

Four camera-type eyes in the earliest vertebrates from the Cambrian Period

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Vertebrate vision is mainly accommodated by a pair of lateral image-forming camera-type eyes and is supplemented in non-mammalian vertebrates by a dorsal pineal complex (pineal and parapineal organs) functioning as photoreceptive and/or endocrine organs¹. The pineal complex shares a common genetic and embryological basis with the lateral eyes, both derived from evaginations during the development of diencephalon². Despite being widely heralded as the ‘third eye’ in crown vertebrates³, the nature of the pineal complex and its presumed visual capability in early vertebrates² remain unknown. Here we describe two pigmented features situated between the lateral eyes in two species of myllokunmingids, the earliest known fossil vertebrates (approximately 518 million years ago), and interpret these as pineal/parapineal organs. In both myllokunmingid species, the pineal complex contains abundant melanin-containing melanosomes identical to those in the retinal pigment epithelium in the lateral eyes, together with a distinctive, regularly ovoid structure interpreted as a lens. Our results indicate that the lateral eyes and pineal complex in myllokunmingids probably functioned as camera-type eyes capable of image formation. Thus, we propose that the four camera-type eyes represent an ancestral vertebrate character, corroborating hypotheses about the deep homology between the eyes and pineal complex.

The origin and evolution of the vertebrate visual system have fascinated scientists since Darwin’s time⁴. In all crown vertebrates, two lateral camera-type eyes are present, although some lizards also possess a parietal eye of camera type, derived from the pineal complex⁵. The lateral camera-type eyes of crown vertebrates are mainly composed of a spherical lens, a retina, an iris, and a set of extraocular muscles. The lens focuses light onto the hemispherical retina, creating visual signals that are transmitted to the brain through the optic nerve⁶. Identifying camera-type eyes in fossil vertebrates is facilitated by distinctive features with high preservation potential: melanin-rich retinal pigment epithelium (RPE)^{7,8} often expressed as dark stains, and the lens, an impression with relief or as a mineral infill owing to its relative resistance to decay⁹. The earliest example of potential camera-type lateral eyes comes from *Metaspriggina walcotti* from the Middle Cambrian Burgess Shale (approximately 505 million years ago (Ma))⁹, in which the preserved retina is recognized as a distinct hemisphere and the lens as a well-defined circular area. This interpretation would be more plausible if melanin or melanosomes were present as evidence for the presence of an RPE. The earliest melanosomes identified in a vertebrate eye have been reported from an anaspid-like fossil, *Euphanerops longaevus*, from the Devonian Escuminac Formation in Canada, where abundant melanosome microbodies are preserved within the lateral eyes, indicating the presence of a retina¹⁰.

Among non-vertebrate chordates, neither cephalochordates nor tunicates possess an organ that can be properly described as a camera-type eye. The cephalochordate *Amphioxus* has both ciliary and rhabdomeric photoreceptors, distributed across four separate non-imaging structures: the frontal ‘eye’, the lamellar body (ciliary), the Joseph cells and the dorsal ocelli². In tunicates, three pigment cup eyes are located in the anterior ventral part of the brain in sexual aggregate blastozooid larvae, whereas only one simple ocellus is observed in asexual solitary oozooid larvae¹¹. During vertebrate embryonic development, the paired lateral eyes emerge from a small region of the anterior neural plate containing cells that give rise to the pineal/parapineal organs. Accordingly, it has been proposed that the three distinct eye fields of sexual larval tunicates may be homologous with the two lateral eyes and the pineal complex of vertebrates within the so-called Olfactores phylogenetic framework¹¹. This inference echoes a long-standing hypothesis that the pineal/parapineal organs evolved through successive degeneration of eye precursors, widely known as the ‘third-eye’ theory¹. However, the spatial relationship between the pineal/parapineal organs and the lateral eyes remains unresolved, as does the degree of visual function these originally photoreceptive—now primarily endocrine—organs possessed when vertebrates first evolved².

We identified a well-developed retina and lens not only in the lateral eyes but also in two smaller dorsally positioned organs that correspond

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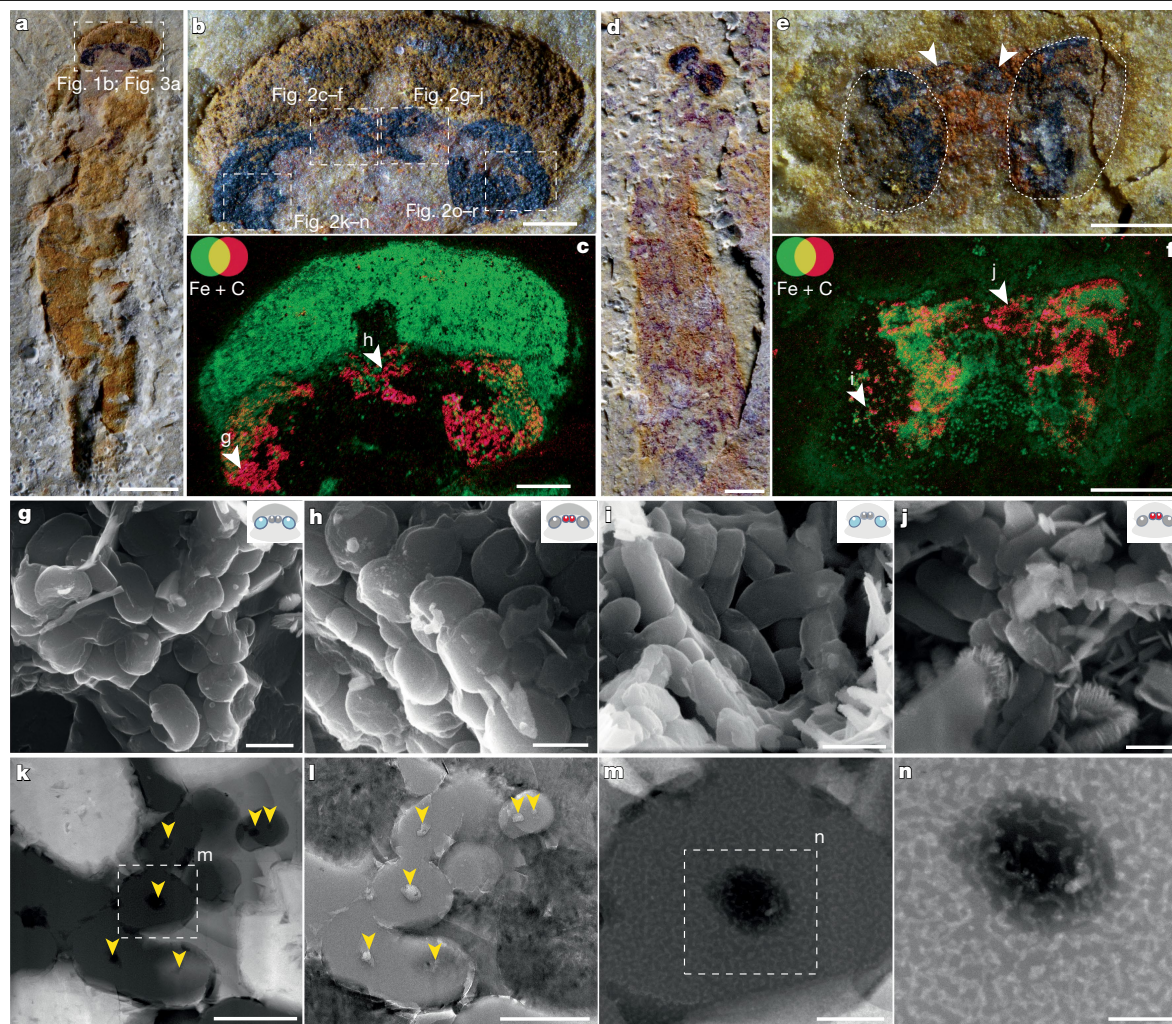


Fig. 1 | General morphology of the lateral eyes and pineal complex with their preserved melanosomes in two species of Myllokunmingidae from the Chengjiang biota. a, b, *H. ercaicunensis* (YNGIP 90281) with its enlarged eye region (b). c, Carbon (C; red) and iron (Fe; green) element mapping of the same region in b. Arrows denote the position of illustrated melanosomes in g and h. d, General morphology of myllokunmingid sp. (YNGIP 90291-b). e, Enlarged eye region of myllokunmingid sp. (YNGIP 90292-a) illustrating lateral eyes (circles in dotted line) and pineal/parapineal organs (arrows). f, Carbon (C; red) and iron (Fe; green) element mapping of the same region in e. Arrows

denote the position of illustrated melanosomes in i and j. g, h, Melanosomes in the eyes (g) and pineal complex (h) of *H. ercaicunensis*. i, j, Melanosomes in the eyes (i) and pineal complex (j) of myllokunmingid sp. k, l, TEM dark-field (k) and bright-field (l) images of melanosome microbodies. m, TEM dark-field image of a single melanosome microbody (boxed in k). n, TEM dark-field image showing details of the central hole (boxed in m), showing the scaffold frame in the central hole and outer shell region. Scale bars, 2 mm (a), 1 mm (d), 200 μ m (b, c, e, f), 500 nm (g-l), 100 nm (m), 50 nm (n).

in location to the pineal complex in two myllokunmingid species from approximately 518 Ma Chengjiang biota. Our results unveiled the origin of the pineal complex and its visual function in vertebrates, demonstrating that the earliest vertebrates possessed four camera-type eyes, showing an unexpected complexity in their image-forming visual systems.

Melanosomes and RPE

Myllokunmingids (such as *Haikouichthys ercaicunensis* and *Myllokunmingia fengjiao*) from the Cambrian (Epoch 2; Age 3) Chengjiang biota are the earliest known vertebrates^{12–16}. Ten specimens representing two distinct taxa were available for this study: six specimens of *H. ercaicunensis*¹³ and four slabs (each with several individuals) of an unnamed myllokunmingid (myllokunmingid sp.)¹⁵. In both species, the head region regularly preserves four anteriorly located black spots (Fig. 1a, b, d, e). Most of the trunk is poorly preserved as a result of post-mortem decay. In both taxa, the larger paired lateral dark spots have been interpreted as eyes, whereas the median pair has been interpreted as nasal sacs^{13,15}

(Fig. 1a, b, d, e and Extended Data Fig. 1a–i). Energy-dispersive X-ray (EDX), Raman spectroscopy and X-ray photoelectron spectroscopy (XPS) analyses indicate that both the lateral eyes and the paired median black spots have carbon-enriched and organic composition (Fig. 1c, f and Extended Data Figs. 2 and 3). Scanning electron microscopy (SEM) investigations reveal that the organic material consists of ovoid, oblong or cylindrical microbodies, with the long axis ranging between 200 nm and 1,200 nm and the short axis between 200 nm and 900 nm (Fig. 1g–j and Extended Data Figs. 4 and 5). The microbodies are associated with, or embedded below, pyrite crystals and the clay-mineral matrix (Extended Data Fig. 6a–f). Most microbodies appear deformed and fused together into a carbonaceous layer (Extended Data Fig. 6c–f).

In the lateral eyes of *H. ercaicunensis*, the microbodies are ovoid in shape (Fig. 1g, h), with the long axis ranging between 250 nm and 900 nm and the short axis between 200 nm and 800 nm. Frequency analyses reveal only one morphotype of these ovoid melanosomes, as denoted by one frequency peak in the spectrum of the length-to-width ratio, with a dominant frequency of 1.04 (Extended Data Fig. 4a).

EDX elemental mapping mounted with transmission electron microscopy (TEM) further confirms that these microbodies, after being sliced with focused ion beam (FIB)–scanning electron microscope, are solid carbonaceous structures with a small hole occurring in the centre (Fig. 1k–n and Extended Data Fig. 6g–i).

The lateral eyes of myllokunmingid sp. have two distinct morphotypes of microbodies (Fig. 1i,j), exhibiting two length-to-width ratio frequency peaks (Extended Data Fig. 4b). The first peak has a dominant length-to-width ratio of 1.12, such as the ovoid microbodies in *H. ercaicunensis*, of which the long and short axes range from 200 nm to 900 nm and from 200 nm to 600 nm, respectively. The second peak is characterized by a length-to-width ratio of 2.00, denoting the cylindrical morphotype that has a larger long-axis range of 400–1,200 nm but a narrower short-axis range of 200–550 nm. Comparisons reveal that the shape and size of these microbodies are identical to the melanosomes reported in both fossil and living vertebrates^{7,17}. This interpretation is further supported by the presence of a central hole in fossil microbodies (Fig. 1k–n and Extended Data Fig. 6g–i), which may represent a phaeomelanin-enriched core because phaeomelanin is less chemically stable and more soluble than eumelanin^{18–20}; its preferential loss could lead to the observed void. This is consistent with the melanin casing/core–shell model²¹, as observed in human neuromelanosomes²² and iridal melanosomes²³. The original infilling of the central hole is evidenced by the observation that the intervening matrix, forming scaffolds (identifiable by its slightly higher electron density) within microbodies, is continuously extending into the hole (Fig. 1m,n and Extended Data Fig. 6g–i). The observed central hole, exclusively reported in extant avian feather melanosomes¹⁸, has been attributed to the loss of an unstable pheomelanin core^{19,23}.

This identification of melanin-bearing melanosomes in the eyes of Chengjiang vertebrates is further corroborated by the results of time-of-flight secondary ion mass spectrometric data (ToF-SIMS) (Fig. 2). The negative secondary ion mass spectra (m/z) obtained from the lateral eyes of the two Chengjiang vertebrates include the feature peaks of melanin at 48, 49, 50, 59, 66, 72, 73, 74, 84, 97 and 121 m/z (ref. 24), but they are slightly obscured or overprinted by strong background signals from the adjacent rock matrix (Extended Data Fig. 7b). Nevertheless, comparison of the feature peaks associated with melanin in the myllokunmingid eye samples reveals that they are highly comparable to melanin from the eyes of living frogs but distinct from those of the Chengjiang sediment samples (Fig. 2b). To further elucidate the composition of the myllokunmingid eye microbodies relative to the Chengjiang matrix, we conducted principal component analysis (PCA) on 54 secondary ions peaks associated with melanin (Extended Data Fig. 7) across a variety of samples, including published fresh, artificially matured, fossil melanin and non-melanin samples²⁴ in addition to our newly collected Chengjiang data. We observed that the Chengjiang vertebrate eye samples do not overlap with the sediment and other negative controls but instead locate within, and adjacent to, the published fossil melanin (Fig. 2a and Extended Data Fig. 7a). These results indicate the presence of a molecular fingerprint consistent with melanin in the microbodies preserved in the eyes of myllokunmingids, although the signal appears comparatively weak.

Unlike experimentally matured melanin, fossil melanin would have experienced a more complex history of diagenetic alteration, late-stage weathering in the subsurface and potential overprinting by more recent organic material. The negative controls of sediments and modern carbon do not overlap with the Chengjiang samples, thereby possibly ruling out the latter two factors. The slightly broader spread of our data points compared with the published fossil melanin (Fig. 2a) may reflect a longer diagenetic history²⁴ (about 518 Ma) and potential difference in their original composition. The two samples from *H. ercaicunensis* containing chiefly ovoid melanosomes are distributed horizontally along the principal component 1 (PC1) axis, whereas the five samples of the (ovoid and cylindrical) melanosomes

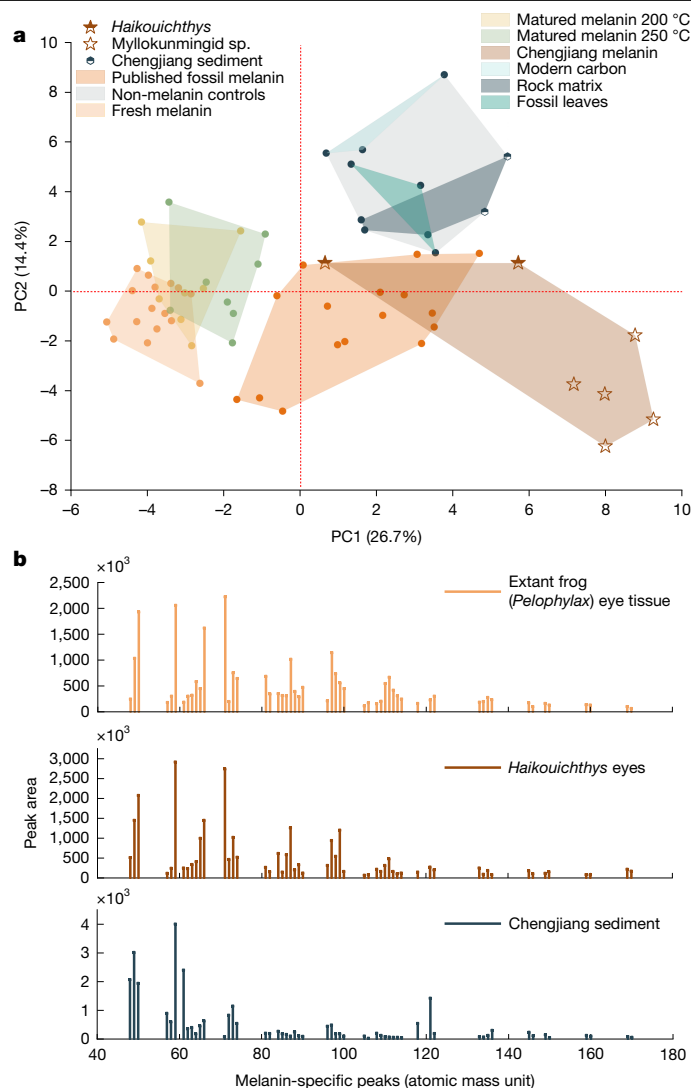


Fig. 2 | Negative ion ToF-SIMS spectra of melanosomes in the eyes of Chengjiang myllokunmingids. **a**, PCA of melanin characteristic peaks from negative ion ToF-SIMS spectra of various sample groups. **b**, Comparison of peak area of 54 melanin characteristic peaks between Chengjiang *H. ercaicunensis* eye sample (middle) and extant frog eye sample (top), with a negative control of the Chengjiang sediment (bottom).

from myllokunmingid sp. spread diagonally in the fourth quadrant of the PCA plot (Fig. 2a). Maturation experiments on modern melanosomes showed that samples of eumelanin-rich (cylindrical) and phaeomelanin-rich (ovoid) separated from each other along the PC2 axis. However, in fossil samples, these two types of melanosomes normally spread along the PC1 axis rather than the PC2 axis²⁴. This could indicate that the observed scattering reflects a signature pertaining to the original composition, rather than being the result of secondary alteration or overprinting.

Melanosomes occur in the iris, choroid and RPE of vertebrate lateral eyes; however, only the RPE shows distinct stratified layers of ovoid and cylindrical types⁷. The lateral eyes of myllokunmingid sp. examined here comprise both abundant ovoid and cylindrical melanosomes (Fig. 1i,j), consistent with an interpretation of RPE. However, our exploration of the six available specimens identified as *H. ercaicunensis* shows that the melanosomes are represented only by an ovoid morphotype. Although this departs from the typical pattern reported in the literature of extant taxa, we note that ovoid melanosome dominance was also observed in the eyes of the cartilaginous fish *Bandringa rayi* from Mazon Creek

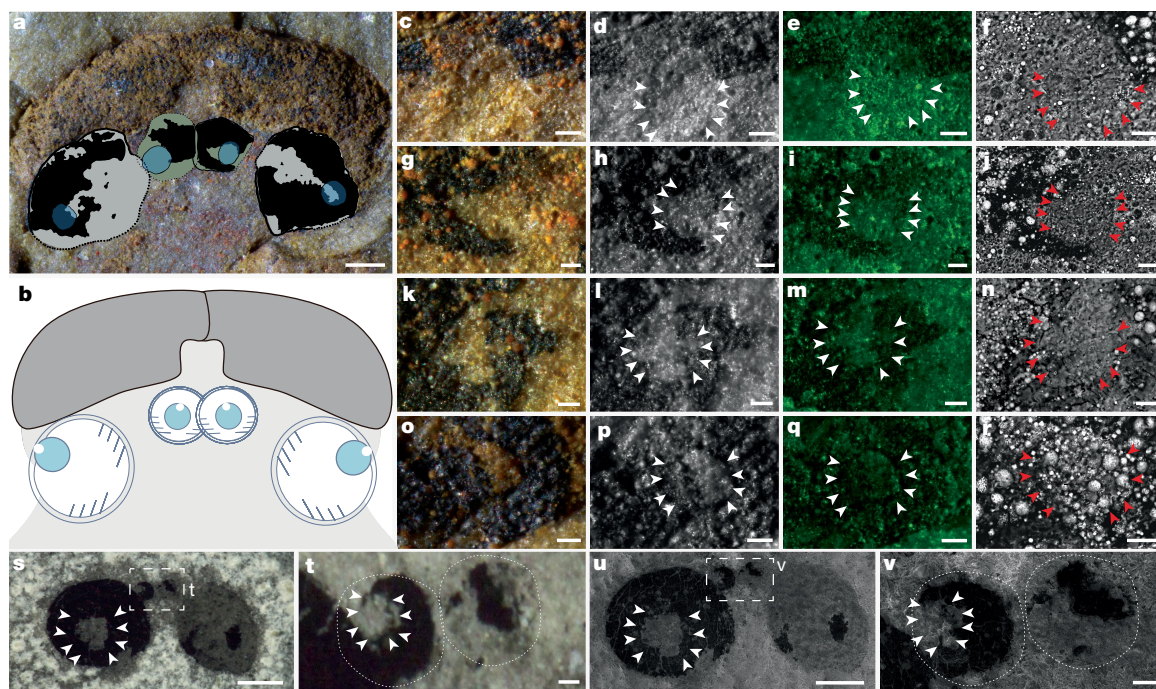


Fig. 3 | Preserved lens in the eyes and pineal complex of Cambrian vertebrates. **a**, Head region of *H. ercaicunensis* (YNGIP 90281) boxed in Fig. 1a, showing the topologies of lateral eyes (grey) and pineal eyes (green). **b**, Schematic reconstruction of the paired lateral eyes and pineal eyes of *H. ercaicunensis* in dorsal view; not to scale. **c–r**, Interpreted lens (circled arrows) in *H. ercaicunensis* (boxed in Fig. 1b), photographed with incident light (**c, d, g, h, k, l, o, p**), fluorescent light (**e, i, m, q**) and SEM (**f, j, n, r**). **c–f**, Lens in the left pineal eye. **g–j**, Lens in the right pineal eye. **k–n**, Lens in the left lateral eye. **o–r**, Lens in the right lateral eye.

s–v, Lens (circled arrows) in the lateral eyes (**s, u**) and pineal complex (**t, v**) in *M. walcotti* from the Burgess Shale (approximately 505 Ma). Image courtesy of J.-B. Caron. **s, t**, Incident light images. Arrows denote lens in the lateral eyes (**s**) and pineal complex (**t**; boxed in **s**). **u, v**, Secondary electron images of SEM. Arrows denote lens in the lateral eyes (**u**) and pineal complex (**v**; boxed in **u**). Scale bars, 200 μm (**a, b, s, u**), 50 μm (**c–r, t, v**). Panels **s–v** adapted with permission from ref. 9, Springer Nature Limited.

(307 Ma; Illinois, USA)⁷ and to some extent in the hagfish *Myxinkela siroka*⁸. The nature and frequency of variation in melanosome morphologies remain to be explored and understood in terms of potential functional or evolutionary importance.

The median paired dark spots in both Chengjiang myllokunmingids are smaller than the lateral eyes, and they are only about 160–240 μm in diameter in *H. ercaicunensis* and 90–120 μm in myllokunmingid sp. They are confirmed to be carbonaceous in composition and to contain melanosome microbodies (Fig. 1c, f). In each species, melanosomes from the median organs are consistent in size and shape with those of the respective lateral eyes; only ovoid melanosomes are identified in the median organs and lateral eyes of *H. ercaicunensis* (Fig. 1g, h and Extended Data Fig. 5), but both ovoid and cylindrical morphotypes are identified in myllokunmingid sp. (Fig. 1i, j). These melanosome data support the presence of RPE in the median organs, which probably functioned in the same way as the lateral eyes.

Lens structures

In addition to the melanosome-containing RPE, a regular ovoid structure is preserved in the lateral eyes of both Chengjiang vertebrate species, which shows consistent size and shape across specimens (Fig. 3a, b, k–r and Extended Data Fig. 1a–i). The structure exhibits a diameter ranging from 80 μm to 110 μm , about one fifth of the diameter of the entire associated eye, as defined by the preserved RPE. This ovoid structure is typically preserved as an impression defined by some relief, indicating an originally decay-resistant composition, which would have displaced adjacent tissues during decay and resisted flattening when the sediment was initially compacted. In other cases, this feature may be preserved as a carbon-rich or carbon-absent spot that is identical to the preserved relief in some specimens (Fig. 3k–n and

Extended Data Fig. 1d, h, i). We interpret this ovoid structure as the eye lens on the basis of its shape, size and position. This lens structure also occurs in the median paired dark spots (Fig. 3c–j). It is similar in size to the lens in the lateral eyes, making it proportionally bigger, about one third to two fifths the diameter of the retinal sphere. The variation in the position of the lens (Fig. 3a–r and Extended Data Fig. 1a–i) is explained by compaction of the organism in a variety of orientations relative to the bedding plane²⁵. The Middle Cambrian vertebrate *M. walcotti* exhibits a similar structure in its lateral eyes, which is also interpreted as a lens⁹ (Fig. 3s, u). In Mazon Creek, lenses are preserved as impressions with some relief, differences in organic material abundance or as mineral infills^{7,9} (Extended Data Fig. 1j–o), reflecting their nature as decay-resistant structures that preserve by forming variation in surface relief, displacing the adjacent RPE melanosomes during compaction/decay or by early infilling of diagenetic minerals.

The median dark spots of Chengjiang myllokunmingids were previously interpreted as nasal sacs or, less likely, as the pineal/parapineal organs¹³. However, in most early vertebrates, such as *Sacabambaspis* and heterostracans, the presence of paired nasal sacs is inferred mainly on the basis of the identified nostrils or their inner impressions in the head shield^{26,27}. Our new evidence demonstrates that the median organs of myllokunmingids are a pair of camera eyes, comprising a melanosome-bearing RPE and a lens. This, instead, invites a reconsideration of their identity as paired pineal/parapineal organs with an original function as camera eyes. Furthermore, in *H. ercaicunensis*, a distinct midline notch cuts into the anterior plate (upper lip) (Fig. 3a, b) in Yunnan Geology, Invertebrate Paleontology (YNGIP) 90281, a feature also seen in previously published specimens of *H. ercaicunensis*¹³. This is potentially an unpaired nasohypophyseal opening.

Another Cambrian vertebrate (*M. walcotti*) from the Middle Cambrian Burgess Shale also bears a pair of small median dark spots between

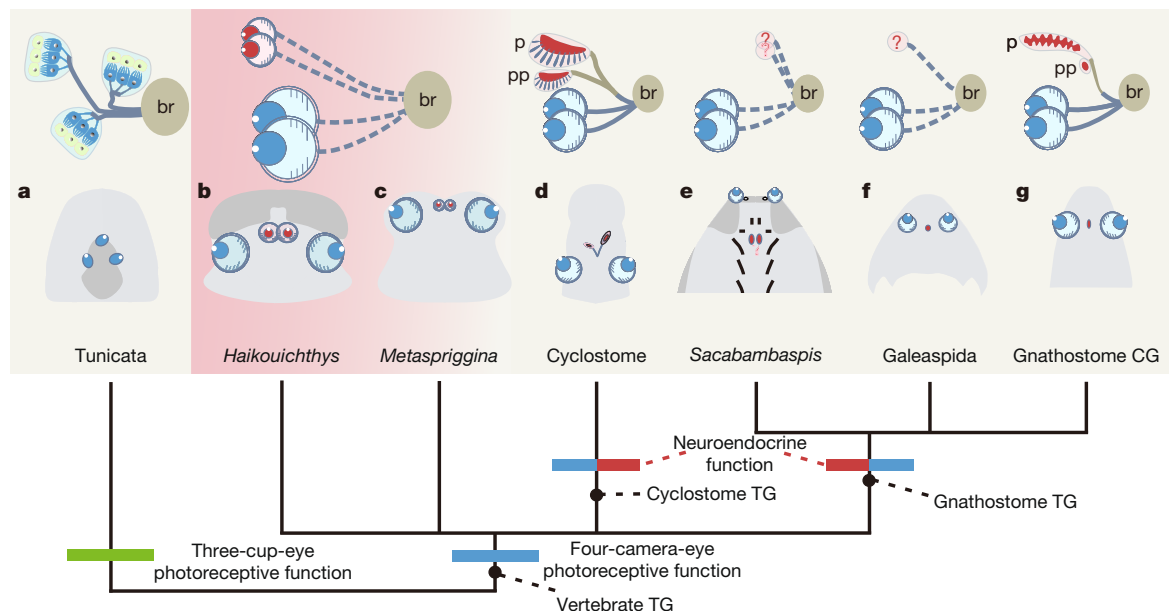


Fig. 4 | Evolutionary scheme of the visual system in early vertebrates.

a, *Thalia* (Tunicata) (adapted from ref. 11, Wiley). **b**, *H. ercaicunensis*. **c**, *M. walcotti* (adapted from ref. 9, Springer Nature Limited). **d**, Generalized cyclostome (adapted from ref. 2, Elsevier, under a Creative Commons CC BY-NC-SA 3.0 licence). **e**, *Sacabambaspis* (adapted from ref. 3, Oxford University Press). **f**, *Shuyu* (adapted from ref. 31, Nature). **g**, *Aphyocharax* (adapted from ref. 42, Wiley). The phylogenetic tree is adapted from the Adams consensus tree (described in

ref. 43, Springer Nature Limited). Note that all schematic reconstructions are not to scale or in strict accordance with biological topologies. The coloured regions show positions of key sensory organs: blue, eyes; red, pineal. The light grey lines represent body outlines. The coloured bars represent the suggested acquisition of key characters. br, brain; CG, crown group; p, pineal; pp, parapineal; TG, total group.

the lateral eyes, preserved as carbonaceous films⁹. Their preservation and topological relationship to eyes resemble the interpreted pineal complex in myllokunmingids, supporting an identification as pineal/parapineal organs. They also preserve as spherical objects⁹ with possible lens-like structures (Fig. 3t,v), indicating that *M. walcotti* has four camera-type eyes.

Discussion

The complex visual systems in stem and crown vertebrates suggest that vision was a key evolutionary innovation in vertebrate evolution. The overall anatomy of the lateral and median pineal eyes in the Chengjiang myllokunmingids described herein shows that camera-type eyes had evolved in vertebrates by the early Cambrian. The extent of the RPE, together with the lens, indicates that the eyes of myllokunmingids contained a larger originally fluid-filled space, which, in combination with the retinal epithelium, defined a large sphere with the lens suspended adjacent to a smaller iris opening. Such a morphology suggests an image-forming capability with a higher degree of acuity, although the extent of subsequent visual processing in the brain cannot be inferred.

In living vertebrates, both photoreceptors (ciliated sensory cells that transduce light) and retinal ganglion cells (which integrate and project photoreceptor signals to the optic nerve) originate from the neurosensory cell precursors of the neural plate, which have a deep homology with visual organs in tunicates²⁸ (Fig. 4). The paired lateral eyes and pineal complex develop from these neurosensory cells. Each arises as an evagination from the developing diencephalon; the developing tissues of the paired eyes form two camera-type eyes, whereas the median section of the evagination develops as the pineal and parapineal organs. This ontogenetic relationship suggests a close homology between the pineal complex and the lateral eyes, forming the basis for positing that the pineal complex had a more complex visual function, such as photoreception and image formation^{2,6}, before its subsequent differentiation.

In crown cyclostomes, lampreys have a pineal eye and a smaller parapineal eye² (Fig. 4d), both of which contain a retina and pinealocytes and are directly photosensory organs responding to changes in light conditions²⁹. Potential pineal eyes may be present in the anaspid-like fossil *Euphanerops longaevus* from the Devonian Escuminac Formation in Canada. An anterior median dark stain is located between two lateral eyes and has been interpreted as an olfactory or neurosensory organ³⁰. However, the observation that the organ contains melanosomes, identical to those in the lateral eyes¹⁰, led us to conclude that it represents the photosensory pineal complex.

In gnathostomes, early osteichthyans normally bear only one pineal (or parietal) foramen, posited to have had a photosensory function¹. This single pineal foramen persisted through the tetrapod stem group into stem amphibians (temnospondyls), stem mammals (synapsids) and various early reptiles⁵, although with some shifts in function. In mammals, the pineal complex is a non-sensory circadian neuroendocrine organ controlled by the day–night cycle. In some lizards, the pineal complex exhibits an evolutionary intermediate photoreceptive ability (although limited) and a well-developed neuroendocrine function⁵. This inferred shift in pinealocyte function across gnathostomes has led to the popular third-eye theory²⁹, which posits that it has an ancestral photoreceptive/visual function. In the stem gnathostome *Sacabambaspis*, two pineal openings have been identified, attributed to both pineal and parapineal eyes³ (Fig. 4e). However, their position (far posterior to the lateral eyes) is odd and requires reassessment. Most other stem gnathostomes (such as Galeaspida) have only one pineal opening^{3,31} (Fig. 4f), indicating that the pineal and/or parapineal eyes in early stem gnathostomes would have evolved a more reduced visual and photosensitive capacity, which later evolved to become more specialized for neuroendocrine regulation in crown gnathostomes²⁹.

The symmetrical pineal organs in Cambrian vertebrates, which we interpret to have functioned as camera-type eyes (Fig. 4b), contrast with the asymmetric pineal/parapineal complex observed in crown vertebrates, indicating that one component may be lost or degraded in

both cyclostome and gnathostome lineages. This disparity highlights a morphological gap that requires further investigation.

The Cambrian explosion was marked by rapid changes in physical environments and intense morphological innovations in animal evolution, which were also driven in part by ecological interactions, most notably the emergence of predation³². Although ultraviolet B radiation in Cambrian shallow marine habitats³³ has been suggested to impose selective pressures on the evolution of vision³⁴, the ensuing arms races driven by the advent of predation could have been equally or probably a more important driver of sensory repertoire innovation in both predators and prey. Cambrian apex predators, including radiodonts and gilled lobopods that reached sizes of more than 1 m (ref. 35), as well as large stem chaetognaths^{36,37}, represent large-bodied taxa in the Cambrian sea, equipped with complex visual systems comprising both compound eyes³⁸ and camera eyes³⁷. By contrast, on the basis of the lack of apparent predatory specializations, the small-bodied myllokunmingids were probably suspension feeding members of the pelagic food chain. In addition, myllokunmingids often occur in dense fossil aggregations (Extended Data Fig. 8), similar in abundance to the occurrence of *M. walcotti* at Marble Canyon in British Columbia, Canada⁹, which may reflect preserved schooling behaviour, a common anti-predatorial response in modern teleosts³⁹. Cambrian stem vertebrates may have been ideal prey for several pelagic predators because they were soft-bodied, active (and hence fairly muscular) and high-calorie prey targets. This would have exerted selective pressure on early vertebrates to evolve visual systems capable of detecting and evading emerging threats. Although visual predators typically evolve high-resolution, narrow binocular vision, prey species tend to exhibit visual systems with a wide field of view, achieved either within a single pair of eyes or through several more distributed visual organs⁴⁰. Hence, the presence of four eyes in stem vertebrates, probably adapted to different visual field perception, orientation and even function, would fit a role in which these visual systems served a purpose for both navigation and predator evasion, aiding survival in the Cambrian 'Dark Forest'⁴¹. In this sense, the inferred gradual reduction in visual complexity and photoreceptive function of the median eyes in crown vertebrates, particularly in gnathostomes, may reflect an evolutionary shift from a mainly prey-based lifestyle to a more predatory one.

Online content

Any methods, additional references, Nature Portfolio 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/s41586-025-09966-0>.

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Methods

Fossils, stratigraphy, photographing and preparation

All investigated specimens were from the *Eoredlichia–Wutingaspis* trilobite zone of Yu'an-shan Formation (Stage 3; Series 2; Cambrian) in Yunnan province in China. The rocks hosting Chengjiang fossils were typically weathered to light yellow and light green, creating a high contrast with the rusty fossils. All specimens were collected during several field trips between 2019 and 2024 in Haikou and Jinning, Kunming, Eastern Yunnan, and are part of the YNGIP collection at Yunnan University. When necessary, the specimens were prepared using a steel needle under a stereomicroscope (Nikon SMZ1000). The illustrated figures of Chengjiang specimens were photographed with a Canon EOS 5D SLR camera equipped with either a Canon MP-E 100 mm or 65 mm (1–59) macrolens. The fluorescence images were produced with a Nikon ECLIPSE Ni-E microscope. Owing to strict requirements for the size of samples in several analyses, the specimens were cut into small sizes of less than $1 \times 1 \times 1 \text{ cm}^3$ (*H. ercaicunensis*) or less than $5 \times 5 \times 5 \text{ mm}^3$ (myllokunmingid sp.), with the latter taken from large slabs that preserve an assemblage of numerous individuals. The fossil eyes were isolated from the specimen using an alcohol-sterilized scalpel and transferred to the sample table for SEM/TEM or wrapped in aluminium foil for the ToF-SIMS analysis. All figures were prepared with Affinity Photo and Designer 2.

SEM and TEM

The morphology and elemental composition of the fossil eyes were acquired using a FEI Quanta 650 SEM with an EDX spectroscopy system. All specimens were uncoated. The secondary electron images of melanosomes were taken in high vacuum using an accelerating voltage of 5–15 keV and a working distance of 10–11 mm. The backscatter electron images and EDX analysis were taken in low vacuum, with an accelerating voltage of 20–25 keV, a spot size of 5.5 and a working distance of 10–11 mm. The melanosome microbodies are common in the eyes of Chengjiang myllokunmingids. Several SEM images were taken from each specimen to measure the size (long and short axes) of melanosomes using ImageJ v.1.54. The TEM specimens were prepared using a focused ion beam–scanning electron microscope (Helios 5 CX). The microstructure of melanosomes and element mappings (high-angle annular dark field) were generated with TEM–EDX imaging (Talos F200X and Spectra 300; Thermo Fisher Scientific), with accelerating voltages of 200 kV or 300 kV.

ToF-SIMS and PCA

The ToF-SIMS analysis was performed on a ToF-SIMS 5-100 (IONTOF GmbH) using 30 keV Bi_3^+ primary ions, consistent with previous studies^{7,24}. The raster-scanned areas were selected between $150 \times 150 \mu\text{m}^2$ and $200 \times 200 \mu\text{m}^2$ with an imaging resolution of 128×128 pixels. All spectra were mass-calibrated using the following fragment series of carbon: C^- , C_2^- , C_3^- , C_4^- , C_5^- , C_6^- , C_7^- , C_8^- and C_9^- . Because of the significant influence of the main mineral components (such as clay minerals and iron oxides) in the sediment on the positive-ion spectra, only negative-ion spectra were selected in this analysis. Following previous studies^{7,24,44,45}, we selected 54 melanin characteristic peaks from each

negative-ion spectrum to compare with those of sediment and other fossils. The data processing and PCA analysis followed the procedures described in previous studies^{7,24}. The PCA plots were generated using OriginPro 2025 (Learning Edition).

XPS and Raman spectroscopy

XPS measurements were performed using a K-Alpha+ spectrometer (Thermo Fisher Scientific). The analysis chamber was maintained at an ultra-high vacuum of approximately 2×10^{-7} mbar. A monochromate Al K α X-ray source (1,486.6 eV) was operated at 12 kV and 6 mA, with a spot size of 400 μm . Spectra were acquired in constant analyser energy mode.

Micro-Raman spectra were collected with a Renishaw inVia Qontor Raman spectrometer. Most Chengjiang fossils have strong fluorescence interference. To minimize the effect of fluorescence background and prevent decomposition of carbon materials in fossils, the laser intensity was maintained at a low level, typically below 5 mW, using 633-nm solid-state lasers and $\times 50$ objectives. The laser spot on the sample was approximately 1.5 μm in diameter.

Reporting summary

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

Data availability

All supporting data of this study are available at Figshare (<https://doi.org/10.6084/m9.figshare.29589761>).

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Author contributions P.C., S.G. and J.V. conceived the project. P.C., X.L., S.Z. and F.W. collected the specimens. X.L. and S.Z. collected and analysed the data and, together with the other authors, wrote the first draft of the paper. X.L. and S.Z. made the figures. P.C. and X.X. secured funding. P.C., J.V., S.G. and X.X. supervised the project and edited the paper.

Competing interests The authors declare no competing interests.

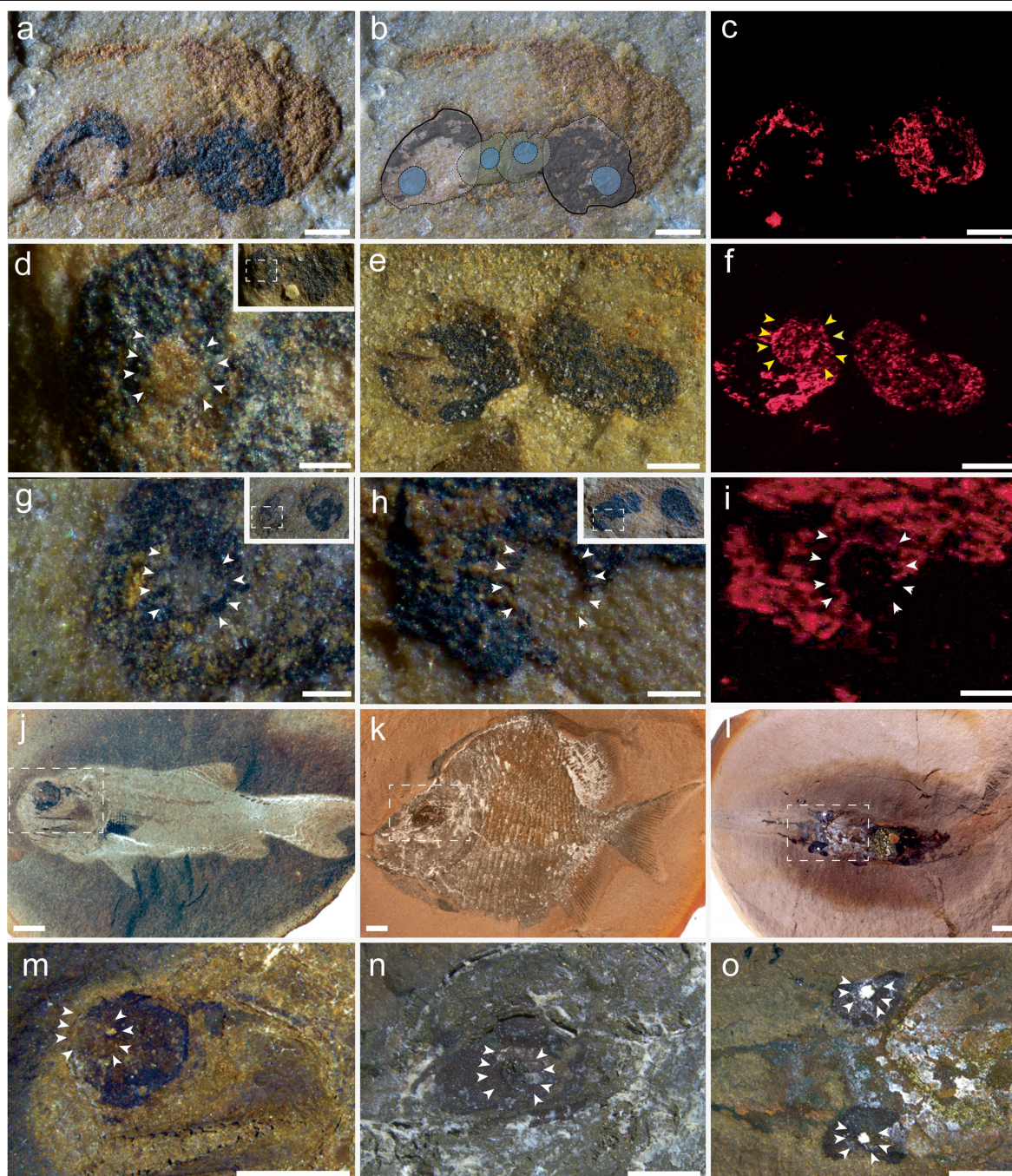
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-025-09966-0>.

Correspondence and requests for materials should be addressed to Peiyun Cong.

Peer review information Nature thanks Per Ahlberg and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

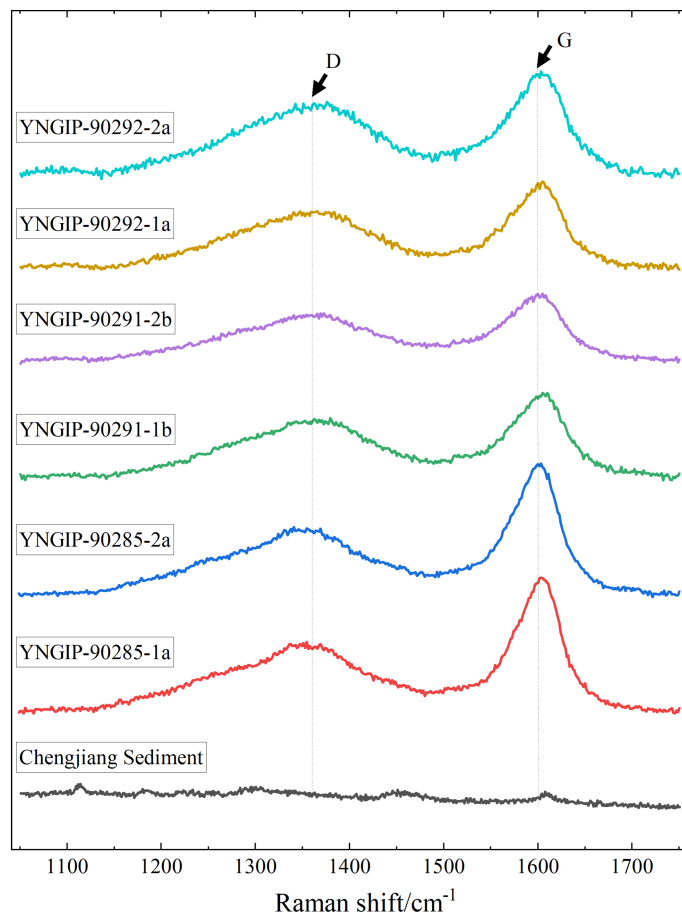
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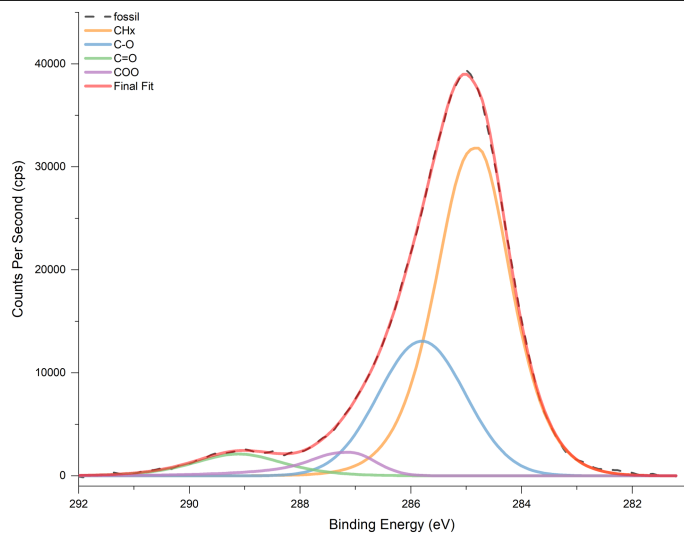
Extended Data Fig. 1 | Preservation of eyes and lens in selected fossil

vertebrates. a-b, *Haikouichthys ercaicunensis* (YNGIP-90285) showing lateral eyes (grey) and pineal eyes (green) with lens (blue). **c,** carbon element map of *H. ercaicunensis* (YNGIP-90285) head. **d,** *H. ercaicunensis* (YNGIP-90284), white arrows showing lens. **e,** *H. ercaicunensis* (YNGIP-90296), yellow arrows indicating left pineal eye. **f,** carbon element map of *H. ercaicunensis* (YNGIP-90296). **g,** (YNGIP-90283), eyes of *H. ercaicunensis* showing lens (white arrows). **h,** eyes

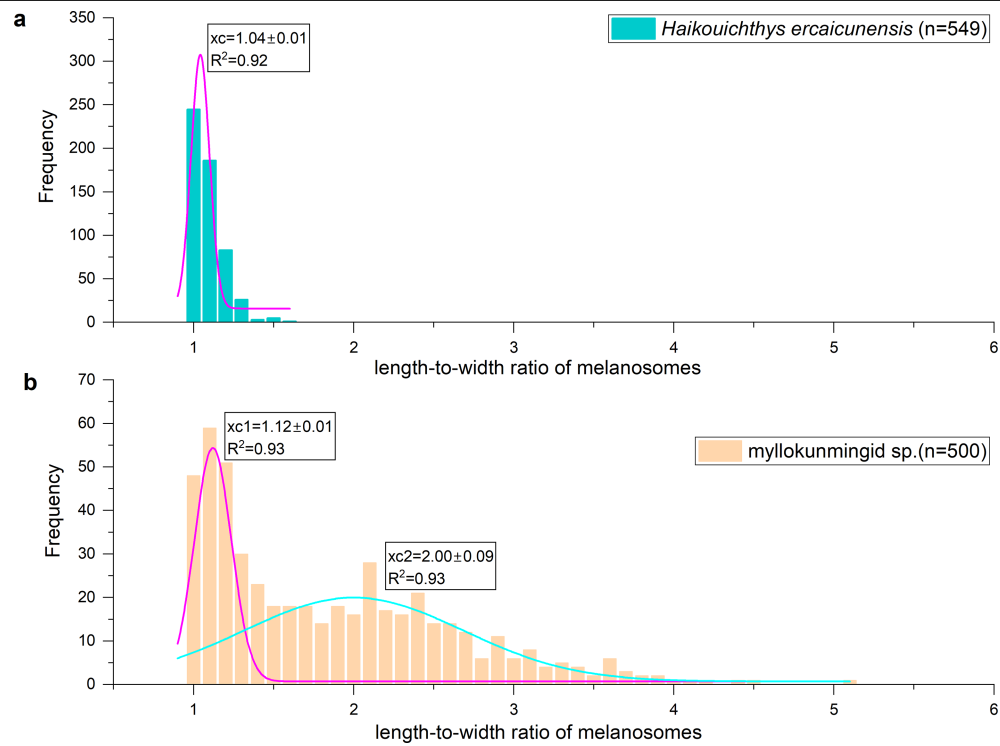
of *H. ercaicunensis* showing lens (white arrows) (YNGIP-90289). **i,** carbon element map of the same region in **h**, note the absence of carbon in the lens (white arrows). **j, m,** lens in *Elonichthys peltigerus* (ROM56794). **k, n,** lens in *Platysomus circularis* (PF7333). **l, o,** lens in *Bandringa rayi* (ROM56789). (j-o) image courtesy of Thomas Clements. Scale bars, 200 μm (a-f); 50 μm (g-i); 5 mm (j-o). Panels j-o reproduced with permission from ref. 7, Springer Nature Limited.



Extended Data Fig. 2 | Raman spectroscopic data across specimens supporting remains in the Chengjiang myllokunmingids eyes are organic material. Note the consistency of Raman spectroscopic data across specimens, which are distinct from the negative control of the Chengjiang sediment. D and G, feature bands of organic materials.

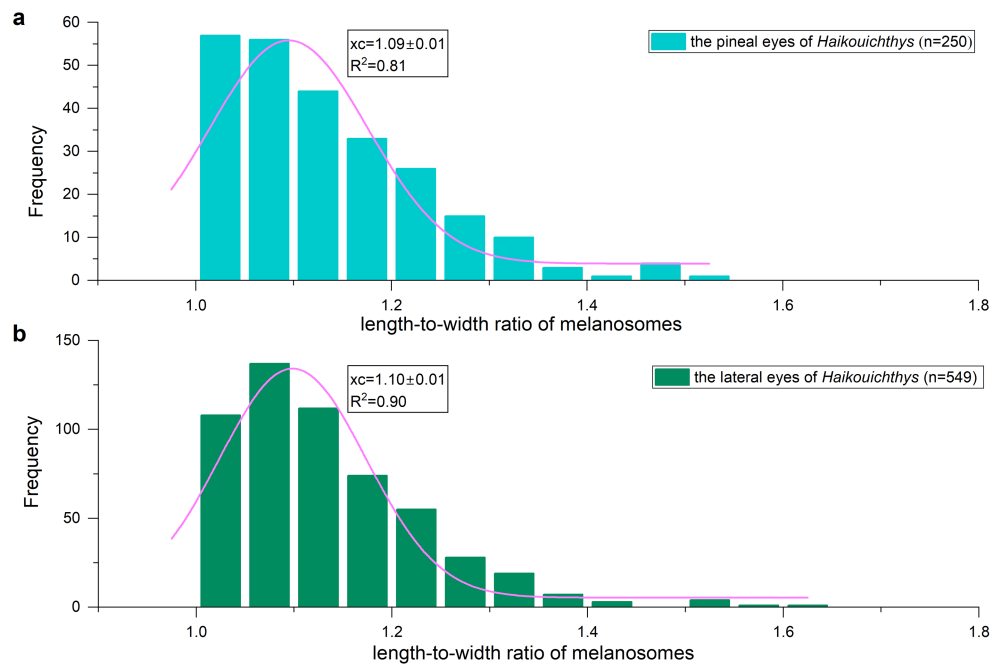


Extended Data Fig. 3 | X-ray photoelectron spectroscopy (XPS) analysis of carbonaceous remains in the eyes of the Chengjiang myllokunmingids (YNGIP90291).



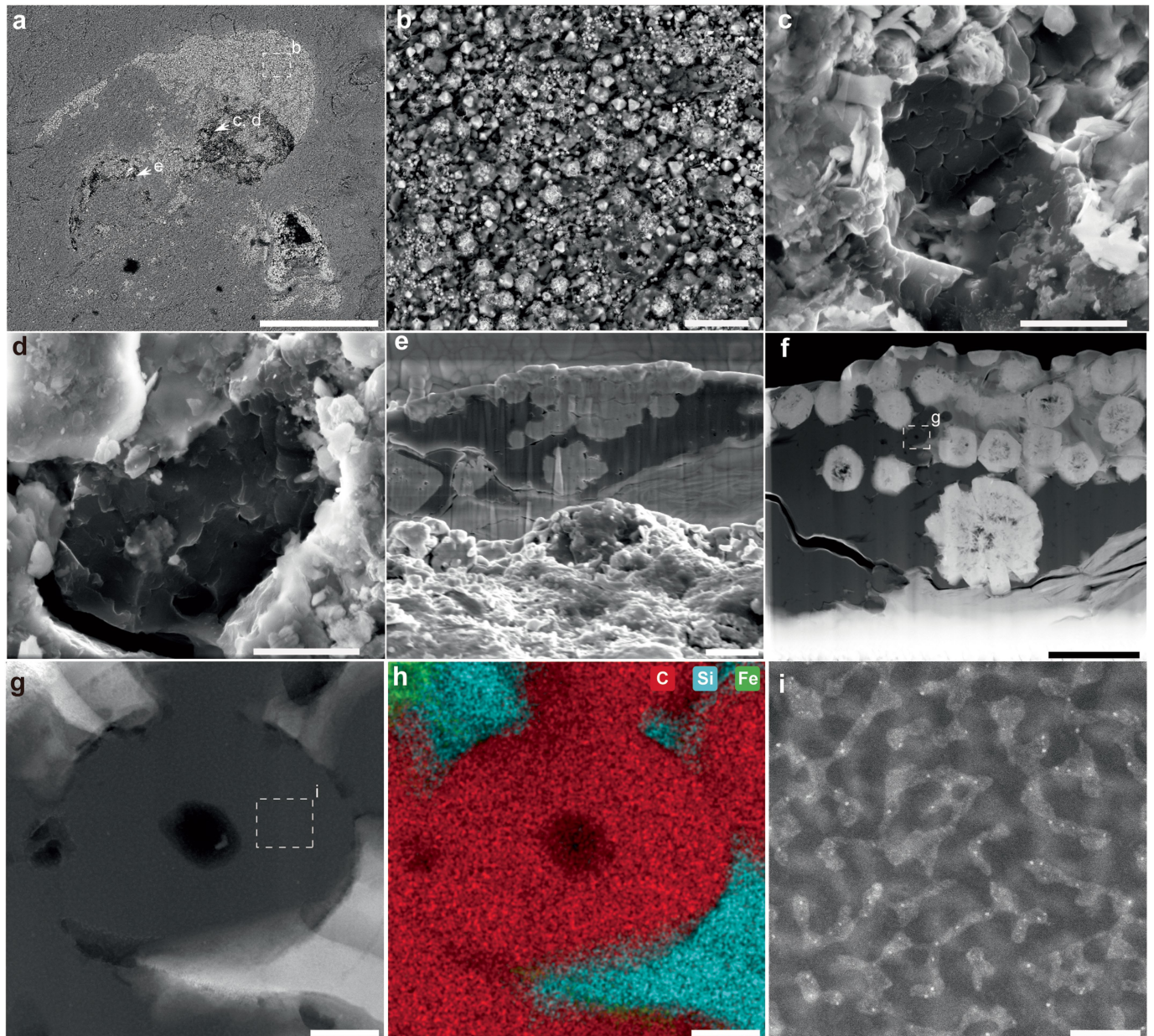
Extended Data Fig. 4 | The frequency distribution and Gaussian fitting of the length-to-width ratio of the melanosomes in the eyes of the Chengjiang vertebrates. a, *Haikouichthys ercaicunensis* (n = 549). b, *myllokunmingid sp.*

(n = 500). n refers to the measured number of melanosome microbodies. xc: peak centre value; R^2 : regression coefficient.



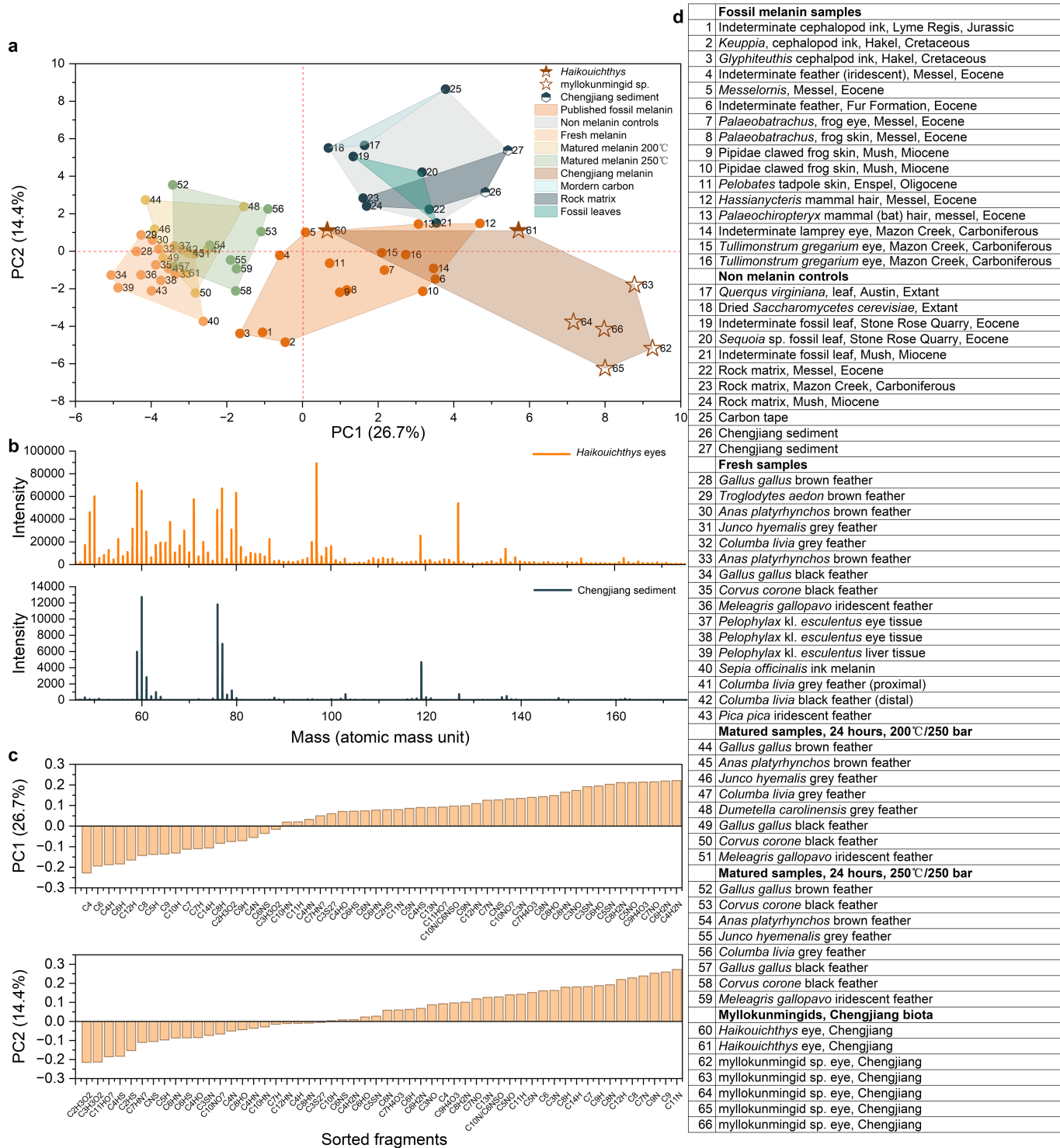
Extended Data Fig. 5 | The frequency distribution and Gaussian fitting of the length-to-width ratio of the melanosomes in the lateral and pineal eyes of *Haikouichthys ercaicunensis*. a, pineal eyes (n = 250). b, lateral eyes (n = 549).

n refers to the measured number of melanosome microbodies. xc: peak centre value; R²: regression coefficient.



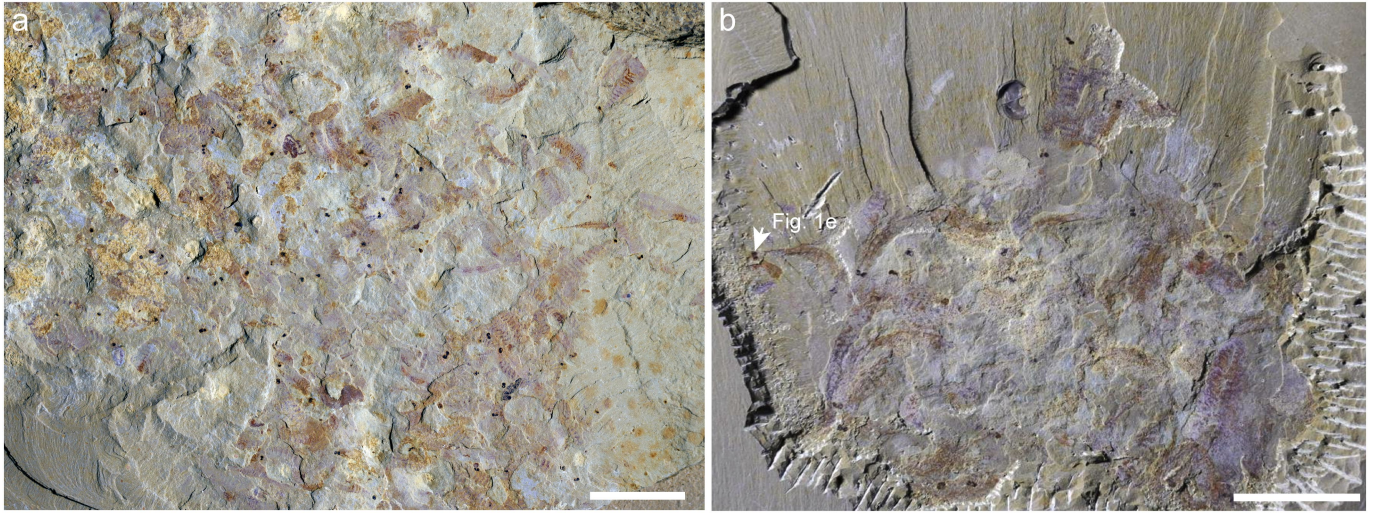
Extended Data Fig. 6 | SEM, FIB and TEM images of the melanosome in the eyes of *Haikouichthys ercaicunensis*. **a-b**, Backscattered electron image (SEM) of *H. ercaicunensis* head. **c-d**, Secondary electron image (SEM) of the melanosome in the eyes (arrowed in **a**) of *H. ercaicunensis*. **e, f**, FIB slicing of the

eye (arrowed in **a**) of *H. ercaicunensis*. **g, i**, TEM images of single melanosomes microbody, with enlarged details in **i** (boxed in **g**). **h**, Carbon (C, red), silicon (Si, blue), and iron (Fe, green) elemental map of a single melanosome as shown in **g**. Scale bars, 500 μm (**a**); 20 μm (**b**); 2 μm (**c-f**); 100 nm (**g, h**); 20 nm (**i**).



Extended Data Fig. 7 | Detailed PCA result of negative ToF-SIMS spectra. **a**, the PCA result of 54 negative secondary ion peaks obtained from the following samples: fresh melanin, artificially matured melanin (aged for 24 h at 200 °C/250 bar and 250 °C/250 bar), fossil melanin, various melanin-negative controls, and specimens of Chengjiang mylokunmingids. **b**, Comparison of

ToF-SIMS full spectra between *Haikouichthys* eyes (top) with a negative control of the host sediment (bottom) from the Chengjiang biota. **c**, loadings for principal component analysis (panel a and Fig. 2a), showing the contribution of specific ion fragments to the positioning of specimens along the x-axis (PC1) and y-axis (PC2), respectively. **d**, the list of details of the numbered samples in a.



Extended Data Fig. 8 | Swarming behaviour of myllokunmingid sp. a, YNGIP-90293, b, YNGIP-90292, with individuals occurring in dense aggregations. Scale bar, 2 cm (a), 1 cm (b).

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☒	☐ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
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Data collection	The measurement of the melanosome microbodies is taken with a free software ImageJ 1.54f (in the interface of Fiji). All figures are prepared with Affinity Photo 2 or Affinity Designer 2.
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Data collection

Timing and spatial scale

Data exclusions

Reproducibility

Randomization

Blinding

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions

Field conditions	later autumn to later spring of next year, with the temperature varying between 0- 25 oC. The specimens were slightly wettish though when split from the mudstone.
Location	The specimens were collected from the Eoredlichia-Wutingaspis trilobite Zone of Yu'an-shan Formation, Cambrian Series 2, Stage 3, in Haikou and Jinning of Kunming, Yunnan, China.
Access & import/export	In accordance with Chinese regulations/laws, all specimens are now and will be in China. No import/export permits are required.
Disturbance	No disturbance is caused during the collecting activities as only hand-tools are used.

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Methods

n/a	Involved in the study
☒	☐ ChIP-seq
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Palaeontology and Archaeology

Specimen provenance	All specimens in this study were collected during the fieldwork seasons between 2019-2024 in accordance with the local regulations/laws. All fossils specimens occur in Yu'an-shan Formation, Cambrian Series 2, Stage 3.
Specimen deposition	The specimens are deposited in the Institute of Palaeontology, Yunnan University, Kunming, Yunnan Province, China
Dating methods	No new dates for the Chengjiang Biota (from which the fossils come) are provided in this study.
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Seed stocks	No seed stocks or other plant material are used in this study.
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