

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- |                                     |   |
|-------------------------------------|---|
| n/a                                 | Confirmed   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated   |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

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

Data collection	<p>Acquisition of the <math>\mu</math>CT data set</p> <p>Synchrotron micro computed tomography was performed at beamline 13-BM-D at the Advanced Photon Source at Argonne National Laboratory. The white X-ray beam from the APS bending magnet was reflected from a Pt-coated mirror at 3 mrad incidence angle, producing an energy spectrum centered at ~25 keV with ~10 keV bandwidth. 3600 projection views were acquired over 180 degrees using a Point Grey Grasshopper 3 camera, with 1920 (H) X 1200 (V) pixels, viewing a LuAG scintillator. The sample-to-scintillator distance was approximately 45 mm. Depending on the sample, the camera was equipped with either a 10X or 5X Mitutoyo long-working distance objective lens. For both objectives, a 175 mm tube was used, resulting in 0.56-micron pixels in object space for the 10X lens and 1.09 micron pixels in object space for the 5X lens. The exposure time was 0.014 seconds per view for the 10X lens and 0.025 seconds per view for the 5X.</p> <p>The reconstruction software is hosted here:<a href="https://github.com/CARS-UChicago/IDL_Tomography">https://github.com/CARS-UChicago/IDL_Tomography</a> This shows the version information (tags).<a href="https://github.com/CARS-UChicago/IDL_Tomography/tags">https://github.com/CARS-UChicago/IDL_Tomography/tags</a>. Versions R0, R1, and R2 were used.</p> <p>Laboratory micro computed tomography was performed on a Phoenix v tome x S 240 from GE (PaleoCT facility, RRID:SCR024763, University of Chicago)</p>
Data analysis	<p>Synchrotron micro computed tomography images were reconstructed using the GSECARS tomography processing software (<a href="https://cars-uchicago.github.io/IDL_Tomography/">https://cars-uchicago.github.io/IDL_Tomography/</a>)14, which dark-current corrects and white-field normalizes the acquired data prior to performing gridding-based image reconstruction. The resulting image voxel sizes are 0.56x0.56x0.56 microns for the 10X configuration and 1.09x1.09x1.09 microns for the 5X configuration. Experiment run at GSECARS; Proposal GUP: 82458; Proposal Title: "Characterizing the origin</p>

and development of the earliest mineralizing tissues in vertebrates using synchrotron microCT"; Experiment Date: 2023-03-21 and 2022-12-02.

Segmentation, reconstruction, and visualization were performed on Amira 3D 2021.1 (©1995-2001 Konrad-Zuse-Zentrum Berlin (ZIB), ©1999-2021 FEI SAS, a part of Thermo Fisher Scientific).

#### Confocal imaging

Three dimensional, whole-tissue volumes were acquired using a combination of Z-stacks and image tiling. Images were processed with Zen (Zeiss) and FIJI21 and VGStudio Max 3.3 was used for three-dimensional visualization of the scans.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

## Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

#### Data Availability:

All original data is on Morphosource, including  $\mu$ CT, Synchrotron and Confocal scans. Data is be publicly available, except where the museum holds the copyright then data are available upon reasonable request: <https://www.morphosource.org/projects/000626244>

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

N/A

Reporting on race, ethnicity, or other socially relevant groupings

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

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

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences

☐ Behavioural & social sciences

☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://nature.com/documents/nr-reporting-summary-flat.pdf)

## Ecological, evolutionary & environmental sciences study design

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

Study description

This study's aim is to qualatativly describe the micro-structure of vertebrate odontodes and invertebrate cuticular structures. No quantitative analysis was performed.

Research sample

The sample size of fossil material was dependent on availability and destructive sampling permissions. Extant taxa sampling was dependent on availability in museum collections and number of animals donated.

Sampling strategy

Sample size was limited to the fossil specimens of appropriate preservation and locality. Extant animal scans were limited to donated specimens or specimens available at museums often (n=1)

Data collection

All Synchrotron data, and  $\mu$ CT data was collected by the first author. Confocal data was collected by the second author.

Timing and spatial scale	Data was collected when synchrotron beam time and $\mu$ CT scantime was allocated.
Data exclusions	No data was excluded.
Reproducibility	$\mu$ CT scans were not subject to reproducibility. Antibody staining was conducted on several specimens not included in this study to verify findings.
Randomization	Randomization was not possible as the limited specimens were selected, scanned, and segmented by the first author.
Blinding	Blinding was not possible as the limited specimens were selected, scanned, and segmented by the first author.
Did the study involve field work?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input type="checkbox"/>	<input checked="" type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

### Antibodies

Antibodies used	(Cy3 donkey anti-mouse IgG, Jackson laboratories, 715-165-150, LOT: 163873; 1:300) // Antibody 3A10 (Registry ID: AB_531874) was used; company: DSHB <a href="https://dshb.biology.uiowa.edu/3A10//4',6-Diamidino-2-Phenylindole, dihydrochloride (DAPI) (Biotium, 40009, Lot: 15D1117;1:1000)">https://dshb.biology.uiowa.edu/3A10//4',6-Diamidino-2-Phenylindole, dihydrochloride (DAPI) (Biotium, 40009, Lot: 15D1117;1:1000)</a>
Validation	Validation from the manufacturer: "Confirmed Species Reactivity: Chicken, Fish, Gecko, Human, Mouse, Planaria, Quail, Rat, Shark, Xenopus, Zebrafish" Validation on Catfish was performed by:  Catfish validation: Hardy et al. 2016 DOI: 10.1098/rspb.2015.2652

### Palaeontology and Archaeology

Specimen provenance	No new material was collected for this study.
Specimen deposition	Specimens figured in this study are deposited at the following institutions:  MPM- Milwaukee Public Museum UWGM- University of Wisconsin Geology Museum GSC- Geological survey of Canada USNM- Smithsonian National Museum of Natural History FMNH- Field Museum of Natural history MBL- Marine Biological laboratory
Dating methods	No new dates are provided
<input type="checkbox"/> Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	No new material was collected for this study. All use, destructive sampling and imaging permissions were provided by the following:  Milwaukee Public Museum- Patricia Coorrough Burke

University of Wisconsin Geology Museum- Carrie A. Eaton  
 Smithsonian National Museum of Natural History- Conrad Labandeira  
 Field Museum of Natural history- William Simpson

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

## Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

### Laboratory animals

Ancistrus sp. (Bristle nose catfish)- 3 weeks  
 Leucoraja erinacea (little skate)- stage 31-33  
 Scyliorhinus retifer (cat sharks)- stage 38

All invertebrates were from the Field Museum of Natural History, or donated as specimens.

Limulus polyphemus (Atlantic horseshoe crab)  
 Hadrurus arizonensis (Hairy desert scorpion)  
 Callinectes sapidus (Atlantic Blue Crab)  
 Petrolisthes galathinus (Porcelain crab)  
 Eupatorus gracilicornis (five-horned rhinoceros beetle)  
 Paracentrotus Lividus (Purple Sea urchin)  
 Asterias Rubens (common starfish)  
 Atya Crassa (Freshwater shrimp)  
 Megabalanus tintinnabulum (Giant purple barnacle)  
 Goniopsis sp. (mangrove crab)  
 Panopeus sp. (mud crab)  
 Cardina sp. (freshwater atyid shrimp)  
 Glycymeris sp. (bittersweet clam)

Anadra sp. (saltwater bivalve)  
 Planorbarius corneus (ramshorn snail)  
 Anadra sp. (saltwater bivalve)  
 Meretrix sp. (saltwater clam)  
 Polites sp. (North American grass skipping butterfly)  
 Planes miutus (Columbus crab)

### Wild animals

The study did not involve wild animals

### Reporting on sex

This information was not collected.

### Field-collected samples

The study did not involve field collected samples

### Ethics oversight

The Institutional Animal Care and Use Committees of the University of Chicago approved the care and breeding of Ancistrus sp. (Bristle nose catfish) (IACUC# 72734). All the procedures were performed at the University of Chicago. The hatchling Leucoraja erinacea (little skate) and hatchling Scyliorhinus retifer (cat sharks) were obtained from the Marine Resources Center, Marine Biological Laboratory, Woods Hole, MA, USA.

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

## Plants

### Seed stocks

N/A

### Novel plant genotypes

N/A

### Authentication

N/A