nature portfolio

Peer Review File

Exceptional brain and ecological diversity in the earliest snakes

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
Overall, this is an interesting submission that has the potential to be of broad interest and to inform on the ecological diversity of early snakes, breaking the now antiquated and ossified “fossorial vs. marine” dichotomy of snake origin ecologies. The writing is generally good, and the figures are excellent. The authors have provided full access to all of their data for review, which is exemplary.

There are minor-to-substantial issues with taxonomic and systematic assignments, anatomical interpretations, data selection for morphometric and phylogenetic analyses, and explanations of materials and methods. There are also errors and inconsistencies in terminology and figure reference within both the main text and the supplemental information section. I discuss these in detail below. Some of these issues can be resolved with simple rewording/text reorganization and the inclusion of additional text for clarification. Others will require reanalysis and likely reconsideration of results. Once these are achieved following additional review, I would think the submission of appropriate impact.

Taxonomy and systematics

The MS provides only a differential diagnosis for Tametara without a single unique apomorphy to recognize the new taxon. This is disappointing given the relative completeness of the specimen, and I suspect it arises from an attempt to differentiate the specimen from the hypodigm of Najash. Comparing the figures of Tametara from the MS with those for Najash from Garberoglio et al. (2019) and Zaher et al. (2009) reveals that the two are very, very similar, and that most of the characters used to differentiate the taxa are subtle or rely on poor preservation. As an example, the presence of a small retroarticular process is reported in Najash but not Tametara (MS pg. 6, lns. 163-164). Comparison with the undistorted right compound bone of Najash (Garberoglio et al. fig. 2A, D) with Tametara (MS fig. 2A-E) shows no difference in the development of the retroarticular process between the two. The morphology of the postfrontal is described as different between Tametara and Najash based on the absence of a parietal process in the former (MS pg. 5, lns. 118-120). However, the shape of both the postfrontal (Garberoglio et al. fig. 1A; MS fig. 2B, D) and the embayment within the anterolateral margin of the parietal to receive it appear very similar

Ultimately, there are only a few characters differentiating Tametara from Najash that can be readily discerned from the data provided in the MS and published accounts of the latter. Nuchal crests of the supraoccipital and slightly greater overlap of the parietal onto the supraoccipital are very thin wire upon which to hang a new genus, and the authors should consider revising the MS to make MPM 420 a new specimen, and arguably a new species, of Najash. This will allow the MS to provide a robust diagnosis for the genus, which is currently lacking, and will not at all detract from the palaeoneurology and ecological inferences of the study.

Neuroanatomy and endocasts

Analysis of reconstructed neuroanatomy based on endocasts, and the implications for inferring early snake habits and locomotory modes, are the key findings of the submission. The results of these analyses are intriguing. However, there are several potentially problematic issues with the shape data used to generate results. These issues include inconsistences in the shapes of reconstructed endocasts, interpretation of the telencephalon shape of Tametara, and inconsistencies in which aspects of endocast morphology are included in the landmarking scheme.

The MS includes a diversity of squamate endocasts as proxies for neuroanatomy, based on established close shape relationships between endocasts and neural tissues. However, the reconstructions for several taxa do not match previously published endocasts, suggesting that the dataset in this study is not sufficiently capturing the range of variation within taxa and/or ecologies. This is exemplified by the endocast of Anilius (Extended Data fig. 7). The telencephalon of the specimen in this study has olfactory bulbs that are anteroposteriorly shorter than the cerebral hemispheres. This contrasts with other published endocasts (Allemand et al., 2017; Scanferla, 2022) in which the bulbs are longer than the hemispheres. This discrepancy is likely due to ontogenetic differences in skull shape; Based on comments in the text and the acknowledgments, it is likely that the Anilius specimen used in the MS is UMMZ 248356, which is a contrast-stained subadult. If the morphometric analysis is comparing different semaphoronts, then it may be creating (at least partially) artificial ecological morphospaces. This may be especially problematic with respect to the non-analog positioning of Dinilysia patagonica (see attachment). The MS should include information about the specimens used and rerun the analysis with an endocast of a representative adult Anilius and appropriate semaphoronts of other taxa.

Tametara is described as having “short, bulky olfactory bulbs with anterior curvature” (MS pg. 7, lns 197-198). The bulb is identified as an anterior extension of the digital endocst(Fig. 2j) which appears to be defined by articulation of the parietal with the posterior margin of the frontal (e.g., Fig. 2e). The endocranial space for the olfactory bulbs extends through the antero-posterior interorbital length of the frontal in squamates (e.g., Allemand et al. 2022), but only a small portion of the posterior frontal is preserved in Tametara, indicating that the full size and shape of the olfactory bulb endocast in the taxon cannot be known. The elongate frontal of nearly identical Najash (e.g., Garberoglio et al., 2019, Fig. 2c) as sister taxon to Tametara suggests that less than 50% of the frontal is preserved in Tametara, and the taxon may have instead possessed a more elongate, anteriorly projected bulb (endocast). The MS needs to revise discussion in light of missing data or justify how they extrapolate the shape of the bulbs, given that the interorbital region is mostly absent.

To illustrate these points, I have digitized an endocast of an adult Anilius scytale and compare It in dorsal and right lateral views to the endocasts of Anilius, Tametara, and Dinilysia from Extended Data fig. 7. The attachment should be included with this review. Arrows indicate the approximate positions of the frontoparietal suture. The position of the articulation for Anilius from fig. 7 is based on the midline position of the suture for UMMZ 248356. I scaled the Anilius endocasts to a common cerebral hemisphere width and Dinilysia and Tametara to approximately a common telencephalon + mesencephalon length.

The differences between the two Anilius specimens are extreme with respect to the size of the olfactory bulbs, with the larger specimen being much more Dinilysia-like in shape. The approximate position of the frontoparietal suture in Tametara demonstrates that there is almost no interorbital region preserved on the specimen and that the olfactory bulbs are not complete. The reconstruction of Dinilysia includes the dorsal border between the mesencephalon and the telencephalon. Previous reconstructions of the same specimen were unable to differentiate this border, and the MS should include some indication of how it was discerned, especially if it may have implications for landmark identification in morphometric analysis.

Morphometric analysis

The absence of a detailed discussion of methods and data makes interpretation of the morphometric analysis, especially the landmarking scheme, difficult. The morphometric analysis of the submission is based on six homologous landmarks distributed across one side of the telencephalon and illustrated for Tametara (Extended data Fig. 8A), and the MS cites Allemand et al. 2022 for their landmarking protocol and some of the data used in analysis (MS pg. 32, lns. 796-806). However, the landmark ordering or even positions illustrated on Tametara for this study do not correspond to those of Allemand et al. 2022 (Fig. 2). As a result, it is not possible to determine what are the anatomical homologies represented by the landmarks and how any morphometric data from Allemand et al. was reordered or converted for this study. The MS needs to be revised to include a more detailed discussion of the landmarking scheme including anatomical homologies and should include the distribution of landmarks on representative extant taxa as well as Dinilysia in a figure similar to Extended Data fig. 7.

Presuming that landmark 1 represents the anterior edge of the telencephalon, the placement of that landmark on Tametara is inaccurate and misrepresents the shape for the taxon. If this is the case, the authors should omit that landmark and redo the GM analysis to focus on the shape of the cerebral hemispheres, which are preserved in all taxa and appear a priori to show differences in lateral and dorsal curvature in fossorial vs. non-fossorial taxa (e.g. Extended Data fig. 7). It is also worth noting that the GMM analysis uses very few landmarks for the telencephalon. There should be more discussion to justify this given that the endocast of Tametara preserves other structures such as the choroid plexus and the pituitary endocast, both of which could be landmarked to provide a more robust representation of brain shape in the sampled squamates.

It is not clear what phylogenetic topology was used to reconstruct the phylomorphospace. Tametara is discussed as close to Xenophidion in the morphospace as evidence of fossorial habits (MS pg. 11), but Xenophidion is not included in the phylogenetic analyses of the MS, and I could not find any indication of where the topologies for the .tre files in the morphometric data folders were sourced. Also, the most comprehensive molecular phylogenetic analyses place Xenophidion as sister taxon to Caenophidia (e.g., Figueroa et al., 2016), not as sister taxon to Boidae as in the .tre files.

Xenophidion is not a uropeltoid as stated in text (MS pg. 11, ln. 317), and its ecology is described as semi-fossorial, not fully fossorial as in uropeltoids (e.g., Quah et al., 2018 contra Feldman [2015]).

Phylogenetic analyses

Phylogenetic analyses, including methods, data availability and presentation/ interpretation of results are comprehensive and readily available, and I commend the authors for this. That being said, topologies resulting from the different analytical runs suggest that the morphological dataset used for inferring key nodes may not be robust. For example, topologies derived from just morphological data (Extended Data Fig. 3) result in interrelationships among snakes that are not corroborated by any prior morphological or molecular studies, including erroneous groupings of fossorial taxa such as the elapid Atractaspis with scolecophidian (s.l.) and uropeltoid taxa, as well as a failure to resolve relationships among and between Constrictores and Caenophidia. Combined analyses also produce conflicting results, including all fossil taxa forming a clade (Extended Data Fig. 4), and the MS relies on results from tip- and node-dating to conclude that Tametara and Najash are stemward of all other known snakes. As a result, molecular data and rate models are doing a lot of heavy lifting to generate the preferred topology of the MS. This is problematic given that previous studies of the interrelationships of included fossil snakes have recovered Dinilysia and/or Sanajeh as stem-ward of Najash (Garberoglio et al., 2019; Zaher et al., 2022) based, in part, on more comprehensive morphological data sets (Zaher et al., 2022). The limitations of the morphological characters are evident when scrutinizing the character justification for the placement of Tametara:

The MS states “The inferred placement of Tametara prior to the split of Dinilysia and all other snake fossils is explained by plesiomorphic skull features, including the presence of a well-developed dorsum sellae on the basisphenoid (absent in later-deriving snakes), absence of a mid-sagittal crest of the basisphenoid (conspicuous among simoliophiids, Wonambi, and many early crown snakes), and, most notably, otoccipitals not meeting in the midline above the foramen magnum (separated by the supraoccipital)” (Pg. 9)

Of these characters, a basisphenoid mid-sagittal crest is absent in Dinilysia, as well as scolecophidians (s.l.) and variably among fossorial alethinophidians within crown snakes (e.g., List, 1966; Zaher and Scanferla, 2012; Strong et al., 2021). The description of the development of the dorsum sellae is contradicted by the extended description of the parabasisphenoid in the supplementary information section where it is described as “weakly developed” (pg. 7, ln. 221) [note, this section also refers to Extended Data figure 1e, but that is the otoccipital, the sellae is visible in figure 1c, where it is labelled as “dorsal sellae” in the figure caption]. The dorsum sellae appears prominent relative to the sella turcica in Tametara, as in non-snake squamates, but that appears due at least in part to the incomplete preservation of the anterior region of the basisphenoid. The more complete parabasisphenoid in Najash also as a more well-developed dorsum sellae than in Dinilysia, but not more so than multiple crown taxa (e.g., Gauthier et al., 2012, pg. 195, character 324). This character is also not figured in the character descriptions and is coded as “?” for Dinilysia in the morphological data nexus files which further undercuts its utility as an indicator of a basal position for Tametara. The MS needs to revise the description and use of this character to be consistent across the text, should code it appropriately for what is known about Dinilysia, and should provide an illustration of how the dorsum sellae is plesiomorphically more developed in Tametara than in Dinilysia and crown snakes.

“The MS also states “Tametara further differs from crown snakes by retaining the basipterygoid process of the basisphenoid, the foramen chorda tympani on the articular, the absence of accessory processes on the prezygapophyses, and the presence of both margo ventralis and lateralis.” (Pg. 9)

Several of these characters are also problematic: Basipterygoid processes are present in multiple crown snake lineages and are coded so in the morphological data matrix of the MS. The matrix is miscoded for Sanajeh, which possesses large basipterygoid processes (Wilson et al., 2010). Both the margo ventralis (subcentral ridges) and margo lateralis (interzygapophyseal ridges) of vertebrae occur across crown snakes (Auffenberg, 1963; Holman, 2000; Szyndlar and Georgalis, 2023- with few exceptions for margo ventralis [Hoffstetter and Gasc, 1969]), and do not differentiate stem from crown taxa. The MS also incorrectly refers to the structures as “interzygantral” ridges (Supplemental Information, pg. 13, ln. 397).

Thus, character support for a basal position of Tametara is not as robust as stated in the text, and the MS should revise this discussion to be limited only to those characters that demonstrate no, or minimal, homoplasy under optimization from the synapomorphies list.

The preferred topology of the MS also indicates profound homoplasy in the evolution of the snake skull roof with respect to Sanajeh. Zaher et al. (2022) demonstrated that Sanajeh retained an upper temporal arcade consisting of the postorbital and squamosal, unlike Najash, Dinilysia, and all crownward snakes. The positioning of Sanajeh as sister taxon to Dinilysia crownward of Tametara+Najash in this study requires either three independent losses of both elements, or redevelopment of the postorbital, squamosal and the arcade they form in Sanajeh. As the MS notes that the preferred topology only requires a single loss of external hindlimbs in the stem-crown transition in snakes, it should also note that it requires multiple losses (or reacquisition) of the upper temporal arcade in the snake stem based on Sanajeh.

(Remarks on code availability)
I did not run the code for analyses, but I examined the associated datasets. Critiques of those are included in the main review. The lead author should be aware that the Datadryad links for Ref. 38 on the Sys. Biol. webpage are invalid and that Datadryad has no available data sets for that reference. That should be fixed before this publication is accepted.

Referee #2

(Remarks to the Author)
Dear authors and editor,
We co-reviewed the manuscript entitled “Exceptional brain and ecological diversity in the earliest snakes” by Simoes and colleagues considered for publication in Nature.
A. Summary of the key results
In this manuscript, the authors describe a new snake fossil Tametara mirim, which is exceptionally well preserved, retaining its 3D shape, including an almost complete skull, numerous vertebrae, and articulated postcranial elements. The authors described the anatomy of the fossil and used its brain and inner ear endocasts, along with bone microstructure to make ecological inferences by comparing it with extant squamates species. Their results suggest that T. mirim was fossorial and possessed a skull morphology distinct from that of other early snake fossils, particularly Dinilysia.
B. Originality and significance: if not novel, please include reference
We consider this manuscript to be of interest to both a large scientific and non-scientific audience. The exceptional preservation of the fossil, combined with its comparison to both extant and extinct species using multiple approaches (3D geometric morphometrics, bone microstructure, and morphological characters), provides convergent evidence for a fossorial ecology. This is particularly valuable in the context of the ongoing debate about the ecological origins of snakes.
C. Data & methodology: validity of approach, quality of data, quality of presentation
The methodology is correct but could be improved, especially regarding the 3D GMM, but overall, the authors made an effort to place their fossil into a biological context using various methods.
D. Appropriate use of statistics and treatment of uncertainties
The statistical methods are sound overall, but some parts of the methodology need to be modified or clarified. The provided R codes and associated files for the endocast analysis are easily usable and results can be replicated but not those for the microstructure analysis (see comments).
E. Conclusions: robustness, validity, reliability
We generally agree with the conclusions of the authors, especially regarding the putative ecology of T. mirim.
F. Suggested improvements: experiments, data for possible revision
List below & in attached file.
G. References: appropriate credit to previous work?
Yes.
H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
We strongly suggest being more careful with the terminology used throughout the manuscript, especially the use of “brain” to refer to the endocst(see comment below). We also feel like the authors oversell their work and results that are interesting per se. For instance, they state that T. mirim is completely different from extant species, but their analyses show the opposite, and if it was truly that different, it would make it hard to infer its ecology. We list below our major and minor comments that need to be addressed before publication.
We must acknowledge that we do not have the necessary background to review the phylogenetic part of the manuscript.
Sincerely,
Marion Segall & Rémi Allemand

PS: The comments below are copy pasted from a Word document so the formatting disappeared but we attached the file for a better readability.

Major comments:
Main text:
- Title: We suggest reconsidering the title, as the use of a superlative, the term 'brain' rather than 'endocast,' and the lack of reference to what seems to be the central focus of the manuscript—a newly discovered, exceptionally well-preserved specimen—may give a slightly misleading impression.
- Terminology: Throughout the manuscript, you may consider using the term “endocast” (or “endocranial morphology”) rather than “brain” when describing features reconstructed from endocranial data. Endocasts only approximate the external brain shape and include non-neural spaces (e.g., meninges, venous sinuses), so this terminology would provide a more accurate description, except when explicitly discussing inferred neural structures.
- Systematic Paleontology: Consider reorganizing the manuscript to facilitate reading. Descriptions of the new species and associated information should be presented clearly and in a consistent order.
- Osteological description: there is no mention of the size of the specimen anywhere. We had to go to the figure to measure its size, which was a big surprise at first since, according to the scale of Fig 1a. the specimen measures about 1m and its skull around 5cm (head length), which is far from being a small specimen. However, we realized that the scale on Fig. 1a. is wrong, it should be about 8.16cm, not 20, according to all other figures for which the scale seems correct and that give a skull length about 2cm (which seems more reasonable for a snake that is supposedly “small-sized”. Scales need to be checked and corrected and a description of the size of the specimen should be added to the main text and discussed, especially since size is the main driver of endocranial shape. Large species have endocranium that are similar to the one of your specimen (anteroposterior elongation etc). But since your specimen is quite small, we agree that it would tend to look like an Anilius or Cylindrophis.
- Allometry: You wrote in your M&M section that you tested for allometry, and running your code, we found out that there is no allometric signal in endocranial shape in your dataset which would be interesting to discuss in the manuscript, especially since in other studies, allometry is usually significant and represent a relatively large amount of the total shape variation. We wonder whether the lack of signal results from the low number of landmarks in your manuscript.
- Olfactory bulbs: These structures are heavily discussed, and a rather large part of the ecological inference is based on its

description. However, as mentioned in the thorough description in the SM, the anterior part of the frontal is missing (l70-71) there is “no facet for a possible prefrontal (but we note that the anterior end of the frontal is mostly missing)” and (l74-75) “due to its incompleteness we cannot determine how far ventromedially 75 it could have extended”. The olfactory bulbs do not seem particularly “bulky” (l197), it is hard to tell whether they are short considering that a part of the frontal is missing, and it is hard to understand what you mean by “anterior curvature” (l198).
- L199 “enlarged cerebral hemispheres”. The cerebral hemispheres do not look enlarged compared to most extant species, but compared to Dinilysia, sure. This statement should be corrected and the discussion adjusted.
- All along the main text, you compare your specimen with many extant species but in the abstract, you state that (l34-35) “Tametara had a brain morphology distinct from both other stem and extant snakes”, while Fig. 4 shows that T. mirim is clearly within the space occupied by extant fossorial.
- L286-288 “Tametara and Dinilysia represent highly divergent brain shapes relative both to each other and to most other snakes and non-ophidian squamates”, while the first part of the sentence is true, the second is not for Tametara. Your specimen is not isolated from fossorial snakes in any of your morphospaces based on endocranial characteristics (Fig. 4, Extended Data Fig. 8, Extended Data Fig. 10).
- L310-313: You wrote that “Quantitative analyses further indicate that the early morphological disparity in snake brains reflects variation in their ecological lifestyles. Specifically, our analyses significantly discriminate how different telencephalon shapes relate to habitat usage, especially between fossorial and non-fossorial species (Fig. 4a).”. We may have missed it, but we do not find the aforementioned quantitative analyses.
- L326-328: “We did not apply geometric morphometrics on the inner ear, however, given contradictory prior results and limited statistical power for reliable palaeoecological inference”. We doubt that all these issues come from the 3D GMM. It is most likely that the inner ear does not bear a strong ecological signal (or at least, a signal that is related to substrate use). However, you based some of your ecological inferences based on the inner ear structure. I would either remove this part of the discussion or be extremely careful in the interpretation.
- L816-817: What shape variables were used? Did you use Procrustes coordinates? Principal Components? If so, how many and how did you choose them?
- L818-821: Why use phylogeny-corrected shapes for the LDA? Why not “raw” shape data? And why 95% of shape variation? Why not all PCs?
- The habitat attributed to some species seems incorrect: Physignathus cocincinus is arboreal same for Basiliscus vittatus, Anguis fragilis is at best semi-fossorial, Thamnophis sirtalis is mostly terrestrial and at best semi-aquatic. In addition, In the Supplementary Text (lines 301–302), Pseudopus is listed among “fossorial limb-reduced non-ophidian squamates,” but it is generally considered terrestrial (you correctly classified it as terrestrial in your analyses). Please correct this for consistency.
- Coloring for the parts of the endocast: The way the endocasts are colored in several figures prevents from seeing the underlying structures. It is too opaque. Plus, you mentioned in the manuscript that, aside from the telencephalon, the rest of the endocast regions are barely distinguishable on your specimen so how did you color it?
- Landmark scheme:
o Please clarify why only six landmarks were used on the telencephalon. This seems limited, especially since Allemand et al. (2023) defined 20 landmarks that can be placed relative to surrounding bones and the labyrinth. Using more landmarks could better capture morphological variation and strengthen the morphometric analyses. Consider justifying the choice of six landmarks, for example using a Landmark Sampling Evaluation Curve (LaSEC; Watanabe 2018, R package LaMBDA).
o We have concerns about how you positioned your first landmark since you mentioned that the anterior part of the frontal is missing.
o Regarding geometric morphometrics, consider whether including all squamates might obscure ecological signals. Would it be more informative to focus on snakes alone for some analysis?
o Provide details on how landmarks were positioned on the endocast of Dinilysia, which shows morphology quite different from other snakes. Could incompleteness affect landmark placement?
o It would be informative to have the labels of your landmarks in Extended Data Fig. 8a, if anyone would like to reproduce your landmark scheme.
- Statistical analysis:
o You tested differences in endocranial shape between ecologies in extant species using pairwise tests but you did not mention these results in the manuscript. Since only fossorial species are significantly different from other ecologies, it would be interesting to mention it since it could strengthen your results.
o The total accuracy of the LDA is 69%. This information does not appear anywhere in the main text. You wrote L314 that Tametara is classified with 96.6% probability within the fossorial group. But how does this number articulate with the overall accuracy of the LDA? It is 96.6% of the overall accuracy (69%)? We recommend giving more details about this method. And again, we suggest using all the PCs, not a subset. We did run your code with all PCs and the overall accuracy of the LDA slightly decreases (63%) but the prediction decreases significantly for Tametara from 96.6 to 66% (and 33% of chances of being aquatic) while the percentage for Dinilysia being a terrestrial species increases from 78.3% to 84.5%. From a statistical point of view, using a subset of PCs that result from only 6 landmarks feels like you are using a very small amount of shape variation to make ecological inferences. You need to convince us that not using all PCs is statistically sound.
- References and figure citations:
o Some references do not match the statements. For example, line 786 cites Mongiardino Koch et al. (2021) regarding published endocasts, but this paper does not include endocasts. Please verify all citations.
o Figure references also need checking. In the Supplementary Text (line 40), the postfrontal is cited as Fig. 1b–f and Extended Data Fig. 1a–e, but Fig. 1d–f shows vertebrae, and no postfrontal appears in Extended Data Fig. 1a–e. Likely the correct references are Fig. 1b,c and Fig. 2b–d.
o Similarly, in the Supplementary Text (line 172), “Extended Data Figure 2” is cited for the otoccipitals, but this figure appears to show postcranial anatomy instead. Please verify and correct.

Minor comments:

Abstract
L34-35: “Quantitative endocast analyses demonstrate that Tametara had a brain morphology distinct from both other stem and extant snakes”. The statement seems too categorical. Some similarities with Anilius and Cylindrophis are also evident and should be acknowledged.
L35-36: “revealing substantial early neuroanatomical disparity—and likely sensory functions—in snake evolution”. The claim appears speculative, as sensory functions were not directly tested in this study. You may consider removing or rephrasing this sentence to better reflect the scope of your results.
L36-38 “Independent evidence from brain shape, inner ear organization, and bone microstructure converges on a fossorial lifestyle for Tametara, contrasting with other stem and early crown snakes”. The end of this sentence is not clear, how Tametara being fossorial is contrasting with other stem and early crown snakes?

Main text:
L34-35: remove space before “mirim”
- Please consider adding references in the main text when comparing with other species
L130-132: “However, the parietal has a short anteromedial process that would have inserted in between the frontals (Fig. 2d), which is absent in the other South American Cretaceous snake”. It is unclear here. In Zaher & Scanferla (2012), the description of the Dinilysia skull indicates that “anterolaterally, the parietal is clasped by the postfrontal, whereas its anterior surface contacts the frontals in a W-shaped, anteriorly concave, and interdigitating suture.”
L192-193: “This allows fossil snake brains to be reliably inferred from endocasts and directly compared with those of extant snakes33,34 » The cited references focus only on endocast studies in extant snakes, so they may not directly support inferences about fossil snakes. You may consider clarifying this point or citing additional studies that specifically address fossil endocast comparisons.
L200-202: “It is followed immediately posteriorly by enlarged cerebral hemispheres in the telencephalon and by a rather undifferentiated mesencephalon. This diverges from the condition observed in most squamates, which display well-expanded cerebral hemispheres that strongly contrast with the mesencephalon and rhombencephalon (= mid- and hindbrain, respectively)32-34”. Please clarify what is meant by “undifferentiated mesencephalon”. Additionally, the sentence is unclear—are you referring to the size of the cerebral hemispheres, or to the differentiation between the cerebral hemispheres and the mesencephalon?
L202-206: The sentences in the main text appear somewhat contradictory to statements made in the abstract.
- Reference number 36 is not cited correctly. It should be: Olori, J. C. (2010). Digital endocasts of the cranial cavity and osseous labyrinth of the burrowing snake Uropeltis woodmasoni (Alethinophidia: Uropeltidae). Ichthyology & Herpetology, 2010(1), 14-26.
- Phylogeny and divergence times: Out of curiosity, did the authors consider alternative phylogenetic hypotheses, such as placing Serpentes as the sister group of anguimorphs?
L258-259: The main text states that Tametara has a “well-developed dorsum sellae” to support its basal placement, but the detailed description notes that it is “the dorsum sella is weakly developed and visible in anterior view” (Line 221 in Supplementary text). Could the authors clarify this apparent discrepancy and specify how they coded or interpreted this character in the phylogenetic analysis?
L259-260: The mid-sagittal crest of the basisphenoid seems only present in adult specimens of large species (similar to the neural crest) in extant species. It says more about your specimen size than of its relationship with other extinct species.
Early evolution of snake brains
L286-287: The statement “We find that the earliest known snake brains of Tametara and Dinilysia represent highly divergent brain shapes relative both to each other and to most other snakes and non-ophidian squamates” could be made more nuanced. The PCA in Fig. 4 shows that Tametara occupies a region of morphospace overlapping with other snakes and squamates, suggesting that its brain shape is not entirely divergent. Please consider revising this sentence to reflect this pattern more accurately.
Palaeoecological origin of snakes
L317-320: “These results support most of our comparative anatomical observations, in which Tametara shares with other fossorial taxa short olfactory bulbs, a weakly developed optic tectum, and morphologically poorly differentiated cerebral hemispheres22,23,32-34 (Extended Data Fig. 7).” The manuscript describes the mesencephalon as both (L211) “undifferentiated” and “unusually elongate”, and the cerebral hemispheres as both “enlarged” and “poorly differentiated”. These descriptions appear contradictory. Please clarify how these observations relate to each other, and specify whether the terms refer to size, shape, or differentiation, to improve clarity and consistency. Additionally, given that the anterior part of the frontal is missing, it would be helpful to clarify whether the olfactory bulbs are completely preserved. If not, this could affect the interpretations.
L324-328: “Interestingly, Tametara further shows inner ear features compatible with fossoriality, such as an enlarged vestibular region and simplified semicircular canal architecture, providing independent support from a different system. We did not apply geometric morphometrics on the inner ear, however, given contradictory prior results19,37 and limited statistical power for reliable37 palaeoecological inference” These sentences are somewhat contradictory. The text claims that Tametara shows inner ear features “compatible with fossoriality” and that this provides “independent support,” yet also acknowledges that no geometric morphometric analysis was performed and that previous results are contradictory. It would be preferable to rephrase this section more cautiously.
L330-332: “The combination of a dorsoventrally shallow lateral profile, poorly differentiated subdivisions, extremely long and curved olfactory bulbs, and narrow cerebral hemispheres is unlike that of any other squamate.” This statement could be made more nuanced. Some similarities can be observed with the endocast of Python molurus and Python bivittatus (Figure S3 Supplementary Material in Allemand, R., Polcyn, M. J., Houssaye, A., Vincent, P., López-Aguirre, C., & Bardet, N. (2024). First Virtual Reconstruction of a Mosasaurid Brain Endocast: Description and Comparison of the Endocast of Tethysaurus nopcsai with Those of Extant Squamates. Diversity, 16(9), 548.)
L343: Reference 45 appears twice.

Figure 4:
• The phylomorphospace was generated using landmarks restricted to the telencephalon. Including miniature reconstructions of the entire brain in this figure may therefore be misleading, as it visually suggests that the morphospace reflects total brain shape rather than telencephalic variation alone. Please consider displaying cropped telencephalon renderings to avoid potential bias in interpretation.
• Many times, you compare Tametara to Anilius and Cylindrophis, it would be nice to have their location on the morphospaces.
Line 646: please remove the coma “Simões, et al.6”
Supplementary Material:
L40: snakes are squamates. Please consider rephrasing
L44: postfrontal rather than “frontal”?
L62: “The supratemporals extend well anteriorly, close to the level of the trigeminal foramen” This description could be reconsidered, as Fig. 2a,b appears to show the supratemporals terminating posterior to the level of the trigeminal foramen rather than extending close to it.
L68: “Frontal” is misspelled
L177: “(hypoglossal) could be identified. is located posterodorsally to CN X” the sentence does not make sense.
L291: We don’t understand the meaning of the end of this sentence: “The brain of Tametara is anteroposteriorly elongate and defined anteriorly by short, 291 bulky olfactory bulbs with anterior curvature”
L301 (and check the rest of the SM & Main text): The refence should be Olori 2010 not Jennifer.
L337-340: “Compared to extant snakes, Tametara and Dinilysia, have remarkably large pituitary glands, although the length of these structures might only correspond to the elongate parietal of the fossils,” I am not sure to understand, could you explain this please?
L378-380: “in reptiles are difficult to determine snakes and other limbless squamates as these are typically defined based on vertebra bearing ribs contacting the sternal plate to discern between cervicals and dorsals, for instance (Hoffstetter & Gasc 1969)” Please consider rephrasing
L783: “Computed-tomographyc”
L795: “of” is italicized
Extended Data Fig. 7: Why choose to show Acrochordus, which is a very peculiar species among snakes in all aspects of its morphology, including its endocranium?
In your Rcode “analysis_microstructure”, the library “ggtree” does not exist and there is no file named “general_pruned_scaled_appended.tre”. Please, make sure we can run the whole code.

(Remarks on code availability)
The code for the endocast analysis runs fine but the code for the microstructure does not (see detailed comments).

Referee #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Referee #4

(Remarks to the Author)
The manuscript is well written and robust, and it does not show deficiences to prevent its publication.
Conclusions are original and convincent.
The results are interesting enough for several researchers in evolutionary biology and vertebrate paleontology.
We were not able to do a code revision since the matrix size is excessive for our hardware devices.

A. Authors present a new species of a Cretaceous stem-snake more or less equivalent (but younger) to Najash rionegrina. Their main goal is to obtain, through high-resolution micro-CT scans new details of the neuroanatomy.
They also recognise a well-defined fossorial lifestyle for Tametara, contrasting with other stem and early crown snakes. This way they demonstrate early major ecological transitions in snake evolution, and that known stem species do not allow us to point the original ecological condition of crown snakes.

B. The paper is original and is significant in demonstrating a well-defined fossorial stem-snake not only by the anatomical analysis of vertebrae but also by features of the neurocranium. This allow authors to discuss with stronger tools the broad range of ecological adaptations in early snakes, something already suggested by the Jurassic material (Caldwell et al., 2015).

C. Data and presentation are optimal. However, there are some unclear detail in the presentation of bones, classifying them in non-mutually excluding categories: i.e., dermatocranium, splanchnocranium, and neurocranium. While dermatocranium refers to the bone origin, splanchno and neurocranium refer to their functionality (for example, pterygoid enters in both dermatocranium and splanchnocranium). We suggest authors to decide for one classification.

D. Appropriate use of statistics and treatment of uncertainties
Nothing to say here
E. Conclusions: robustness, validity, reliability
Conclusions are robust and valid.
F. Suggested improvements: experiments, data for possible revision

There are some notes to state here.
Line 97 (diagnosis). In “strongly overlapping the parietal ventrally” the description of the overlap between parietal and prootic is confusing, see below.
Line 99 (and 125). Where authors refer to quadrate conch in Tametara and other fossil snakes, the argument of its presence is not clearly assessed either here nor in the supplementary (see comment in the supplementary).
Line 100. In “oriented zygosphenes/zygantra”, Oriented how, in respect to what, where?
Line 102. Respect to the presence of parazygantral foramina, they are not visible in the presented material, and what is labelled as such in Extended Data Fig 2 are NOT parazygantral foramina (see below).
Line 106, discussing the ontogenetic stage, we agree in considering the holotype as an adult individual. However, respect to the “high degree of overlap between the supraoccipital and the parietals and prootics”, the overlap between parietal and supraoccipital and great exposure of the latter particularly departs from the typical condition observed in adult snakes. The supraoccipital remains greatly exposed in the skull roof in Tametara, as in Najash and juvenile snakes (Zaher et al., 2009; Scanferla and Bhullar, 2014; Palci et al., 2016; Garberoglio et al., 2019a, 2019b).
Osteological description:
Line 116. As said before, classifying them in non-mutually excluding categories: i.e., dermatocranium, splanchnocranium, and neurocranium, is confussing. While dermatocranium refers to the bone origin, splanchno and neurocranium refer to their functionality (for example, pterygoid enters in both dermatocranium and splanchnocranium). We suggest authors to decide one classification functionally (neuro vs splanchnocranium) or by origin (auto vs dermatochranium). We suggest the former one.
Additionally, besides the quadrates, there is another element of the splanchnocranium that might be actually preserved: a small portion of the left stapes. A small portion of this bone was reconstructed as part of the prootic in Figure 2c,d and Extended Data Figure 1b, which is apparently separated from the main body of the prootic and approaches the ventral side of the suprastapedial process of the quadrate. This matches the stapedial shaft observed in Dinilysia and Najash (Caldwell and Albino, 2002, Zaher and Scanferla, 2012; Garberoglio et al., 2019a).
Line 119. Respect to “The postfrontal has a distinct frontal process and a single distal process”, the postfrontal is described as having two processes, a frontal and ¨single distal process¨, which we find somewhat confusing here. It is more clear in the supplementary that the postrontal is described as having a frontal (=anterior), lacking a parietal (=posterior) process, and having a distal process that is ventral.
Line 133. “the parietal has a short anteromedial process that would have inserted in between the frontals, which is absent in the other South American Cretaceous snake”. The singular in this sentence is incorrect. It refers to the condition in the South American snake (Dinilysia), somewhat ignoring the existence of the other (and better preserved) South American snake (Najash). Anyway, while there is indeed no evidence of such process in Najash (Apesteguía and Zaher, 2006; Zaher et al., 2009; Garberoglio et al., 2019a), a similar anteromedial process of the parietal is arguably present in Dinilysia (visible in specimens where the fronto-parietal suture is well-preserved such as the holotype and MACN-RN 1013), where the parietal contacts the frontals in a W-shaped interdigitating suture (Estes et al., 1970; Caldwell and Albino, 2002: Zaher and Scanferla, 2012).
Line 145. “supraoccipital bears well-developed anterolateral processes that contact and are strongly overlapped by the parietal posterolaterally”. As we read, authors seem to say that the supraoccipital is overlapped by the parietal (implying that is covered by it). This makes sense, as it is common in snakes. Only a small degree of overlap of the supraoccipital by the parietal seems to be the condition in Tametara. It is made clear by a small portion of the posterior margin of the parietal that is missing in the right side, and reveals a small portion of the anterolateral process of the supraoccipital that was not exposed in the skull roof, and was below the parietal: i.e., overlapped by the parietal (Figs. 1c, 2d). However, in the diagnosis it says that the supraoccipital is “strongly overlapping the parietal ventrally”, which implies a different thing or it is incorrect (there is no supraoccipital covering the parietal). Below, it states that the overlap is similar to other snakes (where the supraoccipital is overlapped by the parietal), but greater than in any other snake. This does not make sense, since the last part says correctly that the supraoccipital represents a great portion of the preserved dorsal surface of the skull roof in Tametara, which is the result of less overlap of the supraoccipital by the parietal than in most snakes. For instance, the lesser dorsal exposure of the supraoccipital accounted in the skull roof of adult specimens of Dinilysia is the result of a greater overlapping of the supraoccipital by the parietal than what is observed in juveniles (Scanferla and Bhullar, 2014).
Line 148. Respect to the idea that “the supraoccipital of Tametara represents ca. 25% of the preserved dorsal surface of the skull roof, whereas in adult specimens of Dinilysia this accounts only 8-11% in MACN-Pv RN1013 and MLP 26-410, respectively”, the authors seem to have overlooked previous descriptions of the great exposure of the supraoccipital in Najash (Zaher et al., 2009; Garberoglio et al., 2019a, 2019b), which is approximately in the same range of that observed here in Tametara. Zaher et al. (2009) attributed this feature to a juvenile stage in the only known skull at that time (MPCA-PV 385). But in later descriptions (Garberoglio et al. 2019a, 2019b) demonstrated to be present in all specimens of Najash described afterwards, and is evident now that this don’t reflect a juvenile condition or could even be considered to be a distinct feature of the skull in Najash. In this case, the great exposure of the supraoccpital is not a unique feature of the snake described here, and could be another potential feature in common between the new taxon and Najash, its evident sister group.
Line 152. The reference of “the otoccipitals do not meet each other at the midline, being separated by the posteromedial process of the supraoccipital (Figs. 1c and 2c-e). This configuration is widespread among non-ophidian squamates but was previously known to occur only in Najash among all known fossil and extant snakes”, a posteromedial process of the supraoccipital that separates dorsally the otoccipitals in the skull roof was previously observed not only in Najash, but also

in Haasiophis (Rieppel et al., 2003; Zaher et al.., 2009: Garberoglio et al., 2019a, 2019b). A similar condition also occurs with varying degrees in extant macrostomatans, even with complete dorsal separation of the otoccipitals by the posterior extension of the supraoccipital (Rieppel and Zaher, 2002). However, in such cases the otoccipitals usually meet below the supraoccipital and contact each other above the foramen magnum, but not always (Rieppel and Zaher, 2002). Besides the separation of the otoccipitals in the skull roof, the lack of medial contact of the otoccipitals above the foramen magnum is confirmed by the ct-scan reconstruction in Najash (Garberoglio et al., 2019b) and Tametara, but this remains unknown in Haasiophis. Considering these points, the condition in Najash and Tametara is not necessarily as straightforward plesiomorphic and unknown to occur in snakes, as the statement here implies, which we think it ignores not only previous observations but also the degree of variation of this feature in extinct and extant snakes.
Line 156. Concerning the absence of the crista circumfenestralis, “another plesiomorphic condition occurring among other stem snakes such as Najash, Dinilysia, Yurlunggur, Wonambi, and Sanajeh30”, the citation provided by the authors do not entirely supports this statement, at least not for all the taxa mentioned. To state the absence of the crista circumfenestralis in Najash, Yurlunggur, Wonambi, and Sanajeh they should cite other references, or state here that a crista circumfenestralis sensu Palci and Caldwell (2014) is absent in all of this taxa. The different opinions and criteria about this feature are a lengthy subject, which is apparently ignored here, and described in more detail only in the supplementary (but see comment there).
Line 165. Respect to the absence of the retroarticular process in Tametara, it says that it occurs “as in Dinilysia9,10, whereas Najash exhibits a small one11”. Both Najash and Dinilysia are described as retaining a short retroarticular process (Caldwell and Calvo, 2008: Zaher and Scanferla, 2012; Garberoglio et al., 2019a). Both have indeed only a very short extension of the compound bone passing the glenoid, and there is no much difference between these two and the condition in Tametara. To consider the small process as a present or absent retroarticular process might be subjective. Is actually better understood as with no distinction between the three, and if the authors want to make that case here, it has to be better justified.
Line 184. “Most vertebrae have paracotylar foramina on each side of the cotyle”. Paracotylar fossae are present in the figured vertebra, but seems to be no clear indication of paracotylar foramina inside such fossae in the reconstruction.
Line 185. Concerning the small parazygantral pits on either side of each zygantrum on most vertebrae, there is actually no evidence presented of the presence of parazygantral foramina in Tametara. What is described in the manuscript and labelled in the figured vertebrae as parazygantral foramina (or parazygantral pits) are not in fact parazygantral foramina, since they are located INSIDE the zygantral cavity. On the contrary, parazygantral foramina are located OUTSIDE the zygantral cavity, adjacent to it on each side between the zygantral facet and the postzygapophysial facet (e.g., Rage, 1998; Scanlon, 2005; LaDuke et al., 2010; Hsiou et al., 2014; Gómez et al., 2019; Szyndlar and Georgalis, 2023).
Line 188. Respect to the absence of forelimbs, supported by “the specimen´s articulation, preservation quality, and full exploration with micro-CT scanning”, it makes sense, of course, but aren’t a lot of ribs missing also? In such case, missing small forelimbs seems to be still a possible scenario.
Line 576. Fig. 2. “V.con” labelled in the figure, but not in the caption.
Line 1007. Double .. in TRS.
Line 1070. Extended Data Fig. 1. Cl.Pr labelled in figure, but missing in the caption.

eSupplementary Information
Line 29. Sam problem described for line 116 of the text.
Line 41. Ventral instead of distal?
Line 60. Respect to the description that “Each supratemporal contacts the paroccipital processes of the otoccipitals ventrally and the supratemporal process of the parietal anteromedially”, the supratemporal also have a broad ventral and lateral contact with the prootic.
Line 83. Authors say that “The right side of the parietal is entirely preserved, bearing a large facet for the postfrontal anterolaterally, and a short anteromedial process that would have inserted in between the two frontals, which is absent in Dinilysia”. However, the parietals in Dinilysia are described as having a jagged, w-shaped interdigitating suture with the frontals by several authors (Estes et al., 1970; Cadwell and Albino, 2002; Zaher and Scanferla, 2012). This interdigitating suture is the result of a similar anteromedial process of the parietal which is arguably present in Dinilysia, and is visible in specimens where the fronto-parietal suture is well-preserved, such as the holotype and MACN-RN 1013 (Estes et al., 1970; Caldwell and Albino, 2002; Zaher and Scanferla, 2012), but the contact is not preserved in the specimen described by Caldwell and Calvo (2008).
Line 107. Respect to the conch in the quadrate, it must be said that the quadrate in Dinilsyia shows a notch or concavity on its lateral surface that is flanked anteriorly by an anterior ridge, thus resembling the tympanic crest and tympanic conch in other lizards (Zaher and Scanferla, 2012). However, this crest and concavity are not observed in quadrates attributed to Najash (Zaher et al, 2009; Garberoglio et al., 2019a), and a quadrate lateral conch as in other lizards that have a tympanic chamber has been considered to be absent in both Dinilysia and Najash as in all extant snakes (Gauthier et al., 2012; Zaher and Scanferla, 2012; Garberoglio et al., 2019a; Palci et al, 2019). Therefore, we consider that it is not clear what the authors refers to a well-developed lateral conch in Tametara and the other fossil snakes, and the argument to state its presence in all of these fossil taxa is not particularly well-stablished, since the quadrate in Tametara shows the same condition that in Dinilysia, though differing from that of Najash.
Line 113. The affirmation that there is a well-developed tympanic crest on the quadrate, similarly to Dinilysia (but not preserved or partially broken in all specimens of Najash), seems somewhat contradictory. If the presence of a quadrate conch in fossil snakes is based on the presence of well-developed tympanic crest and a lateral concavity on the quadrate, as is observed in Tametara and posibly Dinilysia, then why they stated before that a quadrate conch is also present in Najash where all this is not actually preserved?
Line 126. Authors say that “along with the anterolateral processes, these have a substantial overlap with the ventral side of the parietal”. However, It results unclear to what is referring with the “these”, while describing the anterolateral processes. A rephrase is necessary.

Line 127. About the overlapping with the ventral side of the parietal, “in other snakes, including the braincase of early snakes such as Dinilysia (Caldwell & Calvo 2008; Zaher & Scanferla 2012) and Najash (Garberoglio et al. 2019a), the extent of this overlap with the prootics and parietals is greater than in any other snake known to us”. It is not clear for us to what refers to with a greater degree of overlap of the supraoccipital in Tametara (See comment in the main text). The supraoccipital is greatly exposed in the skull roof in Tametara, in a very similar way to Najash, resulting from less overlapping by the parietal. The supraoccipital is less exposed on the skull roof in DInilysia, due to a greater overlapping by the parietal.
Line 162. Paired Atlanteal crests instead of nuchal crests?
Line 166. See the comment in the main text for “The same arrangement occurs only in Najash (Garberoglio et al. 2019a) among all snakes known to us”.
Line 169. Respect to the posteriormost end of the paroccipital processes almost at the same level of the posterior end of the supraoccipital, on the right side, the paroccipital process appears to be more complete than in the left side, and in Fig. 2d, e is visible extending well-passing the posterior end of the supraoccipital. On the right side of the skull in Tametara, the situation is quite similar to that of Najash (Garberoglio et al., 2019a).
Line 171. Concerning the length of the paroccipital processes in Dinilysia and Najash, comparatively longer posteriorly, going beyond the level of the occipital condyle, this is true for Dinilysia (Estes et al., 1970; Zaher and Scanferla, 2012), but the paroccipital processes in Najash are not so developed, and at most only reach the occipital condyle. Previous descriptions of Najash stated that they do not project beyond the level of the occipital condyle (Garberoglio et al., 2019a). Moreover, the occipital condyle appears to be not preserved in Tametara, making a comparison to Dinilysia and Najash difficult, and a distinction only an inference. The condition in Tametara is unknown, but judging from the preserved portion of the basioccipital and the posterior end of the supraoccipital, it is likely that the posterior end of the paroccipital process was in fact placed beyond the level of the occipital condyle.
Line 179. “could be identified. is located” . Missing “CN XII is”.
Line 183. “In Najash rionegrina (G.S. pers. obs.)”. Is Weirs personal observation.
Line 190. “border of the crista tuberalis (Figure 1d)”. Wrong figure reference (Figure 1d is a vertebra).
Line 198. “apertura lateralis—a condition shared with several early fossil snakes in which this can be determined (e.g., Najash, Dinilysia, Yurlunggur, Wonambi, and Sanajeh; TRS pers. obs, and Palci and Caldwell (2014).”. Actually, this is the case for Dnilysia, Sanajeh, and the Najash specimen MPCA 500 (Palci and Caldweel, 2014; Garberoglio et al., 2019a; Zaher et al., 2023). But the basioccpital is described as being excluded from the floor of the recessus scalae tympani by the otoccipital in Wonambi, Yurlunggur, and other specimens of Najash (Scanlon, 2005, 2006; Garberoglio et al., 2019a, 2019b).
Line 206. “has a similar trapezoidal shape”. Similar to what?
Line 232. Authors say that “ There is no midsagittal crest on the ventral surface of the basisphenoid ... as also observed in Najash and Dinilysia (Garberoglio et al. 2019a; Zaher & Scanferla 2012),. However, a low midsagittal ridge is present in some specimens of Najash, according to Garberoglio et al. (2019a).
Line 235. basisphenoid (Extended Data Figure 1c,f). Please check correct figure reference. The opening of the Vidian canal is labeled in Extended Fig 1a, and is definitely not visible in Extended Data Fig 1f (which shows only parietal and supraoccipital).
Line 253. “this contact between the prootic and the supraoccipital is a condition also found”. The particular condition in these snakes is that the contact is visible/exposed in the skull roof.
Line 255. Authors declare that the contact between the prootic and the supraoccipital, also found in Dinilysia, Najash and basal alethinophidians was observed by: TRS pers. obs, and Garberoglio et al. 2019a. However, there are other previous descriptions of this feature in the mentioned fossil taxa that are omitted here, such as Estes et al., 1970, and Zaher et al., 2009. Additionally, this condition is not observed in any specimen of Najash, being the contact between prootic and supraoccipital concealed by the parietal in some specimens (Garberoglio et al., 2019a, 2019b).
Line 273. Extra space after fossa.
Line 278. A reference to Extended Data Fig 1h should be inserted after “tympani”.
Line 280. Dot necessary among snakes and The.
Line 280. About the retroarticular process absence, see comment in the main text.
Line 424. Concerning the proposed “small parazygantral pits are present on either side of each zygantrum on most vertebrae”, these foramina are not parazygantral foramina, since they are inside the zygantral cavity.

G. References: appropriate credit to previous work?
Yes.
In line 275 of the supplementary, add Garberoglio et al. (2019a)

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
Abstract is clear but in line 31 should say gen. et sp. nov., not only sp. nov.
In the Introduction, line 45, authors refer that snake evolutionary history extends back to at least the Middle Jurassic. Although we agree, there is competing evidence that proposed Parviraptorids are nor stem-snakes (Benson et al., 2025), and this should be taken into account at least.
In line 57 says borrowing instead of burrowing.
In the etimology section (line 85) the words Tupí-Guaraní both have accent at the end.
Conclusions are ok.

(Remarks on code availability)

Referee #5

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
This is much improved version of the manuscript, and addresses many of my previous critiques and concerns. This is especially the case for the morphometric analysis, and I commend the authors for their very clear documentation of morphometric landmarks, incorporation of appropriate specimens in analysis, and enhanced discussion of methods and results. The diagnosis could be improved and the discussion of character support (and some of the characters themselves) require revision. Importantly, this version has introduced segmented C-T data for Najash (Extended Fig. 8), which allows for easier identification of unambiguous diagnostic morphology for Tametara and would better test for ecological diversity among stem snakes if included in the endocast morphometric analysis. Specific comments and responses to revisions are as follows:

Taxonomy and systematics
In response to my critique of the use of a differential diagnosis to distinguish Tametara, the authors provided a series of counter arguments as well as adding five phylogenetically inferred autapomorphies to the text and Supplementary Information. They provide additional justification and notes for recognizing Tametara as a distinct taxon from Najash in their response to my initial review.
The authors state:
“Differential diagnoses are commonplace in the taxonomy of fossil vertebrates as quite often enough parts are not preserved or not enough specimens are known to provide features exclusively known for a taxon. We note that we do not consider “autapomorphies” to be the most appropriate terminology for this in a species diagnosis, since the term explicitly depends on a phylogenetic context, and homoplastic autapomorphies not only occur but are frequent. Having said that, five phylogenetically inferred autapomorphies were recovered for Tametara and had been listed in our Supplementary Information (synapomorphies list section), four being unambiguous autapomorphies. We note the phylogenetically supported autapomorphies had been listed in our differential diagnosis.”

While it is certainly true that differential diagnoses are common for erecting taxa based on fossils, they are more common for taxa represented by limited remains (e.g., vertebrae), not usually the most anatomically complex portion of the skeleton. And whereas phylogenetic inference is required to determine the diagnostic utility of characters distributed in more than one taxon, an unambiguous autapomorphy is an observation unique to the taxon in question and thus does not require an a priori phylogenetic topology for identification.

The authors go on to state:
“Furthermore, additional features distinguish Tametara from Najash and Dinilysia at the level of neuroanatomical organization, including the configuration and relative proportions of the cerebral hemispheres and the olfactory bulbs (see the comments below on neuroanatomy).”

Extended Data Figure 8 does an excellent job of illustrating the unique morphology of the frontal in Tametara relative to all snake taxa included in the phylogenetic analysis. The frontal is either apomorphically short anteroposteriorly, or it is incomplete anteriorly and the impression of the olfactory bulb is thus short and posteriorly positioned relative to the length of the element. Why not include the anatomy of the frontal in the diagnosis, as it appears to be unique and is key to the ecological interpretations of endocast shape?

With respect to synonymy of Tametara and Najash, they state:
Note 1: We highlight that all the comparisons above were based on personal inspection of nearly all referred materials of Najash and Dinilysia; thus not rely exclusively on the described literature. For greater clarity, all specimens observed are listed in Supplementary Table S1).

Note 2: We also have access to CT scans and the first author has participated in the recent re-description of Najash referred to by the reviewer (Garberoglio et al. 2019a). Therefore, we respectfully disagree with the referee´s concerns and with some of their comments and differences in interpretation of the anatomy of Tametara and Najash: e.g., shape of the postfrontal, especially in the absence of images supporting such statements.

As the authors have C-T data for Najash, I implore them to please include those data in this study (as they have with Fig. 8) and make them available! This will allow for tests of the personal observations of Note 1 and will only increase the impact of the study.

Note 3: in paleontology, one is forced to rely almost exclusively on the morphological criterion for species delimitation. However, geography and chronology can also provide complementary evidence. Tametara is quite different from Najash in both instances: Najash comes from La Buitrera, Upper section of Candeleros Formation, Río Negro province, northern Patagonia, Argentina (Cenomanian; 100.5-99.3Ma). Tametara comes from Adamantina Formation, Bauru Group, Bauru Basin, Presidente Prudente, State of São Paulo, Brazil (late Santonian to early Campanian age; 85–75 million years ago). In other words, they are separated by nearly 3,000 Km and at least 14 million years in time. No other fossil species of snake known to us exhibits such a wide geographical and chronological range combined with such a collection of differential diagnostic features.

As the authors are aware, incorporating geographic and temporal data into taxon identification introduces circularity and bias into evolutionary and biogeographic reconstructions (e.g., Bell et al. 2010) and should be avoided. With respect to the stated context differences between Najash and Tametara, I refer the authors to the fossil records of extant snake genera such as Python, Boa, and Eunectes, all of which possess modern geographic ranges equivalent to, or greater than, the distance between Río Negro and São Paulo and possess diagnostic fossil records of greater temporal duration than the age difference between Najash and Tametara.

In conclusion, not recognizing the numerous differences and criteria used above to draw a distinction between Tametara and Najash would not only be a critical overlook from our part if not recognized here in the erection of new genus, it would also seriously risk a (well-justified) immediate taxonomic revision of Tametara, thereby jeopardizing its significance and the long-term impact of the present study"

Incorporating a robustly diagnostic character/set of characters, such as the morphology of the frontal, would make the taxonomic hypothesis of Tametara more resilient to further revision than the current list of characters (see discussion of the margo lateralis below).

Morphometric analysis/ecological inferences
As stated above, if the authors have access to C-T scans of Najash (as they do from Fig. 8), then it would greatly strengthen the impact of the study to include an endocast of the taxon in the morphometric analyses. Inclusion could either corroborate fossorial habits for Tametara+Najash or demonstrate fossoriality in Tametara as an early experiment in snake ecology.

One important correction remains outstanding with respect to morphospace and ecological interpretation of telencephalon morphology. The authors state:
"On another note, we recognize the incorrect mention of Xenophidion as an uropeltoid in the original text. This error has been corrected now. After re-examining the literature, we also agree that Xenophidion is more appropriately described as semi-fossorial rather than fully fossorial, and the ecological characterization has been updated accordingly in the revised manuscript."

However, MS Pg. 12 lns. 355-356 reads "In fact, Tametara it represents one of the most extreme examples among snakes, clustering near highly modified fossorial extant forms such as Xenophidion (Fig. 4A and Extended Data Fig. 9b)."

I am sure the authors did not intend to retain the description for uropeltids ("highly modified fossorial extant forms") in reference to Xenophidion. And given that they now recognize that Xenophidion is semi-fossorial, the close position in morphospace does not support the hypothesis that Tametara is highly fossorial. I recommend the authors simply delete the sentence altogether. Doing so does not weaken their hypothesis.

Phylogenetic analysis
In my initial review I stated: "Both the margo ventralis (subcentral ridges) and margo lateralis (interzygapophyseal ridges) of vertebrae occur across crown snakes (Auffenberg, 1963; Holman, 2000; Szyndlar and Georgalis, 2023- with few exceptions for margo ventralis [Hoffstetter and Gasc, 1969]), and do not differentiate stem from crown taxa."

To which the authors replied:
"With regards to the polarity of the vertebral characters: almost any given phylogenetic character will undergo homoplasy across different parts of a given tree. Hence, plesiomorphic "absent" character states can still be variably "present" among stem taxa. As long established in phylogenetic inference, it is the ultimate test of character congruence or overall character agreement, that defines the final output and character polarity, not individual examples. The fact that some early snakes bear some character states that are different from Tametara is irrelevant to determining character polarity, since many other early snakes match the morphology of Tamatera, having a stronger influence in ancestral state optimization and, therefore, character polarization."

On this I completely agree with the authors and realize that I was unclear in my statement referring to the distribution of presence of these characters in crown snakes. I was not critiquing character polarity and its use in establishing interrelationships of Tametara. Instead, my observation was to indicate that the character states were miscoded for crown snakes. For example, the margo lateralis is scored in the data matrix of this study (Morpho_166t_394ch_Simple.nex) as absent on posterior presacral vertebrae (character 312) for all examined crown snakes except for Calabaria, Boa, Naja, and Laticauda. I have checked character coding for this character against C-T scans for multiple extant taxa included in the data matrix. Contrary to the codings in the matrix, the margo lateralis is present as a ridge (albeit sometimes reduced) connecting the zygapophyses throughout the posterior presacral and even cloacal vertebrae in Anilius scytale, Cylindrophis ruffus, Tropidophis melanurus, Xenopeltis unicolor, Loxocemus bicolor, Acrochordus javanicus, and Python molurus (I can provide

images if desired). Because the character is more widely distributed than currently scored at least within crown Alethinophidia, the MS needs to reassess codings and impact on determination of synapomorphies or minimally provide a more robust justification/character state delineation for their coding as it stands. Additionally, the MS describes the character for posterior presacral vertebrae, but only illustrates “cervical”, mid-presacral, and dorsal vertebrae for Tametara. Is Extended Fig. 2c a more posterior presacral vertebrae used for coding the character? If so, it should be identified as such. If not, it would be useful to illustrate the character for the taxon to demonstrate how it is different from the condition in crown taxa.

With respect to homoplasy hypotheses required to explain the occurrence of an upper temporal arcade in Sanajeh based on the topology of this analysis, the authors replied to my critique as follows:
“Different data sets analyzed with different algorithms will naturally provide different evolutionary trajectories for specific characters. As dozens of different studies have assessed character evolution in snakes and other squamates, we find that having specific concerns or comparisons focused on Zaher et al. (2023)on the evolution of the upper temporal bar to be of somewhat out of scope and of narrow focus with respect to the intended audience of this study. Furthermore, Zaher et al. (2023) did not apply the phylogenetic methods employed here, making comparisons, even if warranted, not meaningful.”

To be clear, It is not a question of methodological choice in phylogenetic analysis, nor comparisons of different studies- It is the accurate character coding for the upper temporal arcade in Sanajeh in this analysis combined with the chosen phylogenetic topology from this analysis (MS fig. 3) that optimizes the upper temporal arcade as either lost twice or secondarily re-expressed in the early evolution of snakes. As the preferred phylogenetic hypothesis requires homoplastic reorganization of the skull roof, and because this topology is a unique hypothesis for the interrelationships of basalmost snake divergences relative to the lead author’s previous phylogenies (e.g., Garberoglio et al., 2019; Simões et al., 2025), and because, as the authors note in their reply to review, there is not strong morphological support for the chosen topology in the absence of molecular data, the authors absolutely need to recognize the requirement for skull roof homoplasy as a caveat to their listed character support for the interrelationships of Tametara and Najash among stem snakes (MS pg. 10 lns. 282-296). Cautious interpretation and consideration of alternative hypotheses do not necessarily negate the impact of important discovery (e.g., Benson et al. 2025).

Bell, C.J., Gauthier, J.A. and Bever, G.S., 2010. Covert biases, circularity, and apomorphies: a critical look at the North American Quaternary Herpetofaunal Stability Hypothesis. Quaternary International, 217(1-2), pp.30-36.
Benson, R.B., Walsh, S.A., Griffiths, E.F., Kulik, Z.T., Botha, J., Fernandez, V., Head, J.J. and Evans, S.E., 2025. Mosaic anatomy in an early fossil squamate. Nature, 647(8090), pp.673-679.
Simões, T.R., Tollis, M. and Burbrink, F.T., 2025. Origin and Early Evolution of Squamates and Their Kin: From Fossils to Genomes. Annual Review of Ecology, Evolution, and Systematics, 56.

(Remarks on code availability)

Referee #2

(Remarks to the Author)
Dear authors, dear Editor,

We thank the authors for their thorough replies to our comments and for accounting for most of our concerns. We find the manuscript has improved in its newest version. We provide below a few additional suggestions to further improve the manuscript.

Sincerely,
Rémi Allemand & Marion Segall

Title: The two publications you mentioned that use the word “brain” are on birds, which present a good fit between the endocranium and the brain. You could use braincase or brain cast instead of brain. But we suggest to let the editor choose whether he is fine with the word 'brain'.

LDA:
from your response to our previous comment about working on a subset of PCs: ”Regarding the number of PCs used, we retained only the first five components and excluded the remaining ones because they contribute marginally to total shape variance and reduce model performance: a notorious property of PCA on 3D GMM data.”
We would be interested in your references.

Using only a subset of data can be considered cherry picking, it would be the same as getting rid of some landmarks because you consider that they only add noise. I am a strong advocate of working on raw data. This being said, I am not a statistician. I suggest asking a specialist about that issue. I remember Miriam Zelditch advocating to work on whole datasets, either Procrustes coordinates (which themselves are problematic since they violate one of the basic principles of variance analysis which is that the number of variables must be less than the number of individuals, and again. I am not a statistician and I do not know the workaround Adams and colleagues have found but their method is widely used now) or all PCs. PCs are a convenient way of having n-1 observations. PCAs are not biologically oriented, it is a mathematical decomposition of variance. It is nice when one or several PCs bear an ecological signal but if they don't, it does not mean that there is no ecological signal in a dataset.

Again, I would suggest either adding references to support your choice to use a subset of PCs and/or to ask a specialist.

“We explicitly evaluated the impact of PC number on LDA performance. Using PHYLO_PCA scores, 5 PCs yielded the best overall classification results (Accuracy = 0.6504; Kappa = 0.5045), where accuracy reflects the overall predictive performance of the model and Kappa measures classification agreement corrected for chance. This outperforms analyses based on two PCs (Accuracy = 0.5363; Kappa = 0.3326) and all eleven PCs (Accuracy = 0.6146; Kappa = 0.4595).”

We thank the authors for providing the comparison of LDA performance across different numbers of PCs. However, selecting the number of PCs based on classification accuracy alone may introduce bias, as the subset maximizing performance on the dataset does not necessarily reflect the most biologically meaningful representation of shape variation. In this context, the use of five PCs appears primarily driven by optimization of classification results, which may artificially improve performance. Moreover, the difference in performance between five and eleven PCs is relatively small compared with the difference between two and five PCs. This suggests that including additional PCs does not substantially degrade classification and may provide a more complete representation of shape variation. Using the full set of PCs would therefore appear more statistically conservative.
We agree that the classification of the fossil as fossorial is unlikely to change when using all PCs, but relying on the full dataset would strengthen the robustness of the inference.
Regarding the interpretation of Dinilysia switching from terrestrial (5 PCs) to aquatic (all PCs), this pattern may instead reflect the limited discriminatory power of telencephalon shape among most ecological categories. Indeed, the ANOVA results (Supplementary Table 3) indicate that telencephalon shape does not significantly differentiate ecologies for extant taxa, except for fossorial versus all other groups. In particular, aquatic and terrestrial taxa are not distinguishable ($p = 0.687$). Under these conditions, the change in classification of Dinilysia when additional PCs are included is not necessarily evidence that lower-variance PCs add noise, but rather reflects the weak ecological signal in the dataset.

Which leads us to another comment :

Telencephalon as a predictor of ecology:
L314: You state that the telencephalon shape is a “reliable predictor of habitat use in squamates” and cite one of your previous publications. While that study does include analyses of telencephalon shape, it appears to focus on locomotor categories rather than habitat use. The extrapolation from locomotion to habitat therefore seems stronger than what is directly supported by the analyses.
Moreover, the conclusions of that study appear relatively cautious. For instance, you note that: “However, post hoc pairwise comparisons only reveal significant morphological differences between a few locomotor modes (table S4), including between burrowing or facultative burrowing and other limbless movements. This suggests that whole-brain shape parameters may be a weak indicator of squamate locomotion, at least based on the squamate dataset used in this study.” This seems difficult to reconcile with the statement that telencephalon shape is a reliable predictor of habitat use.
The statement at L314 may therefore be too strong and could be revised to better reflect the scope of the cited study. Since the present manuscript directly tests the relationship between telencephalon shape and ecology, the results can stand on their own without relying on this claim. More generally, it may also be preferable to avoid overstating the ecological signal, as the analyses presented here suggest that telencephalon shape primarily distinguishes fossorial taxa from other ecologies, while differences among the remaining categories appear limited.

Squamate versus snakes dataset: “We appreciate the reviewer’s suggestion. However, we do not see a mechanism by which a more inclusive squamate sample would obscure ecological signal in the geometric morphometric analyses. On the contrary, including the full squamate breadth provides a more robust morphological and ecological context for interpreting snake brain shape variation. For this reason, we do not consider it necessary to modify the sample size or composition.

The composition of the sample should reflect the biological question being addressed. Here, the aim appears to be inferring the ecology of a snake fossil rather than testing whether the fossil falls within snakes versus other squamates. Including a broad range of non-snake squamates may therefore introduce large-scale morphological differences that are not directly relevant to the ecological question.
More inclusive sampling can affect morphospace structure by increasing variance driven primarily by major clade-level differences rather than ecological variation. In extreme cases, this may reduce the interpretability of principal components in an ecological framework. For instance, adding very distantly related taxa would likely distort morphospace by emphasizing major anatomical differences unrelated to ecology.
Figure 4b appears to illustrate this issue. The main axis of variation (PC1) seems largely driven by differences between snakes and lizards rather than ecological differences. As a result, PC1 becomes difficult to interpret in terms of ecology for Tametara. This effect appears less pronounced in Figure 4a.
That said, given the strong fossorial signal recovered for Tametara, we do not consider it necessary to modify the sampling. However, we suggest acknowledging that including non-snake squamates may influence the structure of morphospace and reduce the ecological interpretability of the main axes of variation.

Line 123: “Therefore, Tametara is intermediate in skull size, relative to the extant Cylindrophis ruffus (skull length =29.16 mm) and Xenopeltis unicolor (skull length = 36.33 mm).”
This statement relies on a predicted value (~33 mm), for which the method of estimation is unclear, rather than a direct measurement. In addition, the comparison with two extant species is not justified, and the conclusion that Tametara “is intermediate in skull size” appears unsupported. It is unclear what this comparison is intended to demonstrate.
If the goal is simply to provide a size comparison with extant fossorial species, the sentence could be rephrased more cautiously. For example: “Compared to extant fossorial species, the skull size of Tametara would likely fall between

Cylindrophis ruffus (skull length = 29.16 mm) and Xenopeltis unicolor (skull length = 36.33 mm)."
Alternatively, the authors could clarify how the predicted value was obtained and justify the choice of comparative taxa.

Lines 129–138 & 139–154: Lines 129–138 describe the postfrontal, supratemporals, and then the quadrate, whereas lines 139–154 describe the frontal followed by the parietal.
The order in which the bones are described remains confusing and would benefit from a more consistent anatomical sequence.

Lines 152–154: "…it has no 'medial parietal pillar', which typically contributes to the separation between the telencephalon and mesencephalon in snake brains; similar to the condition in Dinilysia, Najash, and scolecophidians (blind snakes)."
Please provide references supporting this statement.

Line 159: We do not understand this sentence. The first part of the paragraph refers to overlap of the supraoccipital by the parietal, whereas the second part of the paragraph discusses the relative size of the supraoccipital. The logical connection between these statements is unclear.

Line 213: "This correspondence enables meaningful quantitative comparisons among extant snakes"
This sentence is unclear. Why does the correspondence between brain and endocast would enable meaningful quantitative comparisons? The two cited references compared endocasts, not brain and not the correspondence between the two. The word "comparison" is vague in the sentence. Do you mean studying the endocast enables to draw conclusion on the ecology of species? If so, you need to rephrase and to remove the first part of the sentence about the brain-endocast correspondence since it is not what either of us have tested, nor what the present work tests.

Lines 214-215: It has also been used both for qualitative assessments of fossil endocasts relative to extant taxa to inform interpretations of brain ecomorphology, and for quantitative comparisons of fossil squamates within a phylogenetic context
This is unclear. How does brain–endocast correspondence allow quantitative comparisons if those comparisons are based only on endocasts?

Lines 227-228: "This diverges from the condition observed in most squamates, including other snakes, which display well-expanded cerebral hemispheres that strongly contrast with the mesencephalon and rhombencephalon (= mid-and hindbrain, respectively)"
A similar pattern is present in other species as well (see Extended Data Fig. 7), especially in fossorial species and large species (see also Allemand et al 2017, 2022, and Fig 4 in Segall et al 2021 showing the allometric pattern in snakes endocranium). This should be clarified.

Lines 230-233: "Interestingly, however, the telencephalon condition of Tametara closely resembles that of species with high degrees of fossoriality, including fossorial snakes such as scolecophidians (e.g., Liotyphlops), Anilius, and Uropeltis, as well as other fossorial, limb reduced non-ophidian squamates such as the amphisbaenian Blanus and the skink Melanoseps (Extended Data Fig. 7)".
This is confusing. The previous sentence states that the condition diverges from most squamates, but here the authors say it resembles certain snakes. The relationship between these statements should be clarified.

Line 239: "The mesencephalon of Tametara is unusually elongated relative to most other squamates"
Could you be more precise? In which direction is it elongated? For example, the condition seems comparable to Thamnophis in Extended Data Fig. 7.

Lines 240-241 : "The mesencephalon of Tametara is unusually elongated relative to most other squamates, including snakes, with a poorly defined optic tectum (= optic lobe). Among extant snakes, the most similar condition to Tametara is seen in Cylindrophis36,40 (Figs. 1i,j and 2j and Extended Data Fig. 1i-k)."
We do not see how it is "unusually elongated". A poorly defined optic tectum is a condition shared by many species of snakes except highly visually oriented species. Consider rephrasing.

Lines 245-246 : "The rhombencephalon of Tametara is characterised by a strongly laterally constrained, hourglass-shaped region (Fig. 2j and Extended Data Fig. 1i,j), as similarly observed in Anilius scytale and Dinilysia".
This morphology appears to be present, to different extent, in most species since it corresponds to the space occupied by the inner ear.

Lines 285-287: "The inferred placement of Tametara prior to the split of Dinilysia and all other snake fossils is explained by plesiomorphic skull features, including the presence of a dorsum sellae on the basisphenoid (absent in later-deriving snakes)"
We are not sure to understand what you mean by "later-deriving snakes"? Are you implying that all extant species of snakes lack a dorsum sellae? If so, the feature is present in most alethinophidian snakes, but not in the Uropeltidea snakes (at least the ones that we checked) or Anilius, and some weird species like Atractaspis. This statement therefore needs clarification.

Line 315 & 322: 'We find that the earliest known snake brain endocasts of Tametara and Dinilysia..." These results indicate that snakes achieved substantial disparity in brain shape'
Only telencephalon shape is tested here, not the whole endocst(even less the brain). Please make sure to only refer to the telencephalon here.

Supplementary data, line 195: Missing period in “T mirim”.

Supplementary L363 (previously flagged in first review):
“Compared to extant snakes, Tametara mirim and Dinilysia, have remarkably large pituitary glands, although the length of these structures might only correspond to the elongate parietal of these species, bearing no significant sensory effect…”
This remains unclear. If the apparent enlargement results from parietal elongation rather than pituitary size, please clarify explicitly how the morphology of the parietal affects the interpretation of pituitary gland size. What do you mean by “bearing no sensory effect”? To our knowledge, the pituitary has a role in hormone secretion, but we may have missed some information.

L235-236: “In the diencephalon, the posterior portion of the pituitary region is notably large (Fig. 2J and Extended Data Fig. 1j), tilting strongly ventrally towards the hypophyseal fossa”.
How do you differentiate the pituitary region from the hypophyseal fossa?

L237-238: “Among extant snakes, fossorial species do not show a markedly large pituitary, while marine and terrestrial forms typically have a more ventrally developed gland”
What do you mean exactly by “large”? Do you mean that the hypophyseal fossa is deep? Or do you mean that, according to the endocranial imprint, the pituitary is wide? According to the end of the sentence, by large, you mean ventrally developed. Some fossorial snakes present a large pituitary, such as Calabaria reinhardtii, Eryx sp., Calamaria lumbricoidea…

L329-332: “This pattern suggests that derived lineages may converge secondarily on brain morphologies resembling ancestral conditions, highlighting an unexpected discrepancy between the transitional skull anatomy of stem snakes and the strongly divergent morphology of their central nervous system”
We found this interpretation unclear. It is not evident which taxa are considered derived, what is meant by “ancestral” brain morphology, or how the results support secondary convergence. The proposed discrepancy between skull and brain morphology should be clarified or the sentence rephrased.

L351: “Kappa value (0.5) indicates a robust classification”
Given that Kappa ranges from 0 to 1, a value of 0.5 may be better described as moderate rather than robust. Similarly, an overall reclassification accuracy of 65% appears relatively limited and may not support a strong claim of classification performance.
It would also be informative to report classification rate by ecological category. As suggested by the ANOVA results, telencephalon shape appears to distinguish fossorial taxa from other ecologies, but provides limited discrimination among the remaining groups. The overall accuracy may therefore mask uneven performance across categories. For example, the model may correctly classify fossorial species at a high rate while showing lower accuracy for other ecological categories. Providing the percentage of correctly classified taxa for each ecology, particularly fossorial species, would help clarify the actual discriminatory power of the model and better support the interpretation.

(Remarks on code availability)

Referee #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Referee #4

(Remarks to the Author)
The manuscript is well written and robust, and it does not show deficiences to prevent its publication.
Conclusions are original.
The results are interesting enough for several researchers in evolutionary biology and vertebrate paleontology.
We were not able to do a code revision since the data matrix is excessive for our hardware devices.

A. Summary of the key results
Authors present a new species of a Cretaceous stem-snake more or less equivalent (but younger) to Najash rionegrina.
Their main goal is to obtain, through high-resolution micro-CT scans new details of the neuroanatomy.
They also recognise a well-defined fossorial lifestyle for Tametara, contrasting with other stem and early crown snakes. This way they demonstrate early major ecological transitions in snake evolution, and that known stem species do not allow us to point the original ecological condition of crown snakes.

B. Originality and significance: if not novel, please include reference
The paper is original and is significant in demonstrating a well-defined fossorial stem-snake not only by the anatomical analysis of vertebrae but also by features of the neurocranium. This allow authors to discuss with stronger tools the broad range of ecological adaptations in early snakes, something already suggested by the Jurassic material (Caldwell et al., 2015).

C. Data & methodology: validity of approach, quality of data, quality of presentation
Data and presentation are optimal.

D. Appropriate use of statistics and treatment of uncertainties
Nothing to say here

E. Conclusions: robustness, validity, reliability
Conclusions are robust and valid.

F. Suggested improvements:
In Line 106, discussing the ontogenetic stage, it was not clear to us what the degree of overlap between parietal and prootic here meant, since the dorsal exposure of the supraoccipital in Tametara is actually greater than in most snakes, specially adults. Additionally, we failed to mention earlier that fully fused neural arches to vertebral pleurocentra is not a clear indicator of adult ontogenetic stage for snakes, since the neural arch and the vertebral pleurocentra are already fully fused in very early ontogenetic stages in snakes, including fossil and extant (Winchester & Bellairs, 1977; Xing et al., 2018).

In Line 116, classifying them in dermatocranium, splanchnocranium, and neurocranium, we accept terms provided by Kardong (2012) but at least in the Kardong vrsion we have, the pterygoid is part of the dermatocranium, not part of the splachnocranium.

Additionally, it seems that the authors misunderstood what was suggested as a possible part of the stapedial shaft. Actually, we are uncertain of the identification of that small fragment of bone in Tametara that is now labelled as the stapes (Sta), but its robust morphology and placement do not particularly match the stapedial shaft of either Najash or Dinilysia (image in attached version). What is circled in red in the image above taken from Fig. 2 of the MS is what was suggested as a possible portion of the stapedial shaf in Tametara by us, rather than being part of the left prootic as reconstructed by the authors. We should have explained our observation with such an image in our past revision (sorry for that). This element is thin and located in contact or close to the suprastapedial process of the prootic. As such, morphology and placement matches the stapedial shaft of Najash and Dinilysia.

Line 133. Not agreed. What the authors are interpreting in the picture above as the fronto-parietal suture in MACN-RN 1013 is, in fact, just part of a crack within the parietal, the actual suture is more anteriorly located, and is clearly W-shaped as in the holotype and mentioned in the previous descriptions. See the picture below including an actual photo of the specimen, and an interpretative drawing of the same from Caldwell and Albino (2002). (image in attached version).

Line 165. Respect to the absence of the retroarticular process in Tametara, We understand that this point is very subjective, and it is now made clear in the MS that the absence in Dinilysia in contrast with previous descriptions is the authors interpretation based on personal observation, which is perfectly fine (reference to observed material of Dinilysia should be added to this character in Supplementary Table 1). However, we still fail to understand what is the rationale behind considering the presence of a small retroarticular process in Najash (in agreement with Garberoglio et al., 2019, but the new Supplementary Table 1 states this feature as previously undescribed for Najash) and to find no evidence of such a process in Tametara, since there it seems to be no distinction between the two. An image of MPCA-500 taken from Garberoglio et al. (2019) is attached to be compared to Tametara from Fig. 2 in the MS.

In Line 255, concerning the contact between the prootic and the supraoccipital in Najash, authors removed the Attribution to Najash. However, this was not necessary. Our original observation (saying “not observed in all”, and not “not observed in any”, don’t know why is changed here) referred that it is visible in some specimens and not in other. Actually, it is described originally for Najash.

G. References: appropriate credit to previous work?
Yes.

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
Abstract is clear.
Conclusions are ok.

(Remarks on code availability)
We were not able to do a code revision since the data matrix is excessive for our hardware devices.

Referee #5

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Version 2:

Reviewer comments:

Referee #1

(Remarks to the Author)
The manuscript continues to improve, and my only outstanding critique remains character coding for the presence of margo lateralis on posterior presacral vertebrae and its use as a component of the diagnosis for Tametara. Otherwise, the authors have either made recommended modifications or justified their original approaches in response. Revisions to the diagnosis and the added discussion of homoplasy in the evolution of the upper temporal bar in snakes required for their preferred topology definitely strengthen the manuscript. One minor fix to the section on the required number of losses of the upper temporal bar- it should be three losses ((Najash+Tametara), Dinilysia, the clade crownward of (Dinilysia+Sanajeh)), following the topology of Fig. 3.
With respect to margo lateralis, in my previous review I noted that it is scored as absent in most extant snakes, but is actually observable in many of the species coded as absent. As a result, I stated that the MS needed to reassess the codings for this character and their impact on determination of synapomorphies, or at least provide a more robust justification for the codings as presented. In reply the authors stated:
“Concerning this point, we simply have a different interpretation of what constitutes the absent condition for this character. As the reviewer acknowledges above in “the margo lateralis is present as a ridge (albeit sometimes reduced)” . Indeed, the margo lateralis can be quite faint and regressed across many species, which we consider a gray zone of interpretation for the absent state, and so the choice of the character state in such situations is likely to change from author to author. On top of this, there is the matter of other sources of intraspecific variation—sexual, ontogenetic, population—that may drive different interpretations depending on the exact specimen examined. The latter can be a major source of variation that is unfortunately still underexplored in morphological systematics. Therefore, although we recognize that some researchers could score a different state for this character for some of the taxa, we have kept our scoring for these taxa based on the specimens we examined and to keep consistency in our interpretation of the morphology across all taxa. We will keep this comment in mind in future expansions of this dataset to look for sources of intraspecific variation that may be driving those differences.

They also state:
“The characters we defined from the posterior presacral vertebrae are interpreted consistently, including in the midsection of the presacral region. Although vertebral shape may vary to some extent (which could be captured by finer-scale linear morphometric measurements), the discrete features remain relatively stable. This is why we illustrate only one dorsal for Tametara in ED Fig. 2c, as all dorsals more posterior to it do not differ substantially—note, however, that many other dorsals are illustrated in Fig. 2 of the main text. So, even if the most posteriorly preserved dorsals in Tametara are not fully posterior, and represent mid-dorsals, that would not change our scoring or description.”

While it is certainly the case that multiple sources of variation can drive character interpretation, the diagnostic utility of the presence of margo lateralis on posterior presacral vertebrae for recognizing Tametara is based on its reported distribution in other snake taxa and therefore coding for the character should be repeatable. To test this, I have compared the dorsal vertebra from Tametara (ED Fig. 2c) with articulated posterior precloacal vertebrae from taxa which are coded as lacking the margo lateralis. This comparison is appropriate following the authors’ above discussion of character continuity along the presacral vertebral column in Tametara. For the included figure, Tametara (A) is compared with Tropidophis haetanius (B), Xenopeltis unicolor (C), Acrochordus javanicus (D), Loxocemus bicolor (E), and Anilius scytale (F). Arrows point to the margo lateralis in three views for extant taxa, and it is identified in Tametara in lateral view from the manuscript.
Although I do not disagree with the authors’ statement that character interpretations can differ based on variation, in this case, the crest is present to the same degree of development in the posterior precloacal vertebrae of extant taxa (which is coded as absent in the data matrices of the study) as it is in the dorsal vertebra in Tametara (which is coded as present for posterior precloacal elements). If the authors’ observations of morphologies in other specimens are not consistent with those figured here, then there is clearly confounding variation in delineating character states and they need to reconsider using the character in phylogenetic analysis as currently coded. At minimum, they should note that this character is variable in their Extended Data section and not uncritically include it in their diagnosis of Tametara. This issue also applies to inconsistent coding of the margo ventralis (character 293), as is visible in the figure.

Referee #2

(Remarks to the Author)
Dear Authors,

We thank you for your thorough replies to our comments and for addressing most of them. We have no further comments and find the manuscript suitable for publication.

Congratulations on such a good work.

Sincerely,

Marion Segall and Rémi Allemand

Referee #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #4

(Remarks to the Author)
Authors have considered our comments and concerns and justified them accordingly.
The manuscript is now completely acceptable for its publication.

Referee #5

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Version 3:

Reviewer comments:

Referee #1

(Remarks to the Author)
The authors have provided a figure to demonstrate their character delineation for the margo lateralis/interzygapophyseal ridge. I cannot repeat the observation that the development of the ridge in Tametara (e.g., Extended Fig. 2C) is more similar to that in Yurlunggur relative to posterior precloacal vertebrae of taxa such as Xenopeltis (for example, compare the degree of lateral offset of the ridge from the lateral margin of the centrum in ventral view from my previous review iteration).

That being said, the authors have revised the MS to address the variability in the character as they define it and have removed both the margo lateralis and ventralis from the formal diagnosis. Therefore, I am happy to recommend acceptance for publication.

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Referees' comments:

Referee #1 (Remarks to the Author):

Overall, this is an interesting submission that has the potential to be of broad interest and to inform on the ecological diversity of early snakes, breaking the now antiquated and ossified “fossorial vs. marine” dichotomy of snake origin ecologies. The writing is generally good, and the figures are excellent. The authors have provided full access to all of their data for review, which is exemplary.

> Thank you for your supportive comment.

There are minor-to-substantial issues with taxonomic and systematic assignments, anatomical interpretations, data selection for morphometric and phylogenetic analyses, and explanations of materials and methods. There are also errors and inconsistencies in terminology and figure reference within both the main text and the supplemental information section. I discuss these in detail below. Some of these issues can be resolved with simple rewording/text reorganization and the inclusion of additional text for clarification. Others will require reanalysis and likely reconsideration of results. Once these are achieved following additional review, I would think the submission of appropriate impact.

Taxonomy and systematics

The MS provides only a differential diagnosis for Tametara without a single unique apomorphy to recognize the new taxon. This is disappointing given the relative completeness of the specimen, and I suspect it arises from an attempt to differentiate the specimen from the hypodigm of Najash. Comparing the figures of Tametara from the MS with those for Najash from Garberoglio et al. (2019) and Zaher et al. (2009) reveals that the two are very, very similar, and that most of the characters used to differentiate the taxa are subtle or rely on poor preservation. As an example, the presence of a small retroarticular process is reported in Najash but not Tametara (MS pg. 6, lns. 163-164). Comparison with the undistorted right compound bone of Najash (Garberoglio et al. fig. 2A, D) with Tametara (MS fig. 2A-E) shows no difference in the development of the retroarticular process between the two. The morphology of the postfrontal is described as different between Tametara and Najash based on the absence of a parietal process in the former (MS pg. 5, lns. 118-120). However, the shape of both the postfrontal (Garberoglio et al. fig. 1A; MS fig. 2B, D) and the embayment within the anterolateral margin of the parietal to receive it appear very similar

Ultimately, there are only a few characters differentiating Tametara from Najash that can be readily discerned from the data provided in the MS and published accounts of the latter. Nuchal crests of the supraoccipital and slightly greater overlap of the parietal onto the supraoccipital are very thin wire upon which to hang a new genus, and the authors should consider revising the MS to make MPM 420 a new specimen, and arguably a new species, of Najash. This will allow the MS to provide a robust diagnosis for the

genus, which is currently lacking, and will not at all detract from the palaeoneurology and ecological inferences of the study.

> Differential diagnoses are commonplace in the taxonomy of fossil vertebrates as quite often enough parts are not preserved or not enough specimens are known to provide features exclusively known for a taxon. We note that we do not consider "autapomorphies" to be the most appropriate terminology for this in a species diagnosis, since the term explicitly depends on a phylogenetic context, and homoplastic autapomorphies not only occur but are frequent.

Having said that, five phylogenetically inferred autapomorphies were recovered for *Tametara* and had been listed in our Supplementary Information (synapomorphies list section), four being unambiguous autapomorphies. We note the phylogenetically supported autapomorphies had been listed in our differential diagnosis.

For greater clarity, we now noted this more explicitly in the Diagnosis section (marked with * therein).

Additional features (previously described in the main text and SI) also discriminate *Tametara* from *Najash* and *Dinilysia*. **For greater clarity**, we have now added a new table (differential diagnosis) summarizing all features that differentiate *Tametara* from *Najash*/*Dinilysia* (new Supplementary Table S1).

In summary, there are seven important differences between them in Supplementary Table S1. This is substantially more than a "few" differences, spanning features of the parietal, supraoccipital, postfrontal, quadrate, compound bone, and vertebrae. Furthermore, additional features distinguish *Tametara* from *Najash* and *Dinilysia* at the level of neuroanatomical organization, including the configuration and relative proportions of the cerebral hemispheres and the olfactory bulbs (see the comments below on neuroanatomy).

Regarding similarities between *Tametara* and *Najash*: we do not disagree that there are important similarities. This was expected given their sister-group relationship. Accordingly, we conducted extensive comparisons with *Najash* in the Main Text (Osteological Description Section) and Supplementary Text . We believe our results and description accurately reflect these similarities and do not undermine them.

Note 1: We highlight that all the comparisons above were based on personal inspection of nearly all referred materials of *Najash* and *Dinilysia*; thus not rely exclusively on the described literature. **For greater clarity**, all specimens observed are listed in Supplementary Table S1).

Note 2: We also have access to CT scans and the first author has participated in the recent re-description of *Najash* referred to by the reviewer (Garberoglio et al. 2019a). Therefore, we respectfully disagree with the referee´s concerns and with some of their comments and differences in interpretation of the anatomy of *Tametara* and *Najash*:

e.g., shape of the postfrontal, especially in the absence of images supporting such statements.

Note 3: in paleontology, one is forced to rely almost exclusively on the morphological criterion for species delimitation. However, geography and chronology can also provide complementary evidence. *Tametara* is quite different from *Najash* in both instances: *Najash* comes from La Buitrera, Upper section of Candeleros Formation, Río Negro province, northern Patagonia, Argentina (Cenomanian; 100.5-99.3Ma). *Tametara* comes from Adamantina Formation, Bauru Group, Bauru Basin, Presidente Prudente, State of São Paulo, Brazil (late Santonian to early Campanian age; 85–75 million years ago). In other words, they are separated by nearly 3,000 Km and at least 14 million years in time. No other fossil species of snake known to us exhibits such a wide geographical and chronological range combined with such a collection of differential diagnostic features.

In conclusion, not recognizing the numerous differences and criteria used above to draw a distinction between Tametara and Najash would not only be a critical overlook from our part if not recognized here in the erection of new genus, it would also seriously risk a (well-justified) immediate taxonomic revision of *Tametara*, thereby jeopardizing its significance and the long-term impact of the present study.

Neuroanatomy and endocasts

Analysis of reconstructed neuroanatomy based on endocasts, and the implications for inferring early snake habits and locomotory modes, are the key findings of the submission. The results of these analyses are intriguing. However, there are several potentially problematic issues with the shape data used to generate results. These issues include inconsistences in the shapes of reconstructed endocasts, interpretation of the telencephalon shape of Tametara, and inconsistencies in which aspects of endocast morphology are included in the landmarking scheme.

The MS includes a diversity of squamate endocasts as proxies for neuroanatomy, based on established close shape relationships between endocasts and neural tissues. However, the reconstructions for several taxa do not match previously published endocasts, suggesting that the dataset in this study is not sufficiently capturing the range of variation within taxa and/or ecologies. This is exemplified by the endocast of Anilius (Extended Data fig. 7). The telencephalon of the specimen in this study has olfactory bulbs that are anteroposteriorly shorter than the cerebral hemispheres. This contrasts with other published endocasts (Allemand et al., 2017; Scanferla, 2022) in which the bulbs are longer than the hemispheres. This discrepancy is likely due to ontogenetic differences in skull shape; Based on comments in the text and the acknowledgments, it is likely that the Anilius specimen used in the MS is UMMZ 248356, which is a contrast-stained subadult. If the morphometric analysis is comparing different semaphoronts, then it may be creating (at least partially) artificial ecological morphospaces. This may be especially problematic with respect to the non-analog positioning of Dinilysia patagonica (see attachment). The MS should include information

about the specimens used and rerun the analysis with an endocast of a representative adult Anilius and appropriate semaphoronts of other taxa.

> We thank this Referee for highlighting this issue and for drawing attention to the morphology of the *Anilius scytale* endocast. We agree that the specimen originally included in the analysis does not fully match the morphology illustrated in previous publications (Allemand et al., 2017; Scanferla, 2022). As noted by the Referee, the *Anilius* specimen initially used in our study was a contrast-stained sample obtained from MorphoSource, for which no explicit information on ontogenetic stage is provided in that source. After further consideration, we agree with their observation on the ontogenetic status of that specimen.

We addressed this by replacing the original *Anilius scytale* endocast with a different specimen (the corresponding MorphoSource identifier is provided in the revised Supplementary Data S3) that closely matches the morphology described in previous studies, notably displaying olfactory bulbs that are anteroposteriorly longer than the cerebral hemispheres (see revised Fig. 3 and ED Fig. 7). This updated specimen is consistent with published adult morphologies and removes the potential ambiguity associated with ontogenetic variation.

In addition, we carefully re-examined the remaining extant taxa included in our dataset to verify that their endocast morphologies are coherent with those reported for the same species in the literature. No other clear discrepancies were identified. A minor adjustment was made to the endocast of *Cylindrophis ruffus* (see revised Fig. 3 and ED Fig. 7), in which the olfactory bulb region was slightly extended to better match previously published reconstructions.

In our updated results (see revised Fig. 4a and ED Fig. 8), both taxa now occupy positions in the morphospace closer to *Dinilysia*, as expected. However, we highlight that these changes did not result in our core findings and conclusions: the uniqueness and quite disparate placement of Dinilysia and Tametara relative to other snakes in morphospace.

Tametara is described as having “short, bulky olfactory bulbs with anterior curvature” (MS pg. 7, lns 197-198). The bulb is identified as an anterior extension of the digital endocst(Fig. 2j) which appears to be defined by articulation of the parietal with the posterior margin of the frontal (e.g., Fig. 2e). The endocranial space for the olfactory bulbs extends through the antero-posterior interorbital length of the frontal in squamates (e.g., Allemand et al. 2022), but only a small portion of the posterior frontal is preserved in Tametara, indicating that the full size and shape of the olfactory bulb endocast in the taxon cannot be known. The elongate frontal of nearly identical Najash (e.g., Garberoglio et al., 2019, Fig. 2c) as sister taxon to Tametara suggests that less than 50% of the frontal is preserved in Tametara, and the taxon may have instead possessed a more elongate, anteriorly projected bulb (endocast). The MS needs to revise discussion in light of missing data or justify how they extrapolate the shape of the bulbs, given that the interorbital region is mostly absent.

To illustrate these points, I have digitized an endocast of an adult Anilius scytale and compare It in dorsal and right lateral views to the endocasts of Anilius, Tametara, and Dinilysia from Extended Data fig. 7. The attachment should be included with this review. Arrows indicate the approximate positions of the frontoparietal suture. The position of the articulation for Anilius from fig. 7 is based on the midline position of the suture for UMMZ 248356. I scaled the Anilius endocasts to a common cerebral hemisphere width and Dinilysia and Tametara to approximately a common telencephalon + mesencephalon length.

The differences between the two Anilius specimens are extreme with respect to the size of the olfactory bulbs, with the larger specimen being much more Dinilysia-like in shape. The approximate position of the frontoparietal suture in Tametara demonstrates that there is almost no interorbital region preserved on the specimen and that the olfactory bulbs are not complete. The reconstruction of Dinilysia includes the dorsal border between the mesencephalon and the telencephalon. Previous reconstructions of the same specimen were unable to differentiate this border, and the MS should include some indication of how it was discerned, especially if it may have implications for landmark identification in morphometric analysis.

> We thank the Referee for raising this important point and for prompting us to clarify an aspect that was insufficiently explained in the original manuscript. We appreciate the opportunity to make our reasoning and methodology clearer.

In the landmarking protocol (Allemand et al. 2023), the most anterior landmark used on the telencephalon corresponds specifically to *"the imprint left by the olfactory bulbs and peduncles on the ventral surface of the frontal bone"*. Our inference of olfactory bulb shape and extent is therefore grounded in the endocranial imprint preserved on the ventral surface of the frontal.

To clarify the points above we now provide a new comparative illustration (ED Fig. 8f-h) showing extant taxa and fossil specimens (including *Dinilysia*, *Tametara*, and *Najash*), which demonstrates that the completeness of the olfactory bulb endocast is not necessarily correlated with the total anteroposterior extent of the frontal bone. As shown in this figure, the proportion of the frontal that may be lost without affecting the preservation of the olfactory bulb imprint varies markedly among squamates.

We note that in some taxa, even minimal truncation of the frontal can result in loss of olfactory bulb information, whereas in others the dorsal surface of the frontal extends far anteriorly relative to the ventral endocranial surface housing the olfactory bulb—e.g. *Blanus*, which is explicitly illustrated in the landmarking protocol by Allemand et al. 2023, including the anterior telencephalic landmark, and *Rhineura*. In these taxa, up to roughly half of the frontal could be missing without compromising the integrity of the olfactory bulb imprint and assignment of this landmark. Importantly, the condition observed in *Tametara* closely resembles that seen in *Xenophidion*, rather than representing an extreme or anomalous morphology.

Similarities in cranial vault configuration and in the morphology of the internal surface of the frontal between *Xenophidion* and *Tametara* can also be appreciated in the revised and expanded ED Fig. 8a,c, which shows how *Xenophidion* possesses a very short frontal (in dorsal view) while still enclosing a complete olfactory bulb. The shape and relative length of these bulbs closely match those observed in *Tametara* when scaled accordingly (relative to the anteroposterior extent of the cerebral hemispheres). This comparison reinforces the conclusion that, regardless of whether the frontal of *Tametara* experienced minimal or more extensive dorsal truncation, the olfactory bulb remains fully contained within the preserved portion of the bone, enabling assignment of landmark 1.

To clarify the points above, we have added these remarks for the placement of landmark 1 into the revised Methods section (MS lines 905-910).

In addition, this configuration of the relative proportions between the cerebral hemispheres and the olfactory bulbs in *Tametara* appears to depart substantially from that of *Najash*, representing an additional feature distinguishing the two fossils at the level of neuroanatomical organization (see ED Fig. 8f-h).

For *Tametara* and *Dinilysia*, we clarify that the colored overlays illustrating brain subdivisions are inferred reconstructions intended solely to provide a spatial representation of major brain regions. For extant taxa, these subdivisions are informed by iodine-stained scans of the same specimens or species, which enable direct visualization of soft neural tissues, allowing a reliable representation of the individual subdivisions on the corresponding endocast. For fossil taxa, the subdivision boundaries are necessarily inferred based on comparative anatomy, but this does not affect our analyses, as the focus of our study is restricted to the telencephalon and the boundaries between subdivisions are not involved in landmark placement.

To clarify the points above, the criteria used to apply the subdivision overlays to the different specimens are now explicitly stated in the Methods section as well as in the corresponding figure legend (see Fig. 3).

Morphometric analysis

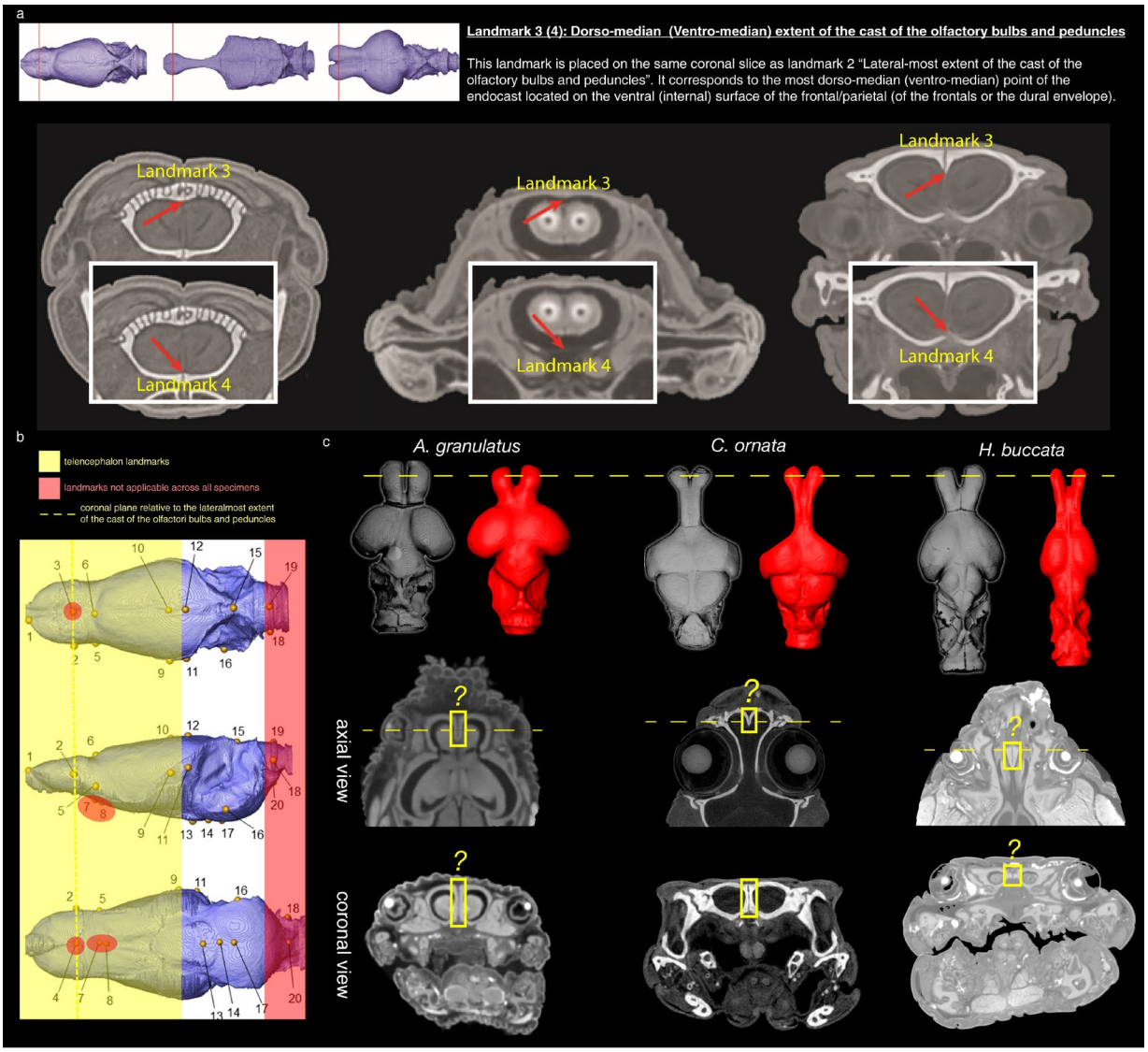
The absence of a detailed discussion of methods and data makes interpretation of the morphometric analysis, especially the landmarking scheme, difficult. The morphometric analysis of the submission is based on six homologous landmarks distributed across one side of the telencephalon and illustrated for Tametara (Extended data Fig. 8A), and the MS cites Allemand et al. 2022 for their landmarking protocol and some of the data used in analysis (MS pg. 32, lns. 796-806). However, the landmark ordering or even positions illustrated on Tametara for this study do not correspond to those of Allemand et al. 2022 (Fig. 2). As a result, it is not possible to determine what are the anatomical homologies represented by the landmarks and how any morphometric data from Allemand et al. was reordered or converted for this study. The MS needs to be revised to include a more detailed discussion of the landmarking scheme including anatomical homologies and should include the distribution of landmarks on representative extant

taxa as well as Dinilysia in a figure similar to Extended Data fig. 7.

Presuming that landmark 1 represents the anterior edge of the telencephalon, the placement of that landmark on Tametara is inaccurate and misrepresents the shape for the taxon. If this is the case, the authors should omit that landmark and redo the GM analysis to focus on the shape of the cerebral hemispheres, which are preserved in all taxa and appear a priori to show differences in lateral and dorsal curvature in fossorial vs. non-fossorial taxa (e.g. Extended Data fig. 7). It is also worth noting that the GMM analysis uses very few landmarks for the telencephalon. There should be more discussion to justify this given that the endocast of Tametara preserves other structures such as the choroid plexus and the pituitary endocast, both of which could be landmarked to provide a more robust representation of brain shape in the sampled squamates.

> We thank this Referee for highlighting this shortcoming in the original Methods section. We appreciate the opportunity to clarify our approach. As indicated in the previous comment, we have updated our Methods (MS lines 878-910), added figures illustrating the landmarking scheme (see ED Fig. 8a-d), and how they are applied to fossil and representative extant taxa. In addition, the figure now includes a table reporting the formal definitions of each landmark, following the original squamate endocast landmarking protocol (Allemand et al. 2023) together with the explicit correspondence between those landmarks and the subset used in this study (see ED Fig. 8e).

In summary: the restricted number of landmarks used, and their confinement to the telencephalic region, are primarily motivated by issues related to fossil preservation and taphonomic distortion. Moreover, we found that landmark #3 of the standardized protocol could not be consistently applied to our dataset. As a result, only 13 of the original 20 landmarks defined in the standardized protocol can be reliably placed across our dataset. These landmarks are distributed across multiple brain regions, but only 6 of them can be consistently digitized on the telencephalon of all specimens, representing the maximum landmark configuration applicable to this region alone. We therefore prioritized a landmark configuration that captures a single, functionally coherent brain region while minimizing the confounding effects of taphonomic distortion. This choice reflects a trade-off between landmark number and anatomical reliability and was made to ensure that the resulting morphospace reflects biological signal rather than artefacts due to sample deformation. These considerations have now been explicitly included in the revised main text and the Methods section (MS lines 878-910).



Anatomical constraints on landmark applicability across squamate endocasts.
(a) Images and definitions/instructions describing landmark 3 and 4 placement extracted from the standardized squamate protocol publication (Allemand et al. 2023). **(b)** Endocast of *Blanus cinereus* illustrating the standardized squamate landmarking protocol (from Allemand *et al.*, 2023). Landmarks highlighted in yellow correspond to those located exclusively on the telencephalon and retained in this study. Landmarks highlighted in red indicate positions that cannot be consistently applied across all specimens in the dataset. **(c)** Examples of taxa with posteriorly divergent olfactory bulbs, shown using endocasts derived from independent studies (Allemand et al., 2017, left, and this study, right), demonstrating that this condition is consistent across datasets and highlighting the absence of suitable midsagittal endocranial space at the coronal level of maximum lateral bulb expansion, which prevents unambiguous landmark placement under the criteria specified in the standardized protocol, as shown in the corresponding axial (upper) and coronal (lower) CT slices.

It is not clear what phylogenetic topology was used to reconstruct the phylomorphospace. Tametara is discussed as close to Xenophidion in the morphospace as evidence of fossorial habits (MS pg. 11), but Xenophidion is not included in the phylogenetic analyses of the MS, and I could not find any indication of where the topologies for the .tre files in the morphometric data folders were sourced. Also, the most comprehensive molecular phylogenetic analyses place Xenophidion as sister taxon to Caenophidia (e.g., Figueroa et al., 2016), not as sister taxon to Boidae as in the .tre files. Xenophidion is not a uropeltoid as stated in text (MS pg. 11, ln. 317), and

its ecology is described as semi-fossorial, not fully fossorial as in uropeltoids (e.g., Quah et al., 2018 contra Feldman [2015]).

> We thank the reviewer for highlighting the need for clarification regarding the phylogenetic topology and the treatment of *Xenophidion*. The phylogeny and protocols used for all phylogenetic comparative methods used herein (both GMM and bone microstructure) are explained in our Methods section "Time-tree for Phylogenetic Comparative Methods" (MS lines 927-931 and 1009-1019).

To clarify, we did not use a mix of various phylogenetic sources, which could result in a chimeric tree. Instead, we have focused the relationships of all extant taxa based on the most recent, comprehensive, and widely adopted topology of Title et al. (2024). Using a single tree source is the standard approach in phylogenetic comparative methods, including similar studies preceding ours, implementing the same techniques—e.g., (Allemand et al. 2023; Macrì et al. 2023).

Following the reasoning above, it is impossible to conciliate a single phylogenetic tree reference with all possible variations on the placement of specific taxa of more ambiguous phylogenetic placement, such as the placement of *Xenophidion* discussed by the Referee (recovered in a slightly different position compared to older analyses).

On another note, we recognize the incorrect mention of *Xenophidion* as an uropeltoid in the original text. This error has been corrected now. After re-examining the literature, we also agree that *Xenophidion* is more appropriately described as semi-fossorial rather than fully fossorial, and the ecological characterization has been updated accordingly in the revised manuscript.

Phylogenetic analyses

Phylogenetic analyses, including methods, data availability and presentation/ interpretation of results are comprehensive and readily available, and I commend the authors for this. That being said, topologies resulting from the different analytical runs suggest that the morphological dataset used for inferring key nodes may not be robust. For example, topologies derived from just morphological data (Extended Data Fig. 3) result in interrelationships among snakes that are not corroborated by any prior morphological or molecular studies, including erroneous groupings of fossorial taxa such as the elapid Atractaspis with scolecophidian (s.l.) and uropeltoid taxa, as well as a failure to resolve relationships among and between Constrictores and Caenophidia.

Combined analyses also produce conflicting results, including all fossil taxa forming a clade (Extended Data Fig. 4), and the MS relies on results from tip- and node-dating to conclude that Tametara and Najash are stemward of all other known snakes. As a result, molecular data and rate models are doing a lot of heavy lifting to generate the preferred topology of the MS. This is problematic given that previous studies of the interrelationships of included fossil snakes have recovered Dinilysia and/or Sanajeh as stem-ward of Najash (Garberoglio et al., 2019; Zaher et al., 2022) based, in part, on

more comprehensive morphological data sets (Zaher et al., 2022). The limitations of the morphological characters are evident when scrutinizing the character justification for the placement of Tametara:

> We are satisfied that the referee is happy with our detailed explanation and availability of data and analytical methods.

Regarding the morphological topology results the reviewer is critical, we agree with them on those specific points. In fact, these points are exactly why none of our final results or conclusions are drawn from the analyses of morphological data alone, nor most state-of-the-art studies in modern phylogenetics. As long established in squamate systematics, including recent reviews on this topic (Simões and Pyron 2021; Simões et al. 2025), morphological data alone have almost never been able retriever key relationships that are now well established by genomic data; This aspect is a broader characteristic of squamate systematics, and far from exclusive to this study. Hence, all our conclusions on the phylogenetic placement of fossils are drawn from the total evidence analysis, not the morphology only analysis. We note that morphology only results are presented here merely for transparency on the morphological signal and bookkeeping, besides the fact this work will be of interest to paleontologists, who most often utilize morphological data only.

Regarding the small variations in results for combined evidence data using non-mechanistic non-clock bayesian inference and clock analyses, these differences among inference methods are not only expected but also common across studies using combined evidence for various methodological reasons discussed over the past decade. We refer the reviewer to discussions of this point by Ronquist et al. (2016); Pyron (2017); Simões et al. (2020a). In this specific case of squamate phylogenetics, such differences are detected in literally all total evidence studies performed to date; e,.g, (Simões et al. 2018a; Simões et al. 2020b; Martínez et al. 2021; Tałanda et al. 2022; Benson et al. 2025). Precisely because of such possible variations, we prefer to embrace and present such variation rather than not conducting these analyses or not presenting their results.

Importantly, regardless of differences across inference methods, it is now well known from simulation and empirical studies that tip dating using mechanistic models in total evidence dataset is the most accurate approach for inferring phylogenies and divergence times, especially for deep time phylogenies; e.g., (Mongiardino Koch et al. 2021, 2023). Hence, we retain these as our final results, from which broader discussions are drawn from.

Finally, we note that the phylogenetic dataset and analyses performed herein replicate those that have already been recently scrutinized and published in a heavily methods-oriented study—Simões et al. (2026), *Systematic Biology*.

The MS states “The inferred placement of Tametara prior to the split of Dinilysia and all other snake fossils is explained by plesiomorphic skull features, including the presence

of a well-developed dorsum sellae on the basisphenoid (absent in later-deriving snakes), absence of a mid-sagittal crest of the basisphenoid (conspicuous among simoliophiids, Wonambi, and many early crown snakes), and, most notably, otoccipitals not meeting in the midline above the foramen magnum (separated by the supraoccipital)" (Pg. 9). Of these characters, a basisphenoid mid-sagittal crest is absent in Dinilysia, as well as scolecophidians (s.l.) and variably among fossorial alethinophidians within crown snakes (e.g., List, 1966; Zaher and Scanferla, 2012; Strong et al., 2021).

> Agreed. Not only absent in *Dinilysia*, but also absent in *Najash*, which supports the placement of *Tametara* close to these stem snakes rather than more derived stem snakes (e.g., simoliophiids).

The description of the development of the dorsum sellae is contradicted by the extended description of the parabasisphenoid in the supplementary information section where it is described as "weakly developed" (pg. 7, ln. 221) [note, this section also refers to Extended Data figure 1e, but that is the otoccipital, the sellae is visible in figure 1c, where it is labelled as "dorsal sellae" in the figure caption]. The dorsum sellae appears prominent relative to the sella turcica in Tametara, as in non-snake squamates, but that appears due at least in part to the incomplete preservation of the anterior region of the basisphenoid. The more complete parabasisphenoid in Najash also as a more well-developed dorsum sellae than in Dinilysia, but not more so than multiple crown taxa (e.g., Gauthier et al., 2012, pg. 195, character 324). This character is also not figured in the character descriptions and is coded as "?" for Dinilysia in the morphological data nexus files which further undercuts its utility as an indicator of a basal position for Tametara. The MS needs to revise the description and use of this character to be consistent across the text, should code it appropriately for what is known about Dinilysia, and should provide an illustration of how the dorsum sellae is plesiomorphically more developed in Tametara than in Dinilysia and crown snakes.

> We agree on the previously ambiguous description of the dorsum sellae. This has now been edited accordingly: it is well-developed relative to most other snakes. We also fixed the figure legend.

We note that does not impact our analyses because we treat this character only as absence and presence of this anatomical structure (not its subjective interpretation on size), which is entirely absent in derived snakes. We also note that despite illustrating 100+ characters in our character list (an effort much beyond the norm in morphological datasets), this particular character was not illustrated because it is readily identifiable by squamate specialists. Besides, it has already been illustrated by Gauthier et al., 2012, pg. 195, character 324, as correctly pointed out by the reviewer.

Regarding the presence of the dorsum sellae in *Dinilysia* (Ch. 181, previously scored as "?"), we have now changed it to "1" (present), following "Zaher & Scanferla 2012, p. 211: "The dorsum sellae is distinct but low…", as suggested.

“The MS also states “*Tametara* further differs from crown snakes by retaining the basipterygoid process of the basisphenoid, the foramen chorda tympani on the articular, the absence of accessory processes on the prezygapophyses, and the presence of both margo ventralis and lateralis.” (Pg. 9). Several of these characters are also problematic: Basipterygoid processes are present in multiple crown snake lineages and are coded so in the morphological data matrix of the MS. The matrix is miscoded for *Sanajeh*, which possesses large basipterygoid processes (Wilson et al., 2010). Both the margo ventralis (subcentral ridges) and margo lateralis (interzygapophyseal ridges) of vertebrae occur across crown snakes (Auffenberg, 1963; Holman, 2000; Szyndlar and Georgalis, 2023- with few exceptions for margo ventralis [Hoffstetter and Gasc, 1969]), and do not differentiate stem from crown taxa. The MS also incorrectly refers to the structures as “interzygantral” ridges (Supplemental Information). Thus, character support for a basal position of *Tametara* is not as robust as stated in the text, and the MS should revise this discussion to be limited only to those characters that demonstrate no, or minimal, homoplasy under optimization from the synapomorphies list.

> Correction: “*The matrix is miscoded for Sanajeh, which possesses large basipterygoid processes (*Wilson et al., 2010*).”. --> Sanajeh* is, in fact, scored as having basipterygoid processes present in our matrix (state “0” means present for this character).

With regards to the polarity of the vertebral characters: almost any given phylogenetic character will undergo homoplasy across different parts of a given tree. Hence, plesiomorphic “absent” character states can still be variably “present” among stem taxa. As long established in phylogenetic inference, it is the ultimate test of character congruence or overall character agreement, that defines the final output and character polarity, not individual examples. The fact that some early snakes bear some character states that are different from *Tametara* is irrelevant to determining character polarity, since many other early snakes match the morphology of *Tamatera*, having a stronger influence in ancestral state optimization and, therefore, character polarization.

Regarding reviewer suggestions, we have edited the SI text accordingly (interzygantral to interzygapophyseal).

The preferred topology of the MS also indicates profound homoplasy in the evolution of the snake skull roof with respect to Sanajeh. Zaher et al. (2022) demonstrated that Sanajeh retained an upper temporal arcade consisting of the postorbital and squamosal, unlike Najash, Dinilysia, and all crownward snakes. The positioning of Sanajeh as sister taxon to Dinilysia crownward of Tametara+Najash in this study requires either three independent losses of both elements, or redevelopment of the postorbital, squamosal and the arcade they form in Sanajeh. As the MS notes that the preferred topology only requires a single loss of external hindlimbs in the stem-crown transition in snakes, it should also note that it requires multiple losses (or reacquisition) of the upper temporal arcade in the snake stem based on Sanajeh.

> Different data sets analyzed with different algorithms will naturally provide different

evolutionary trajectories for specific characters. As dozens of different studies have assessed character evolution in snakes and other squamates, we find that having specific concerns or comparisons focused on Zaher et al. (2023)on the evolution of the upper temporal bar to be of somewhat out of scope and of narrow focus with respect to the intended audience of this study. Furthermore, Zaher et al. (2023) did not apply the phylogenetic methods employed here, making comparisons, even if warranted, not meaningful.

(Remarks on code availability):

I did not run the code for analyses, but I examined the associated datasets. Critiques of those are included in the main review. The lead author should be aware that the Datadryad links for Ref. 38 on the Sys. Biol. webpage are invalid and that Datadryad has no available data sets for that reference. That should be fixed before this publication is accepted.

> We appreciate the feedback on the availability of the dryad link for that paper. We will notify Dryad accordingly.

We note that, since this is an independent study, all of the data and codes used here (even when similar to the ones in the aforementioned SysBio paper) have been made available for review with this submission and will be published on a different repository pending acceptance, as already indicated in our data availability statement:

"as Supplementary Data S1 at Simões Lab Dataverse at https://dataverse.harvard.edu/previewurl.xhtml?token=86ebcd6d-3f65-405a-82ae-488451a354d8 [private link for review only] and in MorphoSource [temporary private link]: https://www.morphosource.org/projects/000780586/temporary_link/tknCvRoDHdwdq3LMiqSYW3ga?locale=en.".

Referee #2 (Remarks to the Author):

Dear authors and editor,
We co-reviewed the manuscript entitled “Exceptional brain and ecological diversity in the earliest snakes” by Simoes and colleagues considered for publication in Nature.

A. Summary of the key results
In this manuscript, the authors describe a new snake fossil Tametara mirim, which is exceptionally well preserved, retaining its 3D shape, including an almost complete skull, numerous vertebrae, and articulated postcranial elements. The authors described the anatomy of the fossil and used its brain and inner ear endocasts, along with bone microstructure to make ecological inferences by comparing it with extant squamates species. Their results suggest that T. mirim was fossorial and possessed a skull morphology distinct from that of other early snake fossils, particularly Dinilysia.

B. Originality and significance: if not novel, please include reference
We consider this manuscript to be of interest to both a large scientific and non-scientific audience. The exceptional preservation of the fossil, combined with its comparison to both extant and extinct species using multiple approaches (3D geometric morphometrics, bone microstructure, and morphological characters), provides convergent evidence for a fossorial ecology. This is particularly valuable in the context of the ongoing debate about the ecological origins of snakes.

C. Data & methodology: validity of approach, quality of data, quality of presentation
The methodology is correct but could be improved, especially regarding the 3D GMM, but overall, the authors made an effort to place their fossil into a biological context using various methods.

D. Appropriate use of statistics and treatment of uncertainties
The statistical methods are sound overall, but some parts of the methodology need to be modified or clarified. The provided R codes and associated files for the endocast analysis are easily usable and results can be replicated but not those for the microstructure analysis (see comments).

E. Conclusions: robustness, validity, reliability
We generally agree with the conclusions of the authors, especially regarding the putative ecology of T. mirim.

F. Suggested improvements: experiments, data for possible revision
List below & in attached file.

G. References: appropriate credit to previous work?
Yes.

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions

We strongly suggest being more careful with the terminology used throughout the manuscript, especially the use of “brain” to refer to the endocst(see comment below). We also feel like the authors oversell their work and results that are interesting per se. For instance, they state that T. mirim is completely different from extant species, but their analyses show the opposite, and if it was truly that different, it would make it hard to infer its ecology. We list below our major and minor comments that need to be addressed before publication.

We must acknowledge that we do not have the necessary background to review the phylogenetic part of the manuscript.

Sincerely,
Marion Segall & Rémi Allemand

> We appreciate the strongly positive feedback! We also thank the reviewers for their shared work in providing detailed and important feedback to improve our manuscript. We have responded to the individual suggestions in the comments below.

Major comments:

Main text:

Title: We suggest reconsidering the title, as the use of a superlative, the term 'brain' rather than 'endocast,' and the lack of reference to what seems to be the central focus of the manuscript—a newly discovered, exceptionally well-preserved specimen—may give a slightly misleading impression.

> We understand your perspective on the title. On the other hand, Nature style titles are expected to be short and communicate well to a broad audience. We are afraid that the term endocast will not be understood by most readers outside of organismal biology. Also, the endocast are proxy for the brain, and utilization of “brain” in such context is in agreement with similar studies in this and other journals when using fossil endocast data—e.g., “Cretaceous bird from Brazil informs the evolution of the avian skull and brain”(Chiappe et al. 2024); “Tempo and Pattern of Avian Brain Size Evolution”(Ksepka et al. 2020), “Evolutionary origins of the avian brain” (Balanoff et al. 2013).

Terminology: Throughout the manuscript, you may consider using the term “endocast” (or “endocranial morphology”) rather than “brain” when describing features reconstructed from endocranial data. Endocasts only approximate the external brain shape and include non-neural spaces (e.g., meninges, venous sinuses), so this terminology would provide a more accurate description, except when explicitly discussing inferred neural structures.

> Similarly to the comment above, we opted to follow the literature standard in multidisciplinary journals by referring to endocast reconstructions of fossil brains as simply “brain” within our main text. To balance this out with field specific terminology, we

also make sure to include the term “endocast” in our abstract (“Endocast analyses…), main text, and methods. Secondly, we made sure to start our brain description section (Brain and inner ear description) by clearly stating that we are using endocasts, and that those provide good correspondence to at least forebrain shape in snakes (MS lines 209-221). To make this even more explicit, several instances of “brain” have now been replaced with “brain endocast” throughout the manuscript. We believe this provides an overall compromise between communicating effectively to a broad scientific audience while maintaining scientific rigor.

Systematic Paleontology: Consider reorganizing the manuscript to facilitate reading. Descriptions of the new species and associated information should be presented clearly and in a consistent order.

> We are unsure as to the specific change requested here. However, we have carefully re-examined the section and believe that its current organization facilitates reading while maintaining taxonomic standards. To further improve clarity (and linked to comments of Referee #1), we have added an explicit statement in the Diagnosis section indicating which features represent phylogenetically inferred autapomorphies and directing readers to Supplementary Table S1 for the detailed differential comparison with other snake fossils.

Osteological description: there is no mention of the size of the specimen anywhere. We had to go to the figure to measure its size, which was a big surprise at first since, according to the scale of Fig 1a. the specimen measures about 1m and its skull around 5cm (head length), which is far from being a small specimen. However, we realized that the scale on Fig. 1a. is wrong, it should be about 8.16cm, not 20, according to all other figures for which the scale seems correct and that give a skull length about 2cm (which seems more reasonable for a snake that is supposedly “small-sized”. Scales need to be checked and corrected and a description of the size of the specimen should be added to the main text and discussed, especially since size is the main driver of endocranial shape. Large species have endocranium that are similar to the one of your specimen (anteroposterior elongation etc). But since your specimen is quite small, we agree that it would tend to look like an *Anilius* or *Cylindrophis*.

> Agreed. Indeed, total specimen size was not initially provided. This is now explicitly mentioned (MS lines 121-125) and we have corrected the scale bar for Fig. 1a. We sincerely appreciate your effort on checking our figures in such detail!

Allometry: You wrote in your M&M section that you tested for allometry, and running your code, we found out that there is no allometric signal in endocranial shape in your dataset which would be interesting to discuss in the manuscript, especially since in other studies, allometry is usually significant and represent a relatively large amount of the total shape variation. We wonder whether the lack of signal results from the low number of landmarks in your manuscript.

> We agree with the Referees that allometry is often a significant component of endocranial shape variation in broader datasets. In the present case, we interpret the absence of a detectable allometric signal as likely related to the specific anatomical focus and landmark configuration adopted in this study. Our morphometric analysis is intentionally restricted to the telencephalon, and it is therefore plausible that size-related shape variation is less strongly expressed than in analyses encompassing the entire endocast or multiple brain regions.

We acknowledge that the limited number of landmarks may reduce sensitivity to subtle allometric effects. However, increasing landmark density was not feasible without compromising homology or introducing noise associated with taphonomic deformation and missing data in the fossil specimens (please see the comment on landmarks below and our response to Referee #1).

To clarify these points, we have now briefly discuss the absence of allometry in light of the restricted anatomical scope of the analysis and the constraints imposed by fossil preservation (MS lines 348-350; 881-910).

Olfactory bulbs: These structures are heavily discussed, and a rather large part of the ecological inference is based on its description. However, as mentioned in the thorough description in the SM, the anterior part of the frontal is missing (l70-71) there is “no facet for a possible prefrontal (but we note that the anterior end of the frontal is mostly missing)” and (l74-75) “due to its incompleteness we cannot determine how far ventromedially it could have extended”. The olfactory bulbs do not seem particularly “bulky” (l197), it is hard to tell whether they are short considering that a part of the frontal is missing, and it is hard to understand what you mean by “anterior curvature” (l198).

> We thank the Referees for this comment and fully agree that the interpretation of the olfactory bulbs in *Tametara* requires careful justification, particularly given the incomplete preservation of the frontal bone. We acknowledge that, as originally presented, our description could appear ambiguous, and we have therefore revised both the text and figures to clarify the anatomical basis of our interpretation (see also the answer to similar comments given to Referee #1).

The characterization of the olfactory bulb in *Tametara* is based on the endocranial imprint left by the olfactory bulbs and peduncles on the ventral surface of the frontal. This imprint defines the most anterior telencephalic landmark used in the standardized endocast landmarking protocol (Allemand et al., 2023), and provides a reliable anatomical reference even when the anterior portion of the frontal is incompletely preserved.

To clarify these points, we have provided the explanation above and in greater detail in the revised Methods section (MS lines 905-910), and added a new comparative illustration (new ED Fig. 8f-h) including both extant and fossil taxa (*Tametara*, *Dinilysia*,

and *Najash*), which demonstrates that the completeness of the olfactory bulb endocast is not necessarily correlated with the anteroposterior extent of the frontal bone.

As already illustrated and implicitly supported in the detailed description of landmark #1 placement in the original standardized landmarking protocol for squamate endocasts (Allemand et al., 2023), this figure shows that, in several squamates, substantial portions of the dorsal frontal may be absent without affecting the preservation of the olfactory bulb imprint on its internal surface. In this comparative framework, *Tametara* shows an olfactory bulb configuration closely resembling that of *Xenophidion*, characterized by a relatively short anteroposterior extent, as can also be appreciated in the revised landmarking scheme (ED Fig. 8a,c). We have also revised the descriptive terminology used in the manuscript. Specifically, we now avoid potentially ambiguous expressions such as “bulky” or “anterior curvature”.

L199 “enlarged cerebral hemispheres”. The cerebral hemispheres do not look enlarged compared to most extant species, but compared to *Dinilysia*, sure. This statement should be corrected and the discussion adjusted.

> Agreed. What we meant here is that it is “enlarged compared to *Dinilysia*”. We agree with the reviewers that it is mildly enlarged compared to most other species of snakes, as indicated in the subsequent sentence: “This diverges from the condition observed in most squamates, which display well-expanded cerebral hemispheres”. We have modified the text accordingly to clarify these points (MS lines 225-229).

All along the main text, you compare your specimen with many extant species but in the abstract, you state that (l34-35) “*Tametara* had a brain morphology distinct from both other stem and extant snakes”, while Fig. 4 shows that *T. mirim* is clearly within the space occupied by extant fossorial.

> Our description section provides many points of comparisons with both fossil and extant snakes in order to provide extensive comparative anatomy of this important new species. The quantitative telencephalon GMM analysis is presented only in Fig. 4a, while the qualitative differences in the rest of the brain (with morphology distinct from both other stem and extant snakes) are also described in the text. These aspects are now explained more clearly in the revised manuscript’s main text, including the abstract where we added the terms “Quantitative and qualitative”.

L286-288 “*Tametara* and *Dinilysia* represent highly divergent brain shapes relative both to each other and to most other snakes and non-ophidian squamates”, while the first part of the sentence is true, the second is not for *Tametara*. Your specimen is not isolated from fossorial snakes in any of your morphospaces based on endocranial characteristics (Fig. 4, Extended Data Fig. 8, Extended Data Fig. 10).

> We appreciate this consideration. Same comment as above: only quantitative aspect for telencephalon (visible on Figures) and qualitative for the rest of the brain (as not

possible to assess by GMM; see comment below and given to Referee #1). We have clarified this point in our main text to avoid ambiguity (revised MS lines 318-325).

L310-313: You wrote that "Quantitative analyses further indicate that the early morphological disparity in snake brains reflects variation in their ecological lifestyles. Specifically, our analyses significantly discriminate how different telencephalon shapes relate to habitat usage, especially between fossorial and non-fossorial species (Fig. 4a).". We may have missed it, but we do not find the aforementioned quantitative analyses.

> We thank the Referees for pointing out this omission. We agree that, as originally presented, the manuscript did not explicitly document the quantitative results underlying this statement. We have now addressed this by adding the results to the Supplementary Tables S2 and S3, which reports the outcomes of the phylogenetic ANOVA and post-hoc pairwise comparisons on telencephalon shape across habitats. These analyses show that the telencephalon morphology of fossorial taxa is significantly distinct from that of all other habitat categories.

L326-328: "We did not apply geometric morphometrics on the inner ear, however, given contradictory prior results and limited statistical power for reliable palaeoecological inference". We doubt that all these issues come from the 3D GMM. It is most likely that the inner ear does not bear a strong ecological signal (or at least, a signal that is related to substrate use). However, you based some of your ecological inferences based on the inner ear structure. I would either remove this part of the discussion or be extremely careful in the interpretation.

> We agree that the issue with the utilization of inner ear has to do with the weak ecological signal from that structure and not necessarily from the nature of the analysis. This is what we meant by lack of statistical power for reliable paleoecological inference. However, in light of this comment, we have slightly rephrased the sentence to make this point clearer (revised MS lines 370-373).

L816-817: What shape variables were used? Did you use Procrustes coordinates? Principal Components? If so, how many and how did you choose them?

> We thank the Referee for this comment and for giving us the opportunity to clarify this point. For the phylogenetic ANOVA, the shape variables used are the Procrustes coordinates obtained after Generalized Procrustes Analysis of the landmark configurations. We have now explicitly stated this in the revised Methods section (revised MS lines 917-931) to avoid any possible ambiguity.

L818-821: Why use phylogeny-corrected shapes for the LDA? Why not "raw" shape data? And why 95% of shape variation? Why not all PCs?

> We performed the LDA on phylogeny-corrected shape variables (PHYLO_PCA scores) because this approach reduces the impact of shared ancestry and better targets

the ecological component of shape variation; a long-established approach in the field of phylogenetic comparative methods (Felsenstein 1985).

For completeness, we also ran the LDA using standard PCA scores and obtained essentially identical results under our main setting (five PCs; see below), indicating that our conclusions are not contingent on the use of PHYLO_PCA.

Regarding the number of PCs used, we retained only the first five components and excluded the remaining ones because they contribute marginally to total shape variance and reduce model performance: a notorious property of PCA on 3D GMM data. Specifically in our dataset, the PHYLO_PCA shows a sharp decline in explained variance after PC5: PCs 1-5 together account for 95.13% of the total variance, whereas each subsequent PC contributes only a very small fraction (PC6 = 2.05%, PC7 = 1.33%, PC8 = 0.81%, PC9 = 0.51%, PC10 = 0.15%, PC11 = 0.03%). Including PCs beyond PC5 therefore mainly adds low-variance dimensions with limited explanatory power.

We explicitly evaluated the impact of PC number on LDA performance. Using PHYLO_PCA scores, 5 PCs yielded the best overall classification results (Accuracy = 0.6504; Kappa = 0.5045), where accuracy reflects the overall predictive performance of the model and Kappa measures classification agreement corrected for chance. This outperforms analyses based on two PCs (Accuracy = 0.5363; Kappa = 0.3326) and all eleven PCs (Accuracy = 0.6146; Kappa = 0.4595).

Importantly, *Tametara* is consistently classified as fossorial across all settings, with very high posterior probabilities (about 0.99), indicating a robust and stable signal. In contrast, *Dinilysia* shows increasing instability when low-variance PCs are included: with five PCs it is predominantly classified as terrestrial, whereas inclusion of all PCs shifts the highest probability toward an aquatic assignment, despite posterior probabilities remaining moderate and never strongly decisive. We interpret this behavior as reflecting noise introduced by near-zero-variance PCs rather than a genuine biological signal.

Furthermore, a complementary picture emerges from the typicality probability analysis (Supplementary Table S5), which we introduce in the revised manuscript (see revised Methods section). This approach evaluates how typical a specimen is of each habitat category based on its position in morphospace, rather than forcing an exclusive classification. Consistent with the LDA results, *Tametara* shows high typicality for the fossorial habitat across different PC subsets, whereas *Dinilysia* displays low typicality values across habitats when higher-order PCs are included, further supporting the interpretation that ecological signal for *Dinilysia* becomes obscured by noise when low-variance PCs are retained.

In summary: these results indicate that retaining five PCs represents a conservative and objective compromise: it captures the vast majority of shape variance, maximizes classification performance, yields stable inference for *Tametara*, and avoids artefactual instability driven by near-zero-variance components. **These points have now been clarified** in the Methods section (revised MS lines 935-939).

The habitat attributed to some species seems incorrect: *Physignathus cocincinus* is arboreal same for *Basiliscus vittatus*, *Anguis fragilis* is at best semi-fossorial, *Thamnophis sirtalis* is mostly terrestrial and at best semi-aquatic. In addition, In the Supplementary Text (lines 301–302), *Pseudopus* is listed among "fossorial limb-reduced non-ophidian squamates," but it is generally considered terrestrial (you correctly classified it as terrestrial in your analyses). Please correct this for consistency.

> We thank the reviewer for raising these points regarding habitat classification and for helping us improve clarity and consistency. However, we respectfully disagree. We followed the life history references provided in our Supplementary Data S3 > DATA > sample_lifestyle_Revised, including the largest available databases for life history traits in squamates—SquamBase (Meiri 2024)—further complemented with additional sources (as in "substrate_metadata" column). We note that discrete habitat categories inevitably simplify complex ecological variation, as many squamate species routinely transition among habitats. Current comparative methods require assigning taxa to a single category rather than representing habitat use as a continuum or proportional preference. Unfortunately, this goes beyond our control and the scope of study.

That said, our final classifications are largely consistent with the Referee´s observations. For instance, *Anguis fragilis* transitions across ground dwelling habitats and cryptic microhabitats and is therefore treated here as semi-fossorial. *Thamnophis sirtalis* is commonly classified as semi-aquatic (Scharf et al. 2015; Hu et al. 2020; Meiri 2024), as we did herein. *Pseudopus* is considered terrestrial in both our analyses and by the Referees, and the incorrect reference to fossoriality in the Supplementary Text has now been corrected. *Physignathus* and *Basiliscus* can be classified both as arboreal and semiaquatic—e.g., for *Physignathus*: "arboreal (e.g., Manthey, 2010; Das, 2010), semi aquatic (Bauer and Jackman 2008), arboreal and aquatic (Jenssen and Sy, 2022)", as reported in SquamBase (Meiri 2024). We further highlight that both genera exhibit well-documented morphological specializations for aquatic locomotion: e.g., tall neural spines along the tail modified for propulsion in water in *Physignathus*, and toe fringes in *Physignathus* and *Basiliscus* (Bauer and Jackman 2008). Accordingly, both have been classified as semi-aquatic in multiple reviews: e.g., "*The agamid Physignathus cocincinus, as well as all species of Hydrosaurus are semi-aquatic and the latter possess toe fringes like those of Basiliscus*." And "*The most striking Neotropical aquatic lizards are the members of the corytophanid genus Basiliscus*." (Bauer and Jackman, 2008). Hence, our final classification for these taxa was in the "semi-aquatic" category. We note that this classification also matches the "semi-aquatic" characterization of several lizard species that are terrestrial, but make frequent incursions into aquatic habitats for food procurement and/or escaping predators—e.g., several arboreal/semi-aquatic gymnophthalmids (Marques-Souza et al. 2018).

To clarify these points and address potential ambiguity, we have now clarified our habitat classification criteria in the Methods (MS lines 862-877). Specifically, taxa coded as terrestrial are restricted to species that do not make regular incursions into aquatic

habitats, whereas species that transition between terrestrial (including arboreal) and aquatic environments for foraging or predator avoidance are classified as semi-aquatic.

Coloring for the parts of the endocast: The way the endocasts are colored in several figures prevents from seeing the underlying structures. It is too opaque. Plus, you mentioned in the manuscript that, aside from the telencephalon, the rest of the endocast regions are barely distinguishable on your specimen so how did you color it?

> We thank the Referees for this comment. We agree that, in the original version of the figures, the opacity of the colored overlays could obscure underlying endocast morphology, and we appreciate this observation. We have now revised all relevant figures by reducing the opacity of the colored overlays.

Regarding the basis for coloring the endocasts, the boundaries between major brain subdivisions for extant taxa are derived from iodine-stained CT scans of the same specimens or species, in which brains are directly visualized and the single subdivisions can be distinguished, providing a reliable anatomical framework that can be transferred to the corresponding endocast. For fossil taxa, subdivision boundaries are necessarily inferred based on comparative anatomy.

The methodology used to apply these overlays is now clearly explained in the corresponding figure legend (see Fig. 3).

Landmark scheme:

Please clarify why only six landmarks were used on the telencephalon. This seems limited, especially since Allemand et al. (2023) defined 20 landmarks that can be placed relative to surrounding bones and the labyrinth. Using more landmarks could better capture morphological variation and strengthen the morphometric analyses. Consider justifying the choice of six landmarks, for example using a Landmark Sampling Evaluation Curve (LaSEC; Watanabe 2018, R package LaMBDA).

We have concerns about how you positioned your first landmark since you mentioned that the anterior part of the frontal is missing.

> We thank the Referees for these comments, which allow us to further clarify the rationale underlying our morphometric approach. These points, which overlap with those raised by Referee #1, are addressed below (see also our detailed response and illustration given to Referee #1 for complementary discussion).

To clarify these points, a detailed discussion for landmark number in choice has been included in our Methods section (revised MS lines 878-910)

In summary: the use of 6 landmarks restricted to the telencephalon is not the result of an *a priori* simplification but reflects the maximum number of landmarks that can be placed consistently, homologically, and reliably across all specimens given the

combined constraints of fossil preservation and taphonomic deformation. Ideally, placing all landmarks defined in the standardized protocol would be preferable. However, under the preservation conditions of the fossil material considered here, this is not feasible. Both fossil endocasts, particularly *Dinilysia*, and to a lesser extent *Tametara*, show clear deformation along the anteroposterior and dorsoventral axes. Including landmarks spanning dorsal and ventral surfaces across the full anteroposterior extent of the endocast would therefore capture deformation-induced variation leading biases in downstream analyses. Restricting the analysis to the telencephalon substantially reduces this problem. Although the telencephalon itself is not entirely free from deformation, this configuration minimizes the inclusion of non-biological variance in the resulting morphospace.

Most anterior landmark: we clarify that, in the standardized protocol used here (Allemand et al., 2023), this landmark is explicitly defined as the anterior limit of the endocranial imprint left by the olfactory bulbs and peduncles on the ventral surface of the frontal bone. As illustrated and discussed in the protocol itself (e.g. the condition shown in *Blanus*), the anteroposterior extent of this endocranial imprint does not necessarily correspond to the dorsal extent of the frontal. In several taxa, a substantial portion of the dorsal frontal may extend far anteriorly beyond the endocranial imprint, such that loss of the anterior dorsal frontal does not imply loss of olfactory bulb information. In *Tametara*, despite incomplete preservation of the frontal, the endocranial imprint of the olfactory bulbs is fully preserved, providing a reliable anatomical reference for placement of the most anterior telencephalic landmark. This interpretation is further supported by comparison with extant taxa such as *Xenophidion*, which possess a very short dorsal frontal while still enclosing a complete olfactory bulb imprint of comparable relative length when scaled to the anteroposterior extent of the cerebral hemispheres (see revised ED Fig. 8a,c). Together, these observations demonstrate that the anterior telencephalic landmark in *Tametara* is placed following the same anatomical criterion applied across taxa, and that its placement does not depend on the preserved dorsal outline of the frontal.

To clarify this point, we provide a more detailed description of the landmarking protocol (revised MS lines 878-910) and a new comparative illustration (new ED Fig. 8f-h).

Using LaSEC: we also acknowledge the Referees' suggestion to assess landmark adequacy using the R function LaSEC. However, LaSEC is designed to evaluate how increasing landmark number improves shape approximation under the assumption that additional homologous landmarks can be meaningfully sampled across the structure of interest. In the present case, landmark number is constrained not by sampling effort but by fossil completeness and taphonomic distortion. As such, the limiting factor is anatomical availability rather than landmark density, and LaSEC would not address the core constraint of this dataset.

Regarding geometric morphometrics, consider whether including all squamates might obscure ecological signals. Would it be more informative to focus on snakes alone for some analysis?

> We appreciate the reviewer's suggestion. However, we do not see a mechanism by which a more inclusive squamate sample would obscure ecological signal in the geometric morphometric analyses. On the contrary, including the full squamate breadth provides a more robust morphological and ecological context for interpreting snake brain shape variation. For this reason, we do not consider it necessary to modify the sample size or composition.

Provide details on how landmarks were positioned on the endocast of *Dinilysia*, which shows morphology quite different from other snakes. Could incompleteness affect landmark placement?

> We thank the Referees for raising this point. We agree that the endocast of *Dinilysia* is incomplete, and this aspect was carefully considered during landmark placement. Closer inspection of the internal surface of the frontal bone reveals, on the better-preserved right side, a clear olfactory bulb imprint extending anteriorly beyond the preserved endocast volume. This indicates that the endocast shown in previous publications—e.g. (Scanferla 2022; Macrì et al. 2023)—and the 3D reconstruction used in this study (from Macrì et al., 2023) is anteriorly truncated. The endocast appears to terminate abruptly at its anterior end due to the absence of the surrounding bony structures required to delimit the endocranial cavity in that region, resulting in the loss of the anteriormost portion of the olfactory bulbs. However, this does not affect landmark placement. The most anterior landmark (landmark 1), as mentioned earlier, is defined by the anterior limit of the endocranial imprint left by the olfactory bulbs and peduncles on the ventral surface of the frontal bone (Allemand et al., 2023).

The remaining telencephalic landmarks are not affected by incompleteness, as the relevant portions of the telencephalon are preserved in *Dinilysia*. Their placement relies on anatomical reference planes that are clearly identifiable on the cranial vault and endocranial surfaces. All landmark positions, with explicit illustration of *Dinilysia*, are now explicitly illustrated in the revised ED Fig. 8a-e, which was added to clarify landmark homologies and positioning across both fossil and extant taxa.

It would be informative to have the labels of your landmarks in Extended Data Fig. 8a, if anyone would like to reproduce your landmark scheme.

> We agree with the Referees that explicit landmark labels are essential for reproducibility. To address this point, we have expanded the landmarking-scheme, figure (see revised ED Fig. 8a-e), which now includes labeled landmarks together with their anatomical reference planes traced directly on the cranial vault bones. The revised figure also shows both fossil taxa (*Dinilysia* and *Tametara*) as well as representative extant species, allowing direct comparison across specimens. In addition, the figure now incorporates a table reporting the formal definitions of each landmark and their correspondence with the standardized squamate endocast landmarking protocol (Allemand et al., 2023). These additions make the landmark scheme fully explicit and reproducible.

Statistical analysis:

You tested differences in endocranial shape between ecologies in extant species using pairwise tests but you did not mention these results in the manuscript. Since only fossorial species are significantly different from other ecologies, it would be interesting to mention it since it could strengthen your results.

> We thank the Referees for highlighting this point. We have now included the results of the pairwise comparisons in Supplementary Table S3 and revised the Results section accordingly (MS lines 345-348). In the updated text, we explicitly state that the significant effect of habitat on telencephalon morphology is driven by fossorial taxa, which differ significantly from all other habitat categories, whereas differences among non-fossorial habitats are not significant.

The total accuracy of the LDA is 69%. This information does not appear anywhere in the main text. You wrote L314 that *Tametara* is classified with 96.6% probability within the fossorial group. But how does this number articulate with the overall accuracy of the LDA? It is 96.6% of the overall accuracy (69%)? We recommend giving more details about this method. And again, we suggest using all the PCs, not a subset. We did run your code with all PCs and the overall accuracy of the LDA slightly decreases (63%) but the prediction decreases significantly for *Tametara* from 96.6 to 66% (and 33% of chances of being aquatic) while the percentage for *Dinilysia* being a terrestrial species increases from 78.3% to 84.5%. From a statistical point of view, using a subset of PCs that result from only 6 landmarks feels like you are using a very small amount of shape variation to make ecological inferences. You need to convince us that not using all PCs is statistically sound.

> We thank the Referees for this comment, which allows us to clarify the interpretation of the LDA results and their relation to overall model accuracy.

In our analyses, overall LDA accuracy reflects the global predictive performance of the model under cross-validation across extant taxa, that is, how often specimens are correctly classified into their ecological categories. Posterior probabilities, instead, quantify the strength of assignment of a given specimen to a particular habitat class, conditional on the trained model. These two metrics therefore describe different and complementary aspects of the analysis and are not meant to be directly combined or interpreted as fractions of one another.

An overall accuracy of 65% (with Kappa = 0.5, which quantifies the level of classification agreement beyond what would be expected by chance alone) indicates that the model captures a meaningful ecological signal rather than random structure.

To clarify this point, we have now clarified the meaning of accuracy and its relationship to posterior probabilities directly in the revised manuscript when presenting the inferential results (MS lines 350-357).

Regarding the suggestion to use all PCs, we refer the Referees to our detailed response above.

References and figure citations:

Some references do not match the statements. For example, line 786 cites Mongiardino Koch et al. (2021) regarding published endocasts, but this paper does not include endocasts. Please verify all citations.

> Edited.

Figure references also need checking. In the Supplementary Text (line 40), the postfrontal is cited as Fig. 1b–f and Extended Data Fig. 1a–e, but Fig. 1d–f shows vertebrae, and no postfrontal appears in Extended Data Fig. 1a–e.Likely the correct references are Fig. 1b,c and Fig. 2b–d.

> Edited.

Similarly, in the Supplementary Text (line 172), “Extended Data Figure 2” is cited for the otoccipitals, but this figure appears to show postcranial anatomy instead. Please verify and correct.

> Edited.

Minor comments:

Abstract

L34-35: “Quantitative endocast analyses demonstrate that *Tametara* had a brain morphology distinct from both other stem and extant snakes”. The statement seems too categorical. Some similarities with *Anilius* and *Cylindrophis* are also evident and should be acknowledged.

> See answer to similar comment above: “Although many individual aspects of its anatomy can (and should) be compared to other similar species, the collection of all characters and skull shape is unlike any other snake, which is the point that we make in the abstract and in our conclusions, and supported by our GMM analyses.”. To improve clarity, we have added the terms “Quantitative and qualitative” from the relevant sentence.

L35-36: “revealing substantial early neuroanatomical disparity—and likely sensory functions—in snake evolution”. The claim appears speculative, as sensory functions were not directly tested in this study. You may consider removing or rephrasing this sentence to better reflect the scope of your results.

>Indeed, hence our usage of “likely”, as we cannot test this directly. In our understanding and perception, it’s nearly impossible for such disparate brain morphologies to not be linked to highly different sensorial functions, even if they cannot be directly tested.

L36-38 “Independent evidence from brain shape, inner ear organization, and bone microstructure converges on a fossorial lifestyle for *Tametara*, contrasting with other stem and early crown snakes”. The end of this sentence is not clear, how *Tametara* being fossorial is contrasting with other stem and early crown snakes?

> The contrast here refers to the stem *Dinilysia* (ground dwelling from our results) and stem snakes that are marine (simoliophiids), besides many early alethinophidians that are not fossorial. However, we agreed that this can be better phrased. We have rewritten the end of this sentence.

<u>Main text:</u>

L34-35: remove space before “*mirim*”

> Edited.

Please consider adding references in the main text when comparing with other species.

> Provided references regarding comparisons with fossil species. On the other hand, most extant species unfortunately lack equally detailed osteological descriptions. Most of our comparisons are based on personal observations of hundreds of specimens in natural history collections across multiple institutions, listed in Supplementary Data S2-S4.

L130-132: “*However, the parietal has a short anteromedial process that would have inserted in between the frontals (Fig. 2d), which is absent in the other South American Cretaceous snake*”. It is unclear here. In Zaher & Scanferla (2012), the description of the *Dinilysia* skull indicates that *“anterolaterally, the parietal is clasped by the postfrontal, whereas its anterior surface contacts the frontals in a W-shaped, anteriorly concave, and interdigitating suture.”*

> Indeed, *Dinilysia* was described as having a wavy (or W-shaped) frontoparietal suture. This seems to be derived from observations on the holotype, but the equivalent region in MACN-RN 1013 (best CT-scanned specimen) is in fact nearly concave. We consider the presence of the process ambiguous at best, or possibly polymorphic. Accordingly, we have made this more careful consideration in our description, and updates the score for this character in Dinilysia (Ch. 112: 0 -> ?).

See also picture and similar response to Referee 4 (below).

L192-193*: "This allows fossil snake brains to be reliably inferred from endocasts and directly compared with those of extant snakes*[33,34] » The cited references focus only on endocast studies in extant snakes, so they may not directly support inferences about fossil snakes. You may consider clarifying this point or citing additional studies that specifically address fossil endocast comparisons.

> What we mean is that the comparison between brain dissections and endocast in extant snakes validates the relationship between endocast and brain shapes (from the sentence preceding this one), thus making it useful to infer brain shape from fossil species. We have revised the sentence and the associated references about previous studies comparing either qualitatively or quantitatively fossil and extant snake/squamate endocasts, to explicitly frame our approach as integrating a qualitative neuroanatomical framework with a quantitative morphometric assessment of the telencephalon (revised MS lines 215-219)

L200-202: "*It is followed immediately posteriorly by enlarged cerebral hemispheres in the telencephalon and by a rather undifferentiated mesencephalon. This diverges from the condition observed in most squamates, which display well-expanded cerebral hemispheres that strongly contrast with the mesencephalon and rhombencephalon (= mid- and hindbrain, respectively)*[32-34]". Please clarify what is meant by *"undifferentiated mesencephalon"*. Additionally, the sentence is unclear—are you referring to the size of the cerebral hemispheres, or to the differentiation between the cerebral hemispheres and the mesencephalon?

> Edited. We mean both: a mesencephalon that is similar in size and shape to the cerebral hemispheres.

L202-206: The sentences in the main text appear somewhat contradictory to statements made in the abstract.

> Please refer to our response above regarding the Abstract. The comparisons and conclusions were based on both quantitative analyses of the telencephalon and qualitative assessments of the whole brain. To avoid potential misinterpretation, we have added the terms "Quantitative and qualitative" and replaced the term "brain" by "telencephalon" from the Abstract, as it could be construed as referring to the entire brain rather than specifically to the telencephalon.

Reference number 36 is not cited correctly. It should be: Olori, J. C. (2010). Digital endocasts of the cranial cavity and osseous labyrinth of the burrowing snake *Uropeltis woodmasoni* (Alethinophidia: Uropeltidae). *Ichthyology & Herpetology*, *2010*(1), 14-26.

> Edited.

Phylogeny and divergence times: Out of curiosity, did the authors consider alternative phylogenetic hypotheses, such as placing Serpentes as the sister group of anguimorphs?

> Bayesian inference analyses already produced thousands of posterior tree topologies and somewhat different summary trees, most of which only disagree with a Serpentes+Anguimorpha hypothesis, similarly to most of the available phylogenomic results—see recent reviews on this topics (Simões and Pyron 2021; Simões et al. 2025).

L258-259: The main text states that *Tametara* has a "*well-developed dorsum sellae*" to support its basal placement, but the detailed description notes that it is "*the dorsum sella is weakly developed and visible in anterior view*" (Line 221 in Supplementary text). Could the authors clarify this apparent discrepancy and specify how they coded or interpreted this character in the phylogenetic analysis?

> Edited in the SI text. We note that this makes no difference to our analyses because the dorsum sella character only codes for its absence or presence, not degree of development. We have removed this size classifier from the description of synapomorphies to avoid this interpretation.

L259-260: The mid-sagittal crest of the basisphenoid seems only present in adult specimens of large species (similar to the neural crest) in extant species. It says more about your specimen size than of its relationship with other extinct species.

> To our knowledge, the ontogenetic variation of this trait has not been quantified and demonstrated in the literature. Secondly, our interpretation on the ontogenetic status of the fossil is that it is skeletally mature, and so it is comparable to the other adult specimens used for phylogenetic scoring.

<u>Early evolution of snake brains</u>

L286-287: The statement *"We find that the earliest known snake brains of Tametara and Dinilysia represent highly divergent brain shapes relative both to each other and to most other snakes and non-ophidian squamates"* could be made more nuanced. The PCA in Fig. 4 shows that *Tametara* occupies a region of morphospace overlapping with other snakes and squamates, suggesting that its brain shape is not entirely divergent. Please consider revising this sentence to reflect this pattern more accurately.

> We have now revised the sentence to only mention that snake fossils occupy extreme positions in the morphospace.

<u>Palaeoecological origin of snakes</u>

L317-320: "*These results support most of our comparative anatomical observations, in which Tametara shares with other fossorial taxa short olfactory bulbs, a weakly developed optic tectum, and morphologically poorly differentiated cerebral hemispheres*[22,23,32-34] *(Extended Data Fig. 7)."* The manuscript describes the mesencephalon as both (L211) *"undifferentiated"* and *"unusually elongate"*, and the cerebral hemispheres as both *"enlarged"* and *"poorly differentiated"*. These descriptions

appear contradictory. Please clarify how these observations relate to each other, and specify whether the terms refer to size, shape, or differentiation, to improve clarity and consistency. Additionally, given that the anterior part of the frontal is missing, it would be helpful to clarify whether the olfactory bulbs are completely preserved. If not, this could affect the interpretations.

> We refer the reviewer to a similar comment above regarding our description of the cerebral hemisphere. We have rephrased this sentence to "mildly enlarged" to more appropriately characterize it as poorly developed relative to most other snakes. We described the mesencephalon as "undifferentiated" because the structure usually most prominent in this region (the optic lobes) are not well-developed in the new taxon (see initial paragraph on Brain description; revised MS lines 227-229 and 360-364). As mentioned above, the term "undifferentiated" for the mesencephalon has now been replaced. Similarly, the mesencephalon is described as elongated in comparison with other squamates, including snakes.

Extension of the olfactory bulbs was based on its impression on the ventral surface of the preserved portion of the frontal (not previously illustrated in the manuscript). We have now provided an illustration of this in the manuscript, matched by similar impressions on the frontal bone of other squamates, including *Najash* and *Dinilysia* (new ED Fig. 8f-h).

L324-328: "*Interestingly, Tametara further shows inner ear features compatible with fossoriality, such as an enlarged vestibular region and simplified semicircular canal architecture, providing independent support from a different system. We did not apply geometric morphometrics on the inner ear, however, given contradictory prior results*[19,37] *and limited statistical power for reliable*[37] *palaeoecological inference*" These sentences are somewhat contradictory. The text claims that *Tametara* shows inner ear features "compatible with fossoriality" and that this provides "independent support," yet also acknowledges that no geometric morphometric analysis was performed and that previous results are contradictory. It would be preferable to rephrase this section more cautiously.

> We agree that the previous wording may have overstated the strength of the inner ear evidence. The inner ear assessment was based solely on qualitative observations, without geometric morphometric analysis. To avoid potential misinterpretation, we have revised the relevant paragraph to clarify this point and removed reference to the inner ear from the Abstract.

L330-332: "*The combination of a dorsoventrally shallow lateral profile, poorly differentiated subdivisions, extremely long and curved olfactory bulbs, and narrow cerebral hemispheres is unlike that of any other squamate."* This statement could be made more nuanced. Some similarities can be observed with the endocast of *Python molurus* and *Python bivittatus* (Figure S3 Supplementary Material in Allemand, R., Polcyn, M. J., Houssaye, A., Vincent, P., López-Aguirre, C., & Bardet, N. (2024). First Virtual Reconstruction of a Mosasaurid Brain Endocast: Description and Comparison of

the Endocast of Tethysaurus nopcsai with Those of Extant Squamates. *Diversity*, *16*(9), 548.)

> Agreed and added.

L343: Reference 45 appears twice.

> Edited.

Figure 4:

The phylomorphospace was generated using landmarks restricted to the telencephalon. Including miniature reconstructions of the entire brain in this figure may therefore be misleading, as it visually suggests that the morphospace reflects total brain shape rather than telencephalic variation alone. Please consider displaying cropped telencephalon renderings to avoid potential bias in interpretation.

> We thank the Referees for highlighting the potential for visual ambiguity in the figure relative to the phylomorphospace. We agree that the morphospace itself is based exclusively on telencephalic landmarks, and this is now explicitly stated in both the revised figure title and legend ("Phylomorphospace representing telencephalon shape distribution…"). Moreover, the telencephalon is intentionally and clearly highlighted in each rendered endocast, so the anatomical focus of the quantitative analysis is visually unambiguous and unlikely to generate interpretative bias.

We therefore chose to retain complete endocast renderings, rather than cropped telencephalic portions, in order to provide anatomical context and to situate telencephalic variation within the broader neuroanatomical diversity of squamates.

Displaying full endocasts also allows readers to appreciate differences in other brain regions among the illustrated taxa, particularly fossorial representatives, and to better contextualize the distinctive morphology of *Tametara*. This is consistent with the manuscript's overall approach, in which quantitative telencephalic analyses are integrated with qualitative discussion of additional endocast regions.

For these reasons, we believe that retaining complete endocasts in this figure is both justified and informative, while maintaining clarity regarding the anatomical basis of the analysis.

Many times, you compare *Tametara* to *Anilius* and *Cylindrophis*, it would be nice to have their location on the morphospaces.

> We agree that visualizing the positions of *Anilius* and *Cylindrophis* would be informative, and their names have now been added to Fig. 4a, along with other key species for comparison.

Line 646: please remove the coma "Simões, et al.[6]"

> Edited.

Supplementary Material:

L40: snakes are squamates. Please consider rephrasing

> Edited.

L44: postfrontal rather than "*frontal*"?

> Edited.

L62: "*The supratemporals extend well anteriorly, close to the level of the trigeminal foramen*" This description could be reconsidered, as Fig. 2a,b appears to show the supratemporals terminating posterior to the level of the trigeminal foramen rather than extending close to it.

> Agreed and edited.

L68: "F**r**ontal" is misspelled

> Edited.

L177: "(hypoglossal) could be identified. is located posterodorsally to CN X" the sentence does not make sense.

> Edited.

L291: We don't understand the meaning of the end of this sentence: "The brain of *Tametara* is anteroposteriorly elongate and defined anteriorly by short, 291 bulky olfactory bulbs with anterior curvature"

> Edited.

L301 (and check the rest of the SM & Main text): The refence should be Olori 2010 not Jennifer.

> Edited.

L337-340: "*Compared to extant snakes, Tametara and Dinilysia, have remarkably large pituitary glands, although the length of these structures might only correspond to the elongate parietal of the fossils*," I am not sure to understand, could you explain this please?

> Edited.

L378-380: "*in reptiles are difficult to determine snakes and other limbless squamates as these are typically defined based on vertebra bearing ribs contacting the sternal plate to discern between cervicals and dorsals, for instance (Hoffstetter & Gasc 1969)*" Please consider rephrasing

> Edited.

L883: "Computed-tomography~~e"~~

> Edited.

L895: "of" is italicized

> Edited.

Extended Data Fig. 7: Why choose to show *Acrochordus*, which is a very peculiar species among snakes in all aspects of its morphology, including its endocranium?

> The goal of this figure is to illustrate a phylogenetically diverse set of snake brains. *Acrochordus* position as part of the earliest deriving group of the major snake clade Caenophidia make it highly relevant.

In your Rcode "analysis_microstructure", the library "ggtree" does not exist and there is no file named "general_pruned_scaled_appended.tre". Please, make sure we can run the whole code.

> This library ended up not being used – we removed it from the script.

(Remarks on code availability):

The code for the endocast analysis runs fine but the code for the microstructure does not (see detailed comments).

> Thank you for taking our codes and noticing the issues with the microstructure code. This has been revised and updated in our repository.

Referee #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

> Responses provided with the main report (above).

Referee #4 (Remarks to the Author):

The manuscript is well written and robust, and it does not show deficiences to prevent its publication.

Conclusions are original and convincent. The results are interesting enough for several researchers in evolutionary biology and vertebrate paleontology. We were not able to do a code revision since the matrix size is excessive for our hardware devices.

A. Authors present a new species of a Cretaceous stem-snake more or less equivalent (but younger) to Najash rionegrina. Their main goal is to obtain, through high-resolution micro-CT scans new details of the neuroanatomy. They also recognise a well-defined fossorial lifestyle for Tametara, contrasting with other stem and early crown snakes. This way they demonstrate early major ecological transitions in snake evolution, and that known stem species do not allow us to point the original ecological condition of crown snakes.

B. The paper is original and is significant in demonstrating a well-defined fossorial stem-snake not only by the anatomical analysis of vertebrae but also by features of the neurocranium. This allow authors to discuss with stronger tools the broad range of ecological adaptations in early snakes, something already suggested by the Jurassic material (Caldwell et al., 2015).

C. Data and presentation are optimal. However, there are some unclear detail in the presentation of bones, classifying them in non-mutually excluding categories: i.e., dermatocranium, splanchnocranium, and neurocranium. While dermatocranium refers to the bone origin, splanchno and neurocranium refer to their functionality (for example, pterygoid enters in both dermatocranium and splanchnocranium). We suggest authors to decide for one classification.

D. Appropriate use of statistics and treatment of uncertainties
Nothing to say here

E. Conclusions: robustness, validity, reliability
Conclusions are robust and valid.

> We appreciate the enthusiastic feedback! We also thank the reviewers for their shared work in providing detailed and important feedback to improve our manuscript. We have responded to the individual suggestions in the comments below.

F. Suggested improvements: experiments, data for possible revision

There are some notes to state here.

Line 97 (diagnosis). In “strongly overlapping the parietal ventrally” the description of the overlap between parietal and prootic is confusing, see below.

> Comments also provided below.

Line 99 (and 125). Where authors refer to quadrate conch in Tametara and other fossil snakes, the argument of its presence is not clearly assessed either here nor in the supplementary (see comment in the supplementary).

> Comment addressed in the osteological description section referred by the reviewers.

Line 100. In “oriented zygosphenes/zygantra”, Oriented how, in respect to what, where?

> Agreed and edited.

Line 102. Respect to the presence of parazygantral foramina, they are not visible in the presented material, and what is labelled as such in Extended Data Fig 2 are NOT parazygantral foramina (see below).

> Comment addressed in the osteological description section referred by the reviewers.

Line 106, discussing the ontogenetic stage, we agree in considering the holotype as an adult individual. However, respect to the “high degree of overlap between the supraoccipital and the parietals and prootics”, the overlap between parietal and supraoccipital and great exposure of the latter particularly departs from the typical condition observed in adult snakes. The supraoccipital remains greatly exposed in the skull roof in Tametara, as in Najash and juvenile snakes (Zaher et al., 2009; Scanferla and Bhullar, 2014; Palci et al., 2016; Garberoglio et al., 2019a, 2019b).

> We all agree on the ontogenetic stage. We also agree that the degree of overlap between bones discussed here is greater than in other snakes. Accordingly, we have mentioned this in our Description and Diagnosis. As for its relevance to ontogenetic stage, a fundamental developmental rule is that bones start condensing and expand in size throughout ontogeny. Therefore, bones must be nonoverlapping in earlier stages, and if any overlap occurs, it can only occur in subsequent stages. Especially considering that the degree of overlap considered here (as mentioned by reviewers) is quite substantial even when compared to other snakes; this provides indirect evidence for a skeletally mature individual.

Osteological description:
Line 116. As said before, classifying them in non-mutually excluding categories: i.e., dermatocranium, splanchnocranium, and neurocranium, is confussing. While dermatocranium refers to the bone origin, splanchno and neurocranium refer to their functionality (for example, pterygoid enters in both dermatocranium and splanchnocranium). We suggest authors to decide one classification functionally (neuro vs splanchnocranium) or by origin (auto vs dermatochranium). We suggest the former one.

> We respectfully disagree. We followed the textbook definition of those terms provided by Kardong (2012) (page 241 and fig.7.1 therein), who divide the skull in 3 parts: dermatochranium, splanchnocranium, and chondrocranium, as in our MS.

Additionally, besides the quadrates, there is another element of the splanchnocranium that might be actually preserved: a small portion of the left stapes. A small portion of this bone was reconstructed as part of the prootic in Figure 2c,d and Extended Data Figure 1b, which is apparently separated from the main body of the prootic and approaches the ventral side of the suprastapedial process of the quadrate. This matches the stapedial shaft observed in Dinilysia and Najash (Caldwell and Albino, 2002, Zaher and Scanferla, 2012; Garberoglio et al., 2019a).

> Agreed. We thank you for noticing this. A small portion of the stapes is preserved, as illustrated in the figures mentioned. It does not have many diagnostic pictures preserved, but we have now included in the detailed description in the SI (section Stapes).

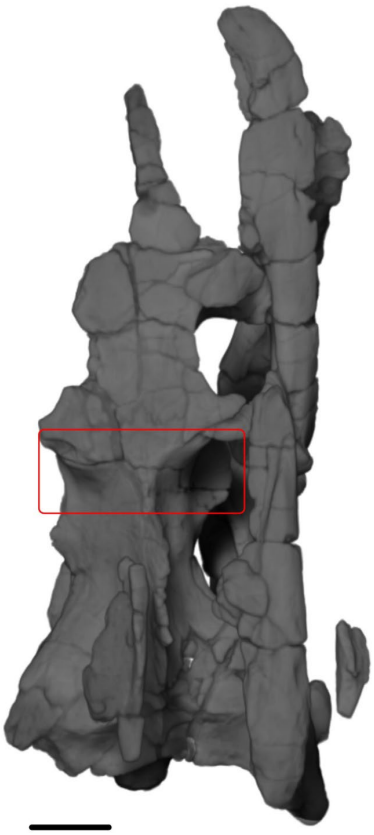
Line 119. Respect to “The postfrontal has a distinct frontal process and a single distal process”, the postfrontal is described as having two processes, a frontal and ¨single distal process¨, which we find somewhat confusing here. It is more clear in the supplementary that the postrontal is described as having a frontal (=anterior), lacking a parietal (=posterior) process, and having a distal process that is ventral.

> Edited in the main text as in the SI: frontal (=anterior) and parietal (= posterior), while still highlighting the absence of the latter in other stem snakes.

Line 133. “the parietal has a short anteromedial process that would have inserted in between the frontals, which is absent in the other South American Cretaceous snake”. The singular in this sentence is incorrect. It refers to the condition in the South American snake (Dinilysia), somewhat ignoring the existence of the other (and better preserved) South American snake (Najash). Anyway, while there is indeed no evidence of such process in Najash (Apesteguía and Zaher, 2006; Zaher et al., 2009; Garberoglio et al., 2019a), a similar anteromedial process of the parietal is arguably present in Dinilysia (visible in specimens where the fronto-parietal suture is well-preserved such as the holotype and MACN-RN 1013), where the parietal contacts the frontals in a W-shaped interdigitating suture (Estes et al., 1970; Caldwell and Albino, 2002: Zaher and Scanferla, 2012).

>Edited from singular to plural to correct the typo.

Indeed, *Dinilysia* was described as having a wavy (or W-shaped) frontoparietal suture. This seems to be derived from observations on the holotype, but the equivalent region in MACN-RN 1013 is in fact nearly concave (see picture below). We consider the presence of the process ambiguous at best, or possibly polymorphic. Accordingly, we have made this more careful consideration in our description, and updates the score for this character in Dinilysia (Ch. 112: 0 -> ?).



Line 145. “supraoccipital bears well-developed anterolateral processes that contact and are strongly overlapped by the parietal posterolaterally”. As we read, authors seem to say that the supraoccipital is overlapped by the parietal (implying that is covered by it). This makes sense, as it is common in snakes. Only a small degree of overlap of the supraoccipital by the parietal seems to be the condition in Tametara. It is made clear by a small portion of the posterior margin of the parietal that is missing in the right side, and reveals a small portion of the anterolateral process of the supraoccipital that was not exposed in the skull roof, and was below the parietal: i.e., overlapped by the parietal (Figs. 1c, 2d). However, in the diagnosis it says that the supraoccipital is “strongly overlapping the parietal ventrally”, which implies a different thing or it is incorrect (there is no supraoccipital covering the parietal). Below, it states that the overlap is similar to other snakes (where the supraoccipital is overlapped by the parietal), but greater than in any other snake. This does not make sense, since the last part says correctly that the supraoccipital represents a great portion of the preserved dorsal surface of the skull roof in Tametara, which is the result of less overlap of the supraoccipital by the parietal than in most snakes. For instance, the lesser dorsal exposure of the supraoccipital accounted in the skull roof of adult specimens of Dinilysia is the result of a greater overlapping of the supraoccipital by the parietal than what is observed in juveniles (Scanferla and Bhullar, 2014).

> Agreed. The supraoccipital overlapped by the parietal (as originally described). The topological relationship between the bones was mistakenly swapped in the diagnosis

and it has been corrected now in the Diagnosis section. Thank you for catching this.

Line 148. Respect to the idea that “the supraoccipital of Tametara represents ca. 25% of the preserved dorsal surface of the skull roof, whereas in adult specimens of Dinilysia this accounts only 8-11% in MACN-Pv RN1013 and MLP 26-410, respectively”, the authors seem to have overlooked previous descriptions of the great exposure of the supraoccipital in Najash (Zaher et al., 2009; Garberoglio et al., 2019a, 2019b), which is approximately in the same range of that observed here in Tametara. Zaher et al. (2009) attributed this feature to a juvenile stage in the only known skull at that time (MPCA-PV 385). But in later descriptions (Garberoglio et al. 2019a, 2019b) demonstrated to be present in all specimens of Najash described afterwards, and is evident now that this don’t reflect a juvenile condition or could even be considered to be a distinct feature of the skull in Najash. In this case, the great exposure of the supraoccpital is not a unique feature of the snake described here, and could be another potential feature in common between the new taxon and Najash, its evident sister group.

> Agreed and excellent observation! We have added the extra comparison with Najash, which had escaped us in this section.

Line 152. The reference of “the otoccipitals do not meet each other at the midline, being separated by the posteromedial process of the supraoccipital (Figs. 1c and 2c-e). This configuration is widespread among non-ophidian squamates but was previously known to occur only in Najash among all known fossil and extant snakes”, a posteromedial process of the supraoccipital that separates dorsally the otoccipitals in the skull roof was previously observed not only in Najash, but also in Haasiophis (Rieppel et al., 2003; Zaher et al.., 2009: Garberoglio et al., 2019a, 2019b). A similar condition also occurs with varying degrees in extant macrostomatans, even with complete dorsal separation of the otoccipitals by the posterior extension of the supraoccipital (Rieppel and Zaher, 2002). However, in such cases the otoccipitals usually meet below the supraoccipital and contact each other above the foramen magnum, but not always (Rieppel and Zaher, 2002). Besides the separation of the otoccipitals in the skull roof, the lack of medial contact of the otoccipitals above the foramen magnum is confirmed by the ct-scan reconstruction in Najash (Garberoglio et al., 2019b) and Tametara, but this remains unknown in Haasiophis. Considering these points, the condition in Najash and Tametara is not necessarily as straightforward plesiomorphic and unknown to occur in snakes, as the statement here implies, which we think it ignores not only previous observations but also the degree of variation of this feature in extinct and extant snakes.

> Agreed. One important point of clarification from our part is that we consider here separation of the otoccipitals when they are totally separated from each other by the supraoccipital (not just partially). This was not clear in our text and we have now clarified it therein. In Haasiophis, as correctly pointed out by the reviewer, it is unknown whether the otoccipitals are in contact beneath the supraoccipital (as in Tametara and Najash), and so it had been scored as unknown in our dataset. Furthermore, we would highlight the fact that the only known specimen of Haasiophis is strongly compressed

and many elements distorted. We will clarify this point in this section accordingly, making explicit mention of Haasiophis.
Line 156. Concerning the absence of the crista circumfenestralis, “another plesiomorphic condition occurring among other stem snakes such as Najash, Dinilysia, Yurlunggur, Wonambi, and Sanajeh30”, the citation provided by the authors do not entirely supports this statement, at least not for all the taxa mentioned. To state the absence of the crista circumfenestralis in Najash, Yurlunggur, Wonambi, and Sanajeh they should cite other references, or state here that a crista circumfenestralis sensu Palci and Caldwell (2014) is absent in all of this taxa. The different opinions and criteria about this feature are a lengthy subject, which is apparently ignored here, and described in more detail only in the supplementary (but see comment there).

> We have added an additional direct reference to this point. We note that our assessment on the crista circumfenestralis is also based on our personal observations of the referred species.

Line 165. Respect to the absence of the retroarticular process in Tametara, it says that it occurs “as in Dinilysia9,10, whereas Najash exhibits a small one11”. Both Najash and Dinilysia are described as retaining a short retroarticular process (Caldwell and Calvo, 2008: Zaher and Scanferla, 2012; Garberoglio et al., 2019a). Both have indeed only a very short extension of the compound bone passing the glenoid, and there is no much difference between these two and the condition in Tametara. To consider the small process as a present or absent retroarticular process might be subjective. Is actually better understood as with no distinction between the three, and if the authors want to make that case here, it has to be better justified.

> Indeed, as with many aspects of osteology, small variations are highly subjective to interpretation (as acknowledged by the reviewer). The description of Tametara and the comparison with the other species are based primarily on our personal assessment of this (and other phylogenetically relevant) characters. We understand that different authors might interpret these differently. We find no extensions beyond the glenoid in Dinilysia (which is quite unambiguous to us), while we agree with the recent re-descriptions of Najash (Garberoglio et al. 2019) with the latter having a small process, which is how we describe this variation in our text. For Tametara, we find no evidence whatsoever of an even small retroarticular process, as in Dinilysia. Given this potential point of subjective disagreement, we have now highlighted our interpretation for greater clarity in the main text.

Line 184. “Most vertebrae have paracotylar foramina on each side of the cotyle”. Paracotylar fossae are present in the figured vertebra, but seems to be no clear indication of paracotylar foramina inside such fossae in the reconstruction.
> These are now indicated more clearly with slices from the CT-scan data (ED Fig. 2d)

Line 185. Concerning the small parazygantral pits on either side of each zygantrum on most vertebrae, there is actually no evidence presented of the presence of parazygantral foramina in Tametara. What is described in the manuscript and labelled in

the figured vertebrae as parazygantral foramina (or parazygantral pits) are not in fact parazygantral foramina, since they are located INSIDE the zygantral cavity. On the contrary, parazygantral foramina are located OUTSIDE the zygantral cavity, adjacent to it on each side between the zygantral facet and the postzygapophysial facet (e.g., Rage, 1998; Scanlon, 2005; LaDuke et al., 2010; Hsiou et al., 2014; Gómez et al., 2019; Szyndlar and Georgalis, 2023).
> These are now indicated more clearly with slices from the CT-scan data (ED Fig. 2d)

Line 188. Respect to the absence of forelimbs, supported by “the specimen´s articulation, preservation quality, and full exploration with micro-CT scanning”, it makes sense, of course, but aren’t a lot of ribs missing also? In such case, missing small forelimbs seems to be still a possible scenario.

> Agreed. We have now also raised this important consideration.

Line 576. Fig. 2. “V.con” labelled in the figure, but not in the caption.

> Edited.

Line 1007. Double .. in TRS.

> Edited.

Line 1070. Extended Data Fig. 1. Cl.Pr labelled in figure, but missing in the caption.

> Edited.

eSupplementary Information

Line 29. Sam problem described for line 116 of the text.

> See our response to this comment above.

Line 41. Ventral instead of distal?

> The most common term is distal as it is ventrolaterally directed in most squamates. It can be unambiguously interpreted based on our illustration for these and other characters in our dataset (illustrations for character 76 in our character list, available as Supplementary Data S1 here and in Simões et al. (2026))

Line 60. Respect to the description that “Each supratemporal contacts the paroccipital processes of the otoccipitals ventrally and the supratemporal process of the parietal anteromedially”, the supratemporal also have a broad ventral and lateral contact with the prootic.

> Agreed and added.

Line 83. Authors say that “The right side of the parietal is entirely preserved, bearing a large facet for the postfrontal anterolaterally, and a short anteromedial process that would have inserted in between the two frontals, which is absent in Dinilysia”. However, the parietals in Dinilysia are described as having a jagged, w-shaped interdigitating suture with the frontals by several authors (Estes et al., 1970; Cadwell and Albino, 2002; Zaher and Scanferla, 2012). This interdigitating suture is the result of a similar anteromedial process of the parietal which is arguably present in Dinilysia, and is visible in specimens where the fronto-parietal suture is well-preserved, such as the holotype and MACN-RN 1013 (Estes et al., 1970; Caldwell and Albino, 2002; Zaher and Scanferla, 2012), but the contact is not preserved in the specimen described by Caldwell and Calvo (2008).

> See our response to this comment above.

Line 107. Respect to the conch in the quadrate, it must be said that the quadrate in Dinilsyia shows a notch or concavity on its lateral surface that is flanked anteriorly by an anterior ridge, thus resembling the tympanic crest and tympanic conch in other lizards (Zaher and Scanferla, 2012). However, this crest and concavity are not observed in quadrates attributed to Najash (Zaher et al, 2009; Garberoglio et al., 2019a), and a quadrate lateral conch as in other lizards that have a tympanic chamber has been considered to be absent in both Dinilysia and Najash as in all extant snakes (Gauthier et al., 2012; Zaher and Scanferla, 2012; Garberoglio et al., 2019a; Palci et al, 2019). Therefore, we consider that it is not clear what the authors refers to a well-developed lateral conch in Tametara and the other fossil snakes, and the argument to state its presence in all of these fossil taxa is not particularly well-stablished, since the quadrate in Tametara shows the same condition that in Dinilysia, though differing from that of Najash.

> Agreed. The statement in the beginning of this paragraph comparing many species to Dinilysia and Najash referred to the emarginated quadrate only, not the conch. However, we agree the text does not make this clear. We have adjusted the text accordingly.

Line 113. The affirmation that there is a well-developed tympanic crest on the quadrate, similarly to Dinilysia (but not preserved or partially broken in all specimens of Najash), seems somewhat contradictory. If the presence of a quadrate conch in fossil snakes is based on the presence of well-developed tympanic crest and a lateral concavity on the quadrate, as is observed in Tametara and posibly Dinilysia, then why they stated before that a quadrate conch is also present in Najash where all this is not actually preserved?
Line 126. Authors say that “along with the anterolateral processes, these have a substantial overlap with the ventral side of the parietal”. However, It results unclear to what is referring with the “these”, while describing the anterolateral processes. A rephrase is necessary.

> See comment above for our text correction regarding the quadrate conch.

Line 127. About the overlapping with the ventral side of the parietal, “in other snakes, including the braincase of early snakes such as Dinilysia (Caldwell & Calvo 2008; Zaher & Scanferla 2012) and Najash (Garberoglio et al. 2019a), the extent of this overlap with the prootics and parietals is greater than in any other snake known to us”. It is not clear for us to what refers to with a greater degree of overlap of the supraoccipital in Tametara (See comment in the main text). The supraoccipital is greatly exposed in the skull roof in Tametara, in a very similar way to Najash, resulting from less overlapping by the parietal. The supraoccipital is less exposed on the skull roof in DInilysia, due to a greater overlapping by the parietal.

> We have now provided a new image where this overlap is clearer, in ED Fig. 1f

Line 162. Paired Atlanteal crests instead of nuchal crests?

> No. Nuchal crests sensu Gauthier et al. (2012) (Ch. 300 therein) and Simões et al. (2018b) (illustration in character list, Ch. 171 herein).

Line 166. See the comment in the main text for “The same arrangement occurs only in Najash (Garberoglio et al. 2019a) among all snakes known to us”.
Line 169. Respect to the posteriormost end of the paroccipital processes almost at the same level of the posterior end of the supraoccipital, on the right side, the paroccipital process appears to be more complete than in the left side, and in Fig. 2d, e is visible extending well-passing the posterior end of the supraoccipital. On the right side of the skull in Tametara, the situation is quite similar to that of Najash (Garberoglio et al., 2019a).

>Edited. See also comment above

Line 171. Concerning the length of the paroccipital processes in Dinilysia and Najash, comparatively longer posteriorly, going beyond the level of the occipital condyle, this is true for Dinilysia (Estes et al., 1970; Zaher and Scanferla, 2012), but the paroccipital processes in Najash are not so developed, and at most only reach the occipital condyle. Previous descriptions of Najash stated that they do not project beyond the level of the occipital condyle (Garberoglio et al., 2019a).

>Edited.

Moreover, the occipital condyle appears to be not preserved in Tametara, making a comparison to Dinilysia and Najash difficult, and a distinction only an inference. The condition in Tametara is unknown, but judging from the preserved portion of the basioccipital and the posterior end of the supraoccipital, it is likely that the posterior end of the paroccipital process was in fact placed beyond the level of the occipital condyle.

> A small part of the base of the occipital condyle, formed by the basioccipital, is preserved in *Tametara*, contacting the otoccipital laterally (ED Fig. 1b).

Line 179. “could be identified. is located” . Missing “CN XII is”.

>Edited.

Line 183. “In Najash rionegrina (G.S. pers. obs.)”. Is Weirs personal observation.

> We did not understand this comment.

Line 190. “border of the crista tuberalis (Figure 1d)”. Wrong figure reference (Figure 1d is a vertebra).

>Edited.

Line 198. “apertura lateralis—a condition shared with several early fossil snakes in which this can be determined (e.g., Najash, Dinilysia, Yurlunggur, Wonambi, and Sanajeh; TRS pers. obs, and Palci and Caldwell (2014).”. Actually, this is the case for Dnilysia, Sanajeh, and the Najash specimen MPCA 500 (Palci and Caldweel, 2014; Garberoglio et al., 2019a; Zaher et al., 2023). But the basioccpital is described as being excluded from the floor of the recessus scalae tympani by the otoccipital in Wonambi, Yurlunggur, and other specimens of Najash (Scanlon, 2005, 2006; Garberoglio et al., 2019a, 2019b).

>Edited.

Line 206. “has a similar trapezoidal shape”. Similar to what?

>Edited.

Line 232. Authors say that “ There is no midsagittal crest on the ventral surface of the basisphenoid ... as also observed in Najash and Dinilysia (Garberoglio et al. 2019a; Zaher & Scanferla 2012),. However, a low midsagittal ridge is present in some specimens of Najash, according to Garberoglio et al. (2019a).

>Edited.

Line 235. basisphenoid (Extended Data Figure 1c,f). Please check correct figure reference. The opening of the Vidian canal is labeled in Extended Fig 1a, and is definitely not visible in Extended Data Fig 1f (which shows only parietal and supraoccipital).

>Edited. Done.

Line 253. “this contact between the prootic and the supraoccipital is a condition also

found". The particular condition in these snakes is that the contact is visible/exposed in the skull roof.

>Edited.

Line 255. Authors declare that the contact between the prootic and the supraoccipital, also found in Dinilysia, Najash and basal alethinophidians was observed by: TRS pers. obs, and Garberoglio et al. 2019a. However, there are other previous descriptions of this feature in the mentioned fossil taxa that are omitted here, such as Estes et al., 1970, and Zaher et al., 2009. Additionally, this condition is not observed in any specimen of Najash, being the contact between prootic and supraoccipital concealed by the parietal in some specimens (Garberoglio et al., 2019a, 2019b).

>Edited. References added. Attribution to Najash removed.

Line 273. Extra space after fossa.

>Edited.

Line 278. A reference to Extended Data Fig 1h should be inserted after "tympani".

>Edited.

Line 280. Dot necessary among snakes and The.

>Edited.

Line 280. About the retroarticular process absence, see comment in the main text.

> See our response to this comment above.

Line 424. Concerning the proposed "small parazygantral pits are present on either side of each zygantrum on most vertebrae", these foramina are not parazygantral foramina, since they are inside the zygantral cavity.

> See our response to this comment above.

G. References: appropriate credit to previous work?

Yes.
In line 275 of the supplementary, add Garberoglio et al. (2019a)

>Added.

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract,

introduction and conclusions

Abstract is clear but in line 31 should say gen. et sp. nov., not only sp. nov.

>We chose this option to fit within word length requirements. But edited now.

In the Introduction, line 45, authors refer that snake evolutionary history extends back to at least the Middle Jurassic. Although we agree, there is competing evidence that proposed Parviraptorids are nor stem-snakes (Benson et al., 2025), and this should be taken into account at least.

> Disagreed. The study above found no consistent placement (3 different hypotheses) for Parviraptorids, which does not shed light on this issue.

In line 57 says borrowing instead of burrowing

>Edited.

In the etimology section (line 85) the words Tupí-Guaraní both have accent at the end.

>Edited.

Conclusions are ok.

Referee #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

> Responses provided with the main report (above).

References above

Allemand, R., C. López-Aguirre, J. Abdul-Sater, W. Khalid, M. M. Lang, S. Macrì, N. Di-Poï, G. Daghfous, and M. T. Silcox. 2023. A landmarking protocol for geometric morphometric analysis of squamate endocasts. The Anatomical Record 306:2425-2442.

Balanoff, A. M., G. S. Bever, T. B. Rowe, and M. A. Norell. 2013. Evolutionary origins of the avian brain. Nature 501:93-96.

Bauer, A. M. and T. Jackman. 2008. Global diversity of lizards in freshwater (Reptilia: Lacertilia). Hydrobiologia 595:581-586.

Benson, R. B. J., S. A. Walsh, E. F. Griffiths, Z. T. Kulik, J. Botha, V. Fernandez, J. J. Head, and S. E. Evans. 2025. Mosaic anatomy in an early fossil squamate. Nature.

Chiappe, L. M., G. Navalón, A. G. Martinelli, I. d. S. Carvalho, R. Miloni Santucci, Y.-H. Wu, and D. J. Field. 2024. Cretaceous bird from Brazil informs the evolution of the avian skull and brain. Nature 635:376-381.

Felsenstein, J. 1985. Phylogenies and the Comparative Method. American Nat. 125:1-15.

Garberoglio, F. F., R. O. Gómez, T. R. Simões, M. W. Caldwell, and S. Apesteguía. 2019. The evolution of the axial skeleton intercentrum system in snakes revealed by new data from the Cretaceous snakes *Dinilysia* and *Najash*. Sci. Rep. 9:1276.

Gauthier, J. A., M. Kearney, J. A. Maisano, O. Rieppel, and A. D. B. Behlke. 2012. Assembling the squamate tree of life: perspectives from the phenotype and the fossil record. Bull. Peabody Mus. Nat. Hist. 53:3-308.

Hu, Y., T. S. Doherty, and T. S. Jessop. 2020. How influential are squamate reptile traits in explaining population responses to environmental disturbances? Wildlife Research 47:249-259.

Kardong, K. V. 2012. Vertebrates : comparative anatomy, function, evolution (6th Edition). McGraw-Hill, New York.

Ksepka, D. T., A. M. Balanoff, N. A. Smith, G. S. Bever, B.-A. S. Bhullar, E. Bourdon, E. L. Braun, J. G. Burleigh, J. A. Clarke, M. W. Colbert, J. R. Corfield, F. J. Degrange, V. L. De Pietri, C. M. Early, D. J. Field, P. M. Gignac, M. E. L. Gold, R. T. Kimball, S. Kawabe, L. Lefebvre, J. Marugán-Lobón, C. S. Mongle, A. Morhardt, M. A. Norell, R. C. Ridgely, R. S. Rothman, R. P. Scofield, C. P. Tambussi, C. R. Torres, M. van Tuinen, S. A. Walsh, A. Watanabe, L. M. Witmer, A. K. Wright, L. E. Zanno, E. D. Jarvis, and J. B. Smaers. 2020. Tempo and Pattern of Avian Brain Size Evolution. Curr. Biol. 30:2026-2036.e2023.

Macrì, S., I.-M. Aalto, R. Allemand, and N. Di-Poï. 2023. Reconstructing the origin and early evolution of the snake brain. Science Advances 9:eadi6888.

Marques-Souza, S., I. Prates, A. Fouquet, A. Camacho, P. J. Kok, P. M. Nunes, F. Dal Vechio, R. S. Recoder, N. Mejia, and M. Teixeira Junior. 2018. Reconquering the water: Evolution and systematics of South and Central American aquatic lizards (Gymnophthalmidae). Zoologica Scripta 47:255-265.

Martínez, R. N., T. R. Simões, G. Sobral, and S. Apesteguía. 2021. A Triassic stem lepidosaur illuminates the origin of lizard-like reptiles. Nature 597:235–238.

Meiri, S. 2024. SquamBase—A database of squamate (Reptilia: Squamata) traits. Global Ecology and Biogeography 33:e13812.

Mongiardino Koch, N., R. J. Garwood, and L. A. Parry. 2021. Fossils improve phylogenetic analyses of morphological characters. Proceedings of the Royal Society B: Biological Sciences 288:20210044.

Mongiardino Koch, N., R. J. Garwood, and L. A. Parry. 2023. Inaccurate fossil placement does not compromise tip-dated divergence times. Palaeontology 66:e12680.

Pyron, R. A. 2017. Novel approaches for phylogenetic inference from morphological data and total-evidence dating in squamate reptiles (lizards, snakes, and amphisbaenians). Systematic Biology 66:38-56.

Ronquist, F., N. Lartillot, and M. J. Phillips. 2016. Closing the gap between rocks and clocks using total-evidence dating. Phil. Trans. R. Soc. B 371:20150136.

Scanferla, A. 2022. A Glimpse into the Evolution of the Ophidian Brain. Pp. 294-315 *in* D. J. Gower, and H. Zaher, eds. The Origin and Early Evolutionary History of Snakes. Cambridge University Press, Cambridge.

Scharf, I., A. Feldman, M. Novosolov, D. Pincheira-Donoso, I. Das, M. Böhm, P. Uetz, O. Torres-Carvajal, A. Bauer, and U. Roll. 2015. Late bloomers and baby boomers: ecological drivers of longevity in squamates and the tuatara. Global Ecology and Biogeography 24:396-405.

Simões, T. R., A. S. Brum, and S. E. Pierce. 2026. Evolutionary rate incongruences in squamates reveal contrasting patterns of evolutionary novelties and innovation. Systematic Biology syaf067:https://doi.org/10.1093/sysbio/syaf1067.

Simões, T. R., M. W. Caldwell, A. Palci, and R. L. Nydam. 2018a. Giant taxon-character matrices II: a response to Laing et al. (2017). Cladistics 34:702-707.

Simões, T. R., M. W. Caldwell, and S. E. Pierce. 2020a. Sphenodontian phylogeny and the impact of model choice in Bayesian morphological clock estimates of divergence times and evolutionary rates. BMC Biology 18:191.

Simões, T. R., M. W. Caldwell, M. Tałanda, M. Bernardi, A. Palci, O. Vernygora, F. Bernardini, L. Mancini, and R. L. Nydam. 2018b. The origin of squamates revealed by a Middle Triassic lizard from the Italian Alps. Nature 557:706-709.

Simões, T. R. and R. A. Pyron. 2021. The Squamate Tree of Life. Bulletin of the Museum of Comparative Zoology 163:47-95.

Simões, T. R., M. Tollis, and F. T. Burbrink. 2025. Origin and Early Evolution of Squamates and Their Kin: From Fossils to Genomes. Annual Review of Ecology, Evolution, and Systematics 56:265-260.

Simões, T. R., O. V. Vernygora, M. W. Caldwell, and S. E. Pierce. 2020b. Megaevolutionary dynamics and the timing of evolutionary innovation in reptiles. Nat. Comm. 11:3322.

Tałanda, M., V. Fernandez, E. Panciroli, S. E. Evans, and R. J. Benson. 2022. Synchrotron tomography of a stem lizard elucidates early squamate anatomy. Nature 611:99-104.

Title, P. O., S. Singhal, M. C. Grundler, G. C. Costa, R. A. Pyron, T. J. Colston, M. R. Grundler, I. Prates, N. Stepanova, M. E. H. Jones, L. B. Q. Cavalcanti, G. R. Colli, N. Di-Poï, S. C. Donnellan, C. Moritz, D. O. Mesquita, E. R. Pianka, S. A. Smith, L. J. Vitt, and D. L. Rabosky. 2024. The macroevolutionary singularity of snakes. Science 383:918-923.

Zaher, H., D. M. Mohabey, F. G. Grazziotin, and J. A. Wilson Mantilla. 2023. The skull of Sanajeh indicus, a Cretaceous snake with an upper temporal bar, and the origin of ophidian wide-gaped feeding. Zoological Journal of the Linnean Society 197:656-697.

Referee #1 (Remarks to the Author):

This is much improved version of the manuscript, and addresses many of my previous critiques and concerns. This is especially the case for the morphometric analysis, and I commend the authors for their very clear documentation of morphometric landmarks, incorporation of appropriate specimens in analysis, and enhanced discussion of methods and results. The diagnosis could be improved and the discussion of character support (and some of the characters themselves) require revision. Importantly, this version has introduced segmented C-T data for Najash (Extended Fig. 8), which allows for easier identification of unambiguous diagnostic morphology for Tametara and would better test for ecological diversity among stem snakes if included in the endocast morphometric analysis. Specific comments and responses to revisions are as follows:

> We appreciate the positive feedback. We address the detailed comments in full below.

Taxonomy and systematics
In response to my critique of the use of a differential diagnosis to distinguish Tametara, the authors provided a series of counter arguments as well as adding five phylogenetically inferred autapomorphies to the text and Supplementary Information. They provide additional justification and notes for recognizing Tametara as a distinct taxon from Najash in their response to my initial review.

The authors state: "Differential diagnoses are commonplace in the taxonomy of fossil vertebrates as quite often enough parts are not preserved or not enough specimens are known to provide features exclusively known for a taxon. We note that we do not consider "autapomorphies" to be the most appropriate terminology for this in a species diagnosis, since the term explicitly depends on a phylogenetic context, and homoplastic autapomorphies not only occur but are frequent. Having said that, five phylogenetically inferred autapomorphies were recovered for Tametara and had been listed in our Supplementary Information (synapomorphies list section), four being unambiguous autapomorphies. We note the phylogenetically supported autapomorphies had been listed in our differential diagnosis."

While it is certainly true that differential diagnoses are common for erecting taxa based on fossils, they are more common for taxa represented by limited remains (e.g., vertebrae), not usually the most anatomically complex portion of the skeleton. And whereas phylogenetic inference is required to determine the diagnostic utility of characters distributed in more

than one taxon, an unambiguous autapomorphy is an observation unique to the taxon in question and thus does not require an a priori phylogenetic identification.

> We respectfully disagree. Collectively, we have authored dozens of squamate fossil species descriptions and re-descriptions with various degrees of specimen preservation in which differential diagnoses have been used, besides by other third-party studies, even if the term "differential diagnosis" was not explicitly stated—e.g., (Evans and Barbadillo 1998; Evans and Barbadillo 1999; Simões et al. 2015; Apesteguía et al. 2016; Simões et al. 2017; Xing et al. 2018). Taxonomic diagnoses are highly variable among authors, with some including more characters in diagnoses to ensure the long-term stability of a species' name, whereas others are more conservative. Having said that, we feel that we have already addressed this point by also including a list of autapomorphies.

The authors go on to state:
"Furthermore, additional features distinguish Tametara from Najash and Dinilysia at the level of neuroanatomical organization, including the configuration and relative proportions of the cerebral hemispheres and the olfactory bulbs (see the comments below on neuroanatomy)."

Extended Data Figure 8 does an excellent job of illustrating the unique morphology of the frontal in Tametara relative to all snake taxa included in the phylogenetic analysis. The frontal is either apomorphically short anteroposteriorly, or it is incomplete anteriorly and the impression of the olfactory bulb is thus short and posteriorly positioned relative to the length of the element. Why not include the anatomy of the frontal in the diagnosis, as it appears to be unique and is key to the ecological interpretations of endocast shape?

> We thank you for the suggestion. We considered it initially, but the frontal is incompletely preserved and the apparently short anteroposterior length is an artifact from preservation, which is why we ultimately did not use it for diagnosis. We note that this was not a problem for identifying the olfactory bulb as this part was preserved, which we highlighted in the previous round of reviews and led to our inclusion of ED Fig. 8. Yet, in order to take this comment into account despite the preservational aspects of the frontal, we have included the morphology of the olfactory bulb and cerebral hemispheres into the diagnosis.

With respect to synonymy of Tametara and Najash, they state:
"Note 1: We highlight that all the comparisons above were based on personal inspection of nearly all referred materials of Najash and Dinilysia; thus not rely exclusively on the described literature. For greater clarity, all specimens observed are listed in Supplementary

Table S1).

Note 2: We also have access to CT scans and the first author has participated in the recent re-description of Najash referred to by the reviewer (Garberoglio et al. 2019a). Therefore, we respectfully disagree with the referee´s concerns and with some of their comments and differences in interpretation of the anatomy of Tametara and Najash: e.g., shape of the postfrontal, especially in the absence of images supporting such statements."

As the authors have C-T data for Najash, I implore them to please include those data in this study (as they have with Fig. 8) and make them available! This will allow for tests of the personal observations of Note 1 and will only increase the impact of the study.

> We would like to first highlight that we are pretty much in favor of public data availability, which we have consistently done in both previous and in the current work. However, we are afraid we are not at the liberty of sharing this specific CT scan data for *Najash*. Although one of us (TRS) is a co-author on the redescription of *Najash*, TRS does not own the copyright to that data and so it cannot be provided in full here without approval of the copyright owners (authors who performed the CT scanning). This is simply beyond our control. Further, we are aware that the copyright owners of that data have continued to develop work on that specimen, including student projects, which prevents us from unilaterally releasing that data without their involvement. Having said that, we highlight that the anatomical description of *Najash* in the publication of its cranial CT scan by Garberoglio et al. (2019) is quite extensive, illustrating key points of taxonomic relevance to *Tametara*, which is the focus of our work here.

"Note 3: in paleontology, one is forced to rely almost exclusively on the morphological criterion for species delimitation. However, geography and chronology can also provide complementary evidence. Tametara is quite different from Najash in both instances: Najash comes from La Buitrera, Upper section of Candeleros Formation, Río Negro province, northern Patagonia, Argentina (Cenomanian; 100.5-99.3Ma). Tametara comes from Adamantina Formation, Bauru Group, Bauru Basin, Presidente Prudente, State of São Paulo, Brazil (late Santonian to early Campanian age; 85–75 million years ago). In other words, they are separated by nearly 3,000 Km and at least 14 million years in time. No other fossil species of snake known to us exhibits such a wide geographical and chronological range combined with such a collection of differential diagnostic features."

As the authors are aware, incorporating geographic and temporal data into taxon identification introduces circularity and bias into evolutionary and biogeographic

reconstructions (e.g., Bell et al. 2010) and should be avoided. With respect to the stated context differences between Najash and Tametara, I refer the authors to the fossil records of extant snake genera such as Python, Boa, and Eunectes, all of which possess modern geographic ranges equivalent to, or greater than, the distance between Río Negro and São Paulo and possess diagnostic fossil records of greater temporal duration than the age difference between Najash and Tametara.

> We note that in the comment referred above by the reviewer (our "Note 3" from the previous round of reviews), we were discussing species delimitation, and not "evolutionary and biogeographic reconstructions". We agree with the reviewer regarding the latter.

"In conclusion, not recognizing the numerous differences and criteria used above to draw a distinction between Tametara and Najash would not only be a critical overlook from our part if not recognized here in the erection of new genus, it would also seriously risk a (well-justified) immediate taxonomic revision of Tametara, thereby jeopardizing its significance and the long-term impact of the present study"

Incorporating a robustly diagnostic character/set of characters, such as the morphology of the frontal, would make the taxonomic hypothesis of Tametara more resilient to further revision than the current list of characters (see discussion of the margo lateralis below).

> We thank the reviewer for their insight regarding species diagnosis. We believe we have already addressed this point in the previous round with the more explicit mention of autapomorphies and inclusion of a diagnostic table. The suggestion of the reviewer for morphological features of the frontal is limited by its limited preservation, as said above, but we have added the unique morphology of the olfactory bulb and cerebral hemispheres in the diagnosis.

Morphometric analysis/ecological inferences
As stated above, if the authors have access to C-T scans of Najash (as they do from Fig. 8), then it would greatly strengthen the impact of the study to include an endocast of the taxon in the morphometric analyses. Inclusion could either corroborate fossorial habits for Tametara+Najash or demonstrate fossoriality in Tametara as an early experiment in snake ecology.

> As mentioned in our comment above, we cannot provide the CT data for *Najash* as we do not own the copyrights to the data. However, part of the frontal bone shown in ED Fig. 8 was in fact reconstructed from the published 3D skull surface model presented in Garberoglio

et al. (2019) (Fig. 2). But most importantly to this particular point, a brain endocast cannot be produced for the specimens of *Najash* the reviewer makes reference to. The specimen we used to illustrate in our ED Fig. 8 is the same published in Garberoglio et al. (2019) (MPCA 500). Although to our knowledge that specimen is the most complete one available for *Najash*, the parietals and frontals are severely damaged and almost entirely broken in dorsal view—see Garberoglio et al. (2019), Fig. 2c. That damage is beyond the point of simple retrodeformation. Hence, important brain components including the cerebral hemispheres (telencephalon), central to our study here, are impossible to reconstruct as endocasts. We are unaware of other specimens with better preserved braincases that could produce brain endocasts currently undergoing study by a separate group of authors, which hopefully will answer the very important points raised above on *Najash*.

One important correction remains outstanding with respect to morphospace and ecological interpretation of telencephalon morphology. The authors state:

"On another note, we recognize the incorrect mention of Xenophidion as an uropeltoid in the original text. This error has been corrected now. After re-examining the literature, we also agree that Xenophidion is more appropriately described as semi-fossorial rather than fully fossorial, and the ecological characterization has been updated accordingly in the revised manuscript."

However, MS Pg. 12 lns. 355-356 reads "In fact, Tametara it represents one of the most extreme examples among snakes, clustering near highly modified fossorial extant forms such as Xenophidion (Fig. 4A and Extended Data Fig. 9b)."

I am sure the authors did not intend to retain the description for uropeltids ("highly modified fossorial extant forms") in reference to Xenophidion. And given that they now recognize that Xenophidion is semi-fossorial, the close position in morphospace does not support the hypothesis that Tametara is highly fossorial. I recommend the authors simply delete the sentence altogether. Doing so does not weaken their hypothesis.

> Thank you for catching this. Indeed, *Xenophidion* should not be described in the main text as highly fossorial, although this taxon is still part of the broader fossorial category (fossorial + semi-fossorial) that we actually used for analyses reported in Fig. 4 and ED Fig. 9. As suggested by the reviewer, we have deleted this part of the sentence (revised MS lines 374-376).

Phylogenetic analysis

In my initial review I stated: "Both the margo ventralis (subcentral ridges) and margo lateralis (interzygapophyseal ridges) of vertebrae occur across crown snakes (Auffenberg,

1963; Holman, 2000; Szyndlar and Georgalis, 2023- with few exceptions for margo ventralis [Hoffstetter and Gasc, 1969]), and do not differentiate stem from crown taxa."

To which the authors replied:
"With regards to the polarity of the vertebral characters: almost any given phylogenetic character will undergo homoplasy across different parts of a given tree. Hence, plesiomorphic "absent" character states can still be variably "present" among stem taxa. As long established in phylogenetic inference, it is the ultimate test of character congruence or overall character agreement, that defines the final output and character polarity, not individual examples. The fact that some early snakes bear some character states that are different from Tametara is irrelevant to determining character polarity, since many other early snakes match the morphology of Tamatera, having a stronger influence in ancestral state optimization and, therefore, character polarization."

On this I completely agree with the authors and realize that I was unclear in my statement referring to the distribution of presence of these characters in crown snakes. I was not critiquing character polarity and its use in establishing interrelationships of Tametara. Instead, my observation was to indicate that the character states were miscoded for crown snakes. For example, the margo lateralis is scored in the data matrix of this study (Morpho_166t_394ch_Simple.nex) as absent on posterior presacral vertebrae (character 312) for all examined crown snakes except for Calabaria, Boa, Naja, and Laticauda. I have checked character coding for this character against C-T scans for multiple extant taxa included in the data matrix. Contrary to the codings in the matrix, the margo lateralis is present as a ridge (albeit sometimes reduced) connecting the zygapophyses throughout the posterior presacral and even cloacal vertebrae in Anilius scytale, Cylindrophis ruffus, Tropidophis melanurus, Xenopeltis unicolor, Loxocemus bicolor, Acrochordus javanicus, and Python molurus (I can provide images if desired). Because the character is more widely distributed than currently scored at least within crown Alethinophidia, the MS needs to reassess codings and impact on determination of synapomorphies or minimally provide a more robust justification/character state delineation for their coding as it stands.

> We thank the reviewer for clarifying the point they had raised previously, which was in fact on their interpretation of the vertebral anatomy for scoring the morphological characters in some crown snakes rather than the distribution of character states in the phylogeny. Concerning this point, we simply have a different interpretation of what constitutes the absent condition for this character. As the reviewer acknowledges above in "the margo lateralis is present as a ridge (albeit sometimes reduced)". Indeed, the margo lateralis can be quite faint and regressed across many species, which we consider a gray zone of

interpretation for the absent state, and so the choice of the character state in such situations is likely to change from author to author. On top of this, there is the matter of other sources of intraspecific variation—sexual, ontogenetic, population—that may drive different interpretations depending on the exact specimen examined. The latter can be a major source of variation that is unfortunately still underexplored in morphological systematics. Therefore, although we recognize that some researchers could score a different state for this character for some of the taxa, we have kept our scoring for these taxa based on the specimens we examined and to keep consistency in our interpretation of the morphology across all taxa. We will keep this comment in mind in future expansions of this dataset to look for sources of intraspecific variation that may be driving those differences.

Additionally, the MS describes the character for posterior presacral vertebrae, but only illustrates "cervical", mid-presacral, and dorsal vertebrae for Tametara. Is Extended Fig. 2c a more posterior presacral vertebrae used for coding the character? If so, it should be identified as such. If not, it would be useful to illustrate the character for the taxon to demonstrate how it is different from the condition in crown taxa.
> The characters we defined from the posterior presacral vertebrae are interpreted consistently, including in the midsection of the presacral region. Although vertebral shape may vary to some extent (which could be captured by finer-scale linear morphometric measurements), the discrete features remain relatively stable. This is why we illustrate only one dorsal for *Tametara* in ED Fig. 2c, as all dorsals more posterior to it do not differ substantially—note, however, that many other dorsals are illustrated in Fig. 2 of the main text. So, even if the most posteriorly preserved dorsals in *Tametara* are not fully posterior, and represent mid-dorsals, that would not change our scoring or description.

On a further note, we hope that our CT data for the postcranium of *Tametara*, made available here, will enable other researchers to look for sources of intra-individual variation and vertebral regionalization in stem snakes, as previously done for crown snakes.

With respect to homoplasy hypotheses required to explain the occurrence of an upper temporal arcade in Sanajeh based on the topology of this analysis, the authors replied to my critique as follows:
"Different data sets analyzed with different algorithms will naturally provide different evolutionary trajectories for specific characters. As dozens of different studies have assessed character evolution in snakes and other squamates, we find that having specific concerns or comparisons focused on Zaher et al. (2023)on the evolution of the upper temporal bar to be of somewhat out of scope and of narrow focus with respect to the

intended audience of this study. Furthermore, Zaher et al. (2023) did not apply the phylogenetic methods employed here, making comparisons, even if warranted, not meaningful."

To be clear, It is not a question of methodological choice in phylogenetic analysis, nor comparisons of different studies- It is the accurate character coding for the upper temporal arcade in Sanajeh in this analysis combined with the chosen phylogenetic topology from this analysis (MS fig. 3) that optimizes the upper temporal arcade as either lost twice or secondarily re-expressed in the early evolution of snakes. As the preferred phylogenetic hypothesis requires homoplastic reorganization of the skull roof, and because this topology is a unique hypothesis for the interrelationships of basalmost snake divergences relative to the lead author's previous phylogenies (e.g., Garberoglio et al., 2019; Simões et al., 2025), and because, as the authors note in their reply to review, there is not strong morphological support for the chosen topology in the absence of molecular data, the authors absolutely need to recognize the requirement for skull roof homoplasy as a caveat to their listed character support for the interrelationships of Tametara and Najash among stem snakes (MS pg. 10 lns. 282-296). Cautious interpretation and consideration of alternative hypotheses do not necessarily negate the impact of important discovery (e.g., Benson et al. 2025).

> Agreed. We have now included a mention and discussion on the homoplastic evolution of the upper temporal bar given our preferred phylogenetic hypothesis and placement of Sanajeh. We tried to keep it brief (revised MS lines 307-316) but hope it can be a future point of discussion in early snake evolution.

##############

Referee #2 (Remarks to the Author):

Dear authors, dear Editor,

We thank the authors for their thorough replies to our comments and for accounting for most of our concerns. We find the manuscript has improved in its newest version. We provide below a few additional suggestions to further improve the manuscript.

Sincerely,
Rémi Allemand & Marion Segall

Title: The two publications you mentioned that use the word “brain” are on birds, which present a good fit between the endocranium and the brain. You could use braincase or brain cast instead of brain. But we suggest to let the editor choose whether he is fine with the word 'brain'.

> Although snake endocasts are not as reliable a proxy for the brain as bird endocasts, squamate endocasts nevertheless provide a variable but informative representation of brain morphology (Allemand et al. 2023). Our title is therefore still appropriate, especially because the abstract clearly states all details. We leave the final decision to the editors.

LDA: from your response to our previous comment about working on a subset of PCs: ”Regarding the number of PCs used, we retained only the first five components and excluded the remaining ones because they contribute marginally to total shape variance and reduce model performance: a notorious property of PCA on 3D GMM data.”
We would be interested in your references.

Using only a subset of data can be considered cherry picking, it would be the same as getting rid of some landmarks because you consider that they only add noise. I am a strong advocate of working on raw data. This being said, I am not a statistician. I suggest asking a specialist about that issue. I remember Miriam Zelditch advocating to work on whole datasets, either Procrustes coordinates (which themselves are problematic since they violate one of the basic principles of variance analysis which is that the number of variables must be less than the number of individuals, and again. I am not a statistician and I do not know the workaround Adams and colleagues have found but their method is widely used now) or all PCs. PCs are a convenient way of having n-1 observations. PCAs are not biologically oriented, it is a mathematical decomposition of variance. It is nice when one or several PCs bear an ecological signal but if they don't, it does not mean that there is no ecological signal in a dataset.

Again, I would suggest either adding references to support your choice to use a subset of PCs and/or to ask a specialist.

“We explicitly evaluated the impact of PC number on LDA performance. Using PHYLO_PCA scores, 5 PCs yielded the best overall classification results (Accuracy = 0.6504; Kappa = 0.5045), where accuracy reflects the overall predictive performance of the model and Kappa measures classification agreement corrected for chance. This outperforms analyses based on two PCs (Accuracy = 0.5363; Kappa = 0.3326) and all eleven PCs (Accuracy = 0.6146; Kappa = 0.4595).”

We thank the authors for providing the comparison of LDA performance across different numbers of PCs. However, selecting the number of PCs based on classification accuracy alone may introduce bias, as the subset maximizing performance on the dataset does not necessarily reflect the most biologically meaningful representation of shape variation. In this context, the use of five PCs appears primarily driven by optimization of classification results, which may artificially improve performance.

Moreover, the difference in performance between five and eleven PCs is relatively small compared with the difference between two and five PCs. This suggests that including additional PCs does not substantially degrade classification and may provide a more complete representation of shape variation. Using the full set of PCs would therefore appear more statistically conservative.
We agree that the classification of the fossil as fossorial is unlikely to change when using all PCs, but relying on the full dataset would strengthen the robustness of the inference. Regarding the interpretation of Dinilysia switching from terrestrial (5 PCs) to aquatic (all PCs), this pattern may instead reflect the limited discriminatory power of telencephalon shape among most ecological categories. Indeed, the ANOVA results (Supplementary Table 3) indicate that telencephalon shape does not significantly differentiate ecologies for extant taxa, except for fossorial versus all other groups. In particular, aquatic and terrestrial taxa are not distinguishable ($p = 0.687$). Under these conditions, the change in classification of Dinilysia when additional PCs are included is not necessarily evidence that lower-variance PCs add noise, but rather reflects the weak ecological signal in the dataset.

> We thank the reviewer for raising this point, which allows us to remove any potential ambiguity regarding the methodological framework adopted.

The issue of dimensionality reduction prior to downstream analyses is well established and represents a long-standing challenge in multivariate statistics. As noted in the literature, the selection of the number of retained PCs represents a critical issue, as both underestimation and overestimation can distort the underlying patterns of variation (Jackson, 1993; Peres-Neto et al., 2005). Accordingly, multiple alternative approaches have been developed (e.g. broken-stick method, variance thresholds, cross-validation, and related stopping rules), none of which provides a universally optimal solution, and which often lead to different dimensionality choices depending on the data (Jackson, 1993; Peres-Neto et al., 2005).

This methodological heterogeneity is also reflected in recent morphometric and paleontological studies, where dimensionality is variably defined using variance-based thresholds, broken-stick approach, cross-validation, or pragmatically selected (without formal justification) numbers of PCs (Allemand et al., 2024; Cornette et al., 2015; Dollion et al., 2015; Dollion et al., 2017; Gold and Watanabe, 2018; Morley et al., 2022; Palci et al., 2017; Palci et al., 2018; Püschel et al., 2018; Vanhoof et al., 2023; Yi and Norell, 2015). In these studies, the number of retained PCs ranged from 2 to 22, and, where reported, typically represented about 80-95% of total variance. Importantly, the constraint that the number of predictors must remain lower than the number of specimens only sets an upper bound and does not uniquely determine how many PCs should be retained. The final choice necessarily involves an additional, dataset-specific decision (e.g. Allemand et al., 2024). Consistently, **none of these studies used the full set of PCs**, and different criteria lead to substantially different retained dimensionalities.

In a classification framework, this point becomes particularly relevant, and the inclusion of weakly informative or near-zero-variance components may degrade model performance by introducing noise and reducing effective discrimination among groups (Kuhn and Johnson, 2013). Accordingly, selecting the number of retained components based on classification performance represents a suitable criterion when PCA is used in conjunction with LDA (Baylac and Frieß, 2005; Bro and Smilde, 2014). In a morphometric framework, several studies have explicitly suggested and adopted the use of performance-based dimensionality selection, retaining the number of PCs that optimizes cross-validated classification, rather than relying solely on variance-based criteria (Baylac and Frieß, 2005; Baylac et al., 2003; Dobigny et al., 2002). This is the rationale adopted in our analyses as well as in several morphometric studies spanning multiple research fields (e.g. Evin et al., 2025; Fernandes et al., 2009; Hemelsdaël et al., 2025; Jeanjean et al., 2025; Lopes et al., 2020; Plomp et al., 2019; Seetah et al., 2014; Vigne et al., 2016; Villemant et al., 2007; Vujić et al., 2020).
In conclusion, the selection of five PCs was not arbitrary as claimed by the reviewers, but based on explicit evaluation of classification performance—an objectively defined statistical criterion. Secondly, in our dataset including additional components beyond the first five worsens the biological interpretation, consistent with the inclusion of weakly informative components in a classification context, a well-known point in the GMM literature, as detailed above. Thirdly, and quite importantly, independently of the number of PC retained, the main biological inference remains unchanged: *Tametara* is consistently classified as fossorial, while the variability observed for other categories likely reflects a lower discriminatory power of telencephalon shape among non-fossorial ecologies. Fourthly, the five retained PCs also account for a high proportion of the total variance

(>95%), placing our choice within the upper part of the range commonly retained in comparable morphometric and paleontological studies cited above.

For all these reasons, the final number of PCs used here is methodologically justified and appropriate for the inferential objective of our study. Far from representing cherry-picking, it reflects a model-based approach that is consistent with both statistical theory and current practice in morphometric analyses. We highlight that these points had all been mentioned in our manuscript before. To satisfy reviewers and editors, we have further expanded these details and added even more references to the Methods section (revised MS lines 938-949).

Which leads us to another comment :

Telencephalon as a predictor of ecology:
L314: You state that the telencephalon shape is a "reliable predictor of habitat use in squamates" and cite one of your previous publications. While that study does include analyses of telencephalon shape, it appears to focus on locomotor categories rather than habitat use. The extrapolation from locomotion to habitat therefore seems stronger than what is directly supported by the analyses. Moreover, the conclusions of that study appear relatively cautious. For instance, you note that: "However, post hoc pairwise comparisons only reveal significant morphological differences between a few locomotor modes (table S4), including between burrowing or facultative burrowing and other limbless movements. This suggests that whole-brain shape parameters may be a weak indicator of squamate locomotion, at least based on the squamate dataset used in this study." This seems difficult to reconcile with the statement that telencephalon shape is a reliable predictor of habitat use. The statement at L314 may therefore be too strong and could be revised to better reflect the scope of the cited study. Since the present manuscript directly tests the relationship between telencephalon shape and ecology, the results can stand on their own without relying on this claim. More generally, it may also be preferable to avoid overstating the ecological signal, as the analyses presented here suggest that telencephalon shape primarily distinguishes fossorial taxa from other ecologies, while differences among the remaining categories appear limited.

> We agree that the original statement may have overstated the scope of the cited study. We have revised the text to remove this claim and to more accurately reflect the anatomical rationale underlying our approach (revised MS lines 327-332).

Squamate versus snakes dataset: "We appreciate the reviewer's suggestion. However, we

do not see a mechanism by which a more inclusive squamate sample would obscure ecological signal in the geometric morphometric analyses. On the contrary, including the full squamate breadth provides a more robust morphological and ecological context for interpreting snake brain shape variation. For this reason, we do not consider it necessary to modify the sample size or composition.

The composition of the sample should reflect the biological question being addressed. Here, the aim appears to be inferring the ecology of a snake fossil rather than testing whether the fossil falls within snakes versus other squamates. Including a broad range of non-snake squamates may therefore introduce large-scale morphological differences that are not directly relevant to the ecological question.

More inclusive sampling can affect morphospace structure by increasing variance driven primarily by major clade-level differences rather than ecological variation. In extreme cases, this may reduce the interpretability of principal components in an ecological framework. For instance, adding very distantly related taxa would likely distort morphospace by emphasizing major anatomical differences unrelated to ecology. Figure 4b appears to illustrate this issue. The main axis of variation (PC1) seems largely driven by differences between snakes and lizards rather than ecological differences. As a result, PC1 becomes difficult to interpret in terms of ecology for Tametara. This effect appears less pronounced in Figure 4a. That said, given the strong fossorial signal recovered for Tametara, we do not consider it necessary to modify the sampling. However, we suggest acknowledging that including non-snake squamates may influence the structure of morphospace and reduce the ecological interpretability of the main axes of variation.

> We recognize that inclusion of a broad non-snake squamate sample may influence morphospace structure and, in some cases, reduce the ecological interpretability of the principal axes. However, our aim was to interpret fossil snake brain shape within a broader comparative framework across all squamates rather than within snakes alone. It is not possible to test whether the telencephalon shape in stem snakes is intermediate between crown snakes and non-ophidian squamates without a broad squamate sampling as performed herein, a point we addressed before. Doing the more restrictive sampling suggested by reviewers here would not allow us to test some of the core questions of this study. We therefore retained the more inclusive sampling strategy, as it provides essential comparative morphological and ecological context for interpreting fossil snake brain shape. As suggested by the Reviewer, we have clarified this point in the revised manuscript (revised MS lines 325-327).

Line 123: “Therefore, Tametara is intermediate in skull size, relative to the extant Cylindrophis ruffus (skull length =29.16 mm) and Xenopeltis unicolor (skull length = 36.33 mm).” This statement relies on a predicted value (~33 mm), for which the method of estimation is unclear, rather than a direct measurement. In addition, the comparison with two extant species is not justified, and the conclusion that Tametara “is intermediate in skull size” appears unsupported. It is unclear what this comparison is intended to demonstrate. If the goal is simply to provide a size comparison with extant fossorial species, the sentence could be rephrased more cautiously. For example: “Compared to extant fossorial species, the skull size of Tametara would likely fall between Cylindrophis ruffus (skull length = 29.16 mm) and Xenopeltis unicolor (skull length = 36.33 mm).” Alternatively, the authors could clarify how the predicted value was obtained and justify the choice of comparative taxa.

> First, we note that the point of the statement was merely to satisfy the reviewers own comments in the previous round of reviews who requested it “Osteological description: there is no mention of the size of the specimen anywhere… But since your specimen is quite small, we agree that it would tend to look like an *Anilius* or *Cylindrophis*.”. As stated above, these comparisons are merely an estimate, to give a general sense of size of the specimen relative to other snakes.

Secondly, as to how it was measured: we cannot make a direct measurement because the skull is broken and missing the anterior half, as clearly illustrated in specimen images.

As common with vertebrate fossils, body sizes are almost always an estimate from preserved elements. This relies on comparisons with closely related species (i.e., phylogenetic bracketing), an approach which is now decades-old in the paleontological literature (Witmer 1997). We had already made this point explicit in our methods (MS lines 976-983): “For missing skull roof segments in fossil specimens, we extrapolated trait expression from the preserved parts. To this end, we reconstructed the proportion of these values by means of phylogenetic bracketing. We based this on the average trait expression encountered in Serpentes, Anguimorpha, and Iguania, weighted at a ratio of 2:1:1. Anteroposterior cranial profile positions were reconstructed based on the average cross section distances for Serpentes.”.

Lines 129–138 & 139–154: Lines 129–138 describe the postfrontal, supratemporals, and then the quadrate, whereas lines 139–154 describe the frontal followed by the parietal.

The order in which the bones are described remains confusing and would benefit from a more consistent anatomical sequence.

> We fail to see the issue with the order of description of those elements. They are consistent with the order they are presented in standard morphological descriptions of new taxa in the paleontological literature, where antorbital elements are described, followed by orbital elements, and followed by dorsal skull roof elements, etc.—e.g., (Evans and Barbadillo 1998; Evans and Barbadillo 1999; Simões et al. 2015; Apesteguía et al. 2016; Simões et al. 2017; Xing et al. 2018).

Lines 152–154: "…it has no 'medial parietal pillar', which typically contributes to the separation between the telencephalon and mesencephalon in snake brains; similar to the condition in Dinilysia, Najash, and scolecophidians (blind snakes)."
Please provide references supporting this statement.

> The supporting reference was already present in the text. As noted by (Scanferla 2022), "The lateral part of this sulcus is occupied by the medial parietal pillar, which separates the cerebral hemisphere from the bony otic region and the optic tectum (Fig. 14.2F)."

Line 159: We do not understand this sentence. The first part of the paragraph refers to overlap of the supraoccipital by the parietal, whereas the second part of the paragraph discusses the relative size of the supraoccipital. The logical connection between these statements is unclear.

> The connection is simple: the relative size of the supraoccipital depends on how much of it is exposed (and actually measured), which is influenced by how much of it is overlapped by the parietal. We have clarified the paragraph (revised MS lines 157-166).

Line 213: "This correspondence enables meaningful quantitative comparisons among extant snakes" This sentence is unclear. Why does the correspondence between brain and endocast would enable meaningful quantitative comparisons? The two cited references compared endocasts, not brain and not the correspondence between the two. The word "comparison" is vague in the sentence. Do you mean studying the endocast enables to draw conclusion on the ecology of species? If so, you need to rephrase and to remove the first part of the sentence about the brain-endocast correspondence since it is not what either of us have tested, nor what the present work tests.

> Both this statement and the term "comparison" are used here in the exact same sense as in the following statement by Allemand et al. (2023), p3, which we do not consider vague: "*By comparing endocasts and corresponding brains of different species, Scanferla (2022) qualitatively showed that snake endocasts represent a realistic representation of brain size and shape*." and also in the abstract: "*Our results indicate that squamate endocasts provide variable representation of the brain, depending on species and neuroanatomical regions*". However, we have revised the paragraph to clarify its meaning and to more accurately reflect the scope of the published comparisons based on endocast morphology (revised MS lines 216-221).

Lines 214-215: It has also been used both for qualitative assessments of fossil endocasts relative to extant taxa to inform interpretations of brain ecomorphology, and for quantitative comparisons of fossil squamates within a phylogenetic context. This is unclear. How does brain–endocast correspondence allow quantitative comparisons if those comparisons are based only on endocasts?

> See the text revision mentioned above.

Lines 227-228: "This diverges from the condition observed in most squamates, including other snakes, which display well-expanded cerebral hemispheres that strongly contrast with the mesencephalon and rhombencephalon (= mid-and hindbrain, respectively)"
A similar pattern is present in other species as well (see Extended Data Fig. 7), especially in fossorial species and large species (see also Allemand et al 2017, 2022, and Fig 4 in Segall et al 2021 showing the allometric pattern in snakes endocranium). This should be clarified.

> We agree and have revised the paragraph to clarify its meaning (revised MS lines 229-238), also incorporating the references mentioned by the reviewer.

Lines 230-233: "Interestingly, however, the telencephalon condition of Tametara closely resembles that of species with high degrees of fossoriality, including fossorial snakes such as scolecophidians (e.g., Liotyphlops), Anilius, and Uropeltis, as well as other fossorial, limb reduced non-ophidian squamates such as the amphisbaenian Blanus and the skink Melanoseps (Extended Data Fig. 7)".
This is confusing. The previous sentence states that the condition diverges from most squamates, but here the authors say it resembles certain snakes. The relationship between these statements should be clarified.

> We have revised the paragraph to clarify its meaning (revised MS lines 229-238).

Line 239: “The mesencephalon of Tametara is unusually elongated relative to most other squamates”
Could you be more precise? In which direction is it elongated? For example, the condition seems comparable to Thamnophis in Extended Data Fig. 7.

> We have revised the sentence accordingly and now specify that the mesencephalon is “posteriorly elongated” (revised MS lines 244-245).

Lines 240-241 : “The mesencephalon of Tametara is unusually elongated relative to most other squamates, including snakes, with a poorly defined optic tectum (= optic lobe). Among extant snakes, the most similar condition to Tametara is seen in Cylindrophis36,40 (Figs. 1i,j and 2j and Extended Data Fig. 1i-k).”
We do not see how it is “unusually elongated”. A poorly defined optic tectum is a condition shared by many species of snakes except highly visually oriented species. Consider rephrasing.

> We have revised the sentence accordingly (revised MS lines 244-245).

Lines 245-246 : “The rhombencephalon of Tametara is characterised by a strongly laterally constrained, hourglass-shaped region (Fig. 2j and Extended Data Fig. 1i,j), as similarly observed in Anilius scytale and Dinilysia”.
This morphology appears to be present, to different extent, in most species since it corresponds to the space occupied by the inner ear.

> We agree that the structure is present in many species but in *Tametara*, *Dinilysia* and *Anilius* it is quite prominent compared to other snakes. This structure has been referred to as choroid plexus of the 4th ventricle or associated structures in different publications, and the inner ear bones actually delimit but do not occupy such endocranial space. In the main text, we now describe the structure as “dorsally projecting”, as an hourglass-shaped region would be expected to be “laterally compressed” (revised MS lines 251-252).

Lines 285-287: “The inferred placement of Tametara prior to the split of Dinilysia and all other snake fossils is explained by plesiomorphic skull features, including the presence of a dorsum sellae on the basisphenoid (absent in later-deriving snakes)”
We are not sure to understand what you mean by “later-deriving snakes”? Are you implying that all extant species of snakes lack a dorsum sellae? If so, the feature is present in most

alethinophidian snakes, but not in the Uropeltidea snakes (at least the ones that we checked) or Anilius, and some weird species like Atractaspis. This statement therefore needs clarification.

> We thank the reviewer for pointing this out. By "later-deriving snakes" we meant taxa positioned more crownward than *Tametara* and *Dinilysia* on the stem, rather than crown snakes as a whole. We did not intend to imply that all extant snakes lack a dorsum sellae. Because this wording was potentially misleading, we have revised the sentence to read "absent in many later-deriving snakes" (MS line 293). We agree that the distribution of this feature is more complex. A full discussion of the evolutionary history of this character is beyond the scope of the present study, but the character optimization can be traced in our phylogenetic dataset provided in the Supplementary Data.

Line 315 & 322: 'We find that the earliest known snake brain endocasts of Tametara and Dinilysia…" These results indicate that snakes achieved substantial disparity in brain shape'
Only telencephalon shape is tested here, not the whole endocst(even less the brain). Please make sure to only refer to the telencephalon here.

> We have rephrased the two sentences accordingly (revised MS lines 333-339).

Supplementary data, line 195: Missing period in "T mirim".

> Edited.

Supplementary L363 (previously flagged in first review):"Compared to extant snakes, Tametara mirim and Dinilysia, have remarkably large pituitary glands, although the length of these structures might only correspond to the elongate parietal of these species, bearing no significant sensory effect…"

This remains unclear. If the apparent enlargement results from parietal elongation rather than pituitary size, please clarify explicitly how the morphology of the parietal affects the interpretation of pituitary gland size. What do you mean by "bearing no sensory effect"? To our knowledge, the pituitary has a role in hormone secretion, but we may have missed some information.

> As now mentioned in the Supplementary text (Supplementary lines 355-360), this corresponds to Allemand et al. (2017)'s measurement #14 "*Length of the pituitary gland*

(LP): distance between the fronto-parietal suture and the most posterior point of the pituitary bulb". We used this proxy when comparing *Tametara*'s pituitary size with that of other snakes, and concluded the length was greater in *Tametara* and *Dinilysia* in comparison to other snakes; however, in these two fossils, the parietal is proportionately longer, and the resulting increased size may be simply the allometric result of this elongation. As for the "sensory effect" we were using the terminology adopted in Allemand et al. (2017), but we recognize it was not the most accurate; since we were discussing potential ecological roles, we have adjusted the language. The sentence now reads (Supplementary lines 360-364): "*Compared to extant snakes, Tametara and Dinilysia, have remarkably long pituitary glands, although the length of these structures might only correspond to the elongate parietal of these species, bearing no significant sensory ecological or hormonal effect*". [allusion to the measurement was included earlier in the paragraph, see two comments below]

L235-236: "In the diencephalon, the posterior portion of the pituitary region is notably large (Fig. 2J and Extended Data Fig. 1j), tilting strongly ventrally towards the hypophyseal fossa". How do you differentiate the pituitary region from the hypophyseal fossa?

> We differentiate between the pituitary region of the endocast and the structure on the dorsal surface of the basisphenoid. We revised the text accordingly (revised MS lines 239-243).

L237-238: "Among extant snakes, fossorial species do not show a markedly large pituitary, while marine and terrestrial forms typically have a more ventrally developed gland"
What do you mean exactly by "large"? Do you mean that the hypophyseal fossa is deep? Or do you mean that, according to the endocranial imprint, the pituitary is wide? According to the end of the sentence, by large, you mean ventrally developed. Some fossorial snakes present a large pituitary, such as Calabaria reinhardtii, Eryx sp., Calamaria lumbricoidea...

> Large here is also referring to measurement #14 of Allemand et al. (2017), because this was the most significant one the analyses regarding this feature. In this comparison with fossorial and marine species, we were alluding to the statistical tests carried out in the aforementioned reference, in which fossorial and marine habits were usually associated with this trait (page 861). We have adjusted the description in both the main text (revised MS lines 239-243) and Supplementary Information (Supplementary lines 355-360): "*Although the anterior portion of the pituitary region of Tametara is missing, the posterior projection is notably long (following measurement #14 of Allemand et al. 2017), tilting strongly ventrally towards the hypophyseal fossa. Among extant snakes, fossorial species*

do not tend to show a markedly long pituitary, while marine and terrestrial taxa tend to have a more ventrally developed gland"

L329-332: "This pattern suggests that derived lineages may converge secondarily on brain morphologies resembling ancestral conditions, highlighting an unexpected discrepancy between the transitional skull anatomy of stem snakes and the strongly divergent morphology of their central nervous system"
We found this interpretation unclear. It is not evident which taxa are considered derived, what is meant by "ancestral" brain morphology, or how the results support secondary convergence. The proposed discrepancy between skull and brain morphology should be clarified or the sentence rephrased.

> We thank the reviewer for this comment. We agree that the original wording was unclear and overstated the inference. We have therefore rephrased the sentence accordingly (revised MS lines 344-349).

L351: "Kappa value (0.5) indicates a robust classification"
Given that Kappa ranges from 0 to 1, a value of 0.5 may be better described as moderate rather than robust. Similarly, an overall reclassification accuracy of 65% appears relatively limited and may not support a strong claim of classification performance.
It would also be informative to report classification rate by ecological category. As suggested by the ANOVA results, telencephalon shape appears to distinguish fossorial taxa from other ecologies, but provides limited discrimination among the remaining groups. The overall accuracy may therefore mask uneven performance across categories. For example, the model may correctly classify fossorial species at a high rate while showing lower accuracy for other ecological categories.
Providing the percentage of correctly classified taxa for each ecology, particularly fossorial species, would help clarify the actual discriminatory power of the model and better support the interpretation.

> We thank the Reviewer for this helpful suggestion. We agree that the previous wording overstated the overall classification performance of the model, and we have removed the word "robust". As suggested by the reviewer, we examined classification performance separately for each ecological category and provided it in the main text. This shows that the overall values mask some differences among groups: in extant taxa, 85.6% of fossorial species are correctly classified, compared with 60.5% of arboreal taxa, 62.5% of terrestrial taxa, and 22.0% of aquatic taxa. Thus, among non-fossorial categories, the clearest deviation from the overall model accuracy is observed in aquatic taxa, whereas arboreal

and terrestrial groups remain comparatively close to the global classification rate. This pattern is consistent with the Procrustes-based ANOVA results, which indicate that telencephalon shape carries its strongest ecological signal in relation to fossoriality, whereas discrimination among the remaining categories is more limited.

Tametara is classified as fossorial with very high posterior probability in the LDA (0.9923) and also shows its highest typicality probability for the fossorial category (0.5744), whereas all alternative ecological assignments are very low. Because fossorial taxa are also the best discriminated category in the extant training set, this supports a robust fossorial interpretation for *Tametara*, as previously reported in the MS.

By contrast, *Dinilysia* is classified as terrestrial by the LDA, but its typicality probabilities are low and diffuse across all categories (highest value = 0.0854 for terrestrial), indicating that it does not fit closely within any extant ecological group. We therefore interpret *Dinilysia* more cautiously as non-fossorial, while avoiding strong confidence in any single alternative ecological assignment. In this sense, our results indicate that the non-fossorial signal is more robust than any specific alternative ecological assignment.

Overall, these results make the main conclusions of the analysis clear: *Tametara* is robustly supported as fossorial, whereas *Dinilysia* is consistently recovered as non-fossorial, even if its precise placement among alternative non-fossorial ecologies remains uncertain. We now report category-specific classification performance (revised MS lines 369-371) and highlight the specificity limits related to *Dinilysia* habitat inference (revised MS lines 393-404). We also describe *Dinilysia* as non-fossorial, rather than assigning it to a more specific ecology, in both the Abstract and the Conclusions (revised MS lines 456-457) of the revised manuscript.

Referee #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

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Referee #4 (Remarks to the Author):

The manuscript is well written and robust, and it does not show deficiences to prevent its publication. Conclusions are original. The results are interesting enough for several

researchers in evolutionary biology and vertebrate paleontology. We were not able to do a code revision since the data matrix is excessive for our hardware devices.

> We thank the reviewers for their comments. We are happy over their excitement over our findings.

F. Suggested improvements:
In Line 106, discussing the ontogenetic stage, it was not clear to us what the degree of overlap between parietal and prootic here meant, since the dorsal exposure of the supraoccipital in Tametara is actually greater than in most snakes, specially adults. Additionally, we failed to mention earlier that fully fused neural arches to vertebral pleurocentra is not a clear indicator of adult ontogenetic stage for snakes, since the neural arch and the vertebral pleurocentra are already fully fused in very early ontogenetic stages in snakes, including fossil and extant (Winchester & Bellairs, 1977; Xing et al., 2018).

> We are happy to clarify this point. Endochondral bones can only grow in degree of overlap during ontogeny, not the other way around. Therefore, a higher degree of overlap is necessarily indicative of later ontogenetic stages, especially for specimens with a substantial degree of overlap compared to most adult extant snakes, as illustrated by *Tametara* (and noted by the reviewers).

As for the fusion of neural arches to vertebral pleurocentra, we agree with the reviewers on their limited value towards establishing ontogenetic stages for snakes, unlike other squamates. On the other hand, other aspects of ontogenetic status can be implied from vertebral elements, such as the relative size of the neural canal and the retention of a notochordal canal in neonate specimens, such as illustrated by Xing et al. (2018). We have now made those considerations in the Ontogenetic stage section (revised MS lines 112-113).

In Line 116, classifying them in dermatocranium, splanchnocranium, and neurocranium, we accept terms provided by Kardong (2012) but at least in the Kardong vrsion we have, the pterygoid is part of the dermatocranium, not part of the splachnocranium.

> Agreed and edited.

Additionally, it seems that the authors misunderstood what was suggested as a possible part of the stapedial shaft. Actually, we are uncertain of the identification of that small fragment of bone in Tametara that is now labelled as the stapes (Sta), but its robust

morphology and placement do not particularly match the stapedial shaft of either Najash or Dinilysia (image in attached version). What is circled in red in the image above taken from Fig. 2 of the MS is what was suggested as a possible portion of the stapedial shaf in Tametara by us, rather than being part of the left prootic as reconstructed by the authors. We should have explained our observation with such an image in our past revision (sorry for that). This element is thin and located in contact or close to the suprastapedial process of the prootic. As such, morphology and placement matches the stapedial shaft of Najash and Dinilysia.

> Thank you for your consideration. However, we note that its placement is not the best way to recognize it as the stapes is frequently shifted in position even in articulated specimens. For the fragmentary element the reviewers have highlighted now, it is equivalent to the posterodorsal extension of the prootic, which extends dorsal to the paroccipital process, on the more complete right side of the specimen (see images below). Secondly, the element is mostly flat and not really matching the shape of a stapedial shaft. Hence, it is unambiguously interpreted by us as part of the prootic.

left: arrow showing posterodorsal extension of the prootic; middle: same in dorsolateral view without supratemporal showing the trough; right: arrow pointing to the broken-off piece identified by reviewers as stapes.

Line 133. Not agreed. What the authors are interpreting in the picture above as the fronto-parietal suture in MACN-RN 1013 is, in fact, just part of a crack within the parietal, the actual suture is more anteriorly located, and is clearly W-shaped as in the holotype and mentioned in the previous descriptions. See the picture below including an actual photo of the specimen, and an interpretative drawing of the same from Caldwell and Albino (2002). (image in attached version).

> Agreed. We see what the reviewers mean now with their image provided. We have re-phrased this part of the description in the main text and SI, following the reviewers own words "...anteromedial process that would have inserted in between the frontals (Fig. 2d),

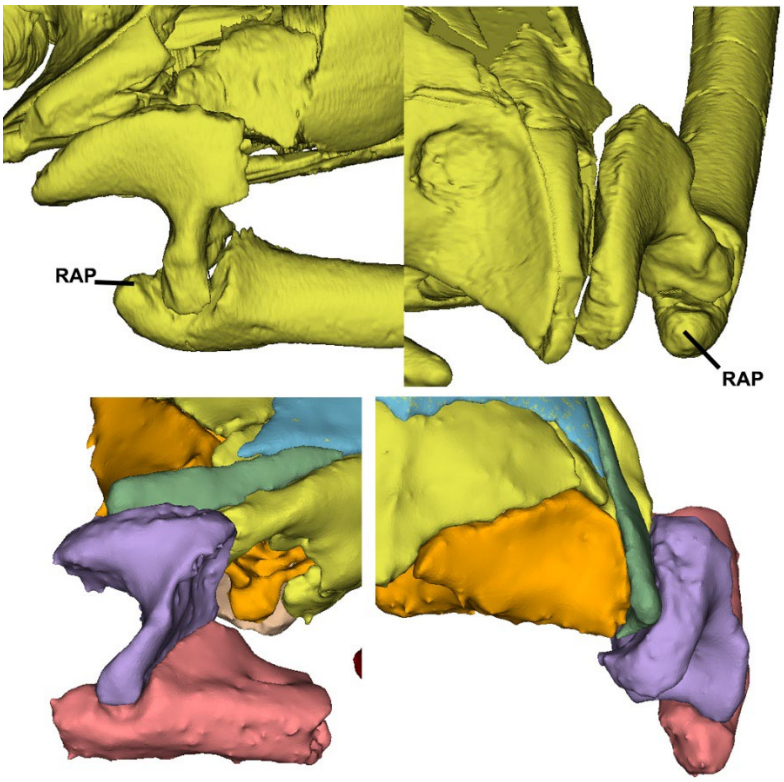
which is absent in *Najash*, but arguably present in *Dinilysia* where the parietal contacts the frontals in a W-shaped interdigitating suture" (revised MS lines 145-147).

A separate matter is whether a W shaped suture is homologous with the anteromedial process of other snakes, besides the quite substantial ontogenetic variation in the shape of this suture among squamates(Bell et al. 2003; Simões et al. 2016). Therefore, for the character dataset we have kept the score for this character in *Dinilysia* as "?", keeping consistent with our change in the last round of review. We hope to have further debate on the homology of this structure across stem snakes with our colleagues in the near future and improve our dataset even further.

Line 165. Respect to the absence of the retroarticular process in Tametara, We understand that this point is very subjective, and it is now made clear in the MS that the absence in Dinilysia in contrast with previous descriptions is the authors interpretation based on personal observation, which is perfectly fine (reference to observed material of Dinilysia should be added to this character in Supplementary Table 1). However, we still fail to understand what is the rationale behind considering the presence of a small retroarticular process in Najash (in agreement with Garberoglio et al., 2019, but the new Supplementary Table 1 states this feature as previously undescribed for Najash) and to find no evidence of such a process in Tametara, since there it seems to be no distinction between the two. An image of MPCA-500 taken from Garberoglio et al. (2019) is attached to be compared to Tametara from Fig. 2 in the MS.

> We have added reference to observed material of *Dinilysia* to Suppl. Table 1, as requested. Regarding our Suppl. Table 1 stating this feature as previously undescribed for *Najash,* what we meant is that the retroarticular process is not textually described in the text of Garberoglio et al. (2019), although it was illustrated therein.

We justify the presence of a retroarticular process (RAP) in *Najash* as the latter has a small but dorsally projecting RAP, clearly beyond the confines of the glenoid border, which in our interpretation is not the case in *Tametara* (see picture below). We have made this criterion of interpretation now explicit in this section of the description as suggested, also acknowledging its difficult interpretation (SI, lines 286-288). We decided to keep the scoring of this character this way for consistency in the way that we have interpreted it across multiple taxa (not just the ones discussed here directly), which is also the way this has been scored in previous versions of this dataset, including its scoring in Garberoglio et al. (2019). We are open to further discussions on potentially more objective ways to delimit the absence/presence of the RAP with our colleagues in the future.



In Line 255, concerning the contact between the prootic and the supraoccipital in Najash, authors removed the Attribution to Najash. However, this was not necessary. Our original observation (saying “not observed in all”, and not “not observed in any”, don’t know why is changed here) referred that it is visible in some specimens and not in other. Actually, it is described originally for Najash.

> OK. This was not entirely clear before. We have now added the attribution to *Najash* back in the SI text (revised SI line 259).

G. References: appropriate credit to previous work?
Yes.

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions
Abstract is clear.
Conclusions are ok.

Referee #4 (Remarks on code availability):

We were not able to do a code revision since the data matrix is excessive for our hardware devices.

Referee #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

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Authors' references:

Allemand, R., J. Abdul-Sater, S. Macrì, N. Di-Poï, G. Daghfous, and M. T. Silcox. 2023. Endocast, brain, and bones: Correspondences and spatial relationships in squamates. The Anatomical Record 306:2443-2465.

Allemand, R., Polcyn, M. J., Houssaye, A., Vincent, P., López-Aguirre, C. and Bardet, N. (2024). First Virtual Reconstruction of a Mosasaurid Brain Endocast: Description and Comparison of the Endocast of Tethysaurus nopcsai with Those of Extant Squamates. Diversity 16, 548.

Apesteguía, S., J. D. Daza, T. R. Simões, and J. C. Rage. 2016. The first iguanian lizard from the Mesozoic of Africa. Royal Society Open Science 3:160462.

Baylac, M. and Frieß, M. (2005). Fourier Descriptors, Procrustes Superimposition, and Data Dimensionality: An Example of Cranial Shape Analysis in Modern Human Populations. In Modern Morphometrics in Physical Anthropology, pp. 145–165. New York: Kluwer Academic Publishers-Plenum Publishers.

Baylac, M., Villemant, C. and Simbolotti, G. (2003). Combining geometric morphometrics with pattern recognition for the investigation of species complexes. Biol. J. Linn. Soc. 80, 89–98.

Bell, C. J., S. E. Evans, and J. A. Maisano. 2003. The skull of the gymnophthalmid lizard Neusticurus ecpleopus (Reptilia: Squamata). Zoological Journal of the Linnean Society 139:283-304.

Bro, R. and Smilde, A. K. (2014). Principal component analysis. Anal. Methods 6, 2812–2831.

Cornette, R., Herrel, A., Stoetzel, E., Moulin, S., Hutterer, R., Denys, C. and Baylac, M. (2015). Specific information levels in relation to fragmentation patterns of shrew mandibles: Do fragments tell the same story? J. Archaeol. Sci. 53, 323–330.

Dobigny, G., Baylac, M. and Denys, C. (2002). Geometric morphometrics, neural networks and diagnosis of sibling Taterillus species (Rodentia, Gerbillinae). Biol. J. Linn. Soc. 77, 319–327.

Dollion, A. Y., Cornette, R., Tolley, K. A., Boistel, R., Euriat, A., Boller, E., Fernandez, V., Stynder, D. and Herrel, A. (2015). Morphometric analysis of chameleon fossil fragments from the Early Pliocene of South Africa: a new piece of the chamaeleonid history. Sci. Nat. 102, 1–14.

Dollion, A. Y., Measey, G. J., Cornette, R., Carne, L., Tolley, K. A., da Silva, J. M., Boistel, R., Fabre, A. and Herrel, A. (2017). Does diet drive the evolution of head shape and bite force in chameleons of the genus Bradypodion ? Funct. Ecol. 31, 671–684.

Evans, S. E. and L. J. Barbadillo. 1998. An unusual lizard (Reptilia: Squamata) from the Early Cretaceous of Las Hoyas, Spain. Zoological Journal of the Linnean Society 124:235-265.

Evans, S. E. and L. J. Barbadillo. 1999. A short-limbed lizard from the Lower Cretaceous of Spain. Special Papers in Palaeontology 60:73-85.

Evin, A., Ameen, C., Brassard, C., Dennis, S., Antipina, E. E., Bonhomme, V., Boudadi-Maligne, M., Britton, K., Cano, F. G., Carden, R. F., et al. (2025). The emergence and diversification of dog morphology. Science (80-.). 390, 741–744.

Fernandes, F. A., Fornel, R., Cordeiro-Estrela, P. and Freitas, T. R. O. (2009). Intra- and interspecific skull variation in two sister species of the subterranean rodent genus Ctenomys (Rodentia, Ctenomyidae): Coupling geometric morphometrics and chromosomal polymorphism. Zool. J. Linn. Soc. 155, 220–237.

Garberoglio, F. F., R. O. Gómez, S. Apesteguía, M. W. Caldwell, M. L. Sánchez, and G. Veiga. 2019. A new specimen with skull and vertebrae of Najash rionegrina (Lepidosauria: Ophidia) from the early Late Cretaceous of Patagonia. Journal of Systematic Palaeontology 17:1533-1550.

Gold, M. E. L. and Watanabe, A. (2018). Flightless birds are not neuroanatomical analogs of non-avian dinosaurs. BMC Evol. Biol. 18, 1–11.

Hemelsdaël, A., Lebrun, R., Guintard, C., Jiménez-Manchón, S., Mureau, C., Jeanjean, M., Guignet, A. and Evin, A. (2025). Skull and inner ear morphometrics in sheep and goats: species and breed differentiation with bioarchaeological applications. R. Soc. Open Sci. 12, e280.

Jackson, D. A. (1993). Stopping Rules in Principal Components Analysis: A Comparison of Heuristical and Statistical Approaches. Ecology 74, 2204–2214.

Jeanjean, M., Mureau, C., Valenzuela-Lamas, S., Nieto-Espinet, A., Gardeisen, A., Colominas, L., Renaud, A., Jiménez-Manchón, S., Segui, M. S. and Evin, A. (2025). Multifactorial temporo-spatial structuring of the morphological diversity of domestic pigs, sheep, and goats between Catalonia (Spain) and Languedoc (France) from the Iron Age to Antiquity. Archaeol. Anthropol. Sci. 17, 25.

Kuhn, M. and Johnson, K. (2013). Applied Predictive Modeling. New York, NY: Springer New York.

Lopes, C. F., Stefanello, F., Bugs, C., Stenert, C., Maltchik, L. and Ribeiro, J. R. I. (2020). Sexual dimorphism in Belostoma angustum Lauck (Insecta: Heteroptera: Belostomatidae) may be related to paternal care. Biol. J. Linn. Soc. 129, 288–314.

Morley, J., Bucchi, A., Lorenzo, C. and Püschel, T. A. (2022). Characterizing the body morphology of the first metacarpal in the Homininae using 3D geometric morphometrics. Am. J. Biol. Anthropol. 177, 748–759.

Palci, A., Hutchinson, M. N., Caldwell, M. W. and Lee, M. S. Y. (2017). The morphology of the inner ear of squamate reptiles and its bearing on the origin of snakes. R. Soc. Open Sci. 4, 170685.

Palci, A., Hutchinson, M. N., Caldwell, M. W., Scanlon, J. D. and Lee, M. S. Y. (2018). Palaeoecological inferences for the fossil Australian snakes Yurlunggur and Wonambi (Serpentes, Madtsoiidae). R. Soc. Open Sci. 5, 172012.

Peres-Neto, P. R., Jackson, D. A. and Somers, K. M. (2005). How many principal components? stopping rules for determining the number of non-trivial axes revisited. Comput. Stat. Data Anal. 49, 974–997.

Plomp, K., Viðarsdóttir, U. S., Dobney, K., Weston, D. and Collard, M. (2019). Potential adaptations for bipedalism in the thoracic and lumbar vertebrae of Homo sapiens: A 3D comparative analysis. J. Hum. Evol. 137, 102693.

Püschel, T. A., Marcé-Nogué, J., Gladman, J. T., Bobe, R. and Sellers, W. I. (2018). Inferring locomotor behaviours in Miocene New World monkeys using finite element analysis, geometric morphometrics and machine-learning classification techniques applied to talar morphology. J. R. Soc. Interface 15, 20180520.

Scanferla, A. 2022. A Glimpse into the Evolution of the Ophidian Brain. Pp. 294-315 in D. J. Gower, and H. Zaher, eds. The Origin and Early Evolutionary History of Snakes. Cambridge University Press, Cambridge.

Seetah, K., Cucchi, T., Dobney, K. and Barker, G. (2014). A geometric morphometric re-evaluation of the use of dental form to explore differences in horse (Equus caballus) populations and its potential zooarchaeological application. J. Archaeol. Sci. 41, 904–910.

Simões, T. R., M. W. Caldwell, and A. W. A. Kellner. 2015. A new Early Cretaceous lizard species from Brazil, and the phylogenetic position of the oldest known South American squamates. Journal of Systematic Palaeontology 13:601-614.

Simões, T. R., M. W. Caldwell, R. L. Nydam, and P. Jiménez-Huidobro. 2017. Osteology, phylogeny, and functional morphology of two Jurassic lizard species and the early evolution of scansoriality in geckoes. Zoological Journal of the Linnean Society 180:216-241.

Simões, T. R., G. F. Funston, B. Vafaeian, R. L. Nydam, M. R. Doschak, and M. W. Caldwell. 2016. Reacquisition of the lower temporal bar in sexually dimorphic fossil lizards provides a rare case of convergent evolution. Scientific Reports 6:24087.

Vanhoof, M. J. M., Croquet, B., De Groote, I. and Vereecke, E. E. (2023). Principal component and linear discriminant analyses for the classification of hominoid primate specimens based on bone shape data. R. Soc. Open Sci. 10, 230950.

Vigne, J. D., Evin, A., Cucchi, T., Dai, L., Yu, C., Hu, S., Soulages, N., Wang, W., Sun, Z., Gao, J., et al. (2016). Earliest "domestic" cats in China identified as leopard cat (Prionailurus bengalensis). PLoS One 11, 1–11.

Villemant, C., Simbolotti, G. and Kenis, M. (2007). Discrimination of Eubazus (Hymenoptera, Braconidae) sibling species using geometric morphometrics analysis of wing venation. Syst. Entomol. 32, 625–634.

Vujić, A., Šašić Zorić, L., Ačanski, J., Likov, L., Radenković, S., Djan, M., Milić, D., Šebić, A., Ranković, M. and Khaghaninia, S. (2020). Hide-and-seek with hoverflies: Merodon aureus – a species, a complex or a subgroup? Zool. J. Linn. Soc. 190, 974–1001.

Witmer, L. M. 1997. The Evolution of the Antorbital Cavity of Archosaurs: A Study in Soft-Tissue Reconstruction in the Fossil Record with an Analysis of the Function of Pneumaticity. Memoir (Society of Vertebrate Paleontology) 3:1-73.

Xing, L., M. W. Caldwell, R. Chen, R. L. Nydam, A. Palci, T. R. Simões, R. C. McKellar, M. S. Y. Lee, Y. Liu, H. Shi, K. Wang, and M. Bai. 2018. A mid-Cretaceous embryonic-to-neonate snake in amber from Myanmar. Science Advances 4:eaat5042.

Yi, H. and Norell, M. A. (2015). Evolutionary Biology: The burrowing origin of modern snakes. Sci. Adv. 1, 3–8.

All changes described below refer to line numbers on the revised MS with tracked changes.

Editorial: comment regarding the olfactory tract endocast.

> We have carefully examined the relevant morphology and confirmed our previous interpretation. We have added text clarifying that, although parts of the anterior frontal region are incomplete, the preserved morphology supports our interpretation, and that comparisons are restricted to structures that can be confidently delimited (MS lines 233-236; SI Text lines 76-78 & 313-319). We would also note that all the reviewers had previously accepted this interpretation during earlier rounds of review, including Reviewer #1, who stated that: "*Extended Data Figure 8 does an excellent job of illustrating the unique morphology of the frontal in Tametara relative to all snake taxa included in the phylogenetic analysis*."

Editorial: You will also need to make some editorial changes to your paper so that it is as brief as possible and complies with our Guide to Authors. We also strongly suggest that your revised manuscript has tracked changes, which is increasingly requested by referees to aid in their re-review.

> We have reduced our main text from 4,960 words to less than 4,300 words to comply with Nature's length guidance for Biological Sciences articles. Most reductions were made in the "Osteological Description" section where detailed information is also provided in the SI Text. We retained discussion points of broader relevance, particularly those added in response to previous reviewers´ feedback. The minor text changes made to explicitly account for the new comments from reviewer #1 are listed with our responses (see below). All changes are marked with track changes. We have also checked the manuscript against the relevant Guide to Authors requirements.

Referee #1 (Remarks to the Author):

The manuscript continues to improve, and my only outstanding critique remains character coding for the presence of margo lateralis on posterior presacral vertebrae and its use as a component of the diagnosis for Tametara. Otherwise, the authors have either made recommended modifications or justified their original approaches in response. Revisions to the diagnosis and the added discussion of homoplasy in the evolution of the upper temporal bar in snakes required for their preferred topology definitely strengthen the manuscript. One minor fix to the section on the required number of losses of the upper

temporal bar- it should be three losses ((Najash+Tametara), Dinilysia, the clade crownward of (Dinilysia+Sanajeh)), following the topology of Fig. 3.

> Thank you for your constructive feedback. We have made the minor fix suggested here (MS line 328).

With respect to margo lateralis, in my previous review I noted that it is scored as absent in most extant snakes, but is actually observable in many of the species coded as absent. As a result, I stated that the MS needed to reassess the codings for this character and their impact on determination of synapomorphies, or at least provide a more robust justification for the codings as presented. In reply the authors stated:
"Concerning this point, we simply have a different interpretation of what constitutes the absent condition for this character. As the reviewer acknowledges above in "the margo lateralis is present as a ridge (albeit sometimes reduced)" . Indeed, the margo lateralis can be quite faint and regressed across many species, which we consider a gray zone of interpretation for the absent state, and so the choice of the character state in such situations is likely to change from author to author. On top of this, there is the matter of other sources of intraspecific variation—sexual, ontogenetic, population—that may drive different interpretations depending on the exact specimen examined. The latter can be a major source of variation that is unfortunately still underexplored in morphological systematics. Therefore, although we recognize that some researchers could score a different state for this character for some of the taxa, we have kept our scoring for these taxa based on the specimens we examined and to keep consistency in our interpretation of the morphology across all taxa. We will keep this comment in mind in future expansions of this dataset to look for sources of intraspecific variation that may be driving those differences.

They also state: "*The characters we defined from the posterior presacral vertebrae are interpreted consistently, including in the midsection of the presacral region. Although vertebral shape may vary to some extent (which could be captured by finer-scale linear morphometric measurements), the discrete features remain relatively stable. This is why we illustrate only one dorsal for Tametara in ED Fig. 2c, as all dorsals more posterior to it do not differ substantially—note, however, that many other dorsals are illustrated in Fig. 2 of the main text. So, even if the most posteriorly preserved dorsals in Tametara are not fully posterior, and represent mid-dorsals, that would not change our scoring or description.*"

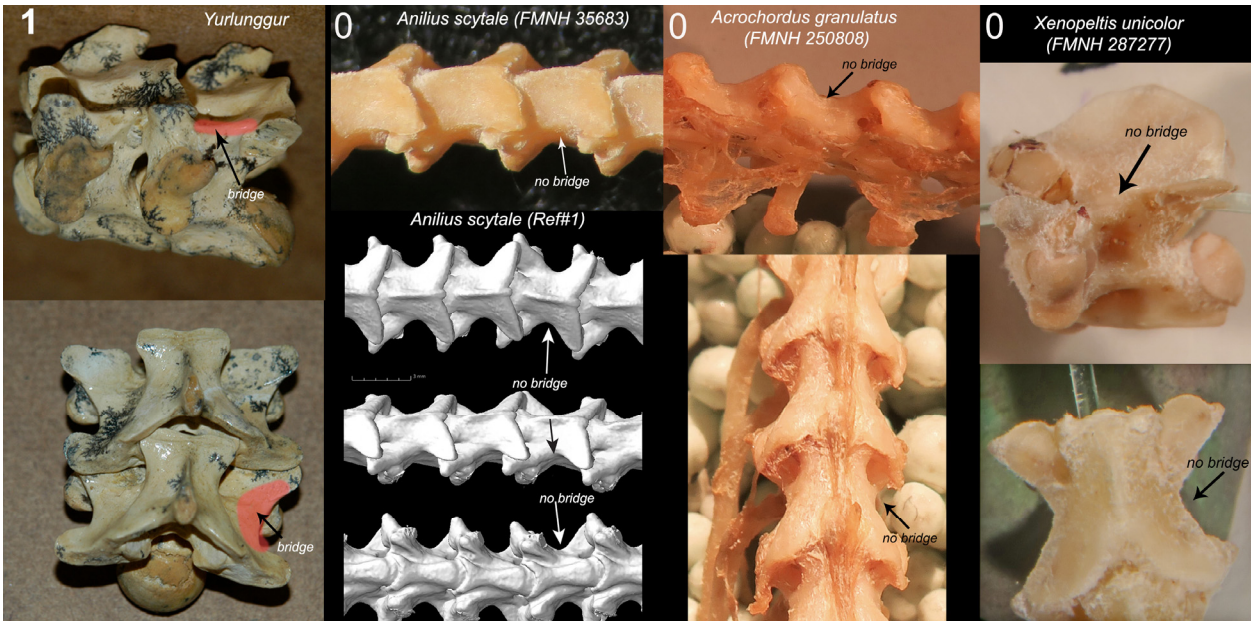
While it is certainly the case that multiple sources of variation can drive character interpretation, the diagnostic utility of the presence of margo lateralis on posterior

presacral vertebrae for recognizing Tametara is based on its reported distribution in other snake taxa and therefore coding for the character should be repeatable. To test this, I have compared the dorsal vertebra from Tametara (ED Fig. 2c) with articulated posterior precloacal vertebrae from taxa which are coded as lacking the margo lateralis. This comparison is appropriate following the authors' above discussion of character continuity along the presacral vertebral column in Tametara. For the included figure, Tametara (A) is compared with Tropidophis haetanius (B), Xenopeltis unicolor (C), Acrochordus javanicus (D), Loxocemus bicolor (E), and Anilius scytale (F). Arrows point to the margo lateralis in three views for extant taxa, and it is identified in Tametara in lateral view from the manuscript.

Although I do not disagree with the authors' statement that character interpretations can differ based on variation, in this case, the crest is present to the same degree of development in the posterior precloacal vertebrae of extant taxa (which is coded as absent in the data matrices of the study) as it is in the dorsal vertebra in Tametara (which is coded as present for posterior precloacal elements). If the authors' observations of morphologies in other specimens are not consistent with those figured here, then there is clearly confounding variation in delineating character states and they need to reconsider using the character in phylogenetic analysis as currently coded. At minimum, they should note that this character is variable in their Extended Data section and not uncritically include it in their diagnosis of Tametara. This issue also applies to inconsistent coding of the margo ventralis (character 293), as is visible in the figure.

> We thank the reviewer for their careful attention to this last standing detail on our manuscript.

We respectfully disagree with some of their interpretations as to how the margo lateralis (ch. 312 in our dataset) should be scored across snakes. We provide the image below to illustrate our point. This is a figure illustrating the character in question, with the different states scored, with each vertebra magnified up close. We have also added a version of this image to the description of ch. 312 in the character list (Ch. 312 in Supplementary Data S2 > Characters (394ch)_Figured.pdf).



The margo lateralis in our dataset was scored as present only when there is a clear bony crest (or "bridge") connecting the pre- and postzygapophysis, as observed in the stem snake *Yurlunggur* (state 1: present), in the image above. We contrast that with the condition in *Anilius* (state 0: absent), where we consider the absence of such bony bridge unambiguous for the mid and posterior precloacal sections. We illustrate using both reviewer #1's own figure, and our own picture of the same specimen used to score this taxon for this character in our dataset (FMNH 35683). Further, we added pictures of some of the specimens we used to score *Acrochordus* (FMNH 250808) and *Xenopeltis* (FMNH 287277) in the present dataset, which also confirmed the absence of the connecting bridge (margo lateralis). We lack images for the exact specimens used for scoring *Loxocemus* and *Tropidophis*, but the cases above illustrate our points: i) what this character represents; ii) how we interpret them; and iii) visual proof of consistency of our scoring matching the character interpretation described above.

On top of the points mentioned above, we cannot stress enough the fundamental problem, as previously raised, of intraspecific and intraindividual variation. Reviewer#1's interpretation on the presence/absence of a margo lateralis (and margo ventralis) might be at least partially influenced by which exact section of the vertebral column or population where those individuals have been sampled from.

From both an epistemological and practical perspective, it is hard (if not impossible) to change taxon scores in a phylogenetic dataset based on the reviewer's external perception rather than the data we collected and morphology we see and illustrated above. We find

no clear support for an alternative interpretation of this character that would warrant, for instance, re-doing all phylogenetic analyses. Furthermore, as the reviewer comments all pertain to crown snakes, not stem snakes (such as *Tametara*), we don't see the direct relevance to either the phylogenetic placement or *Tametara*'s diagnosis; i.e., all taxa more closely related to *Tametara* have the same condition (state 1), so it's not a crucial character to diagnose it. We only included it in the diagnosis because of our differential/more inclusive approach to taxon diagnosis, as already discussed in previous rounds.

However, we would like to expedite the review process and try to find a common ground with this reviewer. The reviewer provides a reasonable suggestion of minimal compromise: "*At minimum, they should note that this character is variable in their Extended Data section and not uncritically include it in their diagnosis of Tametara.*".

Accordingly, we have followed this suggestion and revised the diagnosis of *Tametara* (MS line 107), explicitly acknowledge the variability and ambiguity of the margo lateralis/margo ventralis in the text where relevant, and clarify that expression of these structures varies along the column and among individuals/specimens (MS lines 198-200 & 313-314; SI Text lines 423-427, 482-484 & 495-499). We hope this satisfies this last remaining point.

Referee #2 (Remarks to the Author):

Dear Authors,

We thank you for your thorough replies to our comments and for addressing most of them. We have no further comments and find the manuscript suitable for publication.

Congratulations on such a good work.

Sincerely,

Marion Segall and Rémi Allemand

> Review completed.

Referee #3 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

> Review completed.

Referee #4 (Remarks to the Author):

Authors have considered our comments and concerns and justified them accordingly. The manuscript is now completely acceptable for its publication.

> Review completed.

Referee #5 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

> Review completed.

Response to Decision Letter

Here we provide a point-by-point response to the decision letter.

###

Your manuscript, "Exceptional brain and ecological diversity in the earliest snakes", has now been seen by our referees, and in the light of their advice I am delighted to say that we can in principle offer to publish it. First, however, we would like you to revise your paper to address any remaining points made by the referees, and to make some editorial changes to your paper so that it is as brief as possible and complies with our Guide to Authors. No peer reviewed data should be removed altogether when making these changes.
>There are no pending requests from reviewers, and so we have moved directly to the editorial changes that were highlighted in the decision letter.

SOME SPECIFIC ISSUES
1. Please provide the manuscript in .docx format. Currently it is in pdf format.
> Done.

2. Please remove the main figures from the article file and re-supply them individually in an acceptable format such as EPS, AI, PS, PDF, PPT, PSD or XLS (for graphs) with editable vector files.
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> Every content in all figures were produced by the authors and have no third party rights. This includes the images in figure 1, and animal silhouettes displayed in Fig. 4b and ED Fig 6. We note this information was provided in the figure caption as “Silhouettes

created by TRS." For Fig. 4b and ED Fig. 6. For Fig.1, we have added the statement "specimen photos taken by A.S.H.", one of the senior authors, to clarify our copyrights to the images.

5. Please make sure to provide a third party rights table (more information below) when you resubmit. If Biorender or similar software has been used, please also ensure to provide relevant licenses.
> This table is not applicable to any components of our study.

6. Please ensure that the text size in all figures is at least 5 pt Arial.
>Checked. Note that in phylogenetic tree figure with all species names (e.g., ED Fig.3-6; Fig. 10b) it is impossible to make these 5pt or higher to fit in a single page. We note that this is always the case with large phylogenetic tree figures, but we also provided trees in standard tree file formats (.nex) as Supplementary Data (S2)— as also done during review process—that readers can open and read the tree in any dedicated phylogenetic software.

7. Funding section is provided along the acknowledgment section, please provide it as separate section.
> Done.

8. Please remove the Extended data figures from the article file and re-supply them individually in EPS, JPEG or TIF format.
> Done.

9. The Dataverse web-link provided in the data availability statement (https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi%3A10.7910%2FDVN%2FC6WXMO&version=DRAFT) for all data [CT scan data; Phylogenetic data (morphological and molecular); Input data and output files from geometric morphometric analyses and bone microstructure analyses] is a private weblink for review only. Please provide valid web-link for the same under the data availability statement of the manuscript and in the RS along with public release of the data.
> Dataverse permanent DOI was created and added to 'data availability' and 'data' section of the reporting summary. The repository is published and DOI accessible but the data access is under embargo. The embargo date will be updated once we have the journal's final embargo date.

10. Please provide valid and appropriate Zoobank information in the manuscript under the 'data availability' section as well as in the 'data' section of the reporting summary along with public release of the same.

> Zoobank registrations created and added to ‘data availability' and ‘data’ section of the reporting summary.

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TITLE: Titles cannot exceed 75 characters (including spaces); they must not contain punctuation.
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> N/A

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> Checked.

MAIN TEXT: If further introductory material is necessary, the main text can begin with up to 500 words of introduction expanding on the background to the work (some overlap with the summary is acceptable), before proceeding to a concise, focused account of the findings, and ending with 1 or 2 short paragraphs of discussion. Sections are separated with subheadings (up to 40 characters including spaces) to aid navigation.
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REFERENCES: As a guideline, most papers should include no more than 50 main text references; all additional references can be cited in (and listed after) the Methods section, as detailed below.
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FIGURE LEGENDS: These should be listed sequentially after the main text references and not in the figure files. Each legend should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used. Legends should not exceed 300 words each. Each figure legend should contain, for each panel where relevant, the following information:
* the exact sample size (n) for each experimental group/condition, given as a number, not a range;
* a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc);
* a statement of how many times the experiment shown was replicated;
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* very common tests (e.g. t-test, simple Chi-square tests, Wilcoxon and Mann-Whitney tests) can be identified by name only, but more complex techniques should be described in the Methods;
* whether tests are one-sided or two-sided;
* whether there are adjustments for multiple comparisons;
* the statistical test results (e.g., P values);
* the definition of 'center values' as median or average;
* the definition of error bars as s.d. or s.e.m.
Descriptions that are too long for the figure legend should be included in the Methods section.
> Checked. All legends are compliant (e.g., less than 300 words each).

METHODS: The Methods section, which provides the full, step-by-step instructions that would allow other researchers to replicate the results, is included after the main text figure legends. The Methods section will not appear in print but will appear online in the full-text HTML and PDF versions. The Methods section should be written as concisely as possible but should contain all elements necessary to allow interpretation and reproduction of the results. If there are additional references (in the Methods section, Supplementary Information, etc), their numbering should continue from the last entry in the main text reference list, and they should be listed following the Methods section. Specialized methods that require chemical structures, figures, or tables cannot be accommodated in the Methods section of the main text file. If such information is part of the Methods, the entire Methods section must instead be included within a Supplementary Information text file.
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MAIN TEXT STATEMENTS: Several statements (which will not appear in print but will

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throughout the review process and ensure that only the data most central to the conclusions are presented in the main text figures. Any figures included within the main text file during the review process must be removed from the final main text file and uploaded as separate, individual files; they will be integrated into the main paper in print and online. An overview of the key features of this presentation may be found here.

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> ZooBank LSIDs provided in Data Availability.

* We strongly encourage deposition of 3D morphological data in a suitable repository such as MorphoBank, MorphoSource or similar; the relevant accession numbers should be listed in the Data Availability statement.
> All 3D data is deposited in MorphoSource: final DOI and project ID provided in Data Availability.

We will not send your revised paper for further review. If the revised paper is in our format (as detailed above), in accessible style and of appropriate length, we shall begin the acceptance process.

In order to accept your paper, we require the following electronic files:
* A cover letter describing your response to any editorial comments and detailing any format changes during revision, particularly if the overall length is affected.
> Provided.

* A point-by-point response (preferably in Word) to any remaining issues raised by our referees.
> No further comments to address from reviewers.

* The final version of your text as a Word document. Word Equation Editor/MathType should be used only for formulae that cannot be produced using normal text or symbol font. If this is not possible, the manuscript can be supplied as a single plain vanilla TeX or LaTeX file that includes all references and abbreviations, with no special formatting, as well as a PDF version that is uploaded as a 'related manuscript file'.
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* Production-quality versions of all figures (see above).
> Provided.

* The final version of the Extended Data.
> Provided.

* The final version of any Supplementary Information, presented as one file (ideally a PDF) if feasible, as well as a separate SI Guide.
> Provided.

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> Provided.
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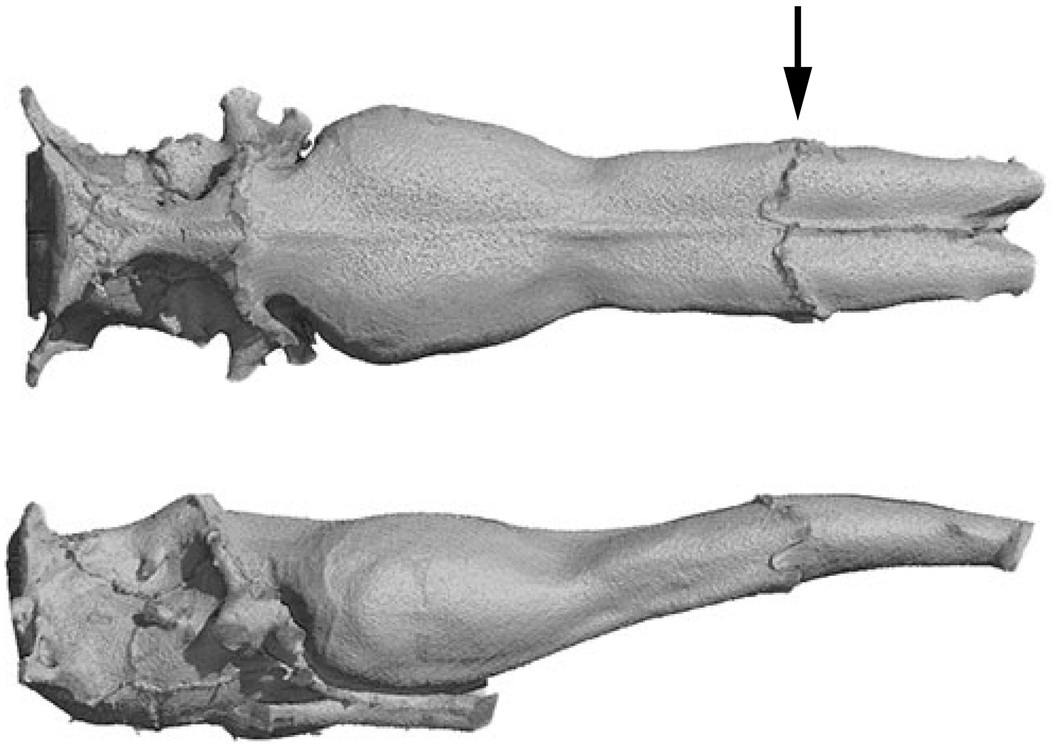
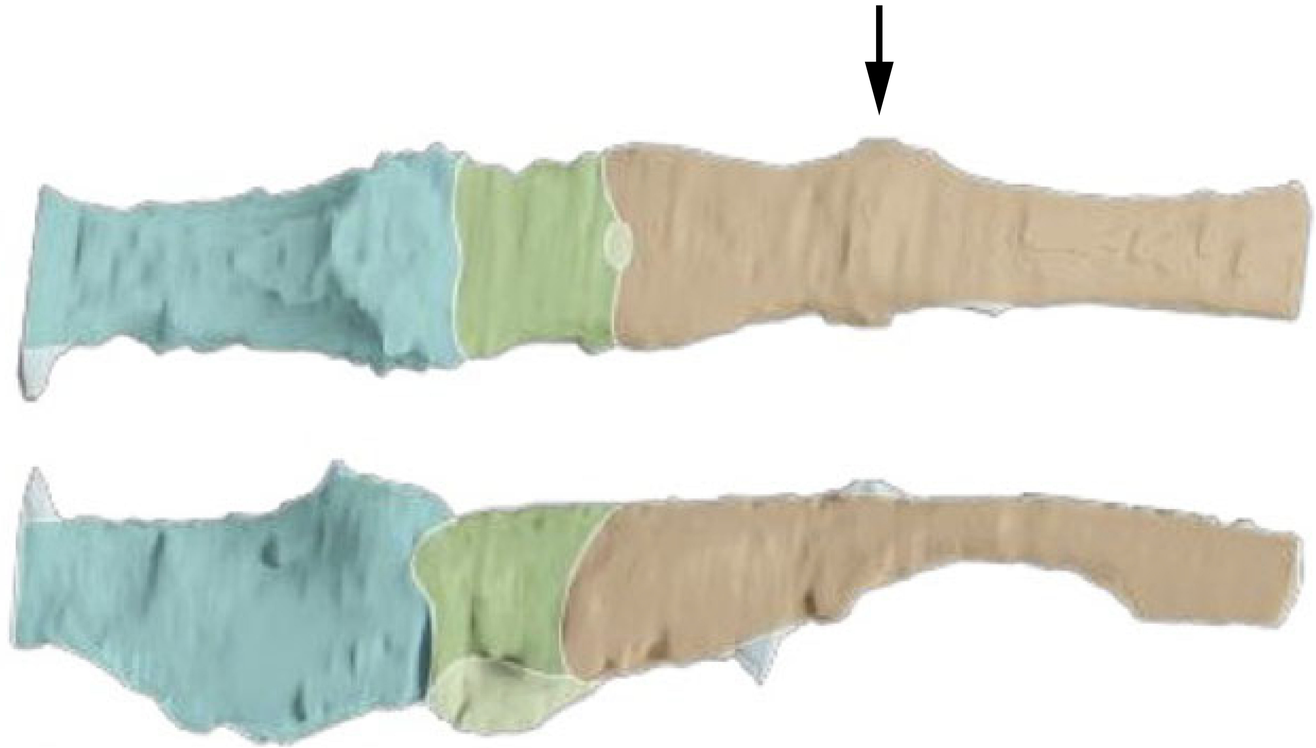
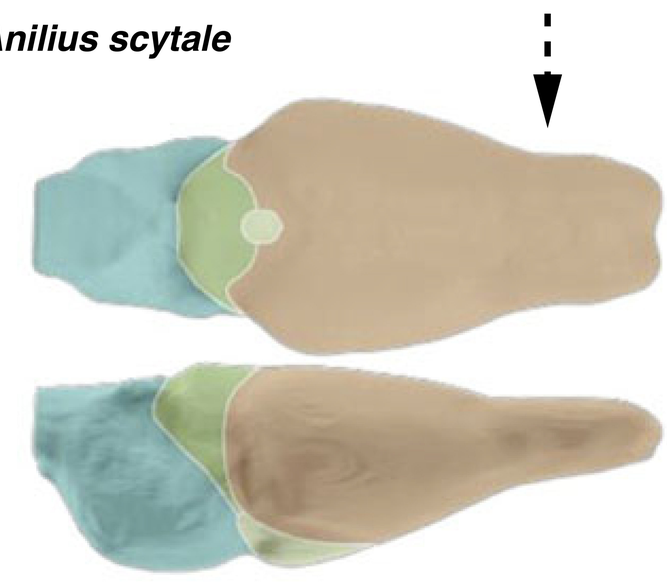


Anilius scytale



Dinilysia patagonica



Anilius scytale (MS)

Tametara

Dear authors and editor,

We co-reviewed the manuscript entitled “Exceptional brain and ecological diversity in the earliest snakes” by Simoes and colleagues considered for publication in Nature.

A. Summary of the key results

In this manuscript, the authors describe a new snake fossil *Tametara mirim*, which is exceptionally well preserved, retaining its 3D shape, including an almost complete skull, numerous vertebrae, and articulated postcranial elements. The authors described the anatomy of the fossil and used its brain and inner ear endocasts, along with bone microstructure to make ecological inferences by comparing it with extant squamates species. Their results suggest that *T. mirim* was fossorial and possessed a skull morphology distinct from that of other early snake fossils, particularly *Dinilysia*.

B. Originality and significance: if not novel, please include reference

We consider this manuscript to be of interest to both a large scientific and non-scientific audience. The exceptional preservation of the fossil, combined with its comparison to both extant and extinct species using multiple approaches (3D geometric morphometrics, bone microstructure, and morphological characters), provides convergent evidence for a fossorial ecology. This is particularly valuable in the context of the ongoing debate about the ecological origins of snakes.

C. Data & methodology: validity of approach, quality of data, quality of presentation

The methodology is correct but could be improved, especially regarding the 3D GMM, but overall, the authors made an effort to place their fossil into a biological context using various methods.

D. Appropriate use of statistics and treatment of uncertainties

The statistical methods are sound overall, but some parts of the methodology need to be modified or clarified. The provided R codes and associated files for the endocast analysis are easily usable and results can be replicated but not those for the microstructure analysis (see comments).

E. Conclusions: robustness, validity, reliability

We generally agree with the conclusions of the authors, especially regarding the putative ecology of *T. mirim*.

F. Suggested improvements: experiments, data for possible revision

List below.

G. References: appropriate credit to previous work?

Yes.

H. Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions

We strongly suggest being more careful with the terminology used throughout the manuscript, especially the use of “brain” to refer to the endocast (see comment below). We also feel like the authors oversell their work and results that are interesting *per se*. For instance, they state that *T. mirim* is completely different from extant species, but their analyses show the opposite, and if it was truly that different, it would make it hard to infer its ecology. We list below our major and minor comments that need to be addressed before publication.

We must acknowledge that we do not have the necessary background to review the phylogenetic part of the manuscript.

Major comments:

Main text:

- Title: We suggest reconsidering the title, as the use of a superlative, the term 'brain' rather than 'endocast,' and the lack of reference to what seems to be the central focus of the manuscript—a newly discovered, exceptionally well-preserved specimen—may give a slightly misleading impression.

- Terminology: Throughout the manuscript, you may consider using the term “endocast” (or “endocranial morphology”) rather than “brain” when describing features reconstructed from endocranial data. Endocasts only approximate the external brain shape and include non-neural spaces (e.g., meninges, venous sinuses), so this terminology would provide a more accurate description, except when explicitly discussing inferred neural structures.

- Systematic Paleontology: Consider reorganizing the manuscript to facilitate reading. Descriptions of the new species and associated information should be presented clearly and in a consistent order.

- Osteological description: there is no mention of the size of the specimen anywhere. We had to go to the figure to measure its size, which was a big surprise at first since, according to the scale of Fig 1a. the specimen measures about 1m and its skull around 5cm (head length), which is far from being a small specimen. However, we realized that the scale on Fig. 1a. is wrong, it should be about 8.16cm, not 20, according to all other figures for which the scale seems correct and that give a skull length about 2cm (which seems more reasonable for a snake that is supposedly “small-sized”. Scales need to be checked and corrected and a description of the size of the specimen should be added to the main text and discussed, especially since size is the main driver of endocranial shape. Large species have endocranium that are similar to the one of your specimen (anteroposterior elongation etc). But since your specimen is quite small, we agree that it would tend to look like an *Anilius* or *Cylindrophis*.

- Allometry: You wrote in your M&M section that you tested for allometry, and running your code, we found out that there is no allometric signal in endocranial shape in your dataset which would be interesting to discuss in the manuscript, especially since in other studies, allometry is usually significant and represent a relatively large amount of the total shape variation. We wonder whether the lack of signal results from the low number of landmarks in your manuscript.

- Olfactory bulbs: These structures are heavily discussed, and a rather large part of the ecological inference is based on its description. However, as mentioned in the thorough description in the SM, the anterior part of the frontal is missing (l70-71) there is “no facet for a possible prefrontal (but we note that <u>the anterior end of the frontal is mostly missing</u>)” and (l74-75) “due to its incompleteness we cannot determine how far

ventromedially 75 it could have extended". The olfactory bulbs do not seem particularly "bulky" (l197), it is hard to tell whether they are short considering that a part of the frontal is missing, and it is hard to understand what you mean by "anterior curvature" (l198).

- L199 "enlarged cerebral hemispheres". The cerebral hemispheres do not look enlarged compared to most extant species, but compared to *Dinilysia*, sure. This statement should be corrected and the discussion adjusted.

- All along the main text, you compare your specimen with many extant species but in the abstract, you state that (l34-35) "*Tametara* had a brain morphology distinct from both other stem and extant snakes", while Fig. 4 shows that *T. mirim* is clearly within the space occupied by extant fossorial.

- L286-288 "*Tametara* and *Dinilysia* represent highly divergent brain shapes relative both to each other and to most other snakes and non-ophidian squamates", while the first part of the sentence is true, the second is not for *Tametara*. Your specimen is not isolated from fossorial snakes in any of your morphospaces based on endocranial characteristics (Fig. 4, Extended Data Fig. 8, Extended Data Fig. 10).

- L310-313: You wrote that "Quantitative analyses further indicate that the early morphological disparity in snake brains reflects variation in their ecological lifestyles. Specifically, our analyses significantly discriminate how different telencephalon shapes relate to habitat usage, especially between fossorial and non-fossorial species (Fig. 4a).". We may have missed it, but we do not find the aforementioned quantitative analyses.

- L326-328: "We did not apply geometric morphometrics on the inner ear, however, given contradictory prior results and limited statistical power for reliable palaeoecological inference". We doubt that all these issues come from the 3D GMM. It is most likely that the inner ear does not bear a strong ecological signal (or at least, a signal that is related to substrate use). However, you based some of your ecological inferences based on the inner ear structure. I would either remove this part of the discussion or be extremely careful in the interpretation.

- L816-817: What shape variables were used? Did you use Procrustes coordinates? Principal Components? If so, how many and how did you choose them?

- L818-821: Why use phylogeny-corrected shapes for the LDA? Why not "raw" shape data? And why 95% of shape variation? Why not all PCs?

- The habitat attributed to some species seems incorrect: *Physignathus cocincinus* is arboreal same for *Basiliscus vittatus*, *Anguis fragilis* is at best semi-fossorial, *Thamnophis sirtalis* is mostly terrestrial and at best semi-aquatic. In addition, In the Supplementary Text (lines 301–302), *Pseudopus* is listed among "fossorial limb-

reduced non-ophidian squamates," but it is generally considered terrestrial (you correctly classified it as terrestrial in your analyses). Please correct this for consistency.

- Coloring for the parts of the endocast: The way the endocasts are colored in several figures prevents from seeing the underlying structures. It is too opaque. Plus, you mentioned in the manuscript that, aside from the telencephalon, the rest of the endocast regions are barely distinguishable on your specimen so how did you color it?

- Landmark scheme:
  - Please clarify why only six landmarks were used on the telencephalon. This seems limited, especially since Allemand et al. (2023) defined 20 landmarks that can be placed relative to surrounding bones and the labyrinth. Using more landmarks could better capture morphological variation and strengthen the morphometric analyses. Consider justifying the choice of six landmarks, for example using a Landmark Sampling Evaluation Curve (LaSEC; Watanabe 2018, R package LaMBDA).
  - We have concerns about how you positioned your first landmark since you mentioned that the anterior part of the frontal is missing.
  - Regarding geometric morphometrics, consider whether including all squamates might obscure ecological signals. Would it be more informative to focus on snakes alone for some analysis?
  - Provide details on how landmarks were positioned on the endocast of *Dinilysia*, which shows morphology quite different from other snakes. Could incompleteness affect landmark placement?
  - It would be informative to have the labels of your landmarks in Extended Data Fig. 8a, if anyone would like to reproduce your landmark scheme.

- **Statistical analysis:**
  - You tested differences in endocranial shape between ecologies in extant species using pairwise tests but you did not mention these results in the manuscript. Since only fossorial species are significantly different from other ecologies, it would be interesting to mention it since it could strengthen your results.
  - The total accuracy of the LDA is 69%. This information does not appear anywhere in the main text. You wrote L314 that *Tametara* is classified with 96.6% probability within the fossorial group. But how does this number articulate with the overall accuracy of the LDA? It is 96.6% of the overall accuracy (69%)? We recommend giving more details about this method. And again, we suggest using all the PCs, not a subset. We did run your code with all PCs and the overall accuracy of the LDA slightly decreases (63%) but the

prediction decreases significantly for *Tametara* from 96.6 to 66% (and 33% of chances of being aquatic) while the percentage for *Dinilysia* being a terrestrial species increases from 78.3% to 84.5%. From a statistical point of view, using a subset of PCs that result from only 6 landmarks feels like you are using a very small amount of shape variation to make ecological inferences. You need to convince us that not using all PCs is statistically sound.

- References and figure citations:
  - Some references do not match the statements. For example, line 786 cites Mongiardino Koch et al. (2021) regarding published endocasts, but this paper does not include endocasts. Please verify all citations.
  - Figure references also need checking. In the Supplementary Text (line 40), the postfrontal is cited as Fig. 1b–f and Extended Data Fig. 1a–e, but Fig. 1d–f shows vertebrae, and no postfrontal appears in Extended Data Fig. 1a–e. Likely the correct references are Fig. 1b,c and Fig. 2b–d.
  - Similarly, in the Supplementary Text (line 172), “Extended Data Figure 2” is cited for the otoccipitals, but this figure appears to show postcranial anatomy instead. Please verify and correct.

Minor comments:

<u>Abstract</u>

L34-35: “Quantitative endocast analyses demonstrate that *Tametara* had a brain morphology distinct from both other stem and extant snakes”. The statement seems too categorical. Some similarities with *Anilius* and *Cylindrophis* are also evident and should be acknowledged.

L35-36: “revealing substantial early neuroanatomical disparity—and likely sensory functions—in snake evolution”. The claim appears speculative, as sensory functions were not directly tested in this study. You may consider removing or rephrasing this sentence to better reflect the scope of your results.

L36-38 “Independent evidence from brain shape, inner ear organization, and bone microstructure converges on a fossorial lifestyle for *Tametara*, contrasting with other stem and early crown snakes”. The end of this sentence is not clear, how *Tametara* being fossorial is contrasting with other stem and early crown snakes?

Main text:

L34-35: remove space before "*mirim*"

- Please consider adding references in the main text when comparing with other species

L130-132: "*However, the parietal has a short anteromedial process that would have inserted in between the frontals (Fig. 2d), which is absent in the other South American Cretaceous snake*". It is unclear here. In Zaher & Scanferla (2012), the description of the *Dinilysia* skull indicates that *"anterolaterally, the parietal is clasped by the postfrontal, whereas its anterior surface contacts the frontals in a W-shaped, anteriorly concave, and interdigitating suture."*

L192-193*: "This allows fossil snake brains to be reliably inferred from endocasts and directly compared with those of extant snakes*[33,34] » The cited references focus only on endocast studies in extant snakes, so they may not directly support inferences about fossil snakes. You may consider clarifying this point or citing additional studies that specifically address fossil endocast comparisons.

L200-202: "*It is followed immediately posteriorly by enlarged cerebral hemispheres in the telencephalon and by a rather undifferentiated mesencephalon. This diverges from the condition observed in most squamates, which display well-expanded cerebral hemispheres that strongly contrast with the mesencephalon and rhombencephalon (= mid- and hindbrain, respectively)*[32-34]". Please clarify what is meant by *"undifferentiated mesencephalon"*. Additionally, the sentence is unclear—are you referring to the size of the cerebral hemispheres, or to the differentiation between the cerebral hemispheres and the mesencephalon?

L202-206: The sentences in the main text appear somewhat contradictory to statements made in the abstract.

- Reference number 36 is not cited correctly. It should be: Olori, J. C. (2010). Digital endocasts of the cranial cavity and osseous labyrinth of the burrowing snake *Uropeltis woodmasoni* (Alethinophidia: Uropeltidae). *Ichthyology & Herpetology*, *2010*(1), 14-26.

- Phylogeny and divergence times: Out of curiosity, did the authors consider alternative phylogenetic hypotheses, such as placing Serpentes as the sister group of anguimorphs?

L258-259: The main text states that *Tametara* has a "*well-developed dorsum sellae*" to support its basal placement, but the detailed description notes that it is "*the dorsum sella is weakly developed and visible in anterior view*" (Line 221 in Supplementary text). Could the authors clarify this apparent discrepancy and specify how they coded or interpreted this character in the phylogenetic analysis?

L259-260: The mid-sagittal crest of the basisphenoid seems only present in adult specimens of large species (similar to the neural crest) in extant species. It says more about your specimen size than of its relationship with other extinct species.

Early evolution of snake brains

L286-287: The statement *"We find that the earliest known snake brains of Tametara and Dinilysia represent highly divergent brain shapes relative both to each other and to most other snakes and non-ophidian squamates"* could be made more nuanced. The PCA in Fig. 4 shows that *Tametara* occupies a region of morphospace overlapping with other snakes and squamates, suggesting that its brain shape is not entirely divergent. Please consider revising this sentence to reflect this pattern more accurately.

Palaeoecological origin of snakes

L317-320: "*These results support most of our comparative anatomical observations, in which Tametara shares with other fossorial taxa short olfactory bulbs, a weakly developed optic tectum, and morphologically poorly differentiated cerebral hemispheres*[22,23,32-34] *(Extended Data Fig. 7)."* The manuscript describes the mesencephalon as both (L211) *"undifferentiated"* and *"unusually elongate"*, and the cerebral hemispheres as both *"enlarged"* and *"poorly differentiated"*. These descriptions appear contradictory. Please clarify how these observations relate to each other, and specify whether the terms refer to size, shape, or differentiation, to improve clarity and consistency. Additionally, given that the anterior part of the frontal is missing, it would be helpful to clarify whether the olfactory bulbs are completely preserved. If not, this could affect the interpretations.

L324-328: "*Interestingly, Tametara further shows inner ear features compatible with fossoriality, such as an enlarged vestibular region and simplified semicircular canal architecture, providing independent support from a different system. We did not apply geometric morphometrics on the inner ear, however, given contradictory prior results*[19,37] *and limited statistical power for reliable*[37] *palaeoecological inference*" These sentences are somewhat contradictory. The text claims that *Tametara* shows inner ear features "compatible with fossoriality" and that this provides "independent support," yet also acknowledges that no geometric morphometric analysis was performed and that previous results are contradictory. It would be preferable to rephrase this section more cautiously.

L330-332: "*The combination of a dorsoventrally shallow lateral profile, poorly differentiated subdivisions, extremely long and curved olfactory bulbs, and narrow cerebral hemispheres is unlike that of any other squamate."* This statement could be made more nuanced. Some similarities can be observed with the endocast of *Python molurus* and *Python bivittatus* (Figure S3 Supplementary Material in Allemand, R., Polcyn, M. J., Houssaye, A., Vincent, P., López-Aguirre, C., & Bardet, N. (2024). First

Virtual Reconstruction of a Mosasaurid Brain Endocast: Description and Comparison of the Endocast of Tethysaurus nopcsai with Those of Extant Squamates. *Diversity*, *16*(9), 548.)

L343: Reference 45 appears twice.

Figure 4:

- The phylomorphospace was generated using landmarks restricted to the telencephalon. Including miniature reconstructions of the entire brain in this figure may therefore be misleading, as it visually suggests that the morphospace reflects total brain shape rather than telencephalic variation alone. Please consider displaying cropped telencephalon renderings to avoid potential bias in interpretation.
- Many times, you compare *Tametara* to *Anilius* and *Cylindrophis*, it would be nice to have their location on the morphospaces.

Line 646: please remove the coma "**Simões, et al.[6]"**

<u>Supplementary Material:</u>

L40: snakes are squamates. Please consider rephrasing

L44: postfrontal rather than "*frontal*"?

L62: "*The supratemporals extend well anteriorly, close to the level of the trigeminal foramen*" This description could be reconsidered, as Fig. 2a,b appears to show the supratemporals terminating posterior to the level of the trigeminal foramen rather than extending close to it.

L68: "F<u>r</u>ontal" is misspelled

L177: "(hypoglossal) could be identified. is located posterodorsally to CN X" the sentence does not make sense.

L291: We don't understand the meaning of the end of this sentence: "The brain of *Tametara* is anteroposteriorly elongate and defined anteriorly by short, 291 bulky olfactory bulbs with anterior curvature"

L301 (and check the rest of the SM & Main text): The refence should be Olori 2010 not Jennifer.

L337-340: "*Compared to extant snakes, Tametara and Dinilysia, have remarkably large pituitary glands, although the length of these structures might only correspond to the elongate parietal of the fossils*," I am not sure to understand, could you explain this please?

L378-380: "*in reptiles are difficult to determine snakes and other limbless squamates as these are typically defined based on vertebra bearing ribs contacting the sternal plate to discern between cervicals and dorsals, for instance (Hoffstetter & Gasc 1969)*" Please consider rephrasing

L783: "Computed-tomography~~c~~"

L795: "of" is italicized

Extended Data Fig. 7: Why choose to show *Acrochordus*, which is a very peculiar species among snakes in all aspects of its morphology, including its endocranium?

In your Rcode "analysis_microstructure", the library "ggtree" does not exist and there is no file named "general_pruned_scaled_appended.tre". Please, make sure we can run the whole code.

Line 106, discussing the ontogenetic stage, we agree in considering the holotype as an adult individual. However, respect to the "high degree of overlap between the supraoccipital and the parietals and prootics", the overlap between parietal and supraoccipital and great exposure of the latter particularly departs from the typical condition observed in adult snakes. The supraoccipital remains greatly exposed in the skull roof in Tametara, as in Najash and juvenile snakes (Zaher et al., 2009; Scanferla and Bhullar, 2014; Palci et al., 2016; Garberoglio et al., 2019a, 2019b).

> We all agree on the ontogenetic stage. We also agree that the degree of overlap between bones discussed here is greater than in other snakes. Accordingly, we have mentioned this in our Description and Diagnosis. As for its relevance to ontogenetic stage, a fundamental developmental rule is that bones start condensing and expand in size throughout ontogeny. Therefore, bones must be nonoverlapping in earlier stages, and if any overlap occurs, it can only occur in subsequent stages. Especially considering that the degree of overlap considered here (as mentioned by reviewers) is quite substantial even when compared to other snakes; this provides indirect evidence for a skeletally mature individual

Agreed, thanks for the clarification, it was not clear to us what the degree of overlap between parietal and prootic here meant, since the dorsal exposure of the supraoccipital in *Tametara* is actually greater than in most snakes, specially adults. Additionally, we failed to mention earlier that fully fused neural arches to vertebral pleurocentra is not a clear indicator of adult ontogenetic stage for snakes, since the neural arch and the vertebral pleurocentra are already fully fused in very early ontogenetic stages in snakes, including fossil and extant (Winchester & Bellairs, 1977; Xing et al., 2018).

Line 116. As said before, classifying them in non-mutually excluding categories: i.e., dermatocranium, splanchnocranium, and neurocranium, is confussing. While dermatocranium refers to the bone origin, splanchno and neurocranium refer to their functionality (for example, pterygoid enters in both dermatocranium and splanchnocranium). We suggest authors to decide one classification functionally (neuro vs splanchnocranium) or by origin (auto vs dermatochranium). We suggest the former one.

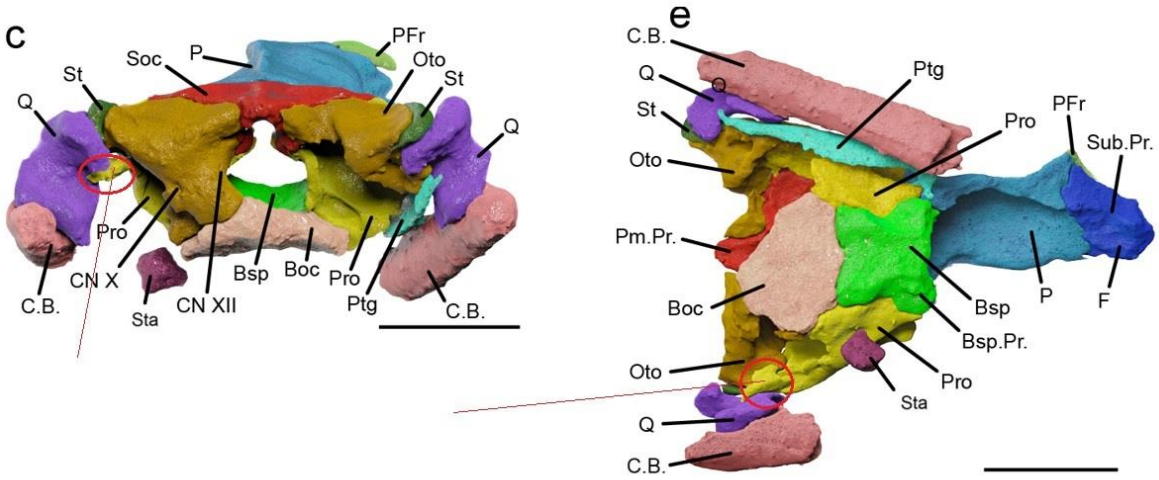
> We respectfully disagree. We followed the textbook definition of those terms provided by Kardong (2012) (page 241 and fig.7.1 therein), who divide the skull in 3 parts: dermatochranium, splanchnocranium, and chondrocranium, as in our MS.

Understood and agreed. However, at least in the Kardong version we have, the pterygoid is part of the dermatocranium, not part of the splachnocranium.

Additionally, besides the quadrates, there is another element of the splanchnocranium that might be actually preserved: a small portion of the left stapes. A small portion of this bone was reconstructed as part of the prootic in Figure 2c,d and Extended Data Figure 1b, which is apparently separated from the main body of the prootic and approaches the ventral side of the suprastapedial process of the quadrate. This matches the stapedial shaft observed in *Dinilysia* and *Najash* (Caldwell and Albino, 2002, Zaher and Scanferla, 2012; Garberoglio et al., 2019a).

> Agreed. We thank you for noticing this. A small portion of the stapes is preserved, as illustrated in the figures mentioned. It does not have many diagnostic pictures preserved, but we have now included in the detailed description in the SI (section Stapes).

Not agreed, it seems that the authors misunderstood what was suggested as a possible part of the stapedial shaft. Actually, we are uncertain of the identification of that small fragment of bone in *Tametara* that is now labelled as the stapes (Sta), but its robust morphology and placement do not particularly match the stapedial shaft of either *Najash* or *Dinilysia.*



What is circled in red in the image above taken from Fig. 2 of the MS is what was suggested as a possible portion of the stapedial shaft in *Tametara* by us, rather than being part of the left prootic as reconstructed by the authors. We should have explained our observation with such an image in our past revision (sorry for that). This element is thin and located in contact or close to the suprastapedial process of the prootic. As such, morphology and placement matches the stapedial shaft of *Najash* and *Dinilysia*.

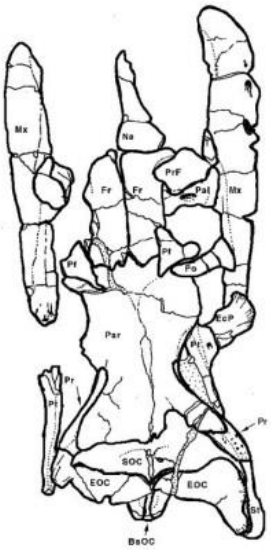
Line 133. “the parietal has a short anteromedial process that would have inserted in between the frontals, which is absent in the other South American Cretaceous snake”. The singular in this sentence is incorrect. It refers to the condition in the South American snake (*Dinilysia*), somewhat ignoring the existence of the other (and better preserved) South American snake (*Najash*). Anyway, while there is indeed no evidence of such process in *Najash* (Apesteguía and Zaher, 2006; Zaher et al., 2009; Garberoglio et al., 2019a), a similar anteromedial process of the parietal is arguably present in *Dinilysia* (visible in specimens where the fronto-parietal suture is well-preserved such as the holotype and MACN-RN 1013), where the parietal contacts the frontals in a W-shaped interdigitating suture (Estes et al., 1970; Caldwell and Albino, 2002: Zaher and Scanferla, 2012).

>Edited from singular to plural to correct the typo.

Indeed, *Dinilysia* was described as having a wavy (or W-shaped) frontoparietal suture. This seems to be derived from observations on the holotype, but the equivalent region in MACN-RN 1013 is in

fact nearly concave (see picture below). We consider the presence of the process ambiguous at best, or possibly polymorphic. Accordingly, we have made this more careful consideration in our description, and updates the score for this character in *Dinilysia* (Ch. 112: 0 -> ?).

Not agreed. What the authors are interpreting in the picture above as the fronto-parietal suture in MACN-RN 1013 is, in fact, just part of a crack within the parietal, the actual suture is more anteriorly located, and is clearly W-shaped as in the holotype and mentioned in the previous descriptions. See the picture below including an actual photo of the specimen, and an interpretative drawing of the same from Caldwell and Albino (2002):

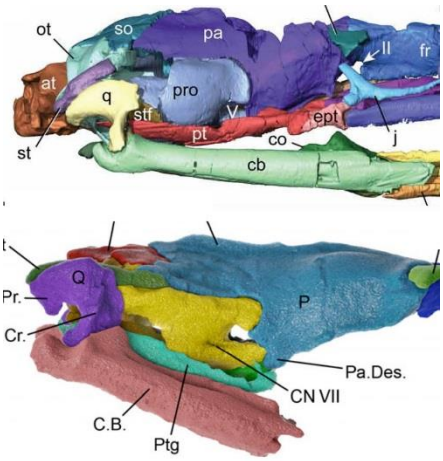


Line 165. Respect to the absence of the retroarticular process in *Tametara*, it says that it occurs "as in *Dinilysia9*,10, whereas *Najash* exhibits a small one11". Both *Najash* and *Dinilysia* are described as retaining a short retroarticular process (Caldwell and Calvo, 2008: Zaher and Scanferla, 2012; Garberoglio et al., 2019a). Both have indeed only a very short extension of the compound bone passing the glenoid, and there is no much difference between these two and the condition in *Tametara*. To consider the small process as a present or absent retroarticular process might be subjective. Is actually better understood as with no distinction between the three, and if the authors want to make that case here, it has to be better justified.

> Indeed, as with many aspects of osteology, small variations are highly subjective to interpretation (as acknowledged by the reviewer). The description of *Tametara* and the comparison with the other species are based primarily on our personal assessment of this (and other phylogenetically relevant) characters. We understand that different authors might interpret these differently. We find no extensions beyond the glenoid in *Dinilysia* (which is quite unambiguous to us), while we agree with the recent redescriptions of *Najash* (Garberoglio et al. 2019) with the latter having a small process, which is how we describe this variation in our text. For *Tametara*, we find no evidence whatsoever of an even small retroarticular process, as in *Dinilysia*. Given this potential point of subjective disagreement, we have now highlighted our interpretation for greater clarity in the main text.

We understand that this point is very subjective, and it is now made clear in the MS that the absence in *Dinilysia* in contrast with previous descriptions is the authors interpretation based on

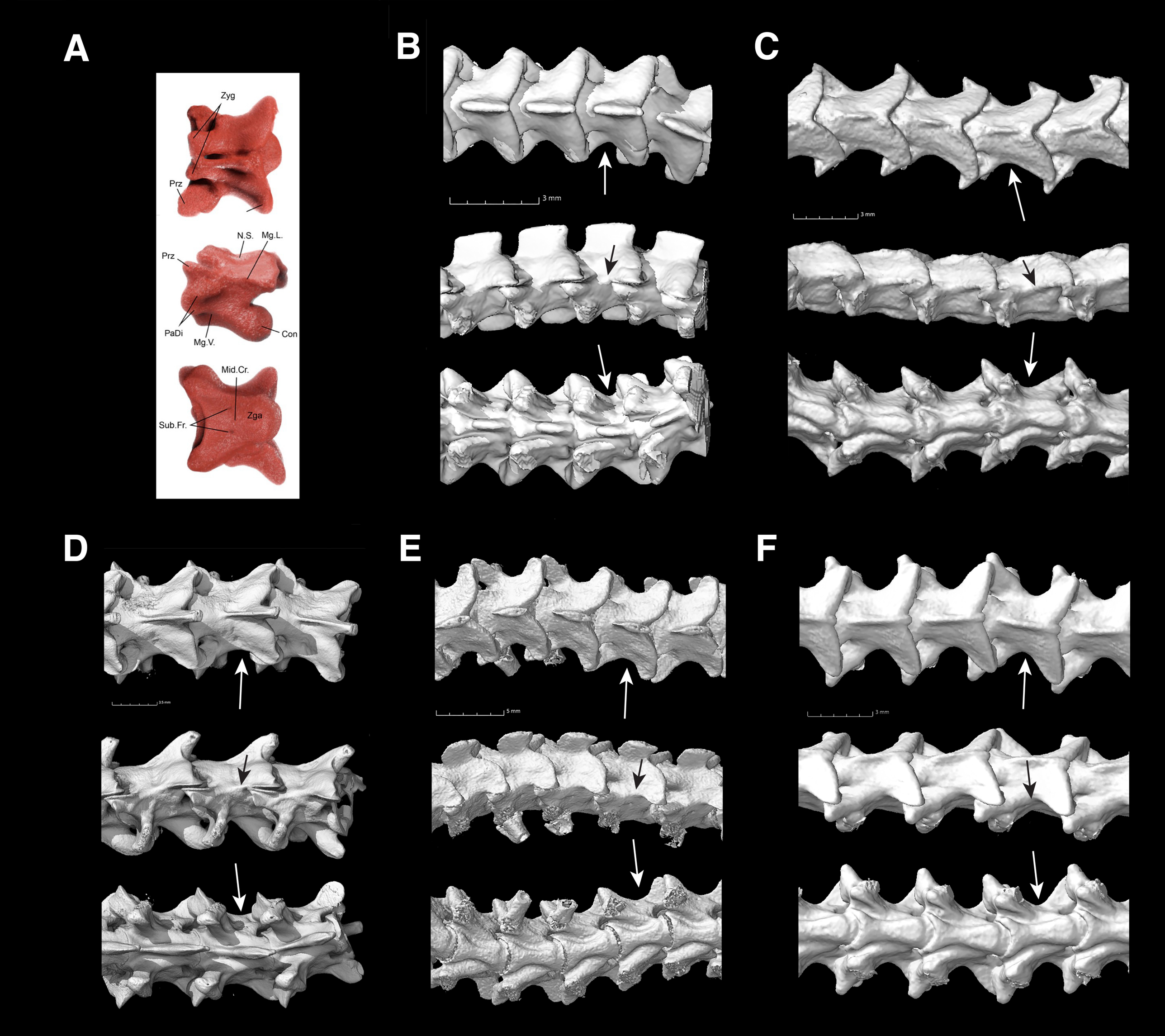
personal observation, which is perfectly fine (reference to observed material of *Dinilysia* should be added to this character in Supplementary Table 1). However, we still fail to understand what is the rationale behind considering the presence of a small retroarticular process in *Najash* (in agreement with Garberoglio et al., 2019, but the new Supplementary Table 1 states this feature as previously undescribed for *Najash*) and to find no evidence of such a process in *Tametara*, since there it seems to be no distinction between the two. See image below of MPCA-500 taken from Garberoglio et al. (2019) compared to *Tametara* from Fig. 2 in the MS:



Line 255. Authors declare that the contact between the prootic and the supraoccipital, also found in *Dinilysia*, *Najash* and basal alethinophidians was observed by: TRS pers. obs, and Garberoglio et al. 2019a. However, there are other previous descriptions of this feature in the mentioned fossil taxa that are omitted here, such as Estes et al., 1970, and Zaher et al., 2009. Additionally, this condition is not observed in any specimen of Najash, being the contact between prootic and supraoccipital concealed by the parietal in some specimens (Garberoglio et al., 2019a, 2019b).

>Edited. References added. Attribution to *Najash* removed.

It was not necessary to remove it all. Our original observation (saying “not observed in all”, and not “not observed in any”, don’t know why is changed here) referred that it is visible in some specimens and not in other. Actually, it is described originally for Najash.


A
Zyg
Prz
N.S.
Mg.L.
Prz
PaDi
Mg.V.
Con
Mid.Cr.
Zga
Sub.Fr.
B
3 mm
C
3 mm
D
3.5 mm
E
5 mm
F
3 mm