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Authors	Yang, Zixiao;Jiang, Baoyu;McNamara, Maria E.;Kearns, Stuart L.;Pittman, Michael;Kaye, Thomas G.;Orr, Patrick J.;Xu, Xing;Benton, Michael J.
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University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

1 **Pterosaur integumentary structures with complex feather-like branching**

2 Zixiao Yang¹, Baoyu Jiang¹, Maria E. McNamara², Stuart L. Kearns³, Michael
3 Pittman⁴, Thomas G. Kaye⁵, Patrick J. Orr⁶, Xing Xu⁷, Michael J. Benton³

4

5 1. Center for Research and Education on Biological Evolution and Environments,

6 School of Earth Sciences and Engineering, Nanjing University, Nanjing 210023,

7 China

8 2. School of Biological, Earth and Environmental Sciences, University College Cork,

9 Cork T23 TK30, Ireland

10 3. Department of Earth Sciences, University of Bristol, Bristol BS8 1RJ, UK

11 4. Vertebrate Palaeontology Laboratory, Department of Earth Sciences, University of

12 Hong Kong, Pokfulam, Hong Kong, China

13 5. Foundation for Scientific Advancement, Sierra Vista, Arizona USA

14 6. UCD School of Earth Sciences, University College Dublin, Belfield, Dublin 4

15 D04V1W8, Ireland

16 7. Key Laboratory of Vertebrate Evolution and Human Origins, Institute of Vertebrate

17 Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044,

18 China

19

20 **Pterosaurs were the first vertebrates to achieve true flapping flight, but in the**
21 **absence of living representatives, many questions concerning their biology and**
22 **lifestyle remain unresolved. Pycnofibres, the integumentary coverings of**
23 **pterosaurs, are particularly enigmatic: although many reconstructions depict**
24 **fur-like coverings composed of pycnofibres, their affinities and function are not**
25 **fully understood. Here we report the preservation in two anurognathid pterosaur**
26 **specimens of morphologically diverse pycnofibres that show diagnostic features**
27 **of feathers, including non-vaned grouped filaments and bilaterally branched**
28 **filaments, hitherto considered unique to maniraptoran dinosaurs, and preserved**
29 **melanosomes with diverse geometries. These findings could imply that feathers**
30 **had deep evolutionary origins in ancestral archosaurs, or that these structures**
31 **arose independently in pterosaurs. The presence of feather-like structures**
32 **suggests that anurognathids, and potentially other pterosaurs, possessed a dense**
33 **filamentous covering that likely functioned in thermoregulation, tactile sensing,**
34 **signalling, and aerodynamics.**

35 Feathers are the most complex integumentary appendages in vertebrates¹. Most
36 feathers in modern birds possess an axial shaft from which branch lateral barbs and
37 barbules. Much is known about the anatomy, developmental biology, and genomic
38 regulation of these structures, but their deep evolutionary origin is controversial²⁻⁴.
39 Feathers and feather-like integumentary structures have been reported in many
40 theropod dinosaurs (including birds)^{3,5} and ornithischians such as *Psittacosaurus*⁶,
41 *Tianyulong*⁷, and *Kulindadromeus*⁸. Feather-like or hair-like structures, termed

42 pycnofibres⁹, have also been reported in several pterosaur specimens⁹⁻¹³, but their
43 nature is not resolved.

44 Here we report remarkably well-preserved pycnofibres in two anurognathid
45 pterosaurs and demonstrate, using evidence from morphology, chemistry and
46 macroevolutionary analyses, that the preserved pycnofibres bear key features of
47 feathers: monofilaments, two types of non-vented grouped filaments, bilaterally
48 branched filaments that were previously considered unique to maniraptoran dinosaurs,
49 and preserved melanosomes with diverse geometries. Both specimens studied are
50 from the Middle–Late Jurassic Yanliao Biota (ca. 165–160 Mya¹⁴). NJU–57003
51 (Nanjing University) is a newly excavated specimen from the Mutoudeng locality and
52 CAGS–Z070 (Institute of Geology, Chinese Academy of Geological Sciences), which
53 has been noted briefly for its feather-like branched pycnofibres¹³, is from the
54 Daohugou locality. Both specimens are near-complete and well-articulated, with
55 extensive soft tissues (Figs. 1 and 2, and Supplementary Figs. 1–5). Both specimens
56 are identified as anurognathids¹⁷ (see Supplementary text for osteological
57 descriptions).

58 Preserved soft tissues include structural fibres (actinofibrils) and pycnofibres.
59 Structural fibres, common in the pterosaur wing membrane^{9,12,18}, are observed only in
60 the posterior portion of the uropatagium in CAGS–Z070 (Fig. 1**o–p**). As reported
61 elsewhere, they are parallel to subparallel and closely packed. Individual fibres are
62 0.08–0.11 mm wide (ca. 5 fibres per mm) and at least 1.9 mm long. Pycnofibres are
63 preserved extensively in both pterosaur specimens (especially CAGS–Z070; Figs. 1

64 and 2, and Supplementary Figs. 1, 4 and 5) and are discriminated from structural
65 fibres based on their curved morphology and overlapping arrangement. In the
66 posterior portion of the uropatagium in CAGS–Z070, pycnofibres co-occur with
67 structural fibres; oblique intersections reflect superposition of these features during
68 decay (Fig. 1o–p).

69 Pycnofibres are categorized here into four types. Type 1 occurs around the head,
70 neck, shoulder, torso, all four limbs and tail of both specimens (Figs. 1c–e, o–p, 2b–c
71 and f). It comprises curved monofilaments that are 3.5–12.8 mm long and 70–430 μm
72 wide. Some short, distally tapering examples discriminate between dark-toned lateral
73 margins and light-toned axial regions, especially near the filament base where the
74 light-toned axis is wider, suggesting a tube-like morphology (Fig. 1c–e). Type 2 is
75 preserved in the neck, proximal forelimb, plantar metatarsus and proximal tail regions
76 of CAGS–Z070. It consists of bundles of curved filaments of similar length that
77 appear to form brush-like structures at the distal ends of thicker filaments (2.0–13.8
78 mm long and 80–180 μm wide) (Fig. 1f–h). The latter may represent individual thick
79 filaments or fused proximal regions of thinner distal filaments. Type 3 occurs around
80 the head of CAGS–Z070. It comprises straight to slightly curved, distally tapered,
81 central filaments (4.5–7.0 mm long and 50–450 μm wide) with short lateral branches
82 that diverge from the central filament near the midpoint (Fig. 1i–k). There are five
83 Type 3 filaments identified on the head, next to five similar filaments likely of the
84 same nature but obscured by overlapping filaments (Supplementary Fig. 5b). Type 4
85 occurs on the wing membrane of both specimens. It comprises tufts of curved

86 filaments (2.5–8.0 mm long and 70–130 μm wide) that diverge proximally (Figs. 11–n
87 and 2d–e), in contrast to the clear separation between Type 1 filaments (Fig. 1o–p).

88 Filamentous integumentary structures in extant and fossil vertebrates commonly
89 contain melanin-bearing organelles (melanosomes). Scanning electron microscopy
90 (SEM) of the filamentous structures of NJU–57003 reveals densely packed
91 microbodies $0.70 \pm 0.11 \mu\text{m}$ long and $0.32 \pm 0.05 \mu\text{m}$ wide (Fig. 2g–h,
92 Supplementary Figs. 4a–f, 6 and 7, and Supplementary Table 2). As with most
93 melanosome-rich fossil feathers¹⁹⁻²¹, energy dispersive X-ray spectroscopy (EDS)
94 spectra of the filaments are dominated by a major peak for carbon (Supplementary
95 Fig. 8). These carbonaceous microbodies resemble fossil melanosomes in terms of
96 their geometry, dense packing, parallel alignment relative to the long axis of the
97 integumentary structure (i.e. barbules in Paraves), and preservation within the matrix
98 of the filament (see Supplementary text). Most of the microbodies are oblate and
99 morphologically similar to those that are usually interpreted as phaeomelanosomes in
100 fossils¹⁹ (Fig. 2h). Rod-shaped examples, usually interpreted as eumelanosomes in
101 fossils¹⁹ (Fig. 2g), are rare.

102 Fourier transform infrared spectroscopy (FTIR) of samples of pterosaur filaments
103 shows four major peaks unique to the filaments (Fig. 2i). These peaks are consistent
104 with the absorption regions of amide I at ca. 1650 cm^{-1} (principally the C=O
105 asymmetric stretching vibration with some C–N bending), amide II at ca. 1540 cm^{-1}
106 (a combination of N–H in-plane bending and C–N and C–C stretching as in indole
107 and pyrrole in melanin and amino acids), and aliphatic C–H stretching at 2850 cm^{-1}

108 and 2918 cm^{-1} ²². These peaks also occur in spectra obtained from extant feathers^{21,23},
109 fossil feathers of the paravian *Anchiornis*²⁰, and melanosomes isolated from human
110 hair²⁴. Further, spectra of the pterosaur filaments more closely resemble those of
111 pheomelanin-rich red human hair in the stronger absorption regions at ca. 2850 cm^{-1}
112 and 2918 cm^{-1} and higher resolution in the region ca. 1500–1700 cm^{-1} than those
113 from eumelanin-rich black human hair and the ink sac of cuttlefish²⁴. This, together
114 with the SEM results, suggests that the densely packed microbodies in the pterosaur
115 filaments are preserved melanosomes. The amide I peak at 1650 cm^{-1} is more
116 consistent with α -keratin (characteristic of extant mammal hair²⁵) than β -keratin (the
117 primary keratin in extant avian feathers^{22,26}). This signal may be original or
118 diagenetic; the molecular configuration of keratin²⁶ and other proteins²⁷ can alter
119 under mechanical stress and changes in hydration levels.

120 The ultrastructural and chemical features of the pterosaur filaments confirm that
121 they are hair-like or feather-like integumentary structures. The four types of filaments
122 described here show distinct distributions and morphologies. They are separated
123 clearly from the sedimentary matrix by sharp boundaries (Supplementary Fig. 4g–i).
124 There is no evidence that one or more filament type(s) were generated
125 taphonomically, e.g. through selective degradation or fossilization, or superimposition
126 of filaments. For instance, although Type 1 and 4 filaments occur widely in both
127 specimens, Type 4 occurs only in the wings, while Type 1 occupies the remaining
128 body regions. Type 1 filaments are thus not degraded products of Type 4, and Type 4
129 filaments do not represent superimposed clusters of Type 1 filaments. Filament types

130 2 and 3 occur only in CAGS–Z070. Type 3 occurs only in the facial area and is
131 associated with Type 1, where Types 2 and 4 are not evident. Type 3 filaments are
132 thus not degraded Type 2 or 4 filaments. Central filaments of Type 3 are
133 morphologically identical to the short, distally tapering filaments of Type 1, but the
134 branching filaments are much thinner ($< 40 \mu\text{m}$ (Type 3) versus $>70 \mu\text{m}$ (Type 1)
135 wide) and shorter ($< 0.6 \text{ mm}$ vs. $> 3.5 \text{ mm}$ long) than the latter. The branching
136 filaments are thus unlikely to reflect superimposition of clusters of Type 1 filaments.
137 In contrast, the distal ends of Type 2 filaments are similar, and have a similar
138 distribution pattern to, Type 1 filaments. An alternative interpretation, that Type 2
139 filaments might represent superimposition of Type 1 filaments at their proximal ends,
140 is unlikely (see detailed discussion in Supplementary text). Feathers and feather-like
141 integumentary structures have been reported in non-avian dinosaurs, although debate
142 continues about their true nature². These structures have been ascribed to several
143 morphotypes, some absent in living birds^{3,5}, and provide a basis to analyse the
144 evolutionary significance of pterosaur pycnofibres. The pterosaur Type 1 filaments
145 resemble monofilaments in the ornithischian dinosaurs *Tianyulong* and *Psittacosaurus*
146 and the coelurosaur *Beipiaosaurus*: unbranched, cylindrical structures with a midline
147 groove that widens towards the base (presumed in *Beipiaosaurus*)^{3,5}. The pterosaur
148 Type 2 filaments resemble the brush-like bundles of filaments in the coelurosaurs
149 *Epidexipteryx* and *Yi*^{3,5,28}: both comprise parallel filaments that unite proximally. The
150 morphology and circum-cranial distribution of pterosaur Type 3 filaments resemble
151 bristles in modern birds¹, but surprisingly do not correspond to any reported

152 morphotype in non-avian dinosaurs. The Type 3 filaments recall bilaterally branched
153 filaments in *Sinornithosaurus*, *Anchiornis*, and *Dilong*, but the latter filaments branch
154 throughout their length rather than halfway along the central filament(s), as in the
155 pterosaur structure^{3,5}. The pterosaur Type 4 filaments are identical to the radially
156 branched, downy feather-like morphotype found widely in coelurosaurs such as
157 *Sinornithosaurus*, *Beipiaosaurus*, *Protarchaeopteryx*, *Caudipteryx*, and *Dilong*^{3,5}.

158 The filamentous integumentary structures in our anurognathid pterosaurs are thus
159 remarkably similar to feathers and feather-like structures in non-avian dinosaurs.
160 Intriguingly, cylindrical (Type 1), radially symmetrical branched (Types 2 and 4) and
161 bilaterally symmetrical branched (Type 3) filaments clearly coexisted in individual
162 animals; these structures may represent transitional forms in the evolution of feathers,
163 as revealed by developmental studies^{3,5}. These new findings warrant revision of the
164 origin of complex feather-like branching integumentary structures from Dinosauria to
165 Avemetatarsalia, the wider clade that includes dinosaurs, pterosaurs, and close
166 relatives^{4,29}. The early evolutionary history of bird feathers and homologous structures
167 in dinosaurs, and the multiple complex pycnofibres of pterosaurs, is enigmatic. A
168 previous study concluded that the common ancestor of these clades bore scales and
169 not filamentous integumentary appendages², but this result emerged only when the
170 filaments of pterosaurs were coded as non-homologous with those of dinosaurs. There
171 are no morphological criteria, however, for such a determination. The presence of
172 multiple pycnofibre types and their morphological, ultrastructural and chemical
173 similarity to feathers and feather-like structures in various dinosaurian clades,

174 confirms their likely homology with filamentous structures in non-avian dinosaurs
175 and birds. Comparative phylogenetic analysis produces equivocal results: maximum
176 likelihood modelling of plausible ancestral states, against various combinations of
177 branch length and character transition models (Supplementary text and
178 Supplementary Fig. 9, Table 3), reveals various potential solutions. The statistically
179 most likely result (Fig. 3 and Supplementary Table 3, highest log-likelihood value)
180 shows that the avemetatarsalian ancestors of dinosaurs and pterosaurs possessed
181 integumentary filaments, with highest likelihood of possessing monofilaments; tufts
182 of filaments, and, especially, brush-type filaments, are less likely ancestral states. This
183 confirms that feather-like structures arose in the Early or Middle Triassic. The
184 alternative tree for Dinosauria, with Ornithischia and Theropoda paired as
185 Ornithoscelida³⁰, produces an identical result.

186 We present these modelling data with caution, however, for two reasons: (1) the
187 tree rooting method can influence the result (Supplementary Table 3), favouring
188 results in which either scales are the basal condition or where non-theropod feather-
189 like structures and feathers evolved independently (Supplementary Figure 9, Table 3),
190 and (2) there is no adequate way to model probabilities of evolution of all six feather
191 types, or to model probabilities of transitions between the six different feather types.

192 The discovery of multiple types of feather-like structures in pterosaurs has broad
193 implications for our understanding of pterosaur biology and the functional origin of
194 feather-like structures in Avemetatarsalia^{31,32}. Potential functions of these structures
195 include insulation, tactile sensing, streamlining and coloration (primarily for

196 camouflage and signalling), as for bristles, down feathers and mammalian hairs³¹⁻³⁴.
197 Type 1, 2 and 4 filaments could shape a filamentous covering around the body and
198 wings (Fig. 4) that might have functioned in streamlining the body surface in order to
199 reduce drag during flight, as for modern bat fur or avian covert feathers^{33,35}. Type 1
200 and 2 filaments occur in considerably high densities, particularly around the neck,
201 shoulder, hindlimb and tail regions where the high degree of superposition prevents
202 easy discrimination of adjacent fibres. This, along with the wide distribution and
203 frayed appearance, resembles mammalian underfur adapted for thermal insulation^{36,35}.
204 Despite the less dense packing of Type 4 filaments on the wings, the morphology of
205 the structures is consistent with a thermoregulatory function: down feathers can
206 achieve similar insulation as mammalian hair with only about half the mass, due to
207 their air-trapping properties and high mechanical resilience, effective in retaining an
208 insulating layer of still air³⁸. This may optimize the encumbrance of the large wing
209 area to wing locomotion¹⁸. Type 3 filaments around the jaw (Fig. 4) may have had
210 tactile functions in e.g. prey handling, information gathering during flight, navigating
211 in nest cavities and on the ground at night, similar to bristles in birds³⁹.

212

213 **Methods**

214 **Sampling.** The specimen NJU-57003 is represented by two fragmented slabs, both
215 containing original bone, fossilized soft tissues, and natural moulds of bones. Each
216 slab was glued together along the fissures by fossil dealers with the fossil on the

217 surfaces untouched. The specimen CAGS–Z070 is represented by a single unbroken
218 slab. Small flakes (1–3 mm wide) of samples with preserved integument and/or
219 enclosing sediments were carefully removed from the inferred integumentary
220 filaments from different parts of NJU–57003 (Supplementary Figs. 1a and 4a–c)
221 using a dissecting scalpel. This method was used to avoid sampling from degraded
222 products of other tissues, such as dermis, epidermis, or even internal organs. Most
223 samples were not treated further; the remainder were sputter-coated with Au to
224 enhance SEM resolution (Fig. 2g–h and Supplementary Figs. 4a–f and 6). All
225 experiments described below were repeated in order to validate the results.

226

227 **SEM.** Samples were examined using a JEOL 8530F Hyperprobe at the School of
228 Earth Sciences, University of Bristol, and a LEO 1530VP scanning electron
229 microscope at the Technical Services Centre, Nanjing Institute of Geology and
230 Palaeontology, Chinese Academy of Sciences. Both instruments were equipped with a
231 secondary electron (SE) detector, a back-scattered electron (BSE) detector and an
232 energy dispersive X-ray spectrometer (EDS).

233

234 **Measurements of melanosomes.** The geometry of melanosomes was measured from
235 SEM images using the image-processing program ImageJ (available for download at
236 <http://rsbweb.nih.gov/ij/>). We measured maximum short and long axis length of
237 melanosomes that were oriented perpendicular to line of sight, and from these data we
238 calculated mean and coefficient of variation (CV) of the long and short axis, and mean

239 aspect ratio (long:short axis). Based on the proposed taphonomic alteration of fossil
240 melanosome size (shrinkage up to ~20% in both length and diameter)^{40,41}, we
241 modelled potential diagenetic alteration by enlarging original measurements by 20%.

242

243 **FTIR microspectroscopy.** Samples of the filamentous tissues and the associated
244 sediments were removed separately from NJU-57003 and placed on a BaF₂ plate
245 without further treatment. The IR absorbance spectra were collected using a Thermo
246 iN10MX infrared microscope with a cooled MCT detector, at the School of Earth
247 Sciences, University of Bristol. The microscope was operated in transmission mode
248 with a 15x15 micron aperture. 10 spectra were obtained from the filamentous tissues.
249 The spectra show consistent results and the example presented in Fig. 2 shows the
250 highest signal to noise ratio and was obtained with 2 cm⁻¹ resolution and 2000 scans.

251

252 **Fluorescence microscopy.** Selected areas with extensive soft tissue preservation in
253 NJU-57003 were investigated and photographed using a Zeiss Axio Imager Z2
254 microscope with a digital camera (AxioCam HRc) and a fluorescence illuminator
255 (514 nm LED) attached, at the Technical Services Centre, Nanjing Institute of
256 Geology and Palaeontology, Chinese Academy of Sciences.

257

258 **Laser-stimulated fluorescence (LSF) imaging and data reduction protocol.** LSF
259 images were collected using the protocol of Kaye et al.^{15,16}. NJU-57003 was imaged
260 with a 405 nm 500 mw laser that was projected into a vertical line by a Laserline

261 Optics Canada lens. The laser line was swept repeatedly over the specimen during the
262 exposure time for each image in a dark room. Images were captured with a Nikon
263 D610 DSLR camera fitted with an appropriate long pass blocking filter in front of the
264 lens to prevent image saturation by the laser. Standard laser safety protocols were
265 followed during laser usage. The images were post processed in Photoshop CS6 for
266 sharpness, colour balance and saturation.

267

268 **Phylogenetic macroevolutionary analysis.** In order to analyse the evolution of
269 feather characters, data were compiled on known integumentary characters across
270 dinosaurs and pterosaurs. The basic data were taken from the Supplementary data of
271 Barrett et al. ², comprising 74 dinosaurs (33 ornithischians, seven sauropods and 44
272 theropods (including four Mesozoic birds)); to this dataset we added four pterosaurs.
273 Barrett et al. ² scored taxa for three integumentary states (scales, filaments, feathers)
274 in their macroevolutionary analyses. We checked and followed these basic categories
275 and added three more; we then cross-referenced these six categories against the
276 feather morphotypes defined by Xu et al. ⁴². The categories used herein are: scales (1;
277 not included in Xu et al. ⁴²), monofilaments (2; morphotypes 1 and 2 in Xu et al. ⁴²),
278 brush-like filaments associated with a planar basal feature (3; morphotypes 4 and 6 in
279 Xu et al. ⁴²), tufts of filaments joined basally (4; morphotype 3 in Xu et al. ⁴²), open
280 pennaceous vane, lacking secondary branching (5; morphotype 5 in Xu et al., ⁴²), and
281 closed pennaceous feathers comprising a rachis-like structure associated with lateral
282 branches (barbs and barbules) (6). There was some uncertainty over feathers coded

283 herein as type 3, which could correspond to morphotype 6, or morphotypes 4 and 6 in
284 Xu et al.⁴². However, the only taxa coded with these as the most derived feather type
285 are *Sordes pilosus* and *Beipiaosaurus inexpectus*. These taxa belong to separate clades
286 and thus the calculation of ancestral states is not affected by how our feather type 3 is
287 coded (i.e. whether treating morphotypes 4 and 6 of Xu et al.⁴² in combination or
288 separately).

289 As in previous studies², we used maximum-likelihood (ML) approaches to
290 explore trait evolution. There are many methods to estimate ancestral states for
291 continuous characters, but choices are more limited for discrete characters, such as
292 here, where only ML estimation of ancestral states is appropriate⁴³. We calculated ML
293 reconstructions of ancestral character states using the ‘ace’ function of the ape R
294 package⁴⁴, with tree branch lengths estimated in terms of time, derived using the
295 ‘timePaleoPhy’ function in the paleotree package⁴⁵ and the ‘DatePhylo’ function in
296 the strap R package⁴⁶. These enabled us to assess results according to three methods
297 of estimating branch lengths, the ‘basic’ method, which makes each internal node in a
298 tree the age of its oldest descendant, the ‘equal branch length’ (equal) method, which
299 adds a pre-determined branch length (often 1 Myr) to the tree root and then evenly
300 distributes zero-length branches at the base of the tree, and the ‘minimum branch
301 length’ (mbl) method, which minimizes inferred branching times and closely
302 resembles the raw, time-calibrated tree. A problem with the ‘basic’ branch length
303 estimation is that it results in many branch lengths of length zero, in cases where
304 many related taxa are of the same age; in these cases, we added a line of code to make

305 such zero branch lengths equal to 1/1000000 of the total tree length. A criticism of the
306 mbl method is that it tends to extend terminal branching events back in time,
307 especially when internal ghost lineages are extensive², but this is not the case here,
308 and the base of the tree barely extends to the Triassic / Jurassic boundary.

309 We ran our analyses using three evolutionary models with different rates of
310 transition between the specified number of character states (six here), namely “ER”,
311 an equal-rates model, "ARD", an all-rates-different model and "SYM", a symmetrical
312 model. These were calculated using the ‘ace’ function in ape² and the
313 ‘add.simmap.legend’ function of the R package ‘phytools’⁴⁷.

314 In a further series of analyses, we attempted to model the macroevolution of all
315 traits, as coded (see Supplementary results), so coding multiple trait values for taxa
316 that preserve multiple feather types. This did not shed much light on patterns of
317 evolution of feather types because the multiple trait codings (e.g. 1,2 or 2,5,6) were
318 each made into a new state, making 14 in all, and these were not linked. Therefore,
319 the six multiply coded taxa that each had feather type 6 were represented as six
320 independent states and their evolution tracked in those terms. Further, we attempted to
321 separate the six characters, so they would track through the tree, whether recorded as
322 singles or multiples in different taxa; however, we did not have the information to
323 enable us to do this with confidence because of gaps in coding. In terms of reality,
324 these multiply coded taxa still represent an incomplete sample of the true presence
325 and absence of character states - by chance, many coelurosaurs are not coded for
326 scales (1) or monofilaments (1), and yet it is likely they all had these epidermal

327 appendages. Therefore, attempting to run such multiple codings, with characters
328 either as groups or coded independently, encounters so many gaps that the result is
329 hard to interpret. Our approach is to code the most derived feather in each taxon, and
330 that too is incomplete because of fossilization gaps, but at least it represents a
331 minimal, or conservative, approach to trait coding and hence to the discoveries of
332 macroevolutionary patterns of feather evolution; complete fossil data might show
333 wider distributions of each feather type and hence deeper hypothesized points of
334 origin. Complete coding of feather types would of course allow each trait to be
335 tracked in a multiple-traits analysis.

336

337 **Data availability**

338 The data that support the findings of this study are available from the corresponding
339 authors upon reasonable request.

340

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453 other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).

454

455 **Supplementary Information** is available in the online version of the paper.

456

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466

467 **Author Contributions**

468 B.Y.J. and M.J.B. designed the research, Z.X.Y., B.Y.J. and X.X. systematically
469 studied the specimens, Z.X.Y., S.L.K., M.E.M, and P.J.O. did the SEM analysis,
470 Z.X.Y. and B.Y.J. did the FTIR analysis, M.P. and T.G.K. did the LSF imaging, data
471 reduction and interpretation, M.J.B. did the maximum likelihood analyses, and
472 Z.X.Y., B.Y.J., M.J.B., M.E.M, X.X. and P.J.O. wrote the paper; all authors approved
473 the final draft of the paper.

474

475 **Author Information**

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477 authors declare no competing financial interests. Correspondence and requests for
478 materials should be addressed to B.Y.J. (byjiang@nju.edu.cn) or M.J.B.
479 (mike.benton@bristol.ac.uk).

480

481 **Figure 1 | Integumentary filamentous structures in CAGS–Z070.** **a**, Overview
482 shows extensive preservation of soft tissues. **b–p**, Details of the integumentary
483 filaments in the regions indicated in **a** on the head and neck (**b–d**, **i–j**), forelimb (**f–g**),
484 wing (**l–m**) and tail (**o–p**), and illustrated reconstructions of the filaments (**e**: Type 1
485 filament; **h**: Type 2 filament; **k**: Type 3 filament; **n**: Type 4 filament). Scale bars: 20
486 mm in **a**; 10 mm in **b**; 500 μm in **c** and **i**; 100 μm in **d**; 1 mm in **f**, **l**, **m** and **p**; 200 μm
487 in **g** and **j**; 5 mm in **o**.

488

489 **Figure 2 | Preservation, microstructure and chemistry of the integumentary**
490 **filamentous structures in NJU–57003.** **a**, Laser-stimulated fluorescence^{6,15,16} image
491 highlights extensive preservation of soft tissues (black areas). **b–f**, Details of the
492 integumentary filaments in the regions indicated in **A** on the head and neck (**b–c**),
493 wing (**d–e**) and tail (**f**). **g–h**, Scanning electron micrographs of the monofilaments on
494 the neck and hindlimb of NJU–57003 (samples 10 and 39, respectively,
495 Supplementary Fig. 1**a**) show densely packed, elongate and oblate melanosomes. **i**,
496 FTIR absorbance spectra of the monofilaments, monofilaments with sediment matrix,
497 and sediment matrix in NJU–57003 (Sample 15, Supplementary Fig. 1**a**) compared
498 with spectra from a feather of *Anchiornis* (from ref. ²⁰), extant Marabou stork feather

499 (from ref. ²¹) and black and red human hair melanosomes (from ref. ²⁴). Scale bars: 20
500 mm in **a**; 1 mm in **b**, **c** and **e**; 5 mm in **d** and **f**; 1 μm in **g** and **h**.

501

502 **Figure 3 | Phylogenetic comparative analysis of integumentary filament and**
503 **feather evolution in pterosaurs and archosaurs.** The phylogeny is scaled to
504 geological time, with recorded terminal character states for each species, and
505 estimated ancestral character states at the lower nodes. The model is the most likely of
506 the maximum likelihood models, based on minimum-branch lengths (mbl) and
507 transitions occurring as all-rates-different (ARD), but other results with lower
508 likelihoods show scales as ancestral. The ancestral state reconstruction shows a
509 combination of monofilaments, tuft-like filaments, and brush-type filaments as the
510 ancestral state for Avemetatarsalia and for Dinosauria. The estimated ancestral state
511 for Theropoda comprises all five feather states. Numbered small vertical arrows
512 indicate earliest occurrences of feather types 2–6. Two hypotheses for timing of avian
513 feather origins are indicated: A, early origin, at the base of Avemetatarsalia in the
514 Early Triassic, or B, late origin, at the base of Maniraptora in the Early–Middle
515 Jurassic.

516

517 **Figure 4 | Reconstruction of one of the studied anurognathid pterosaurs, exhibiting**
518 **diverse types of pycnofibres distributed in different body parts.**