Using linear measurements to diagnose the ecological habitat of *Spinosaurus* (#91634)

First revision

Guidance from your Editor

Please submit by **25 Feb 2024** for the benefit of the authors .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files
Download and review all files from the materials page.

1 Tracked changes manuscript(s)
1 Rebuttal letter(s)
2 Figure file(s)
2 Raw data file(s)
1 Other file(s)

Structure and Criteria

2

✓

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

🗋 You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- ✓ Clear, unambiguous, professional English language used throughout.
- ✓ Intro & background to show context. Literature well referenced & relevant.
- ✓ Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- ✓ Figures are relevant, high quality, well labelled & described.
- ✓ Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- ✓ Original primary research within Scope of the journal.
- ✓ Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- ✓ Rigorous investigation performed to a high technical & ethical standard.
- ✓ Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- ℹ Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- ✓ All underlying data have been provided; they are robust, statistically sound, & controlled.
- ✓ Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Using linear measurements to diagnose the ecological habitat of *Spinosaurus*

Sean Smart [1] , **Manabu Sakamoto** Corresp. [1]

[1] School of Biological Sciences, University of Reading, Reading, United Kingdom

Corresponding Author: Manabu Sakamoto
Email address: m.sakamoto@reading.ac.uk

Much of the ecological discourse surrounding the polarising theropod *Spinosaurus* has centred on qualitative discussions. Using a quantitative multivariate data analytical approach on size-adjusted linear measurements of the skull, we examine patterns in skull shape across a range of sauropsid clades and three ecological habitats (terrestrial, semi-aquatic, and aquatic). We utilise cluster analyses to identify emergent properties of the data which associate properties of skull shape with ecological habitat occupancy. Results revealed terrestrial ecologies to be significantly distinct from both semi- and fully aquatic ecologies, the latter two were not significantly different. Spinosaurids (including *Spinosaurus*) plotted away from theropods in morphospace and close to both marine taxa and wading birds. The position of nares and the degree of rostral elongation had the greatest effect on categorisation. Comparisons of supervised (k-means) and unsupervised clustering demonstrated categorising taxa into three groups (habitats) was inappropriate and suggested instead that cluster division is based on morphological adaptations to feeding on aquatic versus terrestrial food items. The relative position of the nares in longirostrine taxa is associated with which skull bones are elongated. Rostral elongation is observed by either elongating the maxilla and the premaxilla or by elongating the maxilla only. This results in the nares positioned towards the orbits or towards the end of the rostrum respectively, with implications on available feeding methods. Spinosaurids, especially *Spinosaurus*, show elongation in the maxilla-premaxilla complex, achieving similar functional outcomes to elongation of the premaxilla seen in birds, particularly large-bodied piscivorous taxa. Such a skull construction would bolster “stand-and-wait” predation of aquatic prey to a greater extent than serving other feeding methods.

Using linear measurements to diagnose the ecological habitat of *Spinosaurus*

Sean Smart, Manabu Sakamoto*
School of Life Sciences, University of Lincoln, Lincoln, United Kingdom

Abstract:

Much of the ecological discourse surrounding the polarising theropod *Spinosaurus* has centred on qualitative discussions. Using a quantitative multivariate data analytical approach on size-adjusted linear measurements of the skull, we examine patterns in skull shape across a range of sauropsid clades and three ecological habitats (terrestrial, semi-aquatic, and aquatic).

We utilise cluster analyses to identify emergent properties of the data which associate properties of skull shape with ecological habitat occupancy. Results revealed terrestrial ecologies to be significantly distinct from both semi- and fully aquatic ecologies, the latter two were not significantly different. Spinosaurids (including *Spinosaurus*) plotted away from theropods in morphospace and close to both marine taxa and wading birds. The position of nares and the degree of rostral elongation had the greatest effect on categorisation. Comparisons of supervised (k-means) and unsupervised clustering demonstrated categorising taxa into three groups (habitats) was inappropriate and suggested instead that cluster division is based on morphological adaptations to feeding on aquatic versus terrestrial food items. The relative position of the nares in longirostrine taxa is associated with which skull bones are elongated. Rostral elongation is observed by either elongating the maxilla and the premaxilla or by elongating the maxilla only. This results in the nares positioned towards the orbits or towards the end of the rostrum respectively, with implications on available feeding methods. Spinosaurids, especially *Spinosaurus*, show elongation in the maxilla-premaxilla complex, achieving similar functional outcomes to elongation of the premaxilla seen in birds, particularly large-bodied piscivorous taxa. Such a skull construction would bolster “stand-and-wait” predation of aquatic prey to a greater extent than serving other feeding methods.

Introduction:

The enigmatic theropod *Spinosaurus aegyptiacus* (Stromer, 1915) is putatively considered semi-aquatic to some capacity (Aureliano *et al.,* 2018; Henderson, 2018; Ibrahim *et al.,* 2020; Fabbri et al., 2022; Sereno et al., 2022). This is supported by morphological (Ibrahim et al., 2014; Beevor et al., 2021), geographical (Bertin, 2010; Benyoucef et al., 2015), and isotopic (Amiot et al., 2010) evidence. Of specific interest are cranial adaptations to piscivory (itself indicative of aquatic affinities) observed in *Spinosaurus*; conical, interlocking dentition, posteriorly retracted nares, lateral skull compression, and raised orbit position (Ibrahim et al., 2014; Arden et al., 2019; Hone and Holtz, 2021). Partial piscivory has been widely described across Spinosauridae (Charig and Milner, 1997; Allain et al., 2012; Sales and Schultz, 2017; Fabbri et al., 2022a). Spinosaurine spinosaurids show fewer, larger teeth with fluting in place of serrations compared to baryonychine spinosaurids, (Sereno et al., 1998; Sales and Schultz, 2017; Hone and Holtz, 2021), which have been proposed as adaptations to a diet including hard-bodied prey (Massare, 1987; Hone and Holtz, 2021) in spinosaurine spinosaurids. Alternatively, this dentition could be an adaptation to withstand greater bite forces generated by greater body size compared to baryonychine spinosaurids (Sakamoto, 2022), similar selection pressures were suggested by Sereno et al. (2022).

As described, *Spinosaurus* is considered to be semi-aquatic partially due to the shape of its skull and relative position of the orbits (Ibrahim et al., 2014; Arden et al., 2019; Hone and Holtz, 2021). Despite a fragmentary cranial fossil record, sufficient material of multiple spinosaurids (both those considered semi-aquatic and terrestrial) exists to compare these features and their relation to habitat use, both within Spinosauridae and to other taxa where habitat use is undisputed. To this end, cranial linear morphometric analyses (Mosimann, 1970; Sakamoto and Ruta, 2012; Morales and Giannini, 2021) have previously been successful in revealing the taxonomic affinities of unidentified specimens (Blake et al., 2014; Naish et al., 2014). Linear morphometric analysis could thus be a suitable method for categorising the habitat use of *Spinosaurus*.

Within the *Spinosaurus* literature, definitions of 'semi-aquatic' can be varied. We define a 'semi-aquatic' animal to refer to those that utilise aquatic environments for a significant proportion of their nutritional resources and/or spend a significant proportion of time within bodies of water but retain terrestrial locomotory capabilities. However, a more specific diagnosis of the habitat utilisation of *Spinosaurus* beyond semi-aquatic would be advantageous, as there is no consensus thus far (Ibrahim et al., 2014; Hone and Holtz, 2021; Fabbri et al., 2022; Sereno et al., 2022).

Most recent research broadly addresses one of two competing hypotheses: the 'underwater pursuit predator' hypothesis (Ibrahim *et al.*, 2020) and the 'shallow water wading' hypothesis (Hone and Holtz, 2021). The former paints *Spinosaurus* as specialised in actively chasing down prey whilst submerged in the water column, propelled by tail and trunk (Ibrahim et al., 2020). In contrast, the latter hypothesis describes a hunting mode wherein the majority of the animal remains above the waterline, except for portions of the limbs and rostrum, suggesting stork-like feeding behaviours (Paul, 1988; Hone and Holtz, 2017).

The shallow water wading hypothesis has received notable support in recent publications (Hone and Holtz, 2021; Sereno et al., 2022), though discussions remain ongoing (see (Fabbri et al., 2022a; Fabbri et al., 2022b)). Here, we expand on the work by Hone and Holtz (2021) to develop their quantitative approach to analysing cranial morphology in sauropsids across different ecological habitats. To this end, we aim to build upon the work of Hone and Holtz to evaluate whether ecological habitat (terrestrial, semi-aquatic, or aquatic) can be inferred through skull morphometrics based on multiple linear measurements, and apply this to *Spinosaurus*.

Materials and methods:

99 taxa from 8 clades were examined and subdivided by known or inferred ecologies (terrestrial, semi-aquatic (following the definition above), or aquatic). Our dataset expanded upon that of Hone and Holtz (2021), increasing the number of representatives of examined clades and adding the families Ardeidae (herons) and Ciconiidae (storks), to allow morphological comparisons between *Spinosaurus* and these ecological analogues (wading birds) as proposed by Hone and Holtz (2021). Taxa were selected upon the availability of photographs (The Experimental Zoology Group of Wageningen University, 2022), 3D scans

(The University of Texas at Austin, 2022), or reconstructions depicting the skull in dorsal and lateral orientations (Supplemental material).

[Figure 1]

Figure 1. Principal component analysis of 6 size-adjusted linear (Mosimann) variables in 95 representative taxa across 8 sauropsid clades (Squamata and Crurotarsi further divided based on ecology). **(a)** Distribution of taxa in morphospace of principle component (PC) 1 (48.06% of variance) and PC2 (30.10% of variance). Convex hulls delimit K-means (k=3) cluster groupings. Green markers indicate terrestrial ecology, light blue markers indicate a semi-aquatic ecology, dark blue markers represent a fully aquatic ecology, and orange and red represent unknown ecologies. Silhouettes of taxa highlight the naris (blue) and the orbit (green). **(b)** Measurements were taken from the skull in lateral view. Skull height (SH), skull length (SL), distance from naris to anterior margin (NA), distance from naris to dorsal margin (ND), and distance from orbit to dorsal margin (OD). Not pictured: skull width (taken from dorsal view). Modified from (Hone and Holtz, 2021). **(c)** Comparison of skull morphology between *Spinosaurus sp.* (top, modified from (Ibrahim et al., 2014)) and *Pliosaurus kevani* (bottom, modified from (Benson et al., 2013)). Legend silhouettes *Allosaurus* and *Spinosaurus* by Tasman Dixon, *Varanus* by Steven Traver, *Goniopholis*, *Tylosaurus*, *Rhomaleosaurus*, and *Baryonyx* by Scott Hartman, and *Leptoptilos* by L. Shyamal. Skull silhouettes modified from respective sources (see supplemental material).

For each taxon, 6 measurements were taken using ImageJ v. 1.53 (Abràmoff et al., 2004) (figure 1b) following Hone and Holtz (2021). As some variable measurements were 0 (such as when naris lies on the skull anterior margin), to allow these values to be log-transformed, a constant of 1mm was added to all measurements. To account for Isometric scaling due to body size, each measurement was divided by the geometric mean of the skull (Sakamoto and Ruta, 2012). The resultant dimensionless Mosimann shape variables have been demonstrated to out-perform residuals as size-adjusted shape variables, and have the additional benefit of only requiring information from a single specimen (Mosimann, 1970; Sakamoto and Ruta, 2012; Morales and Giannini, 2021). These values were log-transformed (Glazier, 2013), centred on 0 and scaled to unit variance to conform to the assumptions of cluster analysis and to a lesser extent, principal component analysis (PCA). Geometric mean was selected as a proxy for body size over skull length due to the presence of characteristic rostral elongation in multiple taxa examined (Bertin, 2010; Erickson et al., 2012; Fischer et al., 2017). Rostral elongation can interfere with body size estimates derived from skull length due to the allometric relationship between these variables (Therrien and Henderson, 2007).

Data analyses

All data and statistical analyses were conducted in PAST v. 4.03 (Hammer et al., 2001). A principal component analysis (PCA) was performed on the 6 Mosimann shape variables of 95 taxa (those with incomplete information were excluded) using a variance-covariance matrix. Taxa were grouped by clade and ecology. Permutational multivariate analyses of variance (PerMANOVA) were used to assess the overlap in morphospace between clades, and between ecologies. These analyses used a Euclidian similarity index and Bonferroni-corrected p values. To determine the appropriate number and pattern of clustering in morphospace, we used both classical (unsupervised clustering using Ward's method and Euclidian distance measure) and k-means (supervised) cluster analyses to examine the relative effect of ecology compared to phylogeny on skull shape. We chose k=3 for k-means analysis to reflect the number of ecologies.

Results:

Following Principal component analysis (PCA) of linear skull measurements, PC1 and PC2 cumulatively account for 78.16% of the variance in the data, PC1 explaining 48.06% and PC2 explaining 30.10%. A morphospace plot of PC1 against PC2 (figure 1a) reveals that an increase along PC1 is associated with an increase in skull size and a decrease in NA. Whereas increase along PC2 is associated with skull heights approximately equal to skull length, progressively deeper-set orbits (long OD) and nares (long OD), and increasing distance from the naris to the skull anterior margin (long NA) (supplemental material). Ecologies are scattered across morphospace, though an increase along PC1 is associated with increasing terrestriality and higher values of PC2 (both positive and negative) are associated with increased aquatic affinities. When clades contain representatives from multiple ecologies (squamates and crurotarsans), members remain close in morphospace despite their assigned ecologies (figure 1a). Wading birds, spinosaurids, mosasaurs and plesiosaurs occupy the same region of morphospace, with *Spinosaurus* being most similar to *Pliosaurus kevani* (figure 1c).

A one-way PerMANOVA by clade, reveals that all aquatic taxa and semi-aquatic crurotarsans did not occupy significantly different ($p > 0.05$) regions of morphospace. Spinosaurids (excluding *Spinosaurus*) did not show significant differences from any other clade. Storks and herons, terrestrial and semi-aquatic squamates, and semi-aquatic and aquatic crurotarsans could not be differentiated from each other. Non-spinosaurid theropods were distinct from all clades except semi-aquatic squamates and spinosaurids. Likewise, semi-aquatic crurotarsans were distinct from all non-aquatic, non-spinosaurid taxa. A one-way PerMANOVA of the same data grouped by ecology showed all terrestrial ecology pairs as significantly different ($p < 0.001$) but aquatic and semi-aquatic ecologies could not be differentiated.

[Figure 2]

Figure 2. Morphospace plot of principal component scores as in figure 1, showing instead unsupervised cluster analysis groupings.

Supervised clustering using k-means (k=3) cluster analysis did not yield clusters according to the three habitat categories (figure 1a). Cluster 1 is characterised by narrow skulls, elongate rostra, and substantial nares to anterior margin distances (larger NA). Cluster 2 is also associated with rostral elongation but is distinguished from Cluster 1 by anterodorsally elevated nares (smaller OD and NA). Cluster 3 contains all other terrestrial taxa. High PC 1 scores indicate the absence of rostral elongation.

Unsupervised cluster analysis on the other hand produced two clusters (figure 2). Cluster A is formed of taxa with elongate skulls and long nares to anterior margin distances. Taxa in this cluster are all either aquatic (plesiosaurs and mosasaurs) or semi-aquatic (phytosaurs (Crurotarsi) and aves). Cluster B contains taxa with short nares to anterior margin distances, this includes all terrestrial taxa, semi-aquatic squamates, and non-phytosaur crurotarsans.

Discussion

The discrepancy between the number of clusters produced by supervised (3 - figure 1a) and unsupervised (2 - figure 2) cluster analyses demonstrates that the *a priori* categorisation (K=3) based on the three habitats is not supported by our linear morphometric data. Cluster membership did not correspond to specified ecology (terrestrial, semi-aquatic, or aquatic) in either analysis. Taken together, this indicates that ecomorphologies associated with habitat are not emergent properties of our dataset. Instead, unsupervised cluster membership (figure 2) appears to be largely determined by the distance from the nares to the anterior margin (supplemental material), which dictates the relative rostral length often interpreted as adaptations for foraging in water, either semi- or fully submerged. Thus, separation in morphospace may be influenced by factors such as diet (feeding ecology, i.e., what they are eating irrespective of habitat) and to a lesser extent phylogeny (Melstrom et al., 2021). This supports inferences regarding the evolutionary history of derived spinosaurids and feeding ecology in *Spinosaurus*.

Taxa in unsupervised cluster A (figure 2) exhibit proportionally posteriorly retracted nares relative to members of cluster B. Taxa in both clusters display rostral elongation, though this is achieved by elongation of different skull bones in each cluster. Members of cluster A (aves, plesiosaurs, mosasaurs, spinosaurids, and phytosaurs) extend the rostra via elongation of the skeletal elements rostral to the naris, i.e., the maxilla-premaxilla complex. This distinguishes them from taxa in Cluster B (i.e., non-phytosaurian crurotarsans), which elongate the rostrum via increasing the length of the nasal-maxilla complex (supplemental material). These two morphotypes attain the same outcome in terms of elongation of the rostrum (e.g., increased reach), but differ in which part of the rostrum is elongated relative to the nares, anterior (Clade A) or posterior (Clade B). Although increased reach is equally beneficial to taxa in both clusters, the correlated movement of relative nares position results in significant impacts in which feeding modalities are available to these semi- and fully aquatic taxa.

In general, rostral elongation is viewed as an adaptation for both semi- and fully aquatic taxa feeding on aquatic prey. The associated increases in the out-lever distance (distance from jaw joint to bite point) results in greater relative jaw closing speed (Sakamoto, 2010; Ballell et al., 2019; Evans et al., 2019). This would be a desirable trait when feeding on highly mobile aquatic prey (Massare, 1988; McCurry et al., 2017) either fully submerged as in underwater pursuit predation or only submerging a portion of the skull as in the “stand-and-wait” strategy seen in herons and storks (Kushlan, 1976; Willard, 1977; Maheswaran and Rahmani, 2002).

Of these two main strategies proposed to describe feeding behaviour in *Spinosaurus* (Ibrahim *et al.,* 2020; Hone and Holtz, 2021), the position of the nares is beneficial to the “stand-and-wait” strategy whilst being neither beneficial nor detrimental to the efficacy of the underwater pursuit strategy. An increased distance between the tip of the rostrum and the nares allows for a greater portion of the skull to remain submerged without restricting breathing, potentially increasing foraging efficiency (Hone and

Holtz, 2021). In marine mosasaurs and pliosauroid plesiosaurs, rostral elongation is present but not as extreme, especially in plesiosauroid plesiosaurs (supplemental material). In these taxa, nares that are positioned closer to the dorsal margin of the head may have assisted with minimising the portion of the body exposed when surfacing for breath. This benefit has also been suggested for instances when *Spinosaurus* is largely submerged and only extends a small portion of the head above the water, suggesting strong aquatic affinities (Ibrahim et al, 2014; Arden et al, 2019). However, the nares of *Spinosaurus* are not notably closer to the dorsal margin of the head than other spinosaurids for which such strong aquatic affinities are not proposed, and a larger portion of the head would have to be exposed, negating the proposed benefits (Hone and Holtz, 2021; Hone and Holtz, 2022).

Whilst it is difficult to address the sources of natural selection that differentiate the two evolutionary pathways to rostral elongation observed, as this was not directly tested, the relative lengths of the skull bones in *Spinosaurus* presents implications for the impacts of phylogeny on skull shape. Across all theropods examined (both spinosaurids and non-spinosaurids), the relative positions of the naris and orbit to the nasal remain constant (supplemental material). Interestingly, the rostral elongation in *Spinosaurus* is achieved via the lengthening of the skeletal elements anterior to the naris, thus maintaining the theropod configuration of the naris and orbit positions relative to the nasal. This feature is more prominent in the derived *Spinosaurus* than in more basal baryonychine spinosaurids, which show more limited retraction (figure 1a, 1c). Avians display elongation primarily through elongation of the premaxilla as the maxilla is greatly reduced (Bhullar et al., 2015), piscivorous birds in particular have among the largest bills in terms of absolute size. In both birds and spinosaurids, elongation of the skull anterior to the nares serves to considerably increase the striking range, jaw closing speed, and amount of the skull that can be submerged whilst foraging, facilitating foraging on aquatic prey items.

In contrast, Crurotarsi displays both pathways of rostral elongation in phytosaurs (Cluster A) and crocodyliforms (Cluster B), demonstrating less phylogenetic constraint across Crurotarsi as a whole, but distinct effects of phylogeny within Phytosauria and Crocodyliformes respectively, i.e., all phytosaurs are in Cluster A while all crocodyliforms are in Cluster B. The disparity in how rostral elongation is attained across Crurotarsi likely owes to it being a large and diverse clade, with phytosaurs being more basal, representing an older radiation, than crocodyliforms (Nesbitt, 2011). Regardless of what ecological selection pressures may be associated with rostral elongation, it is likely that members within clades attain this trait due to consistent selection pressures from feeding in aquatic environments. However, species engaging with such a feeding ecology are subjected to the mechanical pressures of feeding in a dense fluid medium that restricts viable morphospace (Massare, 1988; Pierce et al., 2008). This combines with fewer modalities of feeding available to large-bodied marine organisms (Taylor, 1987) leading to convergence in skull shape (figure 2) though not necessarily convergence in diet.

The variables investigated here - in particular, the position of the nares relative to the anterior margin of the skull- are largely able to discriminate between taxa that feed terrestrially and those that feed on aquatic prey (figure 1a, 2). However, due to similar biomechanical restrictions, taxa that feed on aquatic prey show substantial skull shape convergence with fully aquatic taxa which prevents definitive categorisation of taxa with uncertain ecology (such as *Spinosaurus*), based on linear measurement of the skull alone. In regards to the feeding behaviour of *Spinosaurus*, elongation of the premaxilla in spinosaurids compared to other theropods results in a nares position which would have been exceedingly beneficial to the “stand-and-wait” predation strategy, whilst not substantially beneficial to the underwater pursuit predation strategy. Further investigations may consider post-cranial data, phylogenetic analyses and dentition, as well as feeding guilds as a categorisation factor. The data we have gathered is not sufficient to completely evaluate the utility of size-adjusted linear measurements of the cranium, but functions as an exploratory study which provides a framework for future studies to develop this line of enquiry. Specifically, we emphasise the importance of comparing supervised and unsupervised clustering to assess if the former is appropriate as the number of groups in supervised clustering can mislead inferences.

Acknowledgements:

Images used are available under the CC BY 4.0 licence https://creativecommons.org/licenses/by/3.0/. Taxa silhouettes sources from PhyloPic.org.

References:

Abràmoff, D.M.D., Magalhães, P.J. and Ram, S.J. (2004) Image Processing with ImageJ. *Biophotonics international*, 11(7) 36–42.

Allain, R., Xaisanavong, T., Richir, P. and Khentavong, B. (2012) The first definitive Asian spinosaurid (Dinosauria: Theropoda) from the early cretaceous of Laos. *Naturwissenschaften*, 99(5) 369–377.

Amiot, R., Buffetaut, E., Lécuyer, C., Wang, X., Boudad, L., Ding, Z., Fourel, F., Hutt, S., Martineau, F., Medeiros, M.A., Mo, J., Simon, L., Suteethorn, V., Sweetman, S., Tong, H., Zhang, F. and Zhou, Z. (2010) Oxygen isotope evidence for semi-aquatic habits among spinosaurid theropods. *Geology*, 38(2) 139–142.

Arden, T.M.S., Klein, C.G., Zouhri, S. and Longrich, N.R. (2019) Aquatic adaptation in the skull of carnivorous dinosaurs (Theropoda: Spinosauridae) and the evolution of aquatic habits in spinosaurids. *Cretaceous Research*, 93 275–284.

Aureliano, T., Ghilardi, A.M., Buck, P.V., Fabbri, M., Samathi, A., Delcourt, R., Fernandes, M.A. and Sander, M. (2018) Semi-aquatic adaptations in a spinosaur from the Lower Cretaceous of Brazil. *Cretaceous Research*, 90 283–295.

Ballell, A., Moon, B.C., Porro, L.B., Benton, M.J. and Rayfield, E.J. (2019) Convergence and functional evolution of longirostry in crocodylomorphs. *Palaeontology*, 62(6) 867–887.

Beevor, T., Quigley, A., Smith, R.E., Smyth, R.S.H., Ibrahim, N., Zouhri, S. and Martill, D.M. (2021) Taphonomic evidence supports an aquatic lifestyle for Spinosaurus. *Cretaceous Research*, 117 104627.

Benson, R.B.J., Evans, M., Smith, A.S., Sassoon, J., Moore-Faye, S., Ketchum, H.F. and Forrest, R. (2013) A Giant Pliosaurid Skull from the Late Jurassic of England. *PLOS ONE*, 8(5) e65989.

Benyoucef, M., Läng, E., Cavin, L., Mebarki, K., Adaci, M. and Bensalah, M. (2015) Overabundance of piscivorous dinosaurs (Theropoda: Spinosauridae) in the mid-Cretaceous of North Africa: The Algerian dilemma. *Cretaceous Research*, 55 44–55.

Bertin, T. (2010) A Catalogue of Material and Review of the Spinosauridae. *PalArch's Journal of Vertebrate Palaeontology*, 7(4) 01–39.

Bestwick, J., Jones, A.S., Purnell, M.A. and Butler, R.J. (2021) Dietary constraints of phytosaurian reptiles revealed by dental microwear textural analysis. *Palaeontology*, 64(1) 119–136.

Bhullar, B.-A.S., Morris, Z.S., Sefton, E.M., Tok, A., Tokita, M., Namkoong, B., Camacho, J., Burnham, D.A. and Abzhanov, A. (2015) A molecular mechanism for the origin of a key evolutionary innovation, the bird beak and palate, revealed by an integrative approach to major transitions in vertebrate history. *Evolution*, 69(7) 1665–1677.

Blake, M., Naish, D., Larson, G., King, C.L., Nowell, G., Sakamoto, M. and Barnett, R., 2014. Multidisciplinary investigation of a 'British big cat': a lynx killed in southern England c. 1903. *Historical Biology*, *26*(4), pp.441-448.

Brusatte, S.L., Sakamoto, M., Montanari, S. and Harcourt Smith, W.E.H. (2012) The evolution of cranial form and function in theropod dinosaurs: insights from geometric morphometrics. *Journal of Evolutionary Biology*, 25(2) 365–377.

Charig, A. and Milner, A.C. (1997) Baryonyx walkeri, a fish-eating dinosaur from the Wealden of Surrey. *Bulletin of the Natural History Museum. Geology series*, 53 11–70.

Erickson, G.M., Gignac, P.M., Steppan, S.J., Lappin, A.K., Vliet, K.A., Brueggen, J.D., Inouye, B.D., Kledzik, D. and Webb, G.J.W. (2012) Insights into the Ecology and Evolutionary Success of Crocodilians Revealed through Bite-Force and Tooth-Pressure Experimentation. *PLOS ONE*, 7(3) e31781.

Evans, K.M., Kim, L.Y., Schubert, B.A. and Albert, J.S. (2019) Ecomorphology of Neotropical Electric Fishes: An Integrative Approach to Testing the Relationships between Form, Function, and Trophic Ecology. *Integrative Organismal Biology*, 1(1) obz015.

Fabbri, M., Navalón, G., Benson, R.B.J., Pol, D., O'Connor, J., Bhullar, B.-A.S., Erickson, G.M., Norell, M.A., Orkney, A., Lamanna, M.C., Zouhri, S., Becker, J., Emke, A., Dal Sasso, C., Bindellini, G., Maganuco, S., Auditore, M. and Ibrahim, N. (2022) Subaqueous foraging among carnivorous dinosaurs. *Nature*, 603(7903) 852–857.

Fabbri, M., Navalón, G., Benson, R.B.J., Pol, D., O'Connor, J., Bhullar, B.-A.S., Erickson, G.M., Norell, M.A., Orkney, A., Lamanna, M.C., Zouhri, S., Becker, J., Sasso, C.D., Bindellini, G., Maganuco, S., Auditore, M. and Ibrahim, N. (2022) *Sinking a giant: quantitative macroevolutionary comparative methods debunk qualitative assumptions*. p.2022.05.05.490811.

Fischer, V., Benson, R.B.J., Zverkov, N.G., Soul, L.C., Arkhangelsky, M.S., Lambert, O., Stenshin, I.M., Uspensky, G.N. and Druckenmiller, P.S. (2017) Plasticity and Convergence in the Evolution of Short-Necked Plesiosaurs. *Current Biology*, 27(11) 1667-1676.e3.

Glazier, D.S. (2013) Log-transformation is useful for examining proportional relationships in allometric scaling. *Journal of Theoretical Biology*, 334 200–203.

Hammer, O., Harper, D.A.T. and Ryan, P.D. (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4(1) 9.

Henderson, D.M. (2018) A buoyancy, balance and stability challenge to the hypothesis of a semi-aquatic Spinosaurus Stromer, 1915 (Dinosauria: Theropoda). *PeerJ*, 6 e5409.

Hone, D.W.E. and Holtz, T.R. (2017) A Century of Spinosaurs - A Review and Revision of the Spinosauridae with Comments on Their Ecology. *Acta Geologica Sinica - English Edition*, 91(3) 1120–1132.

Hone, D.W.E. and Holtz, T.R.J. (2021) Evaluating the ecology of Spinosaurus: Shoreline generalist or aquatic pursuit specialist? *Palaeontologia Electronica*, 24(1) 1–28.

Hone, D.W.E. and Holtz Jr, T.R., 2022. Comment on: Aquatic adaptation in the skull of carnivorous dinosaurs (Theropoda: Spinosauridae) and the evolution of aquatic habits in spinosaurids. 93: 275–284. *Cretaceous Research*, *134*, p.104152.

Ibrahim, N., Sereno, P.C., Sasso, C.D., Maganuco, S., Fabbri, M., Martill, D.M., Zouhri, S., Myhrvold, N. and Iurino, D.A. (2014) Semiaquatic adaptations in a giant predatory dinosaur. *Science*, Available from https://www.science.org/doi/abs/10.1126/science.1258750 [accessed 7 February 2022].

Kushlan, J.A. (1976) Feeding Behavior of North American Herons. *The Auk*, 93(1) 86–94.

Maheswaran, G. and Rahmani, A.R. (2002) Foraging behaviour and feeding success of the black-necked stork (Ephippiorhynchus asiaticus) in Dudwa National Park, Uttar Pradesh, India. *Journal of Zoology*, 258(2) 189–195.

Massare, J.A. (1988) Swimming Capabilities of Mesozoic Marine Reptiles: Implications for Method of Predation. *Paleobiology*, 14(2) 187–205.

Massare, J.A. (1987) Tooth Morphology and Prey Preference of Mesozoic Marine Reptiles. *Journal of Vertebrate Paleontology*, 7(2) 121–137.

McCurry, M.R., Evans, A.R., Fitzgerald, E.M.G., Adams, J.W., Clausen, P.D. and McHenry, C.R. (2017) The remarkable convergence of skull shape in crocodilians and toothed whales. *Proceedings of the Royal Society B: Biological Sciences*, 284(1850) 20162348.

Melstrom, K.M., Angielczyk, K.D., Ritterbush, K.A. and Irmis, R.B. (2021) The limits of convergence: the roles of phylogeny and dietary ecology in shaping non-avian amniote crania. *Royal Society Open Science*, 8(9) 17.

Morales, M.M. and Giannini, N.P. (2021) Pleistocene extinction and geographic singularity explain differences in global felid ensemble structure. *Evolutionary Ecology*, 35(2) 271–289.

Mosimann, J.E. (1970) Size Allometry: Size and Shape Variables with Characterizations of the Lognormal and Generalized Gamma Distributions. *Journal of the American Statistical Association*, 65(330) 930–945.

Naish, D., Sakamoto, M., Hocking, P. and Sanchez, G., 2014. 'Mystery big cats' in the Peruvian Amazon: morphometrics solve a cryptozoological mystery. *PeerJ*, *2*, p.e291.

Nesbitt, S.J. (2011) *The early evolution of archosaurs : relationships and the origin of major clades. (Bulletin of the American Museum of Natural History, no. 352)*. Available from https://digitallibrary.amnh.org/handle/2246/6112 [accessed 16 September 2022].

Paul, G.S. (1988) *Predatory Dinosaurs of the World: a Complete Illustrated Guide*. New York: Simon and Schuster.

Pierce, S.E., Angielczyk, K.D. and Rayfield, E.J. (2008) Patterns of morphospace occupation and mechanical performance in extant crocodilian skulls: A combined geometric morphometric and finite element modeling approach. *Journal of Morphology*, 269(7) 840–864.

Sakamoto, M. (2022) Estimating bite force in extinct dinosaurs using phylogenetically predicted physiological cross-sectional areas of jaw adductor muscles. *PeerJ*, 10 e13731.

Sakamoto, M. (2010) Jaw biomechanics and the evolution of biting performance in theropod dinosaurs. *Proceedings of the Royal Society B: Biological Sciences*, 277(1698) 3327–3333.

Sakamoto, M. and Ruta, M. (2012) Convergence and Divergence in the Evolution of Cat Skulls: Temporal and Spatial Patterns of Morphological Diversity. *PLOS ONE*, 7(7) e39752.

Sales, M.A.F. and Schultz, C.L. (2017) Spinosaur taxonomy and evolution of craniodental features: Evidence from Brazil. *PLOS ONE*, 12(11) e0187070.

Sereno, P., Myhrvold, N., Henderson, D.M., Fish, F.E., Vidal, D., Baumgart, S.L., Keillor, T.M., Formoso, K.K. and Conroy, L.L. (2022) Spinosaurus is not an aquatic dinosaur M. Zhu, C. Rutz, M. Zhu, T.R. Holtz, and D. Hone (eds.). *eLife*, 11 e80092.

Sereno, P.C., Beck, A.L., Dutheil, D.B., Gado, B., Larsson, H.C.E., Lyon, G.H., Marcot, J.D., Rauhut, O.W.M., Sadleir, R.W., Sidor, C.A., Varricchio, D.D., Wilson, G.P. and Wilson, J.A. (1998) A Long-Snouted Predatory Dinosaur from Africa and the Evolution of Spinosaurids. *Science*, 282(5392) 1298–1302.

Stromer, E. (1915) Ergebnisse der Forschungsreisen Prof. E. Stromers in den Wüsten Agyptens. II. Wirbeltier-Reste der Baharije-Stufe (unterstes Cenoman). 3. Das Original des theropoden Spinosaurus aegyptiacus nov. gen., nov. spec. Abhandlungen der Koniglich Bayerischen Akademie der Wissenschaften. *Mathematisch-physikalische Klasse*, 28(3) 1e32.

Taylor, M.A. (1987) How tetrapods feed in water: a functional analysis by paradigm. *Zoological Journal of the Linnean Society*, 91(2) 171–195.

The Experimental Zoology Group of Wageningen University (2022) *Skull Site, Bird Skull Collection*.

The University of Texas at Austin (2022) *Digimorph, Digital Morphology Database*.

Therrien, F. and Henderson, D.M. (2007) My theropod is bigger than yours … or not: estimating body size from skull length in theropods. *Journal of Vertebrate Paleontology*, 27(1) 108–115.

Willard, D.E. (1977) The Feeding Ecology and Behavior of Five Species of Herons in Southeastern New Jersey. *The Condor*, 79(4) 462–470.

Figure 1

Principal component analysis of 6 size-adjusted linear (Mosimann) variables in 95 representative taxa across 8 sauropsid clades (Squamata and Crurotarsi further divided based on ecology).

(a) Distribution of taxa in morphospace of principle component (PC) 1 (48.06% of variance) and PC2 (30.10% of variance). Convex hulls delimit K-means (k=3) cluster groupings. Green markers indicate terrestrial ecology, light blue markers indicate a semi-aquatic ecology, dark blue markers represent a fully aquatic ecology, and orange and red represent unknown ecologies. Silhouettes of taxa highlight the naris (blue) and the orbit (green). **(b)** Measurements were taken from the skull in lateral view. Skull height (SH), skull length (SL), distance from naris to anterior margin (NA), distance from naris to dorsal margin (ND), and distance from orbit to dorsal margin (OD). Not pictured: skull width (taken from dorsal view). Modified from (Hone and Holtz 2021). **(c)** Comparison of skull morphology between *Spinosaurus sp.* (top, modified from (Ibrahim et al 2014)) and *Pliosaurus kevani* (bottom, modified from (Benson et al 2013)). *Allosaurus* and *Spinosaurus* by Tasman Dixon, *Varanus* by Steven Traver, *Goniopholis*, *Tylosaurus*, *Rhomaleosaurus*, and *Baryonyx* by Scott Hartman, and *Leptoptilos* by L. Shyamal.

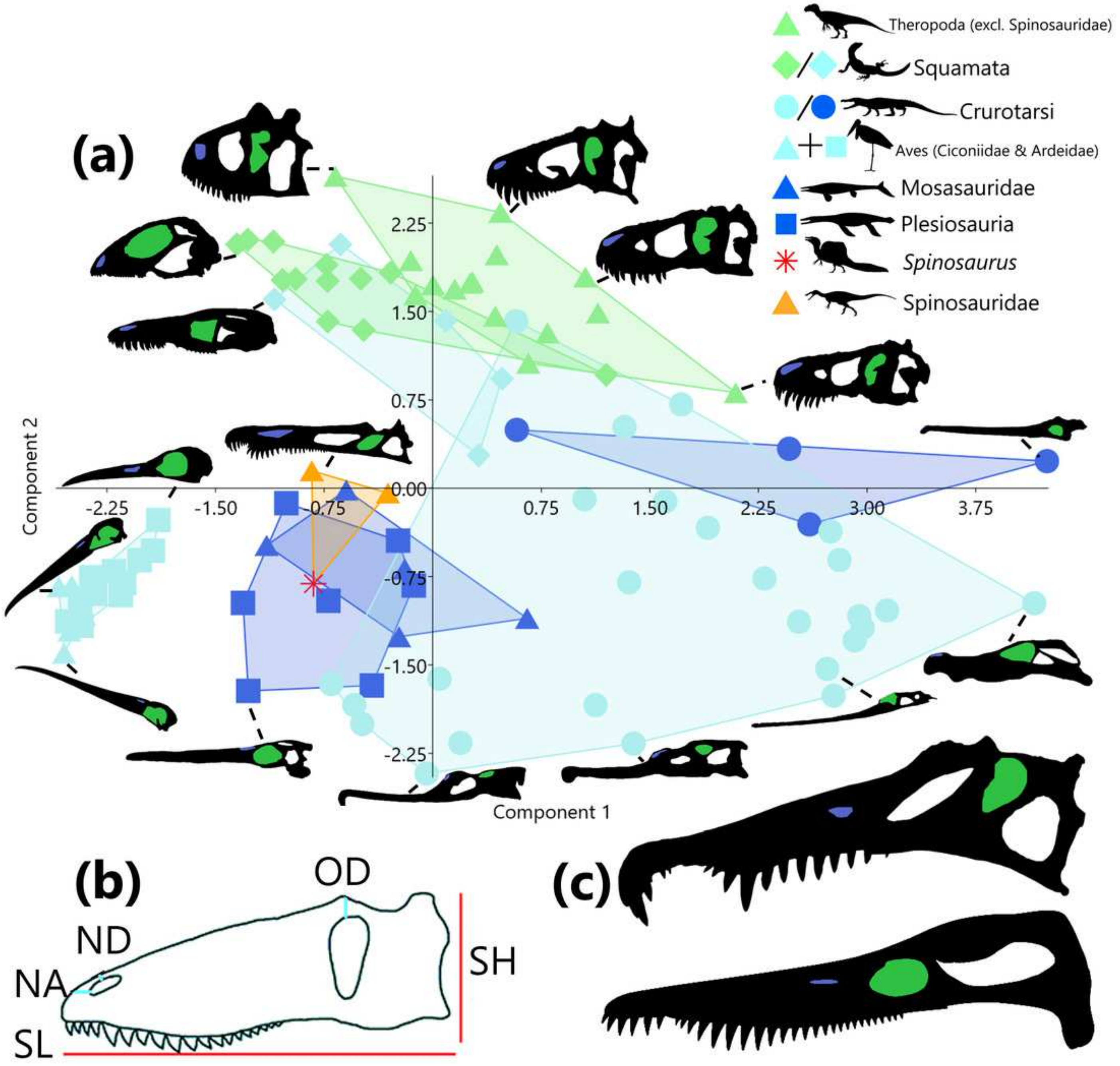
(a)
Theropoda (excl. Spinosauridae)
Squamata
Crurotarsi
Aves (Ciconiidae & Ardeidae)
Mosasauridae
Plesiosauria
Spinosaurus
Spinosauridae
Component 2
Component 1
2.25
1.50
0.75
0.00
-0.75
-1.50
-2.25
-2.25
-1.50
-0.75
0.75
1.50
2.25
3.00
3.75
(b)
OD
ND
NA
SH
SL
(c)

Figure 2

Morphospace plot of principal component scores as in figure 1, showing instead unsupervised cluster analysis groupings.