

Reporting Summary

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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

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

Mimics 20.0 (Materialise Inc.)

Data analysis

PAUP* 4.0a (Sinauer Associates)

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

The digitally reconstructed part and counterpart of *Pipiscius* (Figure 2,e, f) remain under the copyright of the Field Museum of Natural History, Chicago, IL, USA and are available in stl format at DOI: <https://doi.org/10.6084/m9.figshare.13378628.v1>. The original scan data are property of the Field Museum of Natural History (depository: <https://emudata.fieldmuseum.org/>) and available upon request. The cladistic dataset used for our analysis is available as a supplementary file.

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://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

- Sample size** Our description includes: a) all specimens identified as *Priscoomyzon* or *Hardistiella*; and b) all larval specimens pertaining to *Mayomyzon* and *Pipiscius*, and representative adult specimens. Our phylogenetic analysis is based on all fossil taxa within the cyclostome crown group, representative crown-group cyclostomes, representative stem cyclostome and stem gnathostome lineages, and selected outgroups falling outside the vertebrate crown group. The taxon sampling is as broad as reasonably possible for a phylogenetic analysis with its scope on early vertebrate evolution. Each taxon was coded based on type and unambiguously identified specimens with consideration of decay of soft tissue characters.
- Data exclusions** No exclusion was made of specimens or taxa that qualify our criteria above.
- Replication** The specimens described in this paper are curated and accessioned in public depositories and available for study. The computed tomography-based reconstruction (oral apparatus of *Pipiscius*, Fig. 2e, f) and phylogenetic dataset (taxon-character matrix) are both made publicly available, and the original CT data are accessioned at the Field Museum of Natural History. Instructions to access these data are found in the data statement of our paper. Our phylogenetic dataset is a revised version of that from Miyashita et al. (2019, Proc. Nat. Acad. Sci. 116, 2146–2151). Rationales behind each modification are described in Supplementary Information (part B) of this paper.
- Randomization** Randomization is not applicable to our study, as our sample size and the descriptive nature of our study require no such procedure.
- Blinding** Blinding is not applicable to our study, as our sample size and the descriptive nature of our study require no such procedure.

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
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants
- Clinical data

Methods

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

Palaeontology

- Specimen provenance** The specimens of *Priscoomyzon* were obtained during the fieldwork of the second author (R.W.G.) on behalf of the Albany Museum, Makhanda, South Africa. The Waterloo Farm locality is a known fossil site and has been described extensively (e.g., Gess and Trinajstić 2017 PLoS ONE 12(4): e0173169).
- Specimen deposition** Albany Museum (Makhanda, South Africa); Carnegie Museum of Natural History (Pittsburgh, USA); Field Museum of Natural History (Chicago, USA); University of Montana Paleontology Collections (Missoula, USA); Royal Ontario Museum (Toronto, Canada).
- Dating methods** No new dates are provided.
- Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Lamprey (wildtype)
Wild animals	Not applicable.
Field-collected samples	Lamprey hatchlings photographed for Fig. 1r, s were provided by Stephen A. Green (California Institute of Technology).
Ethics oversight	California Institute of Technology

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