PNAS
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Supplementary Information for

The molecular evolution of feathers with direct evidence from fossils

Yanhong Pan[a,1], Wenxia Zheng[b], Roger H. Sawyer[c], Michael W. Penninngton[d], Xiaoting Zheng[e,f], Xiaoli Wang[e,f], Min Wang[g,h], Liang Hu[a,i], Jingmai O'Connor[g,h], Tao Zhao[a], Zhiheng Li[g,h], Elena R. Schroeter[b], Feixiang Wu[g,h], Xing Xu[g,h], Zhonghe Zhou[g,h,I,1], Mary H. Schweitzer[b,j,1]

[a]CAS Key Laboratory of Economic Stratigraphy and Palaeogeography, Nanjing Institute of Geology and Palaeontology and Center for Excellence in Life and Paleoenvironment, Chinese Academy of Sciences, Nanjing 210008, China;

[b]Department of Biological Science, North Carolina State University, Raleigh, NC27695, US;

[c]Department of Biological Sciences, University of South Carolina, Columbia, SC 29205, US;

[d]AmbioPharm Incorporated, North Augusta, SC 29842, US;

[e]Institute of Geology and Paleontology, Lingyi University, Lingyi City, Shandong 27605, China;

[f]Shandong Tianyu Museum of Nature, Pingyi, Shandong 273300, China;

www.pnas.org/cgi/doi/10.1073/pnas.1815703116

[g]CAS Key Laboratory of Vertebrate Evolution and Human Origins of the Chinese Academy of Sciences, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China;
[h]Center for Excellence in Life and Paleoenvironment, Chinese Academy of Sciences, Beijing 100044, China;
[i]College of Earth and Planetary Sciences, University of Chinese Academy of Sciences, Beijing 100049, China;
[j]North Carolina Museum of Natural Sciences, Raleigh, NC 27601, US;

Paste corresponding author name here
Email: yhpan@nigpas.ac.cn; mhschwei@ncsu.edu; Zhouzhonghe@ivpp.ac.cn

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Supplementary text
Figs. S1 to S13
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Supplementary Information Text

Subhead. Type or paste text here. This should be additional explanatory text such as an extended technical description of results, full details of mathematical models, extended lists of acknowledgments, etc.

Material and Methods

Sampling

Samples (0-214-S1) were removed from the specimen of *Anchiornis huxleyi* STM 0-214, housed in the Shandong Tianyu Nature Museum of Nature, using a blade and tweezer sterilized with 75% (vol/vol) ethanol and E-pure water. Specimens were dried under a biological hood, mounted to stubs on carbon tape, and visualized, uncoated, with scanning electron microscopy (SEM). Fossil samples were then removed from the conducting tape and placed into 50% hydrofluoric acid (HF) for 4 hours to dissolve the siliceous minerals of the sediment matrix, then washed with E-pure water five times. Optical microscopy (OM) shows that only a thin film with filamentous structures were left after demineralization. Dehydration and infiltration protocols were applied as described previously (1), then samples were placed in embedding molds, infiltrated with 100% LR White, covered with ACLAR® film, and allowed to polymerize for at least 24 hours at 60°C. A Leica EM UC6 ultramicrotome with DiATOME 45° diamond knives was used to cut sections to desired thickness for immunohistochemistry (IHC), Transmission electron microscopy (TEM), and ChemiSTEM analyses. Chicken (*Gallus gallus*) feathers, prepared separately from fossils, were used as the extant control.

Various aged fossil and extant feathers or related tissues samples for comparing

Feather samples (5-12-S1, 7-144-S1, and 9-5-S1) were removed with a clean blade and tweezer (rinsed with 75% (vol/vol) ethanol and E-pure water) from the specimens STM 5-12, 7-144 and 9-5 respectively (Specimens are housed in the Shandong Tianyu Nature Museum of Nature). The sediments yielding the Oligocene feather were much harder than the other specimens and could not be sampled with blade and tweezer, so it was broken into several smaller pieces (Cenz-f-S1, S2, S3) with a hammer. The clean foils were used to block direct contamination of hammer to the samples. The sample of the feather-like structure from *Shuvuuia deserti*, is one of the embedded specimens within LR white resin (2). The sample of the claw tissue from *Citipati osmolskae*, is one of the embedded specimens within LR white resin (3).

Modern comparable tissues, used as controls, including flight feathers of the chicken, goose, duck, white leghorn chicken, emu; rhamphothecase of the chicken and emu; claws of chicken, emu and ostrich; scales of chicken and ostrich. These were collected from farmers and/or from

markets. Extant tissues were kept separate at all times from fossil specimens and all analyses of extant materials were performed in physically isolated labs, using dedicated instruments and reagents. Fossil samples were never directly exposed to extant materials.

All samples were washed successively in 75% (vol/vol) ethanol and E-pure water, then dried under a biological hood.

Electronic microscopy analyses

For detailed information on SEM, TEM, ChemiSTEM elemental mapping, immunohistochemistry, and immunogold procedures, see Methods section in Pan et al. 2016 (1). The sections for elemental mapping are only stained with uranyl acetate, but not lead citrate, because the k spectrum of Sulfur (S) and Lead (Pb) too close to be differentiated, and the spectrum of lead will cover that of the sulfur. Here we did not include carbon maps because specimens were mounted on carbon-coated grids, and the embedding resin employed (LR White, Electron Microscopy Science Inc. supplies) also contains carbon, making maps in carbon less accurate. Additionally, sections required counterstaining using uranyl acetate, which also contains carbon.

Antigen design

We employed polyclonal antisera in this study, because these sera recognize multiple epitopes of a protein (5); if either evolutionary distance or unequal preservation removed some epitopes, a polyclonal serum would still elicit a positive response from those that remain, whereas if a monoclonal antibody does not bind, it would be impossible to differentiate between a lack of preservation of the epitope, or its lack of evolution in the lineage.

Universally, β-keratin contains three distinct domains: a central domain (34 amino acids segment) that constitutes the β-sheet framework of the ~3nm filament and N- and C-terminal domains that constitute the inter-fibrillar matrix (6, 7). When the amino acids sequences of different subfamilies of β-keratins are aligned, a high degree of homology is notable in the central domain. The N-terminal and C-terminal domains are less conserved, with compositions that vary between appendages (7, 8). The β-keratins comprising extant feather (feather, and feather-like β-keratins) contain the specific N- and C-terminal domains that confer certain biophysical properties (i.e., flexibility and plasticity) (7, 8). Feather and feather-like β-keratins share over 80% homologous residues at both the DNA and amino acid sequence levels, but show less homology with the other β-keratin subfamilies (9). It has been proposed that this particular molecular modification directly contributed to the attainment of powered flight in the theropod-bird lineage (10, 11). Thus, identification of this molecular region in fossils allows us to address broader

evolutionary questions. We capitalized on the homology and variations of the β-keratins, as well as the specificity and sensitivity of the vertebrate immune system, to generate polyclonal antiserum that recognizes all family members of the β-keratins (3, 12) and polyclonal antisera against synthetic peptides that specifically recognize only the feather-type (feather and feather-like) β-keratins (8).

Two amino acids sequences were selected for synthesis of peptides. These peptides were subsequently conjugated to keyhole limpet hemocyanin (KLH), which were then used to generate feather-type β-keratin antisera. The 29 amino acid sequence labeled "Peptide 1" (SCYNPCLPRSSCGPTPLANSCNEPCLFRQ) localizes to the N terminus of the *Emu* feather β-keratin (7) and "Peptide 2" "VGSTTSAAVGSILSEEGVPINSGC" is a 24 amino acid sequence found at the C terminus of the turkey vulture feather β-keratin. Peptide 1 was selected as it rich in Cys residues which are likely to be stabilizing structures in the protein forming disulfide bonds (6, 7, 13). Furthermore, the presence of Pro residues adds flexibility as it helps terminate classical protein secondary structures such as helices and turns (6, 7, 13). Peptide 2 was previously used to produce a feather β-keratin specific antiserum33. Molecular alignments predict that peptide 1 (Table S2), with 7-9 continually matching amino acids to avian claw and scale β-keratins, may show some cross reactivity between tissue types (14). However, negative results in our tests on extant claw and scale tissues from chicken, ostrich, and emu (fig. S5) suggest a low possibility of cross-reactivity to avian claw and scale β-keratins. Both molecular predictions and actual experiments show that the antisera against these peptides are effective in differentiating feather-type β-keratins from other β-keratin subfamilies (Table S3, figs. S5, S6). To control for non-specific cross reactivity of the primary antiserum, we used a non-relevant antiserum (anti-peptidoglycan monoclonal antiserum) not predicted to bind these feather tissues (fig. S7).

Antibody controls

ELISA

To calibrate optimal antibody concentrations and affinity of the antibodies, preliminary ELISA (Enzyme-linked immunosorbent assays) were performed.

ELISA analyses used Immulon 2HB U bottom plates (Thermolyne Labsystem, 96 wells, A-H vs 1-12), read by a ThermoMax microplate reader (Molecular Devices) installed with SoftMax Pro software. Synthesized peptide 1 and peptide 2 were used as antigens, which were each tested at three different concentrations (1000ng, 100ng, 10ng per well) solubilized in 100 μl of PBS. Binding intensities of three antibodies (anti-β-keratin, anti-peptide 1, anti-peptide 2) were

measured against each antigen. For each antigen tested, the primary antibody was omitted from a selection of wells as a negative control to monitor for spurious binding.

The ELISA protocol: 100 μl of antigens (above) were added to a selection of wells, then covered with plastic wrap and incubated 4hrs at RT. Plate was flicked out to remove antigen, then all wells received 200 μl of blocking buffer (5% BSA in PBS with Tween20 and Thimersol) and plate was incubated 4hrs at RT. Blocking buffer was flicked out and wells received 100 μl of one of three primary antibodies (above) diluted in 1:400 in 5% BSA block buffer. Plate was incubated O/N at 4°C, then flicked out and washed 10 times in ELISA wash buffer (1X PBS with 0.1% Tween20) and tapped dry on paper towels. Secondary antibody (Goat Anti-Rabbit IgG(H+L) AP conjugated) diluted 1:2000 in 5% BSA blocking buffer was added to each well (100 μl/well) and incubated 4hrs at RT. Plate was flicked out and washed 10 times in ELISA wash buffer, then wells received 100 μl of a substrate buffer (10 ml of diethanolamine (Pierce) mixed with one p-Nitrophenylphosphate tablet (sigma N-9389). Plate was read on a plate reader at 405 nm.

Preabsorption controls

Preabsorption controls incubate the primary antiserum with excess antigen, which blocks the antibody binding sites specific for that antigen, thus preventing binding to epitopes in tissues to which the preabsorbed antibodies are applied. Four inhibitors are used here for three antibodies: 1) freshly cut extant chicken feather 36mg/ml were used to inhibit antiserum raised against extracted feather proteins (freshly cut feather is used as inhibitor instead of extract feather proteins because the highly basic reagents used for extraction interfere with the antiserum); 2) a mixture of synthesized peptide 1 (1mg/ml) and peptide 2 (1mg/ml) was used to inhibit a second aliguot of antiserum raised against extract feather proteins; 3) synthesized peptide 1 (1mg/ml) was used to inhibit peptide 1 antiserum; 4) synthesized peptide 2 (1mg/ml) was used to inhibit peptide 2.

Preabsorbed antibodies were applied to tissue samples as negative controls following the IHC protocol detailed in Pan et al., 2016 (1).

Enzyme digestion

Oligocene fossil feather and extant duck wing feather were washed with E-pure water five times, dried at RT, then incubated in 10% Shout (a detergent product of S. C. Johnson & Son Inc.) with stirring for 12 hours at RT. Detergent was removed by washing with E-pure water five times, then samples were dried at 37°C. Samples (extant feather was cut into similar size to the fossil sample) were then incubated in 0.1% NaOH overnight at 70°C with shaking, then in 2mg/ml Keratinase in 0.1% SDS (adjusted to pH 9 using Na_2HPO_4), overnight, with shaking.

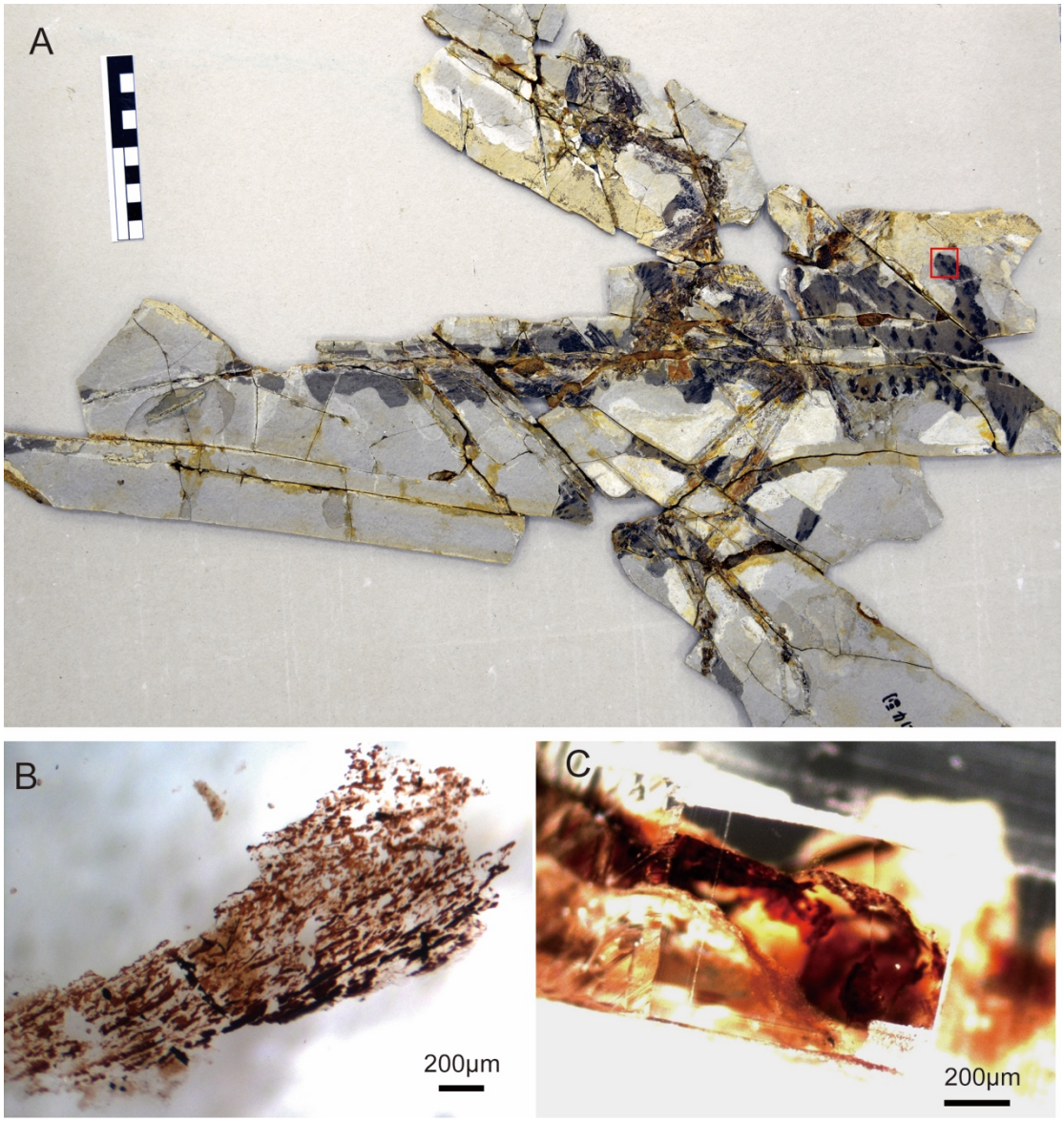

Fig. S1. (A) The *Archiornis* (STM0-214) specimen used in this study, collected from Jianchang, western Liaoning, location of sample is marked by red box. The sample was collected from wing feathers attached to the right forelimb. (B) Filamentous remnants of feathers remaining after incubation in 50% HF (see methods) were collected and embedded with known orientation in resin. The cutting surface of the embedded sample is shown in (C).

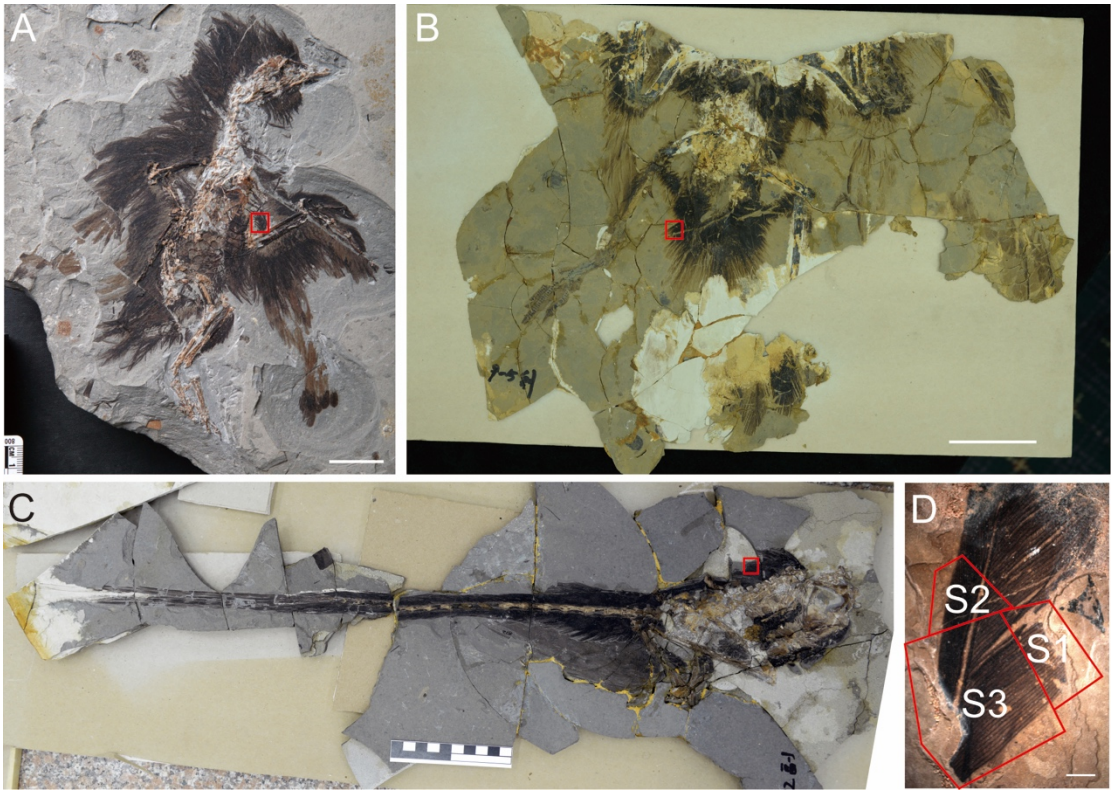

Fig. S2. Showing the sampling locations (red box) of the selected samples of the other geological younger feathered dinosaurs and birds, except for *Anchiornis*, from China for analyses. (A) 7-144-S1 sampled from the wing feather near the right forelimb, between the humerus and the radius of *Eoconfuciusornis* (STM7-144); (B) 9-5-S1 sampled from the tail feathers, near the distal end of the left pubis of the *Yanornis* (STM9-5); (C) 5-12-S1 sampled from the wing feathers possibly belonging to the left forelimb of the Dromaeosauridae indet. (STM5-12); (D) Cenz-f-S1, S2, and S3 sampled from the isolated Oligocene feather (DY 1502006). Scale bars: A, 2 cm; B, 5 cm; C, 10 cm; D, 2 mm.

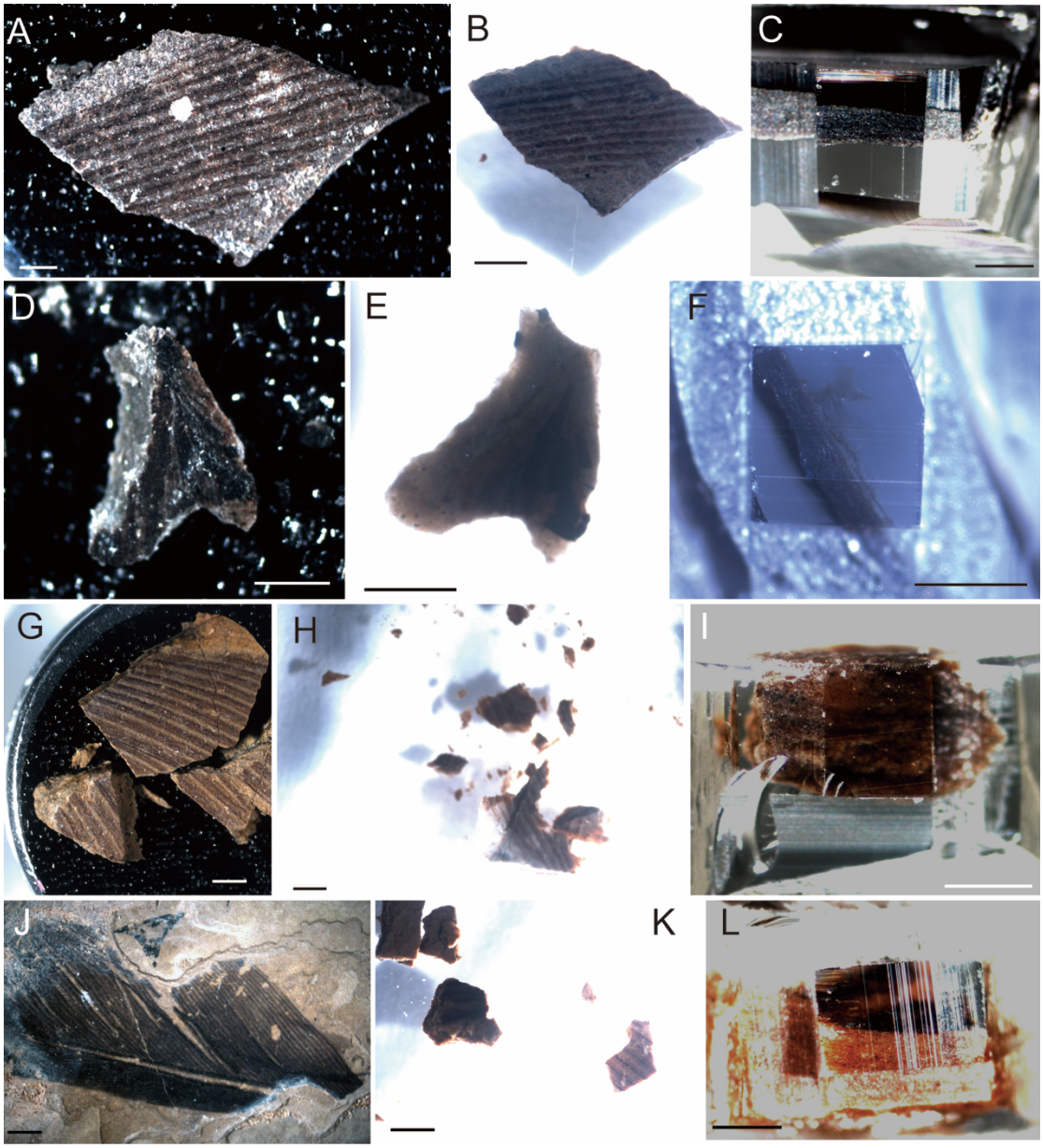

Fig. S3. Preparing the LR white sections of the other fossil feathers for the TEM and immunohistochemistry. (A, D, G, J) fossil samples were documented with optical light microscope; (B, E, H, K) treated in 50% HF for 4 hours, then embedded with a known orientation; (C, F, I, L) cutting surface of the embedded samples. (A-C), Dromaeosauridae indet. 5-12-S1; (D-F), *Eoconfuciusornis* 7-144-S1; (G-I), *Yanornis* 9-5-S1; (J-L), isolated Oligocene feather Cenz-f-S1. Scale bars: A, B, D, E, G, H, K, 1 mm; C, F, I, L, 500 μm; J, 2 mm.

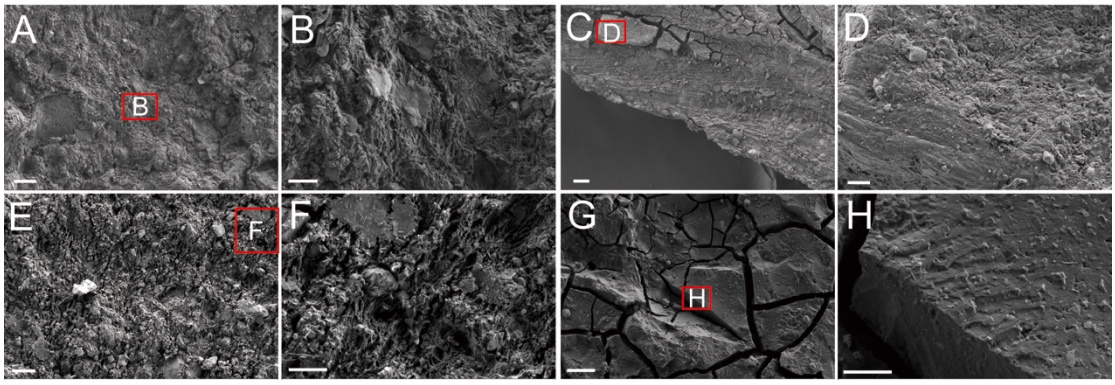

Fig. S4. SEM images of the other studied geological younger fossil feathers. (A-B) SEM images of feather from Dromaeosauridae indet.; (C-D) SEM images of feather from *Eoconfuciusornis*; (E-F) SEM images of feather from *Yanornis*; (G-H) SEM images of the isolated Oligocene feather. Scale bars: A, C, E, G, 20 μm; B, D, F, H, 2 μm.

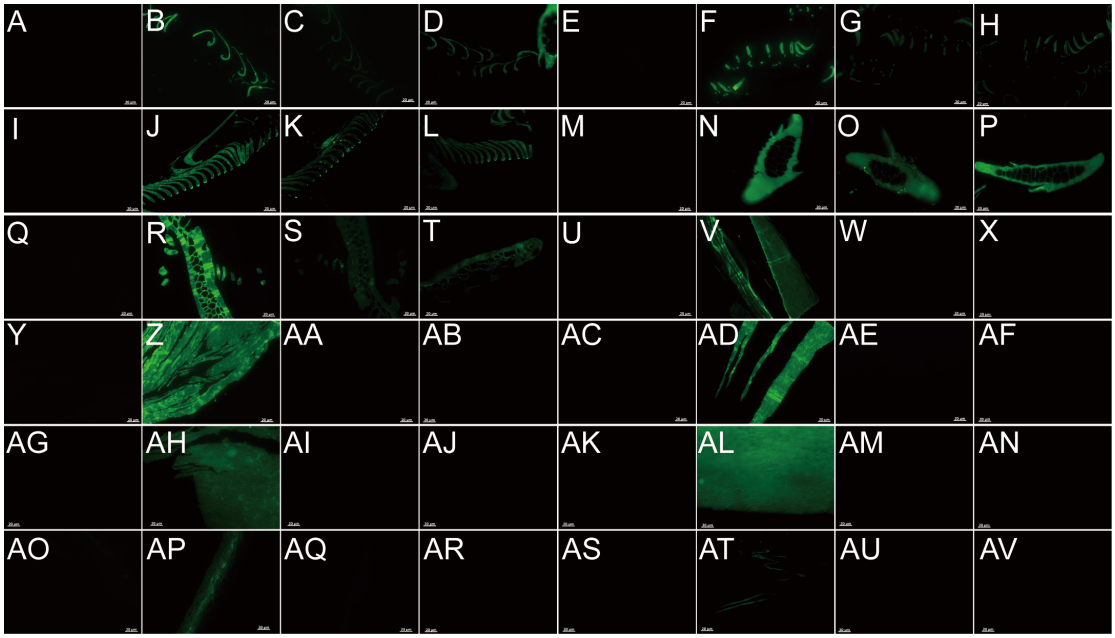

Fig. S5. Specificity tests of three antisera (against extracted feather proteins, Peptide 1, and Peptide 2 respectively) on modern known tissues. (A, E, I, M, Q, U, Y, AC, AG, AK, AO, AS) represent negative controls, where no primary antibody is applied but all the other steps are identical; (B-D) show antibody-antigen (ab-ag) complexes localized to chicken feather; (F-H) show ab-ag complexes localized to duck feather; (J-L) show ab-ag complexes localized to goose feather; (N-P) show ab-ag complexes localized to white leghorn feather; (R-T) show ab-ag complexes localized to emu feather; (V, Z, AD, AH, AL, AP, AT) demonstrates positive reactivity of the more general antiserum against extracted feather proteins to chicken rhamphothecase, emu rhamphothecase, emu claw, ostrich claw, chicken claw, ostrich scale, and chicken scale tissues; (W-X, AA-AB, AE-AF, AI-AJ, AM-AN, AQ-AR, AT-AV) do not show binding to the peptides antisera of these tissues. Scale bars: 20 μm

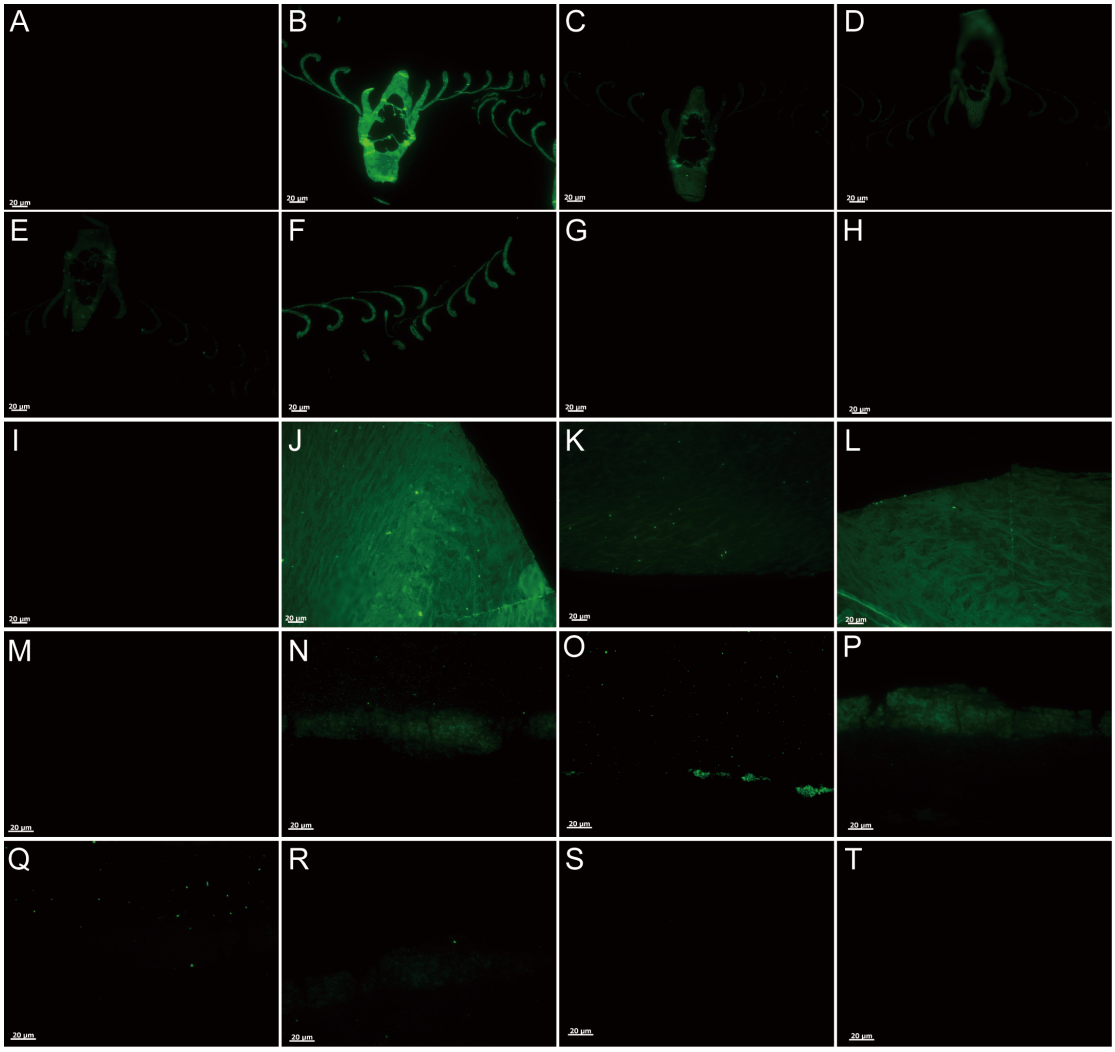

Fig. S6. Preabsorption controls on modern and fossil tissues. (A-H) extant chicken feather; (I-L) extant chicken claw; (M-T) fossil feathers (7-144-S1). (A, I, M) represent negative controls, where no primary antibody is applied but all the other steps are identical; (B, J, N) represent positive controls of the antiserum against extracted feather proteins; (C, O) represent positive controls of the antiserum against Peptide 1; (D, P) represent positive controls of the antiserum against Peptide 2; (E, K, Q) freshly cut extant chicken feather 36mg/ml was used to inhibit antiserum raised against extract feather proteins, show much weakened reactions; (F, L, R) a mixture of synthesized peptide 1 (1mg/ml) and peptide 2 (1mg/ml) was used to inhibit a second aliguot of antiserum raised against extract feather proteins, panel (F) and (L) exhibit strong positive signals, while panel (R) show a bit weaker positive signals; (G, S) synthesized peptide 1 (1mg/ml) was used to inhibit peptide 1 antiserum, show negative results; (H, T) synthesized peptide 2 (1mg/ml) was used to inhibit peptide 2, show negative results. Scale bars, 20 μm.

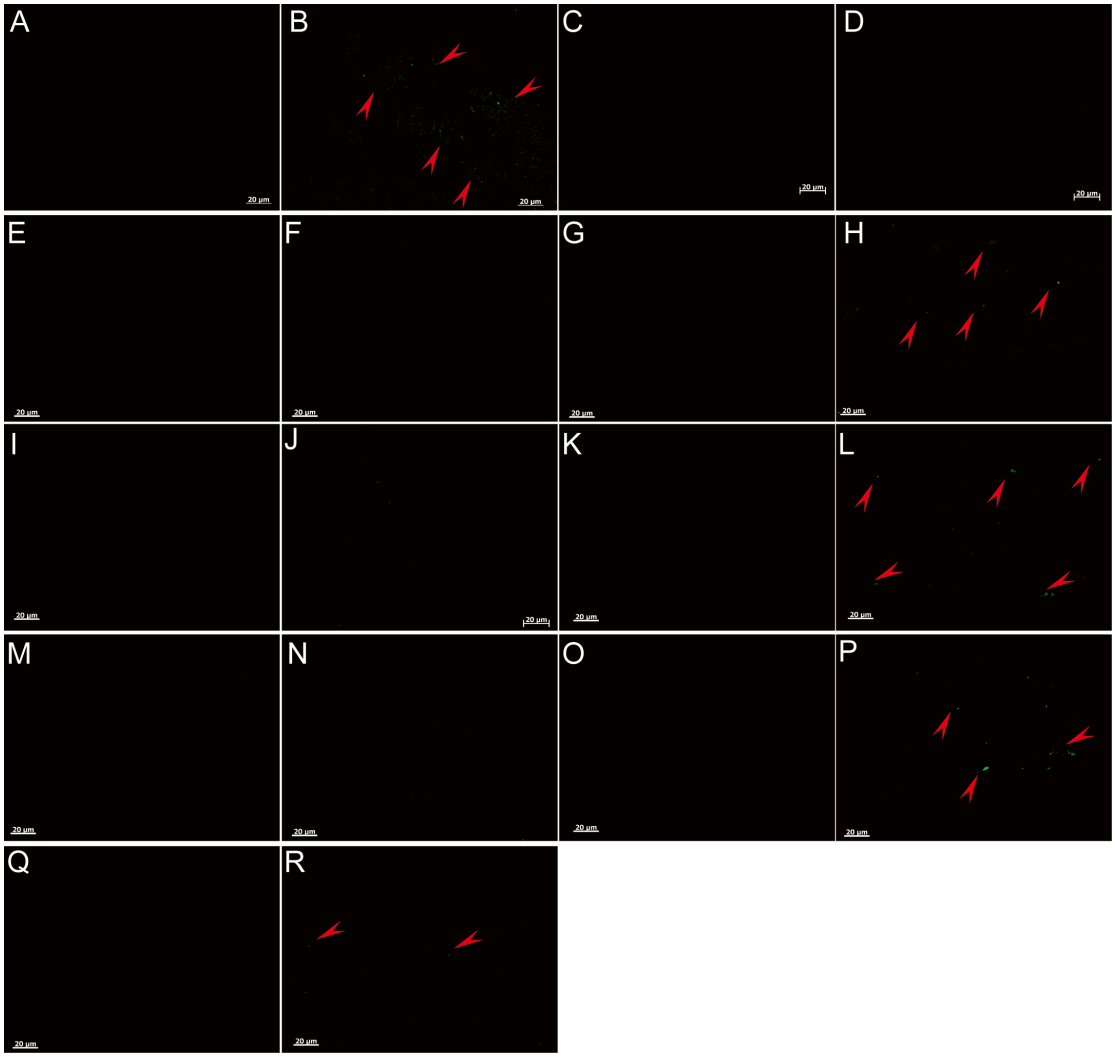

Fig. S7. Immunofluorescence (IF) controls of non-relevant anti-body (anti-peptidoglycan monoclonal antiserum), most of the fossil tissues showing negative results, some exhibiting positive signals but the pattern is completely different from the feather pattern. (A, B) tests on *Bacillus cereus* biofilm; (C, D) tests on extant chicken feathers; (E, F) tests on claw tissues from *Citipati osmaolskae*; (G, H) tests on feather-like structures from *Shuvuuia deserti*; (I, J) tests on STM0-214-S1; (K, L) tests on STM5-12-S1; (M, N) tests on STM7-144-S1; (O, P) tests on STM9-5-S1; (Q, R) tests on Cenz-f-S1. (A, C, E, G, I, K, M, O, Q) represent negative controls, where no primary antibody is applied but all the other steps are identical. Scale bars, 20 μm.

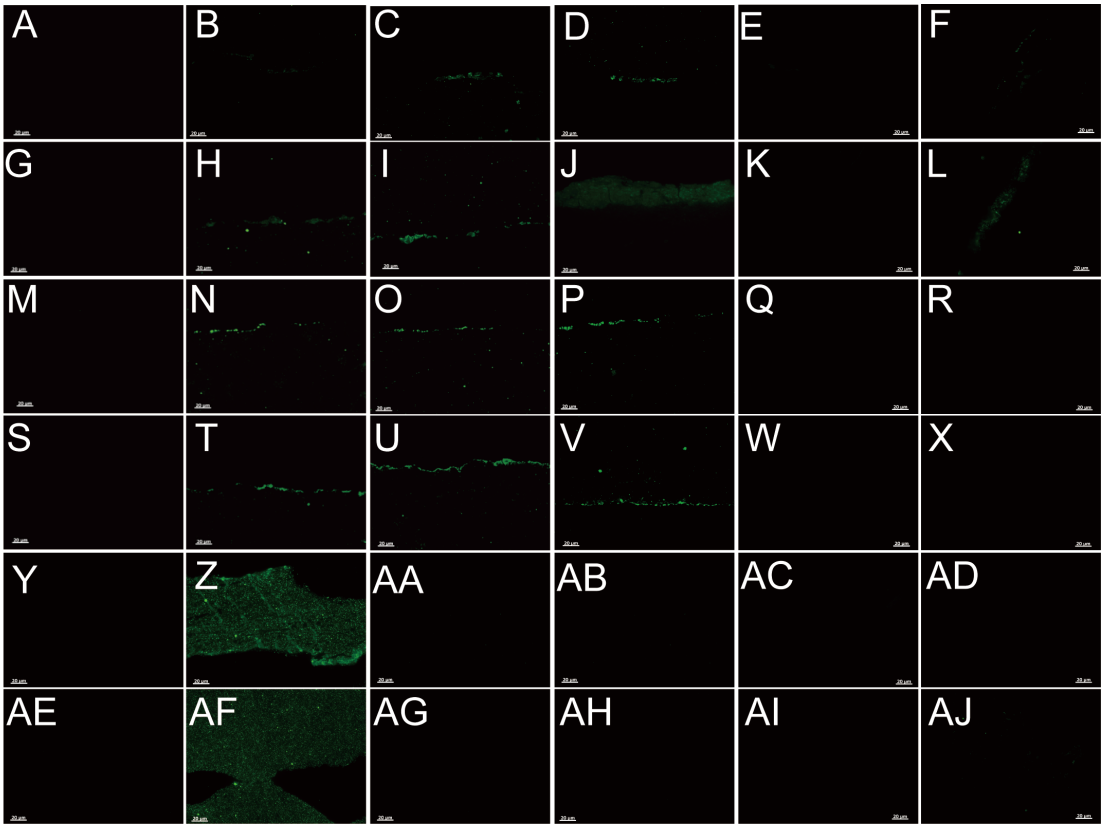

Fig. S8. *In situ* immunofluorescence (IF) using three antisera (against extracted feather proteins, Peptide 1, and Peptide 2 respectively) and AE1/AE3 +5D3 antiserum on the other geological younger fossil specimens. (A, G, M, S, Y, AE) represent negative controls for the three designed antisera, where no primary antibody is applied but all other steps kept identical to test conditions; (B-D), (H-J), (N-P), (T-V), show antibody-antigen (ab-ag) complexes localized to feather tissues from Dromaeosauridae indet., *Eoconfuciusornis*, *Yanornis*, the Oligocene isolated feather respectively. (E, K, Q, W, AC, AI) represent negative controls for the AE1/AE3 +5D3 antiserum, (F) and (L), showing ab-ag complexes localized to the feather tissues from Dromaeosauridae indet. and Eoconfuciusornis; (R) and (X) feather tissues from *Yanornis*, the Oligocene isolated feather do not show binding to the antiserum. As controls, panel (AF) demonstrates positive reactivity of the more general antiserum against extracted feather proteins to claw tissues from *Citipati*; panels (AG) and (AH) do not show binding to the peptides antisera; panel (AJ) showing weak signals binding to the AE1/AE3 +5D3 antiserum. Similarly, positive reactivity of the more general antiserum against extracted feather proteins to the feather-like structures from *Shuvuuia* is seen in panel (Z), but the feather-type peptide antisera do not bind these tissues as seen in panel (AA) and (AB), as well as the AE1/AE3 +5D3 antiserum do not bind in panel (AD). Scale bar is 20 μm.

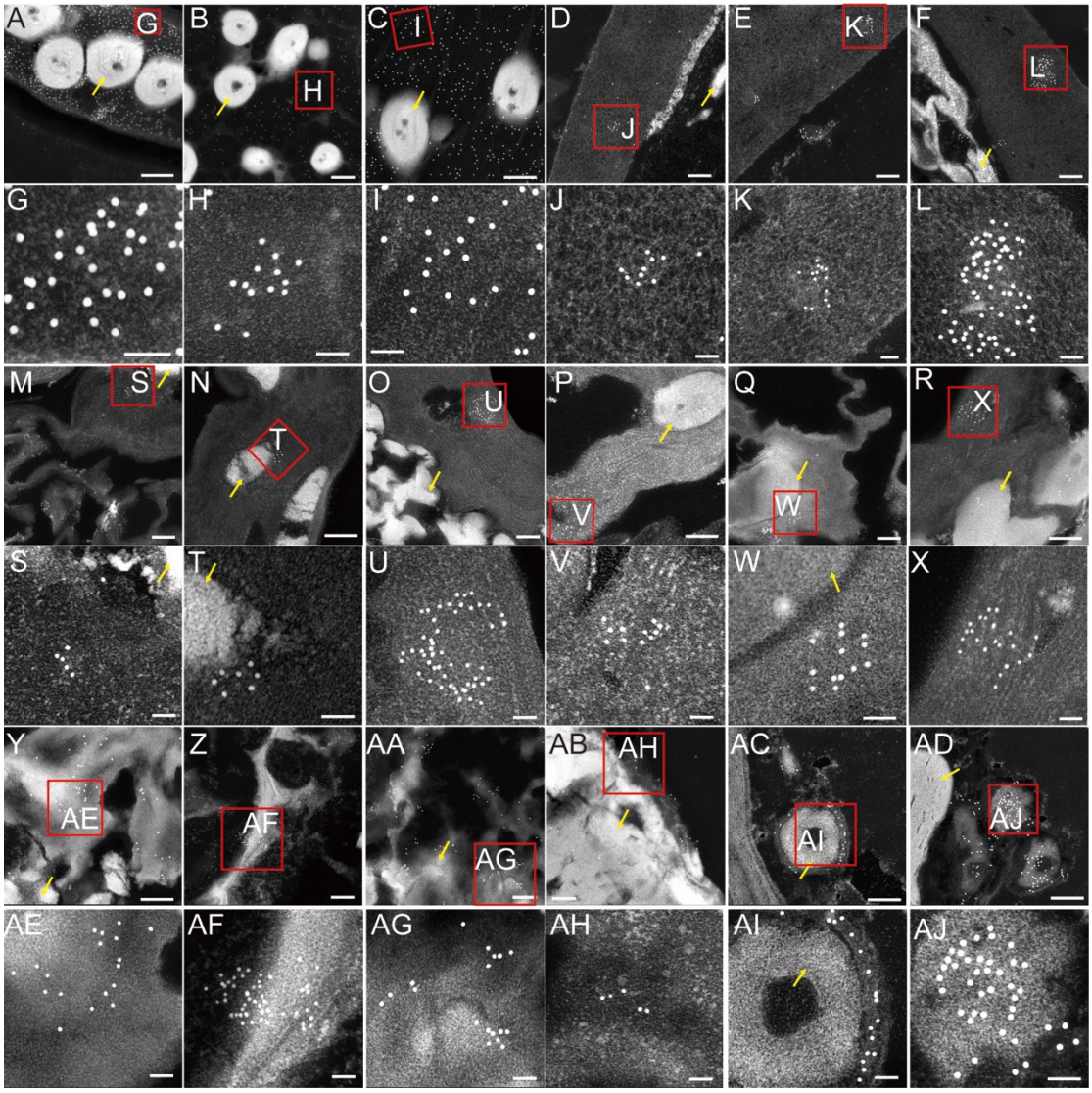

Fig. S9. In situ immunogold (IG) assays showing bind of three antisera (against extracted feather proteins, Peptide 1, and Peptide 2 respectively) on fossil specimens compared with results on extant feather. Positive binding of antisera to extant feather tissues from *G. gallus*, as revealed by localized, electron dense gold beads, is seen in panels (A-C, G-I). Panels (D-F, J-L), (M-O, S-U), (P-Q, V-X), (Y-AA, AE-AG), (AB-AD, AH-AJ) show antibody-antigen (ab-ag) localization to feathers from *Archiornis*, Dromaeosauridae indet., *Eoconfuciusornis*, *Yanornis* and the Oligocene isolated feather respectively. Yellow arrows indicate melanosomes. Scale bars: A-F, M-R, Y-AD, 200 nm; G-L, S-X, AE-Aj, 50 nm.

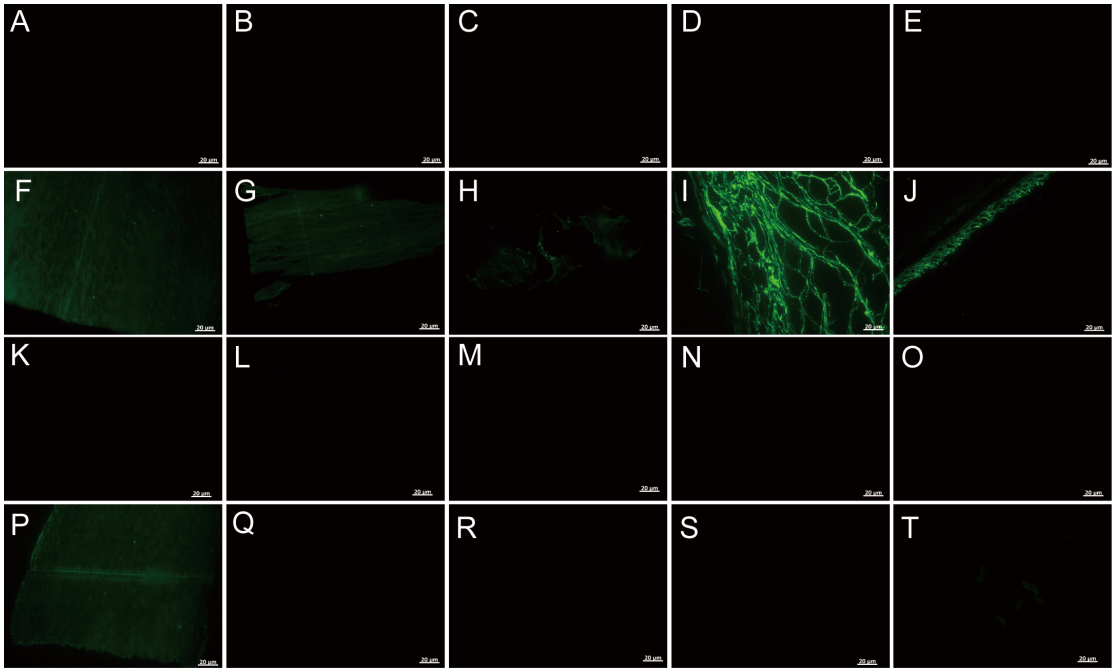

Fig. S10. Specificity tests of AE1/AE3 +5D3 α-keratin antiserum on modern known tissues. (A-E, K-O) represent negative controls, where no primary antibody is applied but all the other steps are identical; (F) show antibody-antigen (ab-ag) complexes localized to chicken feather; (F-J, P) demonstrates positive reactivity of the antiserum to chicken claw, emu claw, turtle claw, chicken scale, turtle skin, chicken rhamphothecase; (Q-S) do not show binding to the antiserum of the emu rhamphothecase, chicken feather, and duck feather; (T) indicating a weak signal binding to the antiserum of the emu feather. Scale bars: 20 μm.

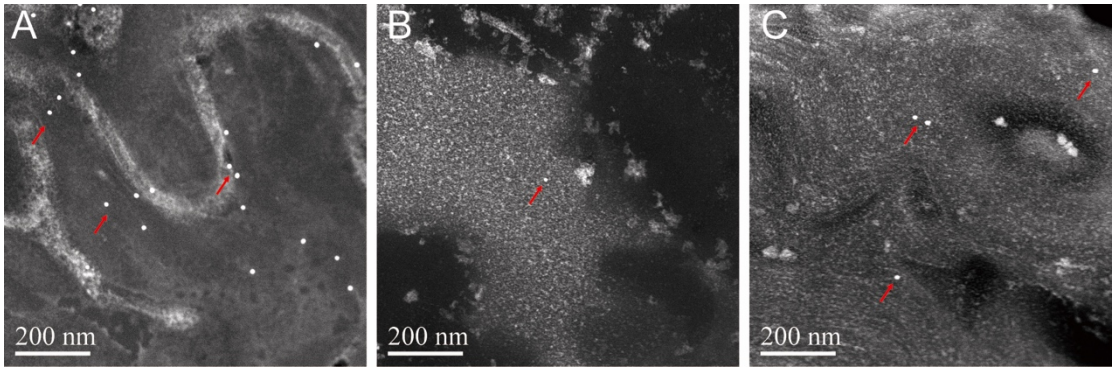

Fig. S11. In situ immunogold (IG) assays showing bind of the AE1/AE3 +5D3 antiserum on fossil specimens compared with results on extant chicken claw. Positive binding of antiserum to extant claw tissues from *G. gallus* (A), and fossil feather tissues from Dromaeosauridae indet. (B), *Eoconfuciusornis* (C) as revealed by localized, electron dense gold beads (red arrowed) is seen. Scale bars: 200nm.

Fig. S12. Keratinase digestion tests on the Oligocene fossil feather (Cenz-f-S2) and extant duck wing feather. (A) extant and fossil feathers were treated in 10% shout; (B) extant and fossil feathers were treated in 0.1% NaOH; (C) extant and fossil feathers after overnight treating in 0.1% NaOH; (D) extant and fossil feathers after overnight keratinase digestion; (E) image of controlled fossil sample (Cenz-f-S3) putting in E-pure water instead of all the digesting dilutions; (F) image of the fossil feather (Cenz-f-S2) after keratinase digestion; (G) SEM image of controlled fossil sample (Cenz-f-S3); (H) SEM image of keratinase digested fossil feather (Cenz-f-S2); (I) image of the untreated extant duck feather; (J) the extant feather was cut into similar size of the Oligocene fossil before adding 0.1% NaOH; (K) the extant feather after keratinase digestion. Scale bars: E, F, 1 mm; G, H, 100 μm; I-K, 1 cm.

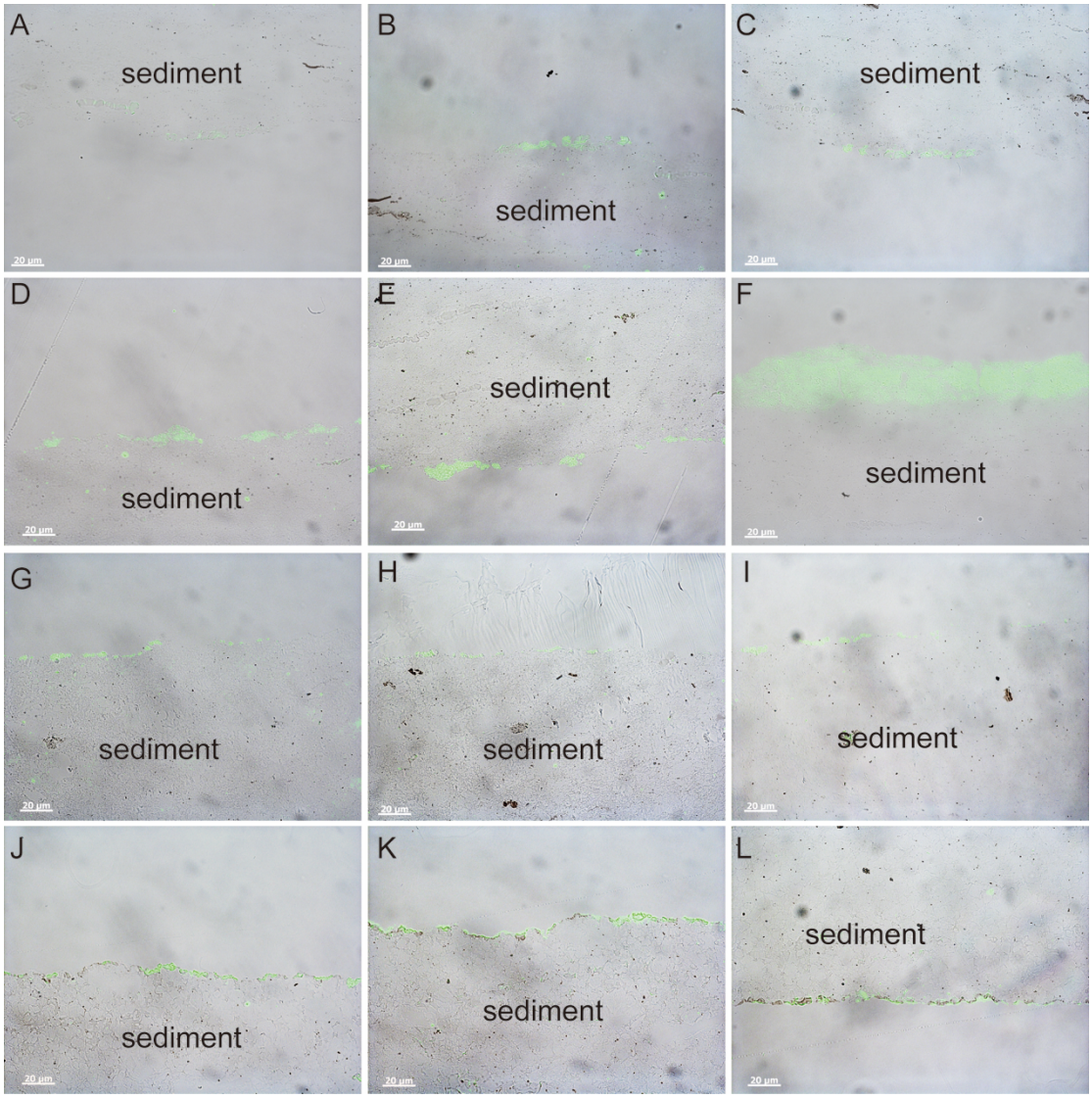

Fig. S13. Overlying images show where three antisera (against extracted feather proteins, Peptide 1, and Peptide 2 respectively) bind to the fossil feather tissues, which represented by the green fluorescence of the FITC label, while the sediment is not labelled. (A-C), sample from Dromaeosauridae indet.; (D-F) sample form *Eoconfuciusornis*; (G-I) sample from *Yanornis*; (J-L) sample from the Oligocene isolated feather. Scale bars: 20 μm.

Tissue type	Sampled taxa	Specimen number	Geological age	Locality
Pennaceous feather	*Anchiornis**	STM0-214	Middle-Upper Jurassic (~160Ma) (38)	Jianchang, western Liaoning
Pennaceous feather	Dromaeosauridae indet.*	STM5-12	Lower Cretaceous (~130Ma) (45)	Fengning, northern Hebei
Wing feather	*Eoconfuciusornis**	STM7-144	Lower Cretaceous (~130Ma) (45)	Fengning, northern Hebei
Tail feather	*Yanornis**	STM9-5	Lower Cretaceous (~120Ma) (46)	Chaoyang, western Liaoning;
Flight feather	Isolated feather**	DY 1502006	Oligocene (26-23Ma) (47)	Lunpola, Tibet
Short fiber	*Shuvuuia deserti****	IGM 100/977	~ 75 Ma (4)	Mongolia
Claw	*Citipati osmolskae****	MPC-D 100/979	~ 75 Ma (4)	Mongolia

*specimens housed in Shangdong Tianyu Museum of Nature, Shandong, China

**specimen housed in IVPP, Beijing, China

***specimens housed in American Museum of Natural History, New York, USA

Table S1. Detailed information of the fossil feather and related fossil tissues analyzed in this work.

	Amino Acid Sequence	Best Alignment	Contiguous Amino Acids
Peptide 1	Emu Feather	89%	20
Peptide 1	Feather-like, Duck	62%	13
Peptide 1	Scale, Parakeet	44%	7
Peptide 1	Claw, Swift	41%	7
Peptide 1	Alligator, AMI_BK_B	37%	9
Peptide 1	Crocodile	34%	7
Peptide 1	Anolis, ACA_	41%	3
Peptide 1	KTN, Ostrich	31%	5
Peptide 1	Chicken Scale	44%	7
Peptide 1	Ostrich Scale	51%	7
Peptide 1	Emu Claw	48%	7
Peptide 1	Ostrich Claw	44%	6
Peptide 1	Chicken Claw, Clade1	44%	6
Peptide 1	Chicken Claw, Clade2	58%	9
Peptide 2	Emu Feather	73%	10
Peptide 2	Feather-like, ostrich	91%	20
Peptide 2	Scale, Kea	17%	3
Peptide 2	Claw, Woodpecker	21%	5
Peptide 2	Alligator, AMI_BK_H	30%	6
Peptide 2	Crocodile	30%	6
Peptide 2	Anolis, ACA_Ac29	30%	5
Peptide 2	KTN, Eagle	17%	5
Peptide 2	Chicken Scale	30%	<5
Peptide 2	Ostrich Scale	30%	<5
Peptide 2	Emu Claw	21%	<5
Peptide 2	Ostrich Claw	47%	<5
Peptide 2	Chicken Claw, Clade1	34%	<5
Peptide 2	Chicken Claw, Clade2	34%	<5

Table S2. Best alignment of synthetic peptide 1 and peptide 2 with known amino acid sequences of avian and crocodilian β-keratins.

Antigen\ Antisera	2^o	2^o	Anti-pep. 1	Anti-pep. 1	Anti-pep. 2	Anti-pep. 2	Anti-feather	Anti-feather
Pep. 1 10 ng	-0.001	-0.003	0.653	0.402	0.263	0.217	1.046	0.802
Pep. 1 100 ng	-0.002	-0.004	0.777	1.050	0.186	0.130	0.982	1.096
Pep. 1 1000 ng	-0.002	0.021	1.093	0.904	0.011	0.026	0.986	0.976
Pep. 2 1000 ng	-0.004	-0.001	-0.002	0.001	0.848	0.804	0.807	1.018
Pep. 2 100 ng	-0.004	-0.002	0.005	-0.002	1.169	0.754	0.754	0.746
Pep.2 10 ng	-0.003	-0.005	-0.001	-0.001	0.886	0.867	0.872	0.672
PBS	-0.004	0.001	-0.001	-0.001	0.007	-0.001	0.002	0.005
Blank	0.007	-0.003	0.004	-0.004	-0.002	-0.001	0.003	-0.001

Table S3. ELISA Tests on the specificity and affinity of the antisera. Pep.1: synthetic peptide 1; Pep. 2: synthetic peptide 2; Anti-pep.1: antiserum against synthetic peptide 1; Anti-pep.2: antiserum against synthetic peptide 2; Anti-feather: antiserum against extracted feather proteins. The concentration of the antisera in the tests is 1:400.

Extant avian tissues	General β-keratin antiserum	Peptide 1 antiserum	Peptide 2 antiserum
Chicken feather	+	+	+
Duck feather	+	+	+
Goose feather	+	+	+
White leghorn feather	+	+	+
Emu feather	+	+	+
Chicken rhamphothecase	+	-	-
Emu rhamphothecase	+	-	-
Emu claw	+	-	-
Ostrich claw	+	-	-
Chicken claw	+	-	-
Ostrich scale	+	-	-
Chicken scale	+	-	-

Table S4. IHC responses of various extant avian tissues to three antisera (against extracted feather proteins, Peptide 1, and Peptide 2 respectively. "+" : Positive reaction; "-" : Negative reaction.

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