PhD project proposal

XL Cycle

Neuroanatomy of the early alligatoroid crocodylian *Diplocynodon*: phylogenetic, ecological and biogeographical implications

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State of the art

Until the end of the last century, information on the internal skull structure of fossil taxa were available only thanks to the rare finding of natural endocasts of the cranium, via the use of highly invasive techniques or, most commonly, with the realization of latex endocasts: unfortunately, this last method is not always viable and also poses lots of risks to the fossil [1]. Today, with the advent of computed tomography (=CT scanning), a non-invasive method is available to know more about the internal cranial structures of extinct taxa, giving a great boost to paleoneurology [1,2].

All the information obtained in this way can be useful for multiple purposes: firstly this kind of data can reveal important information to obtain phylogenetic trees based on characters from both external and internal cranial anatomy [3]; the internal cranial structures are also fundamental to gain key ecological and behavioural information thanks to the analyses of several sensory systems regarding hearing, vision, olfaction, balance, etc. [3]; lastly, knowing the volume of the brain and the body mass, it is also possible to infer cognitive capabilities [4,5].

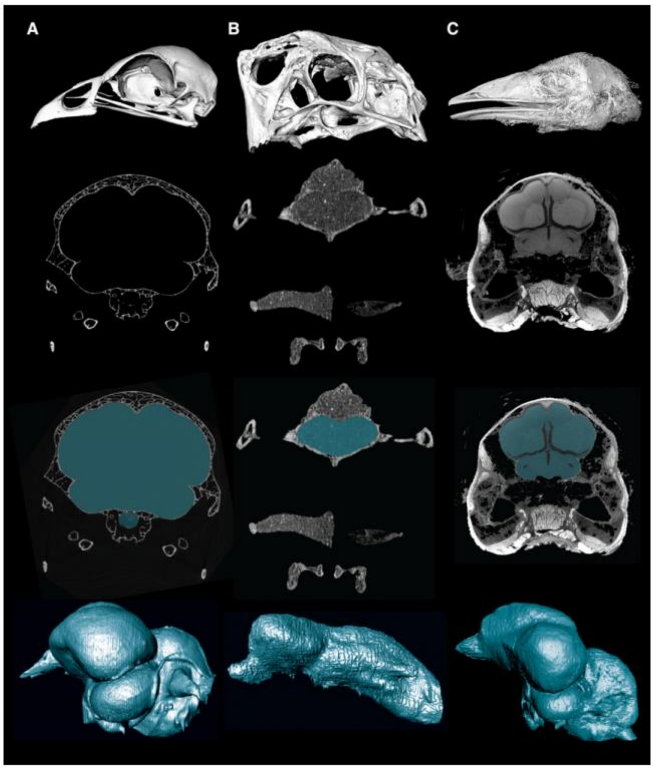


Fig. 1. CT images and cranial endocasts from different specimen types and preparations.
A) An extant galliform bird, *Alectura lathami*.
B) An oviraptorosaur dinosaur, *Citipati osmolskae*.
C) An extant paleognathous bird *Dromaius novaehollandiae*.
(Balanoff et al., 2016 [6])

This kind of analysis can be extremely useful in the study of an often-neglected group like Crocodylomorpha, which has a long evolutionary history of around 230 million years and still comprises some extant species that can be used to make important comparisons when inferring characteristics in fossil taxa. In this project the focus will be on the early alligatoroid genus *Diplocynodon* (Fig. 2).

Diplocynodon is one of the earliest diverging groups of the superfamily Alligatoroidea [e.g., 7,8,9] whose extant members are alligatorines and caimanines, occurring only in the Americas, apart from *Alligator sinensis* which is endemic to eastern China. *Diplocynodon* was instead seemingly endemic to Europe and also the last known alligatoroid group to have inhabited the continent, spanning from the late Paleocene to the middle Miocene [9,10,11]. Some specimens found in North America and Africa have also been previously referred to *Diplocynodon* [12,13,14,15]: even if most of these identifications have been disproved, further works are crucial to shed new light on this matter.

Currently, nine species of *Diplocynodon* are considered valid, and although there is a general consensus on it being an early diverging alligatoroid genus, the interrelationships among the species are not clear. *Diplocynodon* is not always recovered as monophyletic: Rio & Mannion (2021) [9] found *Diplocynodon* as a paraphyletic group, whereas in a previous work, in one of the four recovered trees, *Diplocynodon remensis* was placed outside of *Diplocynodon* in a more basal position [16]. This taxon, despite its stratigraphically earlier appearance than all other *Diplocynodon* species [10], in Rio & Mannion (2021) [9] is recovered in the most crownward position of all *Diplocynodon* species.

Fig. 2. *Diplocynodon ratelii* reconstruction in a typical ambush predator behaviour. (By José Antonio Peñas, SINC).

To date only one neuroanatomical analysis has been conducted for this genus (Fig. 3) [17] but, since the specimen from which the brain endocast has been reconstructed is only partially preserved, a lot of the internal structures had to be estimated or could not be observed at all (i.e. the semicircular canals of the inner ear, the dorsal limit of the cerebellar region, both crucial points for ecological inferences; see Barrios et al., 2022 [3]). The fact that an endocast reconstruction has been done only for one of the nine accepted species also makes it impossible to solve the phylogenetic doubts surrounding the interrelationships among the members of the taxon.

Despite them being usually found in freshwater environments, the discovery of a *Diplocynodon* specimen from a typical marine sedimentary environment in Romania [18] has raised doubts about the possible marine adaptations of some species. Thus, during their long evolutionary history, *Diplocynodon* species could have inhabited different habitats at different times, but this possibility requires further investigation.

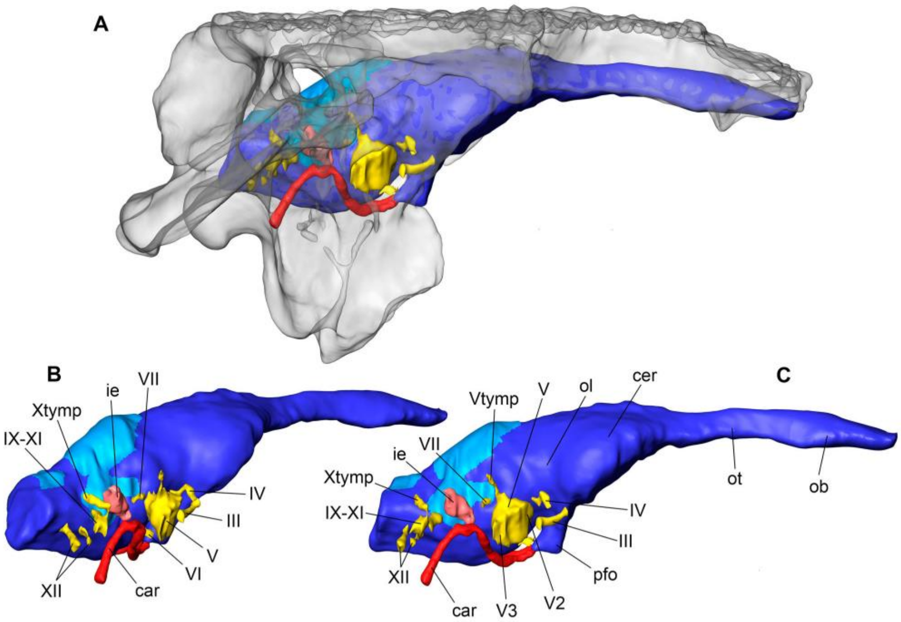


Fig. 3. Three-dimensional reconstruction of the endocranial cavities of *Diplocynodon tormis* STUS-344. (Brain in blue; cranial nerves in yellow; arteries in red; inner ears in pink; estimated parts in light blue) (Serrano-Martinez et al., 2019 [17]).

General objective

Broadening of the knowledge of the endocranial anatomy in extinct Crocodylomorpha taxa with phylogenetic, ecological and biogeographical implications.

Specific objectives

- Reconstruction of the neuroanatomy of several species of *Diplocynodon* based on CT scan data, to better understand the evolution of the brain and inner ear in extinct alligatoroids (implications for ecology and ontogeny).
- Formulation of new phylogenetic characters based on neuroanatomical data and incorporation of them into an existing crocodylian data matrix, with the aim to reassess the interrelationships among the nine species of *Diplocynodon* and relationships with other alligatoroids.
- Combination of the ecological information derived from the endocasts with paleoenvironmental and biogeographical data, to better understand the group's ecological niche and its evolution through time.

Implications and applications

The results obtained from this work will be important for future studies in different ways. Firstly, a lot of morphological data on the neauroanatomy of Crocodylomorpha will be collected that will be crucial to better understand the phylogenetic significance of this traits, a topic of great interest that is being studied recently and that would benefit from the information obtained from different endocast reconstructions within the same taxon.

The use of Ecological Niche Modelling on an extinct taxon is difficult to perform due to the need of an abundant quantity of data which is rarely obtained in palaeontological studies: this results in very few examples of this kind of works. Since this group existed for such a long and climatically diverse period, the information on the modifications of its climatic niche through time could potentially also be useful to predict extant crocodilian species' responses to the present climatic crisis.

Research activity

Work plan

The research activity will start with an extensive analysis of the literature on neuroanatomy in fossil taxa and on the latest information about *Diplocynodon* and alligatoroids in general.

Firstly, the focus will be on the most recent advancements in the field of endocast reconstruction to strengthen confidence in the application of this technique; the research will also explore the current understanding of the braincase anatomy of Crocodylomorpha and its evolution through time.

The research will then shift to the newest data regarding *Diplocynodon*, with a focus on its phylogenetic position within the crocodylian evolutionary tree, its paleobiology and paleoecology. An analysis of all the papers describing specimens putatively belonging to the taxon will be conducted, crucial for the biogeographical and paleoenvironmental inferences that will be drawn afterward.

The next phase will involve selecting sufficiently complete specimens for a detailed neuroanatomical reconstruction. The specimens will be sourced from the Natural History Museum of London, UK, and from other museums in Europe, mainly in Spain, France and Germany.

The selected specimens, when needed, will be sent to specific research centres to obtain a three-dimensional reconstruction of the skull with computed tomography (CT): this consists of a 3D reproduction of the specimen based on a series of digitally acquired two-dimensional images known as tomograms. Then, the work will focus on the reconstruction of the endocasts with the software Avizo: this will be done by manually highlighting the areas of interest in the 2D slides, to then obtain a 3D endocast of the brain and the other internal cranial structures by interpolating the selected areas. The process of endocast reconstruction and analysis takes approximately between three and four weeks for every specimen.

The reconstructions will be then analysed to draw inferences on the ecological adaptations of the species and compared to one another, to obtain information regarding the variability of the internal structures within the genus. The data will then be used to formulate new phylogenetic characters to be incorporated into an existing crocodylian data matrix, with the aim of reassessing the interrelationships among the nine species of *Diplocynodon* and their relationships with other early alligatoroid species. This process will require the use of the specialized software Mesquite, MrBayes (Bayesian inference method) and TNT (maximum parsimony criterion).

The palaeoecological details obtained from the endocasts will be combined with the available information regarding the paleoenvironment (acquired from the analysis of the sedimentary deposits) to better understand the ecological niche of this taxon and its modifications throughout the 40 million years long existence of the genus. Ecological Niche Modelling will be used to reconstruct the past distribution of the species, starting from multiple climatic variables extracted from paleoclimate models. This will allow to observe the fluctuations of *Diplocynodon*'s geographical distribution through time and to analyse if there is a correlation between its extinction and climatic change. These results will also provide a solid foundation for biogeographical analysis: understanding the paleoecology and distribution of the genus is fundamental to draw conclusions on its dispersal capacity, shedding light on possible transcontinental movements. This phase of the study will require specialized software like R and Maxent.

The material hosted in the Natural History Museum of London will allow to draw some implications also regarding the ontogeny of the group, by studying specimens of different ontogenetical stages.

Milestones

In the first year, the reconstruction of the endocasts will begin and the obtained data will be analysed to achieve the first results; the work will be focused at first on the specimens hosted in the Natural History Museum of London. In the second year other specimens will be studied, then the research will focus on a more complete phylogenetic analysis and the first results will be published. Concurrently, after obtaining all the necessary data, a biogeographical evaluation will be carried out. The third year will be mainly dedicated to a final synthesis, drafting of the final dissertation and publication of the results.

Dissemination of the results

The results of the research will be actively disseminated throughout the three years through scientific publications in specialist sector journals and participating to national and international conferences: for example, the annual conference Progressive Palaeontology (ProgPal) and the ones organized by the Italian Palaeontological Society (SPI).

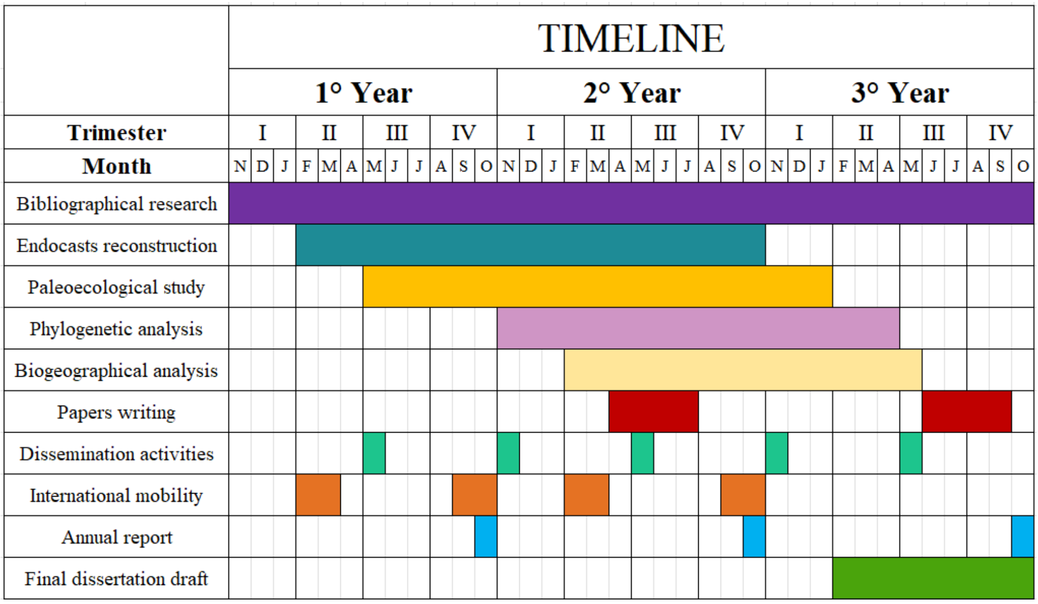
Training activities

During the first year of the PhD, I will attend courses organized by the Earth Science Department of La Sapienza University that will strengthen my knowledge in the field of palaeoenvironment reconstruction and will help manage the challenges of a PhD with professionality. I will also attend many other seminars, webinars and workshops such as those organized by the SPI.

International mobility

This project will be carried out in collaboration with the University College London (UCL) under the supervision of Prof. Philip Mannion. During the three years, part of the international mobility period will be spent in London to study the specimens from Natural History Museum and to conduct the endocast reconstructions. I expect to spend between three and six months in the UK during the first two years of the PhD. To strengthen the knowledge regarding the taxon under investigation, some of the specimens will be personally analysed by visiting the museums that host them in other foreign countries like Spain, France and Germany (for a total of approximately two/three more months).

Time schedule



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