X-ray microtomography of fossil types in natural history collections

Loïc Costeur[a], Thore Friesenhagen[a], and Georg Schulz[b,c]

[a]Natural History Museum Basel. Augustinergasse 2, 4001 Basel, Switzerland
[b]Core Facility Micro- and Nanotomography, Department of Biomedical Engineering, University of Basel, Hegenheimermattweg 167 C, 4123, Allschwil, Switzerland
[c]Biomaterials Science Center, Department of Biomedical Engineering, University of Basel, Hegenheimermattweg 167 C, 4123, Allschwil, Switzerland

ABSTRACT

Imaging natural history collections is becoming an important conservation tool that also serves research purposes. Herbaria are at the forefront of this new area, where automatic conveyor belts can scan thousands of sheets per day. The production of high quality images is used as a tool for inventory, monitoring, communication, data exchange between scientists and new taxonomic identifications. Microtomography of collection items with these aims is much more time-consuming and expensive. While it has been so far limited to rare and important specimens such as types or reference specimens (i.e., historically or scientifically important specimens; see in Ref. [1]), the data generated takes conservation to another level. This is because nit captures not only surface features, but also very fine texture and internal structures are digitally recorded, depicting the object in almost all its complexity and dimensions. Generating this kind of data helps researchers achieve their goals, provides first-hand scientific data, limits further handling of sometimes fragile specimens and, can help reduce the ecological footprint of scientific travel. In this work, we illustrate the power of microtomography in conservation work by imaging fossil type specimens (i.e. remains of extinct organisms used to designate new species) which are witnesses of past life on our planet. They provide information on how today's biodiversity has evolved and are a good indicator for the past climates. In addition, they often fascinate a wide audience and are therefore good ambassadors for communicating scientific findings. Recording them with the help of X-ray microtomography should therefore be a general goal, which we illustrate here with examples.

Keywords: holotypes, paleontology, imaging, X-ray microtomography, natural history collection

1. INTRODUCTION

Describing and ascribing a new fossil specimen to an animal, plant or other organism's taxon requires comparisons with existing resources, and above all with type material. Types are published permanent reference specimens linked to the designation of a new species. Several categories of types from name-bearing holotypes to type series, including among others the accompanying paratypes, exist and help in the description of a taxon, often encompassing its morphological variability. Fossil type specimens are the primary source of past biodiversity data and are the most important specimens in paleontology [2]); they are primarily accessible through the scientific literature. Most type specimens are hosted in public museum or university collections. However, few institutions provide a full list of their types, and even less so include images. A quick survey of the websites of the largest, and arguably two of the best digitized fossil collections in the world, that of the National Museum of Natural History of the United States (NMNH, `https://collections.nmnh.si.edu/search/paleo/` and that of the Natural History Museum in London (NHM, `https://data.nhm.ac.uk`) was made on June 14th, 2024. It shows that about 18.5% of all fossil types are available as a record on the public database of the NMNH. About 40% of

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Further author information: (Send correspondence to L.C.)
L.C.: E-mail: loic.costeur@bs.ch, Telephone: +41 61 266 55 87
T.C.: E-mail: thore.friesenhagen@bs.ch, Telephone: +41 61 266 55 93
G.S.: E-mail: georg.schulz@unibas.ch, Telephone: +41 61 207 5437

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all recorded 5,000 fossil holotypes have images associated, often only one, but the figure drops to less than 5% when other type categories are searched for (e.g., about 4% for paratypes). Overall, types are better represented than other specimens online since only about 5% of the estimated 14 million occurrences of fossils at the NMNH are indeed available in the public database, and that is not taking images only into account. The NHM has over 7,000 fossil holotypes in its public database, the majority of which having a picture attached, but often only of the original entry in the catalogue, while a little less than half of the records figure the specimen itself, which is still a big milestone. The situation at the NMNH or at the NHM portrays maybe the state-of-the-art in terms of sharing images of types. Both being national museums with large resources compared to others, they represent exceptions to the rule, where most museums have very little of their type specimens available publicly and much less so with images.

Considering the great scientific importance of types and the correlated reluctance of curators to lend them to limit handling, damage or other risks, a massive effort should be undertaken to make them available as images to the broadest public. In addition, when it comes to high quality images that would give access to more than just a surface view of fossil types, i.e., a mere standard picture showing the state of the specimen, the amount of data publicly available becomes insignificant but is most wanted (see [3]). X-ray microtomographic data have long proven incredibly relevant to the scientific study of fossil specimens because they provide a non-invasive approach to often critical internal structures. Producing computed-tomography scans of specimens is a time consuming and expensive task but more and more devices are available in universities or collection-holding institutions, making the process easier for curators.

In 2021, after a national survey showing that only 17% of all 60 million specimens (extant and extinct) in Swiss collection-holding institutions were inventoried in digital databases, Switzerland has invested in a national project with the aim of making natural history specimens more available online. The Swiss Natural History Collections Network, or SwissColNet project run by the Swiss Academy of Natural Sciences and funded by the Swiss Confederation, has financed over 65 projects nationwide in the past four years. We present here one aspect of project SCN125-BS aiming at imaging fossil reference specimens (not only types) in a network of 10 partner institutions. While the project aimed at imaging specimens with different imaging techniques, from classical photography to surface scanning via X-ray microtomography, the aspect presented here revolves around the use of the latter and shows 3 examples of types, which were scanned in the course of the project. Challenges and issues associated to this technique for mass-digitization projects like this one will be discussed here.

2. MATERIALS AND METHODS

The project involves 10 Swiss collection-holding institutions. Individual curators were asked to choose specimens appropriate for X-ray microtomography (hereafter also referred to as CT-scan). Fossil type specimens were selected for CT scans if the objects to be examined were complete. When internal structures of large scientific interest were supposed to be present and preserved. When internal structures of large scientific interest were supposed to be present and preserved, X-ray microtomography was preferred over more classical imaging techniques. Specimens like fossil vertebrate skulls or jaws, or even bones for which the internal structure informs the understanding of specific paleoecological adaptations, paleobiological features or phylogenetic information were systematically chosen for CT-scanning. “Invertebrates”, or representatives of groups without a vertebral column (e.g., molluscs, decapods, arthropods, echinoderms, etc) were chosen again on the same basis, in the hope that preserved internal structures would bring an added value to the specimen.

The project spans the period November 2022 to September 2024. To date (July 2024), CT-scans were essentially carried out for specimens from the Natural History Museum Basel, The Palaeontological Institute and Museum of Zurich, The Nature Museum of Solothurn and the Natureum of Lausanne.

Most of the X-ray microtomography measurements were performed at the Core Facility Micro- and Nanotomography at the Department of Biomedical Engineering of the University of Basel using a nanotom® (phoenix|x-ray, GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) which is equipped with a $180\,kV$ / $15\,W$ nanofocus X-ray source. Tomographic scans with 1000 - 2000 equiangular projections over 360° were performed. The specimens were mounted on the precision rotation stage. For the acquisition acceleration voltages between 110 and 170 kV and beam currents between 30 and 120 μA were used, where the mean photon energy was increased by adding 0.25 mm - 0.50 mm Cu filters. The pixel sizes were between 8 and 25 μm. The scanning time for each specimen was about two hours. The individual scanning parameters can be found in Table 1. A second microtomography system, an xradia 610 Versa (Zeiss, Oberkochen, Germany) provided scans for smaller and less dense specimens.

Table 1. Scanning parameters.

Specimen	**voltage** $[kV]$	**current** $[\mu A]$	**filter** $[mm]$	**pixel size** $[\mu m]$	**exp. time** $[s]$	**proj.**
NMB Sau1692	110	120	Cu 0.25	8	8	1200
MGL21908	110	120	Cu 0.25	11	3	1000
NMB F1662	170	30	Cu 0.50	25	6	2000

3. RESULTS

From November 2022 to July 2024 over 400 specimens were CT-scanned during the course of this project. A total of about 200 were actual types, both primary and secondary (holotypes, syntypes, lectotypes, paratypes

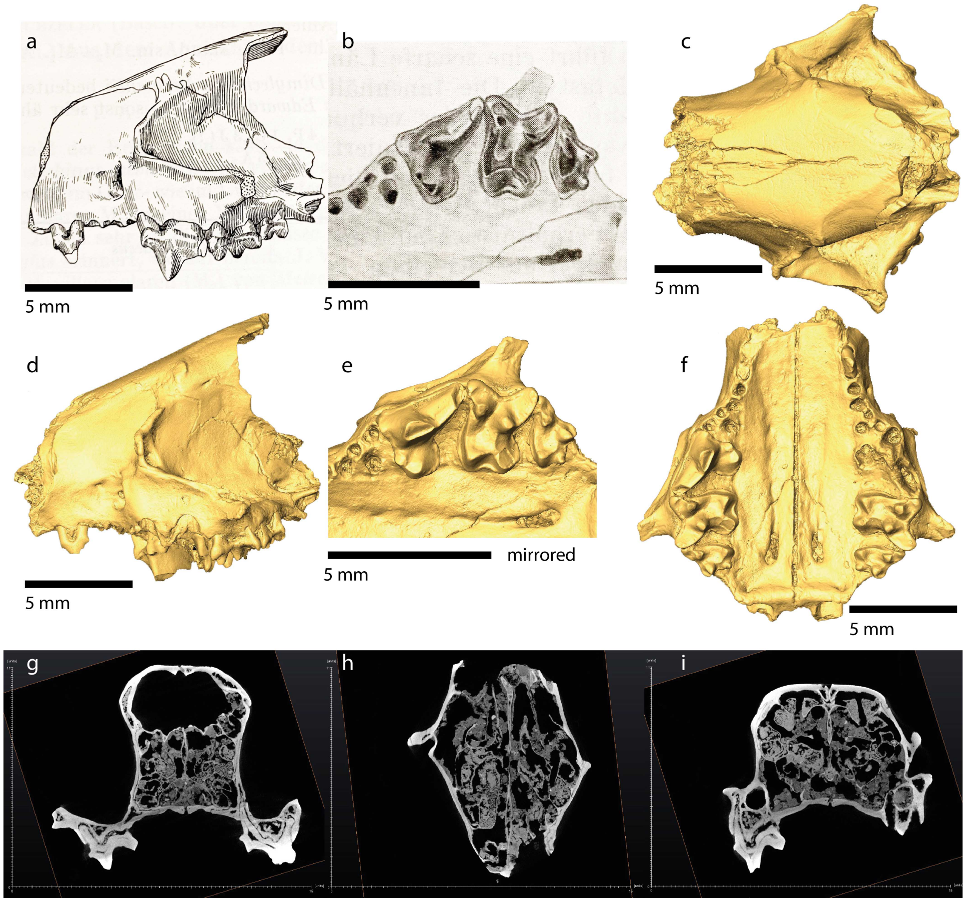

Figure 1. NMB Sau.1692, holotype skull of *Dimylechinus bernoulli*, Hürzeler, 1944. a-b, original figure in Hürzeler, 1944. c-f, surface model reconstructed from the CT-scanned data of the skull, c. dorsal view; d, lateral view; e, ventral view with mirrored close up of right tooth row; f, ventral view. g-i, coronal (g, i) and horizontal (h) slices through the skull where the turbinals are visible inside the nose region.

and paralectotypes). For types only, an average of about 11 specimens were CT-scanned per month representing about 20 hours of beam time, and an equivalent of 3,000.- CHF per month with the current hourly price of the scanning lab. Digital models were produced using the visualisation and segmentation editors of AVIZO 9.0 at the Natural History Museum Basel. Not all scanned specimens were 3D-rendered as surface models yet due to the large processing time required for several hundred specimens. We present here some example as flagships for the project.

3.1 NMB Sau.1692 *Dimylechinus bernoulli*, Hürzeler 1944

The holotype skull of the fossil hedgehog *Dimylechinus bernoulli* NMB Sau.1692 was published by Johannes Hürzeler in 1944 [4]. The specimen is a proximal part of a skull that serves as type for the genus *Dimylechinus* as well as for the species *D. bernoulli*. It comes from the locality Saulcet in central France (Allier basin) and is dated to about 23 million years ago. The original description figures good interpretative drawings of the specimen (Fig. 1a, b) and of its accompanying paratypes (upper and lower jaws) or associated material. It classically describes the external anatomy of the skull as well the detailed morphology of its teeth, no description of internal structures was then possible. No picture is given. CT-scan data of this specimen show more details than those given on the original drawings (Fig. 1c-f) and also revealed the presence of the fully preserved anterior part of the olfactory system of the animal (Fig. 1g-i)), known as maxilloturbinals. The maxilla- and nasoturbinals of small mammals have been shown to be related to heat and moisture conservation and/or to be correlated to specific ecological adaptations [5,6]. Access to the turbinals in all their complex dimensions (size, surface, 3D

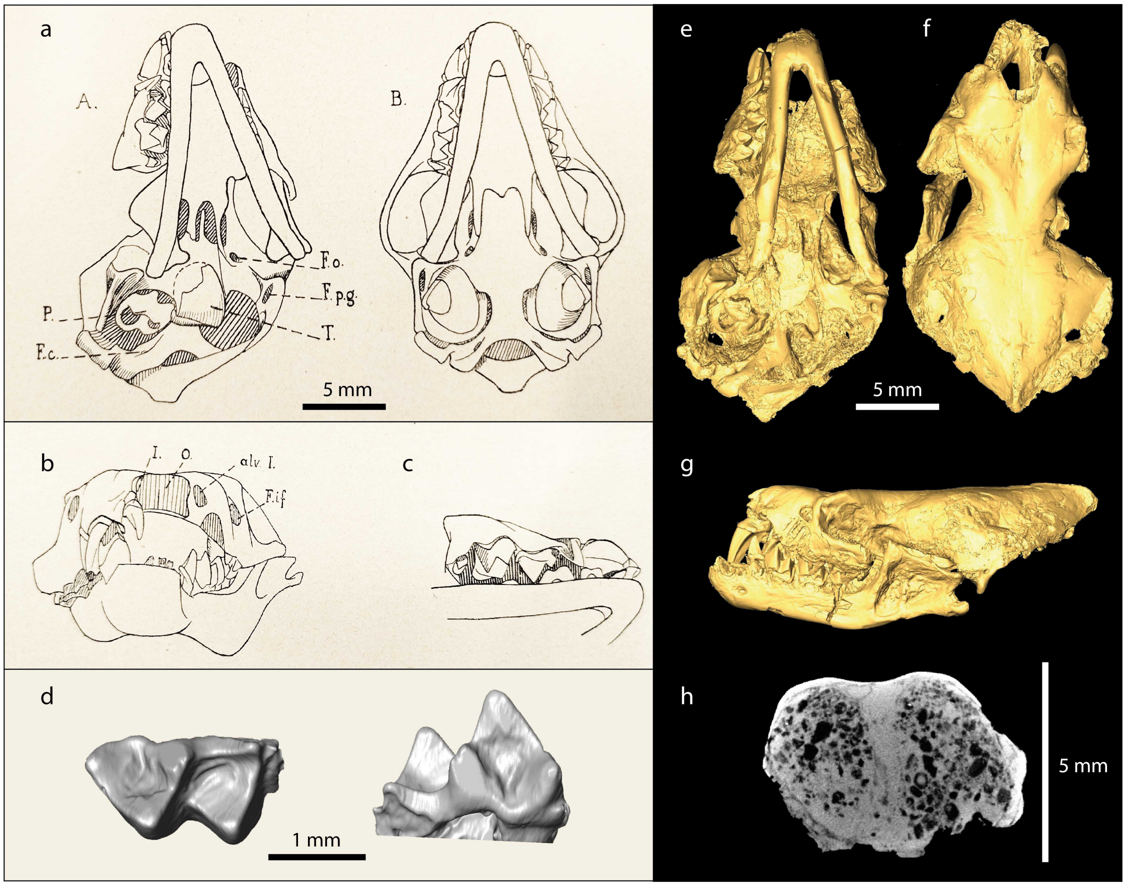

Figure 2. NMGL 21908, holotype skull of *Samonycteris majori*, Revillod, 1922. a, original figure 51A, B in Revillod, 1922. b, original figure 53 in Revillod, 1922. c, original figure 54 in Revillod, 1922. d, surface model of the left lower first molar, occlusal (left) and lingual (right) views reconstructed from the CT-scanned data of the skull. e. skull in ventral view. f, skull in doral view. g, skull in lateral view. h, coronal slice through the endocast of the olfactory bulbs.

complexity) both in living and extinct species is only possible through X-ray microtomography. The presence of the turbinals in this holotype brings this specimen to another level of interest, not only providing a 3D Model that can be shared and ensures long term conservation through less handling, but also providing new critical data for a whole mammalian clade for which still little is known [6].

3.2 MGL21908 *Samonycteris majori Revillod*, 1922

Samonycteris is a rather poorly known genus of fossil bats included in the family Verspertilionidae [7]). It was described over a hundred years ago based on a skull articulated to its mandible from the famous late Miocene Greek locality of Mytilini, Samos, dated to about 8 million years ago [8]. The specimen was classified in a new taxon by Revillod (1922) [9] who published numerous informative drawings, pictures, and a detailed description of the skull. Fig. 54 in [9] (here Fig. 2c) shows the upper molars of the skull that are largely concealed by the mandible so that little can actually be seen. The same is true for a later photographic illustration of the skull [10]. No other material was found or described since then although isolated tooth material possible from Suchomasty 3 in the Czech Republic is mentioned [11]. The genus and species are still valid and the holotype is thus is only known specimen available for the taxon.

Computed tomography data generated from this specimen give a full access to its teeth morphology, which were until now only partly known (Fig. 2d-e). Likewise the brain endocast is preserved including the olfactory bulbs. 3D data of fossil bats can resolve many question revolving around their biological adaptations and the evolution of those, including echolocation, flight control (balance), or olfaction [12] or [13].

3.3 NMB F1662 *Hepatus lineatinus* Todd & Collins, 2005

The new species *Hepatus lineatinus* was described using specimen NMB F1662 from the Natural History Museum Basel as a holotype [14]. It comes from the Escudo de Veraguas geological Formation in the Late Pliocene of

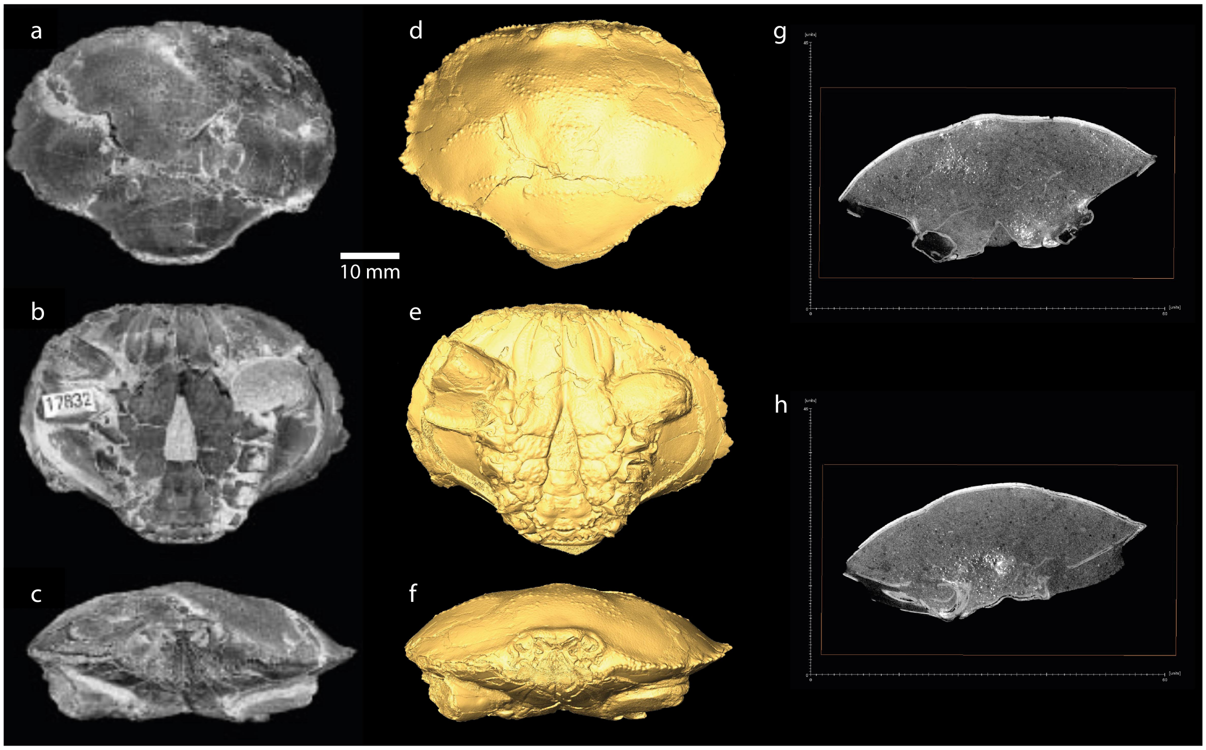

Figure 3. NMB F1662, holotype of the decapod *Hepatus lineatinus* Collins & Todd, 2005. a-c, original pictures in Todd & Collins, 2005. s-f, same views of the surface model reconstructed from the CT-scanned data of the specimen. g-h., coronal slices though the holotype showing visible internal structures.

Panama (north-central coast of Escudo de Veraguas, Bocas del Toro Province) and was collected in 1988 by members of the Panama Paleontology Project (hereafter PPP). The original publication is on open access and a figure gives 3 views of the specimen with a poor resolution when downloaded online ([14], p. Plate 2, Fig. 8a-c; see Fig. 3a-c and Fig. 3d-f for their new digital counterparts). The decapod crustacean fossil record of the Caribbean area was poorly known before the start of the PPP despite large amount of fossils (see [15] for a review). Collecting and taxonomic efforts have led to a better understanding of their past biodiversity in relation to the tectonically active zone of the Panama Isthmus which eventually closed by the end of the Pliocene. This event lead to a disconnection of the water flow between the Pacific Ocean and Caribbean Sea and to local to regional extinctions. Knowledge of this important event for the biodiversity of this geographic area is based on the description of species discovered in the sediments dated to the Pliocene and Pleistocene such as this specimen.

Preservation of decapod fossils is often limited to claws and a good part of the systematics of fossil decapods relies on claws [15]. Fossil of "superior preservation" [15] are rarer. They obviously provide more information and preserve internal structures that are revealed by X-ray microtomography (Fig. 3g-h). Producing 3D data of this holotype allows to share them easily, and increase the amount of morphological and taxonomic information available.

4. DISCUSSION AND CONCLUSIONS

Digitizing natural history collection items is becoming an increasingly important task for curators. Natural history specimens represent a fundamental biodiversity resource with probably over 2 billion objects in the collection-holding institutions of the world. Indeed about 1.15 billion objects were recorded in the 73 largest collection-holding institutions according to an international survey [16]); likewise according to the overarching digitization programs iDigBio in North America and DiSSCO in Europe (see websites below), over 1 billion specimens are curated in the North American collections and over 1.5 billion in European ones.

Several initiatives are being developed to mobilize this biodiversity information at different levels, including that of digitization, i.e., producing image/media data of specimens in collection-holding institutions. iDigBio is one of those, where most of the 1600 collections across the United States of America started digitizing their specimens. To date over 56 million specimens have been imaged and are shared within the scientific community for scientific purposes (see `https://www.idigbio.org`, consulted on July 5th, 2024). By being made available on an open access website, they can also be used for educational purposes. The European DiSSCO platform (`https://www.dissco.eu`) seeks to organize the European natural history collections in the same way in order to reach the same goal of unifying, sharing and homogenizing this vast resource that is currently only barely available. The Global Biodiversity Information Facility (GBIF, `https://www.gbif.org/fr/`) is a database gathering inventory and digitization data of millions of biological and paleontological specimens making them also available to the community.

The Swiss Natural History Collections Network financed by the Swiss Academy of Sciences is also one such project (`https://swisscollnet.scnat.ch/en`) on a national level. It aims at revising, producing and sharing data from natural history collections, and eventually integrating them to the above-mentioned international initiatives.

CT-scanning specimens using X-ray microtomography is a very special case of digitization for collection-holding institutions. Contrary to classical imaging that gives an idea of specimen state and, in the case of high quality pictures also gives access to scientifically relevant morphological characters, CT-scanning is more scientifically oriented in the sense that it produces a full 3D image of a specimen including its internal, otherwise not visible, structures (e.g. [17]). This kind of data has become one of the most used resource for various disciplines such as evolutionary biology, paleontology or organismal biology. It requires a long imaging time, from a few tens of minutes to several hours and it necessitates a sometimes long post-processing to generate a 3D model or even more so when segmentation of certain interesting parts is involved. In that sense, it does not really match mass digitization projects such as those known for herbaria sheets using conveyor belts. In the latter projects, thousands of sheets can be photographed at high resolution per day (e.g., [18], [19]). Some museums have applied this technique to imaging entomological collections like the Museum für Naturkunde in

Berlin or the Digitarium in Finland. Here several million specimens will be imaged using conveyor belts for small prices per object (from 0.5 to 1.5 dollars per object; [20]. It is an immense effort requiring several people at a time who are preparing the objects, labelling the pictures and putting back the specimens in their cabinets. This represents a large financial investment, not even mentioning the saving space required to store all this digital data.

Ongoing large-scale CT-scanning projects like the openVertebrate (oVert) Thematic Collections Network ([21], https://www.floridamuseum.ufl.edu/overt/) financed by the National Science Foundation have yielded 3D data for over 13'000 vertebrate specimens in public collections. This project benefited to scientists with more than 200 publications involving about 700 authors and several thousand specimens recorded since 2017 [21], They also show how the society started interacting with the datasets: artists or education institutions used the resources for their very own purposes (from artistic projects to science exhibitions for the general public).

This project where types of fossil taxa are being CT-scanned represents a great effort of the Swiss community of collection-holding institutions to make extremely important data available. The three specimens shown here are just examples of what is achieved and why it is important. Over the course of the last year and a half, about 20% of the produced data for types has already been requested for study by scientists in Switzerland and mostly abroad, while no online portal for direct download is available yet. The major drawbacks of such a project are the time consuming and costly procedure of X-ray microtomography, including the costs of storing this large amount of data on servers, especially for small institutions with limited financial means. Other more technical drawbacks should be taken into account before starting large-scale CT-scanning. Indeed, X-ray microtomography should be used with caution on relatively recent material that can be dated using dating techniques involving heating the specimens. The results can be influenced or made unusable by X-ray beams. It is thus not recommended for objects younger than a few hundred thousand years that fall in the range of dating possibilities using these techniques. This time frame also encompasses the time frame in which our own species evolved, so potentially relevant specimens for anthropologists and paleoanthropologists in dire need of absolute dating. Synchrotron radiation-based microCT should also be used with caution on amber. It can cause brownish discolorations, which may become irreversible depending on the type of amber and the used energy level during the scan [22].

The benefits are clear with strong requirements in the scientific community to access such data on an open access basis. In addition providing CT-scans reduces travel expenses and their accompanying environmental footprint, increases the relevance of keeping digital copies of irreplaceable specimens, and limits future handling and thus possible damage. Mass digitization projects involving X-ray microtomography are still rare and mostly run on a collaborative basis (e.g. the oVert project) to share resources and maximize the outcomes. Accessing public funding from large national financing bodies is a strong limiting factor to digitization [23] and thus somehow represents a prerequisite to such projects. Political stakeholders require more and more digitization to the institutes under their authorities, they should thus now mobilize perennial financial means in order for the museum's staff to create the conditions of the future Natural History Collections sensu [24].

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