Paleobiology of ancient amphibians: analysis of unique fossilized soft tissues through state-of-the-art synchrotron X-ray imaging

Context

Amphibians are one of the most diverse clades of tetrapods (four limbed vertebrates) with 8431 species known in 2022 (https://amphibiaweb.org) and they are unique in being able to metamorphose. Metamorphosis is defined as a hormonally induced phenotypic remodelling resulting in a condensation of developmental events (Alberch, 1989). In extant amphibians, this shift often results in a transition from aquatic to (semi-)terrestrial lifestyle, as well as feeding, physiological and ontogenetic changes. Metamorphosis requires a lot of energy. It results in a time lapse when the animal cannot feed and becomes an easy prey for predators. Despite this, metamorphosis, even though diverse in intensity among the different clades of extant amphibians, is widely spread and crucial. Is metamorphosis a derived feature of extant amphibians or was it inherited from a common ancestor?

Metamorphosis induces important modifications in the *respiratory apparatus* (with a loss of gills in favour of a pulmonary/cutaneous respiration), in the *muscular system* with a shift towards a more terrestrial/burial lifestyle and in the *digestive system* with a clear shift in prey hunting. These changes in soft tissues and skeleton are the results of deep hormonal stimuli. Soft tissues are therefore crucial for characterizing the metamorphosis process. However, so far only skeletal developmental trajectories could be investigated from the fossil record of amphibians (e.g. Steyer, 2000; Schoch, 2009; Sanchez et al., 2010). Consequently, metamorphosis remains a very debated question in fossil amphibians.

Concerning fossil amphibians, two clades compete for the status of sister-group to the modern amphibians: the temnospondyls (e.g., Anderson et al., 2008) and the lepospondyls (e.g., Marjanović & Laurin, 2019). A temnospondyl origin has been hypothesized within the dissorophoidea, a clade of small amphibians that originated during the late Carboniferous (300 Ma) and survived until the early Triassic (260 Ma) (e.g., Schoch, 2019). A lepospondyl origin has also been suggested within the clade of lysorophia known from the Carboniferous and the Permian (300-250 Ma).

The collections of the MNHN host exceptional specimens of both clades (Heyler, 1994; Werneburg, 2019) preserved in unique conditions in nodules. They come from the Carboniferous *Lagerstätte* of Montceau-les-Mines, France. Thousands of dissorophoid temnospondyls have been fossilized in sideritic nodules, as well as several lepospondyl specimens. They constitute a unique collection of 300-million-year-old tetrapods with body soft tissues preserved. So far 70 temnospondyls from this locality present soft tissues (e.g., gills, muscular and dermic tissues, intestines/stomach contents).

This PhD project aims at analysing these exquisite specimens using state-of-the-art synchrotron X-ray imaging techniques (microtomography and mapping of chemical elements by fluorescence) (e.g., Sanchez et al., 2016). The candidate will have to 1) characterize the changes affecting the organs of various extant amphibians (through the literature and through X-ray synchrotron imaging if necessary); 2) image the fossil internal soft anatomy over growth series to locate whether drastic anatomical and chemical changes occurred in these fossil amphibians; 3) identify biosignatures and how hormones are distributed in the body of extant amphibians over the metamorphosis (through the literature); 4) interpret this distribution in terms of chemical elements that can be retrieved in the fossil record through fluorescence mapping and locate the organs stimulated by metamorphosis hormones.

Material and goals

Approximately 15 dissorophoid temnospondyls and 2 lepospondyls from the MNHN collections have been selected because they preserve a nearly complete set of soft tissues (i.e. dermal tissues, respiratory apparatus, digestive apparatus, muscular tissues) as well as the skeleton. Numeric data will be acquired through synchrotron X-ray imaging. Several specimens have already been scanned: they show a powerful range of information that ensure the feasibility of this PhD project. Comparisons with soft tissues of extant amphibians will be conducted (ex. the dissorophoids will be compared with salamanders because their external and skeletal morphologies are analogous).

3D dissection of Paleozoic amphibians: The analysis of the dissorophoids and lepospondyls from Montceau-les-Mines through X-ray imaging will lead to a better visualisation of the skeleton, notably the skull, which will allow for a better systematic identification of these taxa. The project will permit building a detailed reconstitution of the internal morphology of these early amphibians in 3D, including their breathing, digestive, muscular and dermal system. Each internal organ will be reconstructed in 3D. This model will allow for a better understanding of the paleobiology of these extinct animals.

Paleobiology: The proposed project is to use state-of-the-art X-ray imaging techniques beyond the morphological study of fossils to also exploit the 3D density and 2D elemental contrasts they provide as new sources of information: this will allow to determine the ontogenetic stages of the studied specimens and to identify the associated developmental changes (Fig. 1). In addition, this study will focus on soft tissues that have never been thoroughly analysed to our knowledge and described in Paleozoic tetrapods. Ontogenetic trajectories of these soft tissues (e.g., branchial apparatus versus pulmonary/cutaneous system; muscular architecture; dermal composition) will be highlighted and compared with extant species. This will give interesting new data on the paleobiology and evolution of early amphibians.

Paleophysiology: The next step will be to study the distribution of metamorphosis hormones in the soft remnants of these 300 million-year-old fossils. This original approach will be based on the identification of chemical elements produced during this developmental event, and their mapping in 2D. This will be conducted in collaboration with Dr. Pierre Gueriau who has recently developed and applied fluorescence mapping on fossils.

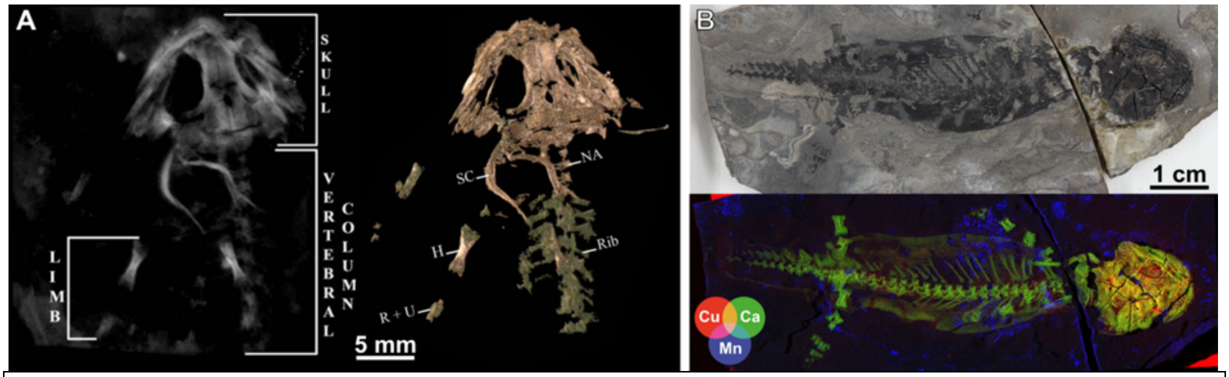

Figure 1. (A) X-ray microtomography can uncover distinct density properties related to different degrees of ossification (calcified cartilage *vs.* bone) in axial and cranial elements of 3D-preserved fossils such as this Carboniferous-Permian branchiosaurid from the Autun Basin, France (Sanchez et al. 2010). (B) The distribution and abundance of elements such as Ca, imaged using X-ray fluorescence, can track degrees of ossification in flat fossils such as this temnospondyl from the Permian of France (Gueriau *et al.* in prep.).

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Feasibility

Access to the material: The material comes from the MNHN collections housed in the Muséum of Natural History of Autun (MHNA). Previous appointments with the Collection Manager of the MNHA have allowed the selection of specimens of great interest for the study. Moreover, the soft tissues preserved on the specimens have been inventoried in 2017 and the recent revision of 2019 also served as a basis for the selection of the material.

Access to synchrotron analyses: X-ray microtomography will be performed at different synchrotron beamlines: at the European Synchrotron Radiation Facility, Grenoble (BM5) and the Synchrotron SOLEIL, Paris Saclay (PSICHE, PUMA, ANATOMIX) depending on the size of the fossils and the needed resolution. X-ray fluorescence will be made at the PUMA beamline (dedicated to ancient materials including paleontology) in collaboration with Dr. Pierre Gueriau. Beamtime will be obtained through peer-review proposal applications and through P. Gueriau's own beamtime. Note that some specimens have already been scanned by P. Gueriau and another collaborator, Dr. Damien Germain, ensuring data is available at the start of the project.

In case of a Covid lockdown: Several specimens from the MNHN/MHNA collections have already been borrowed. The access to the Paris-Saclay synchrotron may be delayed for a maximum of 6 months but we have ensured that the student could start working on the few specimens already scanned. Note that the access to the Grenoble synchrotron has not been delayed during the last year of the pandemic thanks to a sanitary route they have set up.

Setting of the project within both laboratories

This project perfectly fits within the teams of both directors J.-S. Steyer (Team FOSFO for "Shapes and Functions" of the CR2P; Centre de Recherches en Paléontologie – Paris) and of S. Sanchez (program of Evolution and Development, Department of Organismal Biology). The FOSFO team (Paris) deals with paleobiology, anatomical and physiological innovations in vertebrate evolution. The program of Evolution and Development (Uppsala) integrates both paleontological and molecular aspects of vertebrate evolution, with a major interest in 3D virtual histology. The student will be based at the CR2P (Paris) but he/she will make some scans at Paris Saclay and Grenoble, with complementary analyses on a workstation with multiple 3D software licences available in Sweden for histological analyses. J.-S. Steyer, director Nr.1, will bring his expertise on fossil amphibians systematics, phylogeny and paleoecology. S. Sanchez, director Nr.2, will bring her expertise on developmental biology, paleophysiology and paleohistology. P. Gueriau, a close collaborator, will bring his expertise on methodology, taphonomy and paleoenvironements. The student will have a very favourable working context which is unique in the world, in term of both state-of-the-art methodologies (3D platform, reconstruction), scientific material and expertise (paleontological collections of the MNHN, competences and collaborative networks within the CR2P and internationally, European facilities).

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Student profile

We are looking for a student with a Master degree in Paleontology or Evolutionnary Sciences or Biology and with a good knowledge in Paleozoic tetrapods and/or imaging methodologies.