers, but also characterises mammal hair relative to mammal skin. Further, our morphological evidence for clearly branching structures that correspond to Stage IIIa of feather development in the Prum evo-devo model, plus our ancestral state reconstruction, provide compelling evidence that the pterosaur structures are not simply feather homologues but that they are feathers.*

Comment 3: Thus—I think that is the main, groundbreaking, novel result of this study. I think it should be highlighted more in the title, abstract, intro, and discussion. The authors frame the opening of their paper as if pterosaurs unequivocally had feathers, and this is accepted fact. But it’s not. I think they’re missing a tact here: by framing their results as the most definitive evidence yet for pterosaur feathers, this paper would have even more novelty and impact for a wider audience of scientists.

*Response: We agree and we have followed most of the reviewer’s suggestions here. In the revised manuscript we provide further background on the debate surrounding pterosaur integumentary structures. We take particular care to avoid a priori interpretations as feathers without consideration of alternative perspectives. We have placed greater emphasis on the implications of our discovery of branched feathers in a new pterosaur specimen. As suggested, we have modified the abstract (lines 18–20), introduction (lines 34–36) and discussion (lines 88–92). Modification of the title is more difficult because of the tight constraints imposed by the character count (indeed the title of the original version already exceeded the character count!). We have, however, made efforts to shorten and modify the title and we feel that the revised title succeeds in placing greater emphasis on the feathers themselves without detracting from the melanosome aspects (which, as the reviewer points out below in Comment 7, should be the “main thrust” of the paper).*

Comment 4: A few other points to consider for revision: The debate over pterosaur ‘feathers’ needs to be acknowledged more.

*Response: Agreed – see response to Comment 3.*

Comment 5: Yes, I do think these things are feathers, but it does come down in a sense to terminology and homology, and alternative ideas of independent origins of strand-like integuments could be explored in this paper.

*Response: We fundamentally agree with the reviewer that the heart of this issue – what are pterosaur integumentary filamentous structures? – boils down to terminology and interpretations of homology. The current paper, however, does not offer the scope to examine this debate on terminology and nomenclature with an expanded level of detail. As suggested, however, the revised manuscript includes new/additional comments on the alternative hypothesis that the structures in dinosaurs and pterosaurs arose independently (lines 123–125).*

Comment 6: What would the interpretations of the new data on color patterning be if these were not feathers in the avian homology sense?

*Response: Interesting thought experiment! If the pterosaur structures are not feathers (or feather homologues), then they represent a third type of vertebrate integumentary outgrowth that is capable of imparting, and varying, coloration. We include a brief reference to this alternative hypothesis in the revised manuscript (lines 169–172).*

Comment 7: The discovery of greater melanosome diversity in these structures, relative to the simple melanosomes of the few previously studied pterosaurs, is really interesting. It is and should be a main thrust of the paper. To me, that is a huge line of evidence supporting homology with avian feathers. But the authors spend more time interpreting this finding to address a debate over whether ‘the ability to vary melanosome geometry and thus control the color of integumentary appendages arose independently in birds and mammals, or is an ancestral feature that originated in a common amniote ancestor’. They come down heavily on the latter interpretation. However, I don’t see how the new fossil adds anything new here. Diverse melanosome geometries were already known in the dinosaur/bird and mammal lineages, and this new finding just moves their origin slightly further down the bird line. Parsimony optimizations would not change, although perhaps Bayesian probability optimizations would, with the addition of a pterosaur (=earlier or more ‘basal’ member of the bird lineage) with diverse melanosomes.

*Response: We appreciate these insights and we agree that our text may have been somewhat overzealous. We agree that our primary interpretations regarding potential common ancestry of the ability to vary melanosome geometry for coloration relate directly to Avemetatarsalia, not to amniotes – although as the reviewer points out, the probability of this feature being more basal is likely to increase. We have modified the text accordingly (lines 206–214).*

Comment 8: Which brings me to my main point: the authors should underpin this discussion of ancestral amniote melanosomes with the sort of character optimization and modelling analysis that they use to support the homology between dinosaur and pterosaur feathers (figure 2 and supplementary figures 4-6). Provide an analysis that actually demonstrates that this new discovery adds evidence that diverse melanosomes go back to the amniote common ancestor. If such an analysis cannot show this, then I suggest losing this line of framing altogether, and focusing on the pterosaur/bird feather homology theme.

**Response:** Excellent idea. We have done this, and the results show (lines 208–210 and Extended Data Fig. 11) that the most parsimonious scenario is that feathers in the avemetatarsalian ancestor had melanosomes with different geometries.

Comment 9: Some of these feathers are associated with the cranial crest. This strikes me as interesting. In life, were they attached to the crest? Or was there another association? Have integumentary filaments been found previously in close association with a pterosaur cranial crest? Does this change our understanding of the structure and function of the crests, or what they would have looked like in life?

**Response:** This comment arises in part from the language we use in the original abstract, which refers to “simple and branched feathers associated with the cranial crest”. It is now apparent that this description, although accurate, is somewhat misleading as it suggests that the preserved feathers are located on the soft tissue part of the crest, when in fact they are located immediately dorsal and ventral of the occipital extension of the crest – within 15 mm of the bone. The feathers do not occur on the soft tissue part of the cranial crest. Where the reviewer refers to “integumentary filaments”, we are not sure whether he is referring to feathers or to dermal fibres. Feathers have been reported from cranial regions of other pterosaurs (Yang et al. 2019) but not from cranial crests. Elongate fibrous structures have been reported from the cranial crests of other specimens of *Tupandactylus*, but have not been described in detail (e.g. using high-resolution light microscopy or SEM). Regarding the reviewer’s questions about the structure and function of the crest, a comprehensive treatment of the morphology, ultrastructure, chemistry and taphonomy of the various soft tissue features of the soft tissue part of the cranial crest itself is beyond the scope of the current manuscript. Indeed, we do not feel that we currently have sufficient data on these aspects to provide a definitive answer to the reviewer’s questions at this time, but they will form the basis of a separate manuscript. We have amended the abstract to remove the ambiguity regarding the location of the feathers.

Comment 10: It is wonderful to see this Brazilian fossil rescued from the illegal fossil trade, repatriated to Brazil, and described with the glory it deserves. I just note that it is not in Brazil yet. The repatriation process is still taking place. I’ll leave it to the editors to determine whether this may be an issue with the timing of publishing this paper. Just make sure this fossil gets to Brazil.

**Response:** The paperwork required for the repatriation of the fossil to the Geological Survey of Porto Alegre in Brazil is finalised and the official repatriation will occur in early 2022. It is planned that the embassy of Brazil in Belgium will be invited for an official repatriation ceremony in Brussels. The current resubmission includes official confirmation from the Brazilian embassy in Brussels that the repatriation process has been initiated (the documents are in French and Portuguese).

Comment 11: Supplementary figures 4-6 seem to have the new fossil with a dark blue color—corresponding to feather stage 4. Whereas figure 2 in the main text has the new fossil with a light blue color—corresponding to feather stage 5, which is what is described in the text. Check these figures to make sure they are accurate.

**Response:** We apologize for the confusion. Thanks for pointing this out. We have corrected figure 2. Note that the correct label colour for SGB-PA PZ010 should be light blue (open pennaceous vane lacking secondary branching).

**Referee #3** (Remarks to the Author):

Comment 1: Cincotta and coauthors report the discoveries of feather-like structures and a diverse melanosome from these structures and also from skin preserved in an Early Cretaceous pterosaur fossil, which have not previously been reported. The new melanosome data is unexpected, providing significant new information on our understanding of feather evolution, both morphologically and functionally. I believe this is an important contribution to the field, and it will not only interest paleontologists focusing on Mesozoic ecosystem, but also experts on integumentary development and evolution. For these reasons, I recommend the publication of this ms in Nature, pending on addressing some minor issues detailed below.

*Response: We have incorporated all of the reviewer's suggestions into the revised manuscript.*

Comment 2: Lines 29-30: The genes  $\alpha$ -MSH, ASIP and MC1R should be fully spelled out here (also should be italic).

*Response: Reference to these genes has been removed from the abstract. The genes are spelled in full where they are mentioned in the discussion (lines 197–198 and 204–205).*

Comment 3: Lines 31-32: Is the evidence enough to infer the presence of the melanin-based coloration genomic regulatory system in the most recent common ancestor of birds and mammals? If the authors want to make such an inference, they need make an ancestral state reconstruction (see below)

*Response: We agree with the reviewer and have performed an ancestral state analysis for melanosome geometry. Our results show that the most parsimonious scenario is that feathers in the avemetatarsalian ancestor had melanosomes with different geometries (lines 208–210).*

Comment 4: Line 32: Should here be “the most recent common ancestor” rather than “the common ancestor”

*Response: Text has been deleted.*

Comment 5: Line 39: “feathers evolved not in dinosaurs but in the avemetatarsalian ancestor of pterosaurs and dinosaurs in the Early Triassic” is confusing and need be rephrased

*Response: We apologize for the confusion and have modified our text (lines 33–35).*

Comment 6: Line 45: change “more basal taxa” to “earlier-diverging taxa”

*Response: Text has been amended throughout the manuscript, lines 40, 140 and 214.*

Comment 7: Line 46 change “non-maniraptoran dinosaurs” to “non-coelurosaurian dinosaurs” given relatively good integumentary data from several non-maniraptoran coelurosaurian groups such as compsognathids and tyrannosaurs

*Response: Text has been amended line 41.*

Comment 8: Lines 57-63: Here it is not clear what exactly the authors want to express. Ref. 5 suggests two shifts of melanosome diversity at the base of pennaraptoran theropods and mammals, respectively, and all other amniotes including other dinosaurian groups such as ornithischians and several non-pennaraptoran groups display low melanosome diversity. If this pattern is true, the authors' discovery of an increase in melanosome diversity in pterosaurs will add a third independent shift, rather than provide evidence for a single origin.

**Response:** We are a little confused here as the subject of the reviewer's comment – shifts in melanosome diversity – is not the same as the subject of lines 57–63 in the original version of the manuscript. In the latter section of text, we summarise previous literature on melanosomes in extant reptile skin and pterosaur feathers. This literature shows that both tissue types contain low-diversity, ovoid melanosomes and no evidence for spheroidal or elongate melanosomes. Based on these data we hypothesise that the presence of low-diversity melanosomes is thus an ancestral condition. We do not refer to shifts in melanosome diversity that are known to occur in theropods and mammals. On that particular issue (treated in the revised manuscript on lines 51–55), we acknowledge that the reviewer correctly points out the evidence for a shift towards more diverse melanosomes in both the mammal and theropod lineages. Given our discovery of a similar shift in pterosaurs, we feel that it is unlikely that the same trends in melanosome evolution would arise independently in three closely related groups. Instead, we feel that this is more likely to reflect the presence of a shared genetic machinery facilitating melanosome shape plasticity in dinosaurs, pterosaurs and mammals. This could in turn reflect the evolution of this common genetic regulatory network earlier, in the amniote ancestor of mammals and avemetatarsalians. It's effectively a pre-adaptation: the genes may have been already present, and functioning in other aspects of melanisation, but were available to be co-opted into varying melanosome shape and thus geometry later in the evolution of the three groups. Text has been modified to clarify our interpretation (lines 203–208).

Comment 9: Line 65: Change “the Early Cretaceous Crato Formation” to “the Lower Cretaceous Crato Formation”

**Response:** Text has been amended, line 69.

Comment 10: Lines 71-72: the tissue-specific partitioning (particularly between skin and feathers) of melanosome geometry in this fossil seems to represent the first fossil evidence, and thus deserves more discussions and should be highlighted in the abstract.

**Response:** Tissue-specific melanosome geometries have been reported previously by one of the authors (MMN) and colleagues in several studies (McNamara et al., 2018; Rossi et al., 2019, 2020; Rogers et al., 2019). In particular, partitioning of melanosome geometry between integumentary tissues such as skin and feathers has been reported for extant birds, but not feathered fossil taxa. We have included additional discussion of this feature in the revised text (lines 160–163).

Comment 11: Line 77: "cf" should not be italicized in “Tupandactylus cf. imperator”

**Response:** Thanks for pointing this out. We have corrected that typo in the revised manuscript (line 68).

Comment 12: Lines 90-97: I am a little confused by the description and the discussion, and the authors need provide some clarifications. Seems to me, figure 1 shows some morphologies different from what the authors describe. First, the central shaft seems not to be a basal calamus, but a rachis (only rachis is known to have barbs along its basal-apical length). Second, the authors suggest that Type 2 feathers in this specimen represent Stage II feathers in Prum (1999) Model, but stage II feathers display a morphology radically different from Type 2 feathers described here. Stage II feathers are basically a radially radiated structure (i.e., barbs radiating from the distal edge of the calamus). However, Type 2 feathers in this pterosaur seems to be a bilaterally symmetrical structure with barbs branching from both sides of the central shaft along the whole length, or less likely but possible, to be a radially radiated structure with barbs branching along the whole length of the central shaft rather than along the distal edge of the calamus if the compression leads to the

bilateral symmetry in this fossil. If the former is true, Type 2 feathers in this pterosaur resemble Stage IIIa feathers; if the latter is true, Type 2 feathers in this pterosaur represent a morphotype that is not predicted by Prum Model. Nevertheless, the authors need clarify what exactly Type 2 feathers in this fossil look like (maybe providing an illustrative drawing to show the morphology). Also unusual is that the central shaft is extremely thick comparing to the barbs, and any interpretation?

*Response: We apologise for the confusion here – we made a mistake in the figure and text. The Tupandactylus branched feathers correspond to Stage IIIa of the Prum evo-devo model (consistent with the reviewer’s descriptions), not Stage II as we erroneously indicated. We have fixed this in both the text and figure. We agree that a schematic illustration would be useful and we have included a new figure in Extended Data Fig. 4 to better illustrate the morphology of the feathers reported in this specimen and in other pterosaurs. In addition, the new figure provides clear assignments of these feather morphotypes to defined evo-devo stages (as per the Prum model) and to feather morphotypes as defined by Xu (2020) and will thus allow easy cross-reference of morphology and terminology as per the two different nomenclature systems.*

*It is not possible to accurately determine the width of the shaft in most of the branched feathers as the barbs seem very closely spaced and the point at which the barbs branch from the rachis is difficult to identify with confidence. The shaft is, however, visible in one of the branched feathers that is curved (arrows in Extended Data Fig. 3b, c). Immediately proximal of the inflection point, the orientation of the splayed barbs changes and the rachis here is clearly very thin.*

Comment 13: Lines 125-126: this is confusing: the authors refer the Type 2 feathers to Stage II feathers in Prum Model in earlier paragraph, but here refer them to open pennaceous vane lacking secondary branching (i.e., Stage III or other more advanced stage)?

*Response: See response to Comment 12; the pterosaur branched feathers correspond to Stage IIIa of the Prum model.*

Comment 14: Line 128: Rephrase “a basal feature of pterosaur”: ‘basal’ is not normally used for describing a feature.

*Response: Text has been deleted.*

Comment 15: Lines 135-139: Need clarifications. If the authors mean to discuss the trend of increasing complexity in avemetatarsalian integumentary evolution, the Tupandactylus discovery is not directly relevant (it shows only the increasing complexity in pterosaurian integumentary evolution).

*Response: We agree that our primary interpretations regarding complexity of integumentary structures should be toned down as the Tupandactylus structures relate directly to pterosaur feather evolution, not to Avemetatarsalia. Text has been amended (lines 123 – 131).*

Comment 16: Line 149: change “some dinosaurs and basal birds” to “some non-avian dinosaurs and early-diverging/stem birds”

*Response: Text has been amended (line 140).*

Comment 17: Lines 152-153: the authors mentioned Pinheiro et al (2019)’s discovery of melanosomes in another Tupandactylus specimen, and it will be nice for the authors to provide information on what is new from this study compared to the earlier study (maybe put it in Supp.)

*Response: As requested, the revised text includes additional details of the melanosomes reported in Pinheiro et al. (2019); we also highlight the novel aspects of the melanosomes in our study. The*



*absence of multiple distinct populations of melanosomes in the specimen studied by Pinheiro et al. 2011, 2012, 2019 almost certainly reflects limited sampling: their 2019 paper shows a cluster of four samples in the most anteroventral region of the crest and one sample in the anterodorsal region. The latter region broadly corresponds to the region in sample #22 (Extended Data Fig. 1) of our specimen. The specimen studied in Pinheiro et al., however, lacks the ventral part of the crest, which was extensively sampled in our specimen, and which demonstrates variability in melanosome geometry. Despite the presence of filamentous integumentary structures interpreted as possible pycnofibres in that specimen, however, samples and melanosomes are not reported from these soft tissue regions (see lines 146–148 and Fig. 4a in Pinheiro et al., 2011).*

Comment 18: Lines 171-172: “rods and spheres were reported previously only from mammalian hair and avian feathers”? There are reports of rod-shaped and sphere-shaped melanosomes in non-avian dinosaurs such as Microraptor and Caihong, and do the authors mean elongate melanosomes with a specific range of aspect ratio?

**Response:** *Thank you for pointing this out – we didn’t mean to exclude non-avian dinosaurs – text has been amended. The second point raised by the reviewer is interesting because although there is a common terminology in use for describing melanosome geometries in fossils, this terminology is not underpinned by defined categories with specific size range data. We include a figure in supplementary data (that will be uploaded onto a data repository at a later stage) with proposed categories for different terms used to describe melanosome geometry (see supplementary data file).*

Comment 19: Lines 177-179: see above.

**Response:** *We are not sure what the reviewer would like changed here. We have modified the text to refer to the regulatory mechanisms that underpin variation in melanosome geometry, rather than visible colour. Towards the end of the manuscript we discuss the genomic basis of melanogenesis in some detail and how the new specimen informs on the evolution of those systems (lines 196–208). In addition, we have performed a new ancestral states estimation of melanosome geometries across pterosaurs and dinosaurs, which shows that variation in melanosome ancestry is ancestral to pterosaurs and dinosaurs (lines 208–210).*

Comment 20: Line 192: change “integumentary appendages (feathers)” to “integumentary appendages (feathers or hairs)”

**Response:** *Agreed; text has been amended (line 190).*

Comment 21: Lines 205-210: I doubt that a parsimonious analysis will produce results showing a single origin of this feature (see above). Instead, it does suggest the more complex integumentary structures, associated melanosomes, and the underlying genetic machinery have independently evolved in some pterosaurs as represented by Tupandactylus, birds, and mammals. Particularly, the genetic mechanisms responsible for producing the tissue-specific partitioning of melanosome morphology and for melanin-based visual communication represent a deep homology, and it is something like the Pax6 gene for the eye development: eyes are independently evolved in multiple lineages, but genetic mechanisms have a deep homology across different groups

**Response:** *We acknowledge that the presence of homologous morphological structures in different taxa does not automatically imply that those structures share a single common origin, even where the underlying GRNs share deep homology, e.g. evolution of the eye. It is unclear whether similar processes (translation of genotype to phenotype) would also apply to the evolution of more derived anatomical features such as feathers. We have included additional text in the Discussion (lines 203–208) where we outline the three possible evolutionary scenarios that could explain the presence of*

*multiple melanosome geometries in theropods and pterosaurs. Our new ancestral state reconstruction (Extended Data Fig. 11) demonstrates differentiated melanosome geometries are the most likely ancestral state, supporting our interpretations of progressive increase in melanosome diversity in Avemetatarsalia.*

Comment 22: Line 210: change “basal amniotes” to “early-diverging amniotes”.

**Response:** *Text has been amended (line 214).*

## Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The Response document shows careful consideration of all critical comments by myself and by the other referees. I appreciate the care taken in explaining and overhauling the nomenclature of feather types, which is now much clearer and applicable in palaeontological and evo-devo settings. The authors have clarified other aspects of the description and they add a new analysis on ancestral states of melanosomes among avemetatarsalians. The language and illustrations now thoroughly and convincingly present the data from the new fossil and, like referee 3, I appreciate their actions in ensuring the specimen returns to Brazil.

On the last point, I'd say the evidence the repatriation is underway is sufficient to suggest that publication can proceed without waiting for the act to occur. **It is good to be able to cite the repository number for the specimen in the Porto Alegre collection, looking to future readers of the paper.** Words concerning repatriation (Supplementary, page 1) can be modified to indicate this has occurred.

Small, final suggested corrections

Line 64: the ancestral avemetatarsalians were probably Early to Middle Triassic in age – based on the age of basal aphanosaurians and silesaurids (recent papers by Nesbitt; e.g. <https://www.nature.com/articles/s41586-020-3011-4>).

Line 119: Tupandactylus branched feathers = branched feathers in Tupandactylus

Lines 160–172: This caveat, that acceptance of the most parsimonious phylogenetic conclusion does not exclude alternative, more information-costly, models, has already been made at lines 123–124. Maybe it's appropriate to retain both caveats.

Referee #2 (Remarks to the Author):

I am happy to see this revised manuscript and feel that the authors have addressed my comments suitably. I particularly appreciate how the paper is framed with more discussion and acknowledgement of the pterosaur feather debate, which increases the importance and novelty of this study. I also am glad to see the issue of deep amniote ancestral characters toned down, and the focus now more on avemetatarsalians and their ancestral characters and evolutionary trends. This is a remarkable specimen, a well-reasoned and provocative paper, and I look forward to seeing it published.

Steve Brusatte

Referee #3 (Remarks to the Author):

i think the authors did a great job in addressing some issues raised by myself and other referees, and the revised ms is ready for publication in my opinion.

## Author Rebuttals to First Revision:

### Response to referee comments:

#### Referee #1:

Comment 1: The Response document shows careful consideration of all critical comments by myself and by the other referees. I appreciate the care taken in explaining and overhauling the nomenclature of feather types, which is now much clearer and applicable in palaeontological and evo-devo settings. The authors have clarified other aspects of the description and they add a new analysis on ancestral states of melanosomes among avemetatarsalians. The language and illustrations now thoroughly and convincingly present the data from the new fossil and, like referee 3, I appreciate their actions in ensuring the specimen returns to Brazil.

On the last point, I'd say the evidence the repatriation is underway is sufficient to suggest that publication can proceed without waiting for the act to occur. It is good to be able to cite the repository number for the specimen in the **Porto Alegre collection**, looking to future readers of the paper. Words concerning repatriation (Supplementary, page 1) can be modified to indicate this has occurred.

**Response:** *The fossil has now been repatriated to the Museum of Earth Sciences in Rio de Janeiro, Brazil, and text has been amended in the supplementary information to reflect this.*

Comment 2: Line 64: the ancestral avemetatarsalians were probably Early to Middle Triassic in age – based on the age of basal aphanosaurians and silesaurids (recent papers by Nesbitt; e.g. <https://www.nature.com/articles/s41586-020-3011-4>).

**Response:** *Agreed – text has been modified (lines 63–64).*

Comment 3: Line 119: Tupandactylus branched feathers = branched feathers in Tupandactylus.

**Response:** *Agreed - text has been modified (line 117).*

Comment 4: Lines 160–172: This caveat, that acceptance of the most parsimonious phylogenetic conclusion does not exclude alternative, more information-costly, models, has already been made at lines 123–124. Maybe it's appropriate to retain both caveats.

**Response:** *The two statements are retained as they relate to different arguments. The first (lines 122 – 123) acknowledges the possibility that integumentary filaments in pterosaurs and theropods may have separate origins. The second statement (lines 168 – 171) develops this caveat further, focusing on the implications for colour tuning: in the (unlikely) scenario that the pterosaur structures are not feathers, then three separate types of integumentary structure have evolved that imparted the ability to vary melanic coloration.*

Referees #2 and #3 did not request any further changes to the manuscript.