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Exploring How Micro-Computed Tomography Imaging Technology Impacts the Preservation of Paleontological Heritage

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Abstract

Museums play an essential role in preserving both cultural and natural heritage, safeguarding samples that offer invaluable insights into our history and scientific understanding. The integration of micro-computed tomography (micro-CT) has significantly advanced the study, restoration, and conservation of these priceless objects. This work explores the application of micro-CT across three critical areas of museum practice: sample virtualization, restoration assessment, and the analysis of fossil specimens. Specifically, micro-CT scanning was applied to fossils stored in the G.A.M.P.S. collection (Scandicci, Italy), enabling the creation of highly detailed non-invasive 3D models for digital archiving and virtual exhibitions. At the Opificio delle Pietre Dure in Florence, micro-CT was employed to evaluate fossil bone restoration treatments, focusing on the internal impact of menthol as a consolidant and its effects on the structural integrity of the material. Furthermore, micro-CT was utilized to investigate a sealed bee preserved in its cocoon within a paleosol in Costa Vicentina (Portugal), providing unprecedented insights into its internal anatomy and state of preservation, all while maintaining the integrity of the specimen. The results of this study underscore the versatility of micro-CT as a powerful non-destructive tool for advancing the fields of conservation, restoration, and scientific analysis of cultural and natural heritage. By integrating high-resolution imaging with both virtual and hands-on conservation strategies, micro-CT empowers museums to enhance research capabilities, improve preservation methodologies, and foster greater public engagement with their collections.

Keywords: micro-CT; museums; collection; restoration; microstructure; fossils; virtualization; valorization

1. Introduction

Micro-computed tomography or micro-CT (μ CT) is a high-resolution X-ray imaging technique that enables the non-destructive investigation of an object's internal structure [1,2]. Developed in the early 1980s [3,4], micro-CT operates by measuring X-ray transmission through an object from multiple angles and reconstructing a series of high-resolution transaxial slices using tomographic algorithms [2,4].

The result is a three-dimensional volume reconstructed from numerous two-dimensional images (known as slices), offering spatial resolution at the sub-millimeter scale, with achievable precision down to $0.2\ \mu\text{m}$ [1,5].

Initially developed for biomedical applications [6,7], micro-CT subsequently expanded to several different fields, including engineering [8], materials science [9], and cultural heritage [10–13] research. While its clinical use has been limited due to long scan times and challenges in stabilizing living subjects [14], its ability to analyze materials and fossil specimens [15,16] with high precision while preserving their integrity has proven invaluable. This innovative technique allows for the non-invasive and non-destructive internal analysis of samples, providing information about the hidden architecture and state of preservation. Its use in conservation fields has demonstrated the importance of detecting micro-fractures and hidden weaknesses in fragile objects, enabling restorers to implement preventive conservation strategies before any physical deterioration develops [17–21]. Additionally, in the case of the presence of sediments or encrustations on the object, it is still possible to use micro-CT to observe the internal structure, providing critical information without the need for physical intervention [5,22–26]. Finally, another benefit of this technology is its ability to create virtual bi- or three-dimensional models, greatly aiding in the digital preservation and sharing of fossil and cultural heritage specimens. By integrating with other existing digital techniques, micro-CT enables the production of high-quality datasets that are frequently uploaded to online repositories, increasing their accessibility for research, education, and conservation purposes. These digital representations can assist museums in conducting more detailed analyses of artifacts and serve as important educational resources for institutions [27–30].

The use of micro-CT technology in cultural heritage has introduced innovative methods for the non-invasive evaluation and preservation of objects. This research explores the benefits of micro-CT across three main aspects: sample analysis, restoration evaluation, and object virtualization. Each of these areas leverages micro-CT's high-resolution three-dimensional imaging capabilities to tackle particular challenges in the conservation and examination of historical materials. By focusing on these three aspects, the study highlights the versatility of micro-CT imaging as a means to enhance scientific research related to cultural assets. The ongoing investigation examines the various applications of micro-CT, including object virtualization, restoration work, and thorough fossil analysis in paleontological heritage conservation.

1.1. Micro-CT Imaging for Ichnology

Modern technology is revolutionizing how museums study and preserve fossils, revealing details once invisible to the naked eye. Among these advancements, micro-CT enables researchers to explore the internal structures of fossils non-destructively, offering new insights into ancient life. This is particularly valuable in ichnology, the study of fossilized traces left by organisms—such as Precambrian traces, Miocene echinoid burrows,

and Pleistocene elephant tracks—which serve as crucial records of past behaviors and ecosystems [31–33].

The application of micro-CT to ichnology presents both challenges and opportunities, enhancing our ability to analyze trace fossils and their broader implications. These fossils provide direct evidence of extinct organisms' behavior and the environmental conditions in which they were formed. By offering high-resolution three-dimensional imaging, CT scanning overcomes many complexities inherent in ichnological studies, allowing for non-destructive analysis of trace fossils and their associated body fossils—such as tracemakers preserved within burrows or undigested inclusions within coprolites. Although the potential of CT scanning for ichnological research was recognized as early as the 1990s by Fu and colleagues [34], its widespread application has only gained momentum in recent years [35]. Marengo and Bottjer [36] provided one of the first reviews on its use in studying biogenic structures in unconsolidated sediments, demonstrating its value in calculating burrow volumes and generating realistic three-dimensional reconstructions. Despite its ability to reveal both internal and external morphologies of trace fossils with unprecedented detail, its adoption remained limited for decades. This delay is particularly surprising given that traditional methods, such as sectioning rock samples, typically alter or damage specimens, whereas CT scanning offers a non-destructive alternative.

Each category of ichnofossils—bioturbation, bioerosion, biodeposition, and biostratification—poses specific challenges for micro-CT imaging due to differences in preservation, composition, and taphonomic pathways. Bioturbation structures, which result from organisms disturbing sediment layers, disrupt pre-existing stratification and fabric [31]. Examples of bioturbation structures include burrows attributed to trilobite activities and vertically oriented tunnels produced by polychaete annelids. An important challenge in imaging these structures lies in the minimal density contrast between disturbed and undisturbed sediment. Many organisms, such as echinoids, use a backfilling technique, moving sediment from front to rear as they burrow [37]. Since these backfilled tunnels are composed of material nearly identical to their surroundings, they can be difficult to detect by means of CT scanning [33].

In contrast, bioerosion traces, which are mechanically or biochemically excavated into rigid substrates by organisms, often exhibit a clearer density contrast. These perforated structures, such as predation drillholes created by gastropods and borings created by bivalves and sponges, are more readily imaged through CT analysis [17,38]. Biodeposition structures, on the other hand, reflect the accumulation or concentration of sediment through biological activity [31]. Among biodeposition structures, bromalites, fossilized digestive products, are particularly significant for micro-CT studies as they can encapsulate fossilized remnants of undigested material. Coprolites, representing fossilized fecal material, are a characteristic example of bromalites. Their role as microenvironments for fossilization provides valuable insights into the diets and behaviors of ancient organisms.

Biostratification structures, formed by biological activity imparting stratification patterns onto sediment, present yet another area where micro-CT scanning proves valuable [31]. Stromatolites and microbially induced sedimentary structures (MISSs) [39] often consist of alternating layers of different materials, which create distinct density contrasts. This layered architecture makes them particularly well-suited for micro-CT imaging, allowing researchers to extract detailed structural information about ancient microbial ecosystems [40].

In this study, we explore the application of micro-CT to analyze a fossilized bee preserved within its cocoon. The specimen was recovered from sedimentary deposits along the coastal Atlantic cliffs of Costa Vicentina, southwestern Portugal, and has been previously described in detail by Neto de Carvalho and colleagues [41]. The cocoons correspond to the ichnofossil *Palmiraichnus castellanosi* [41]. This non-destructive imaging technique allowed

for high-resolution visualization of both external and internal morphological features, providing new insights into the preservation of sealed bee cells.

1.2. Micro-CT for Restoring Samples

The preservation of cultural heritage requires rigorous conservation strategies, where advanced imaging techniques play an increasingly significant role. Micro-CT has become an essential tool for restorers and researchers, enabling non-destructive analysis of restored samples. Assessing restoration efficacy requires something more than a surface examination; micro-CT provides detailed insights into internal structures, detecting fractures, voids, and inconsistencies introduced during conservation treatments. This technology has proven particularly useful in numismatics, where ancient coins often suffer from corrosion and encrustation that obscure critical details [23]. By revealing lithic inclusions, voids in the clay matrix, and variations in wall thickness, micro-CT provides key insights into ancient production methods. Beyond ceramics and metalwork, it has been employed in paleontological conservation, playing a crucial role in assessing the safety and effectiveness of novel consolidants, such as menthol, in fossil preservation.

At the Opificio delle Pietre Dure (O.P.D.) in Florence, Italy, micro-CT was employed to evaluate menthol as a temporary consolidant, as described by Zhang et al. (2022) [42], on fossilized remains provided by the Department of Earth Sciences, University of Florence. Menthol, a naturally derived organic compound obtained from *Mentha piperita* L., is a whitish crystalline solid that has widespread applications in pharmaceuticals, cosmetics, and food science. Only recently has it been introduced into restoration practices, where its ability to sublime at room temperature without leaving residues makes it particularly effective for temporary applications [43]. With a low melting temperature of 41–44 °C, menthol can be applied in liquid form, penetrating the porosity of an object and forming a water-repellent crystalline film. This property allows conservators to perform delicate operations while preserving the artifact's structural integrity. The speed and reversibility of its application make it especially useful in emergency situations, such as in situ excavations, where immediate stabilization is required to prevent damage [44].

Micro-CT plays a crucial role in evaluating conservation materials, providing a means to study how different substances interact with an artifact's internal structure. This is essential for porous or fragile materials, such as archaeological textiles, leather, wood, and fossilized bone. By offering high-resolution three-dimensional imaging, micro-CT enables researchers to track structural changes over time, verifying the long-term stability of treated samples. In this study, micro-CT was employed to investigate menthol's interactions with various materials and assess any potential impact on internal bone structures, reinforcing its value as a research tool in the field of restoration science.

1.3. Micro-CT for Digitalizing Samples

Beyond internal structural analysis, micro-CT plays a critical role in museum settings by enabling the generation of high-resolution digital models, facilitating both external and internal visualization without compromising the integrity of specimens. This capability is essential for conservation, research, and public engagement as it allows for detailed archiving, educational applications, and remote access to cultural heritage and online dissemination. The integration of micro-CT and 3D technologies is increasingly shaping museum practices, particularly through the creation of digital models that can be used for 3D printing. This approach minimizes the handling of fragile artifacts, reducing the risk of deterioration while enhancing accessibility for educational and research purposes [27,45].

A notable example of micro-CT's application in museum collections is the permanent geo-paleontological exhibit of "Gruppo Avis Mineralogia e Paleontologia Scandicci" (G.A.M.P.S.) in Scandicci (Florence, Italy), where micro-CT scanning is employed for the digital archiving of fragile fossils. The resulting 3D reconstructions may contribute significantly to scientific research and public dissemination through interactive digital models. To test the efficacy of this technology, micro-CT scanning was applied to various specimens from the museum's collection, including shark teeth, bivalves, gastropods, and the ear bones of a dolphin. These digitized fossils, which belong to an extensive collection of Pliocene-aged marine vertebrate and invertebrate remains from Tuscany (central Italy), provide valuable data for scientific study while facilitating knowledge sharing within the research community.

2. Materials and Methods

2.1. Micro-CT Devices

In this study, the primary tool employed for the non-invasive analysis of specimens was the Cheetah EVO X-ray inspection system manufactured by Comet Yxlon GmbH (Hamburg, Germany) [46]. This micro-CT system is equipped with a Multifocus X-ray tube (FeinFocus FXT-160.51, Comet Yxlon GmbH, Hamburg, Germany) with a tungsten target and a 1004 px × 1004 px flat-panel X-ray detector featuring a pixel pitch of 127 µm. The system operates with a voltage range of 25–160 kV and a current of up to 1 mA, offering a maximum spatial resolution of approximately 0.9 µm. Tomographic scans were performed with 1420 projections at a 0.25° angular step, covering 360° for a complete scan. The projection images were subsequently processed and reconstructed using VG Studio Max® 3.5 software, enabling the extraction of 2D slices and 3D visualizations [47].

The micro-CT system served as the main imaging tool throughout the study as it was particularly valuable for examining smaller, more delicate specimens, such as sealed bee cells, where high-resolution imaging of internal structures was required. These samples were analyzed using micro-CT at the ICTP ElettramicroCT facility [41], providing detailed 3D reconstructions that allowed for the visualization of intricate internal features without damaging the specimens.

2.2. Investigated Samples

2.2.1. Sealed Bee Cells

The coastal Atlantic cliffs between São Torpes Beach, south of Cape Sines, and Cape São Vicente (Costa Vicentina, Portugal) have been systematically surveyed for Quaternary palaeosols between 2017 and 2020. Notably, Praia das Furnas Sul and Carreira Brava have yielded subfossil cells produced by eucerid bees. While both locations revealed these structures, it was only at Carreira Brava that bees were commonly found preserved inside the sealed brood cells.

This exceptional preservation allowed for detailed study of the bee biology and the conditions that led to their entombment [41]. The collected specimens from these sites are preserved in the collections of DISTAV at the University of Genova, designated with the prefix DISTAVCB, and the Museum of the Centro Português de Geoistória e Pré-História, marked with the prefix CPGP.20.22. A sealed bee cell underwent x-ray micro-computed tomography at the ICTP ElettramicroCT facility, enabling a non-destructive three-dimensional visualization of its internal structure (Figure 1).

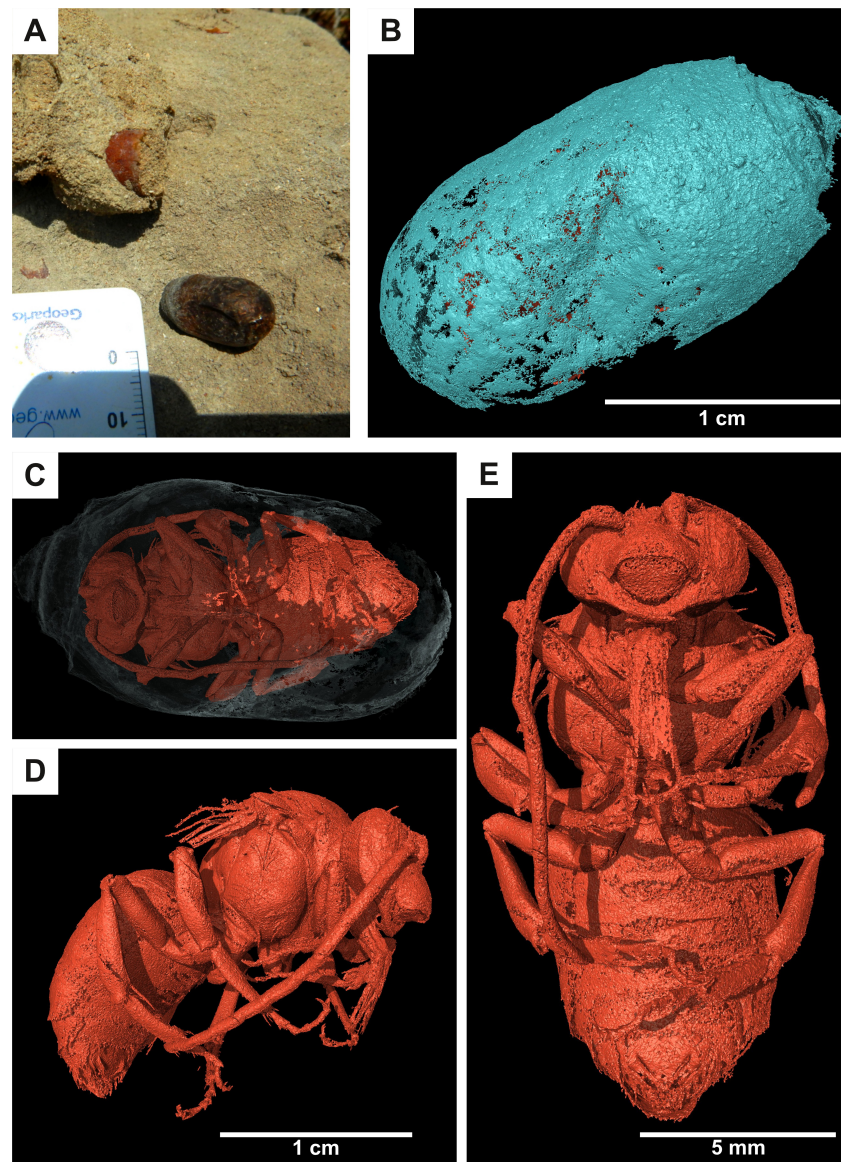


Figure 1. Holocene bee cells from Carreira Brava paleosol in Portugal, featuring field photography and micro-CT analysis. (A) Subfossil bee cocoon captured in situ after excavation, with a scale in millimeters. (B–D) Micro-CT scans of a sealed brood cell (specimen DISTAVCB.4), revealing morphological features: smooth outer wall of the bee cell (B); adult bee inside the cell (C); lateral view of the eucerid bee highlighting the prominent clypeus (D); ventral view of the adult bee within the brood cell (E). GeologyOracle [48] has been used for AI-powered language revision of the text related to bee cocoons.

2.2.2. O.P.D. Institute of Conservation and Restoration

Micro-CT analysis was performed in the framework of an experimental thesis at the O.P.D. Institute (Scuola di Alta Formazione e Studio S.A.F.S.).

The consolidation treatment with menthol was applied on a skull of *Canis mosbachensis* (field-labeled as DE11-1) (Figure 2A) from the Early Pleistocene site of Pirro Nord (Apricena, Foggia) [49,50], whose dorsal surface was found attached to a rock block. The parietal bones of the skull are very fragile; as a consequence, the application of menthol was necessary as it can create a sort of armor to make them more resistant to detachment and micro-excavation actions.

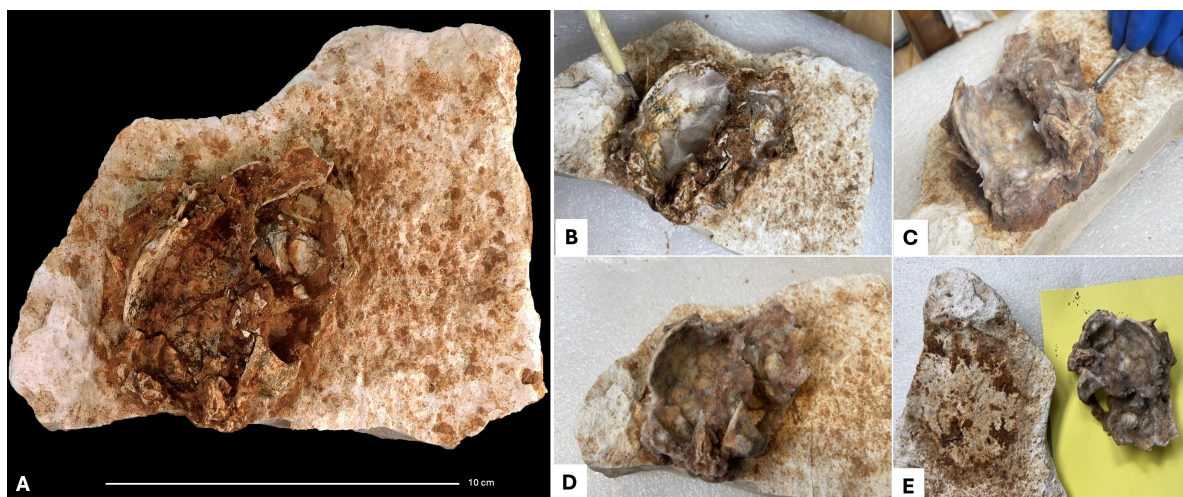


Figure 2. (A) The Early Pleistocene partial skull of *Canis mosbachensis* from Pirro Nord, its dorsal surface still contacting its original substrate. (B) Application of fiberglass fabric between the surface of the specimen and the first layer of menthol. (C) Cerebral surface of the skull after the layered application of menthol. (D,E) The partial skull freed from sediment.

The main problem with menthol is its tendency to form needle-like crystals, which can damage the underlying substrate on which menthol was applied by exerting mechanical pressure. Therefore, it was necessary to test the material on a sample before application. The choice of sample was a Pleistocene mammal rib fragment (Figure 3) (field-labeled as DE12.2-2), also from the Pirro Nord site (Apricena, Foggia).

The goal was to confirm that the internal bone structures remained intact, ensuring that the menthol applied during crystallization did not affect their integrity. Therefore, a micro-CT scan was performed on the rib fragment before the application of menthol, during its application in layers, and after the sublimation of menthol.



Figure 3. Small fragment of an Early Pleistocene mammal rib from Pirro Nord (Apricena, Foggia) before the application of menthol (A) and after the application (B). The sample is shown in its crystallization phase (B).

2.2.3. G.A.M.P.S. Collection

Micro-CT was employed in the study of paleontological samples from the G.A.M.P.S. permanent geo-paleontological exhibit collection in Scandicci. Here, micro-CT scanning was utilized to digitally preserve delicate paleontological specimens, including marine vertebrate and invertebrate fossils dating from Pliocene. This non-invasive technique allowed for detailed 3D modeling and comprehensive analysis of the specimens. Among the studied specimens were three shark teeth from different species (*Carcharhinus plumbeus*, *Carcharodon carcharias*, and *Chlamydoselachus lawleyi*) as well as a gastropod shell and a *Goneplax* sp. fossilized crab specimen. These specimens contributed to the creation of 3D reconstructions.

The tooth of *Carcharhinus plumbeus*, a living sandbar shark belonging to the requiem shark family Carcharhinidae, was discovered near Castelfiorentino, Florence Province. The tooth of the modern white shark, *Carcharodon carcharias*, was found in Volterra, Pisa Province [51]. Finally, the tooth of the extinct species *Chlamydoselachus lawleyi* was uncovered at Castelnuovo Berardenga, Pisa Province, Italy [52]. This species is closely related to the extant frilled shark *Chlamydoselachus anguineus*.

Additionally, micro-CT images were collected for ear bones (periotic and tympanic bulla) from a partial skeleton of the extinct dolphin *Etruridelphis giulii*, which was found at Lucciolabella, Siena Province [53].

Micro-CT analysis was performed on all specimens to image their internal structures. Certain specimens, specifically the fossilized *Goneplax* sp., the gastropod shell, the dolphin tympanic bulla, and the two shark teeth from *Chlamydoselachus lawleyi* and *Carcharhinus plumbeus*, were further utilized to create the 3D reconstructions.

3. Results

The micro-CT analysis yielded significant findings across three key areas: restoration assessment, ichnological studies, and digital modeling in museum collections. Each section details the specific contributions of micro-CT, from detecting structural changes in restored fossil specimens to analyzing ichnofossil morphology and enhancing the digital preservation of paleontological specimens.

3.1. The Case Study of Sealed Bee Cells

A successful application of micro-CT in ichnology regards the identification of the biological producers within bioturbation ichnofossils. A notable example is reported in Figure 1A, a solitary bee species preserved within its cocoon in Costa Vicentina, Portugal. The CT-scan data discussed in this paper have been previously published by Neto de Carvalho and colleagues [41]. This case exemplifies an extreme density contrast that facilitated successful micro-CT imaging as it involved three distinct components: the organic-walled cocoon preserved sealed within the burrowed cell (Figure 1B), the air inside (Figure 1C), and the bee itself (Figure 1D,E). Through this stark contrast, a clear visualization of the bee was possible.

3.2. The Case Study of Menthol in Restoration Artifacts

To evaluate the safety of crystallized menthol when applied to internal bone structures, a rib fragment from the Pirro Nord site was analyzed. Micro-CT imaging was conducted across three stages: untreated, post-menthol application, and post-sublimation. The results revealed limited penetration of menthol into the trabecular network, likely due to the heterogeneous structure of the bone, which hindered uniform diffusion (Figure 4A,B). Despite this shallow infiltration, the bone remained structurally intact. Comparative

analysis of the scans showed no significant morphological alterations, supporting the reversibility and compatibility of menthol as a temporary consolidant (Figure 4C–E).

These findings confirmed that, at the morphological level, no changes occurred between the untreated state and the post-sublimation stage. As such, the consolidant was considered safe for further application. The menthol was heated to a molten state and applied to the ventral side of the Early Pleistocene skull of *Canis mosbachensis* (Figure 2A), facilitating the micro-excavation needed to detach the fossil from the surrounding limestone matrix and to expose the previously covered dorsal portion (Figure 2B–E).

The application of menthol, reinforced with a fiberglass sheet (Figure 2B,C), enabled the successful detachment of the fossil and exposure of the dorsal side (Figure 2D,E). The consolidant provided structural support to the ventral surface throughout the micro-excavation, preserving the spatial arrangement of the fragments. After sublimation, the skull remained stable, requiring only minor filling to ensure safe handling (Figure 5A,B).

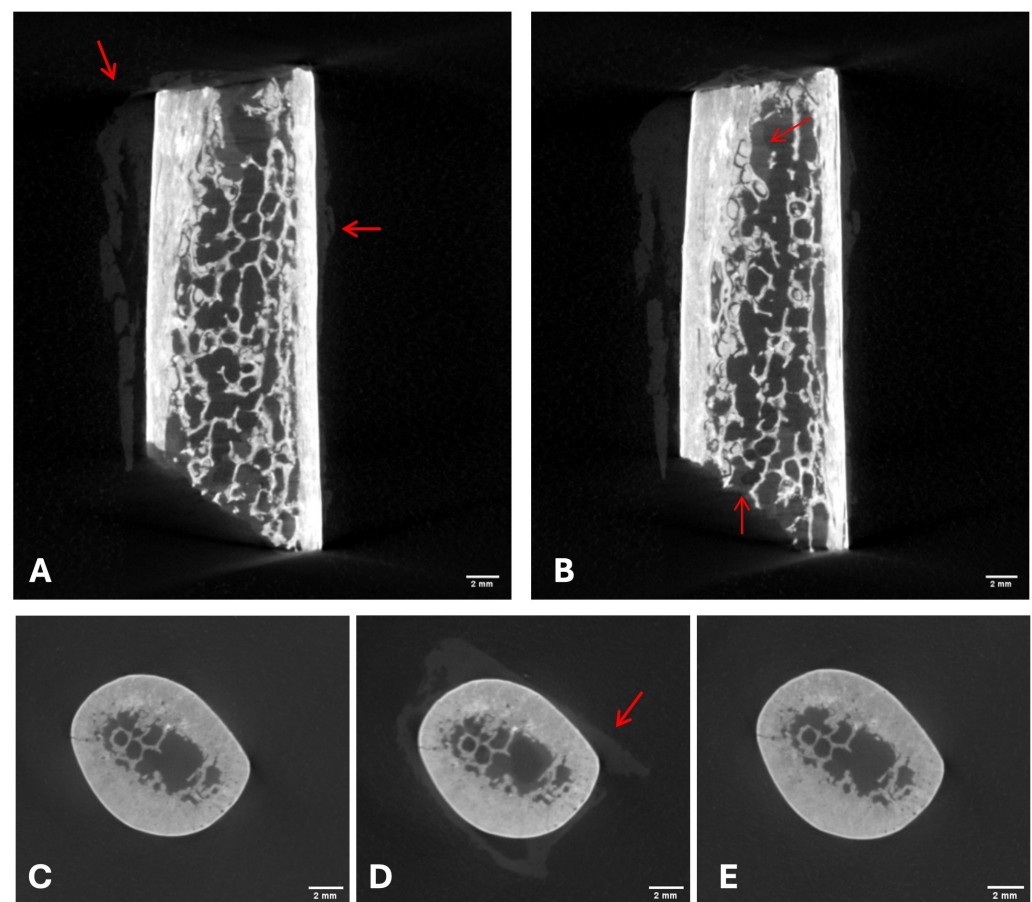


Figure 4. Micro-CT scans of the Early Pleistocene rib fragment from Pirro Nord. (A,B). The presence of menthol is clear externally, while within the cancellous bone the material does not appear to be evenly distributed. A comparative analysis of micro-CT scans of trabecular structure of the rib before menthol application (C), during menthol crystallization (D), and after sublimation (E). Red arrows indicate the distribution of menthol (gray material) both externally and within the internal structure.

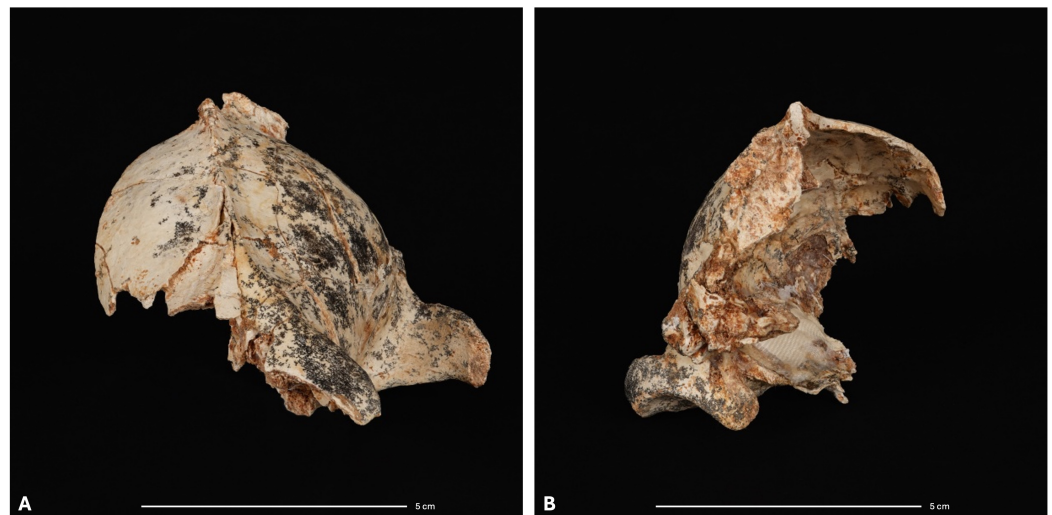


Figure 5. The Early Pleistocene skull of *Canis mosbachensis* from Pirro Nord after restoration. (A) Anterior and (B) posterior view of the skull. The detachment of the skull from the stone substrate allowed the specimen to be studied. The remains were confirmed to belong to a young individual thanks to the identification of porous bone tissue typical of developing bones.

3.3. The G.A.M.P.S. Case Study

To showcase the full potential of micro-CT technology and the subsequent development of high-resolution 3D models, the technique was applied to selected specimens from the G.A.M.P.S. collection. Particular attention was given to shark teeth as this collection is recognized as one of the most significant of its kind in Italy [54]. It includes geolocalized specimens of exceptional scientific value, including rare and unique finds such as the *Chlamydoselachus lawleyi* tooth from Castelnuovo Berardenga. The remarkable richness and diversity of the G.A.M.P.S. collection not only provide an invaluable resource for paleontological research but also offer an ideal case study for demonstrating how advanced imaging techniques can enhance the documentation, analysis, and dissemination of fossil samples.

Micro-CT scanning was employed to investigate the internal architecture of selected shark teeth, providing insights into their histological types and structural organization (Figures 6 and 7), avoiding more invasive but still important methods such as thin sectioning [55]. All the micro-CT images were acquired using a YXLON CHEETAH EVO scanner [46], with a spatial resolution of approximately 2 µm, allowing for detailed discrimination of internal dental tissues. All the image analyses and 3D reconstructions (Figure 7D) were conducted using VGSTUDIO MAX software (version 3.5.0) [47].

The scan of the *C. plumbeus* tooth (Figure 6B), a representative of the Carcharhiniformes—and as such of the orthodont histotypes—reveals a central pulp cavity surrounded by a distinct layer of orthodentine, with the outer crown portion being composed of enameloid and the root being composed of osteodentine. The orthodentine clearly encapsulates the pulp cavity, which remains open, which is consistent with the definition of orthodonty [56–58].

In contrast, the *C. carcharias* tooth (Figure 6D), a representative of the Lamniformes as well as of the osteodont histotype, lacks an open pulp cavity. The scan shows the crown and root being entirely filled by osteodentine, with no evidence of orthodentine. This homogenous internal filling is typical of osteodont teeth, where the pulp cavity is gradually replaced during development [59,60]. The difference between enameloid and osteodentine is evident in the scan, the outer enameloid layer being clearly delineated from the less dense inner core of osteodentine.

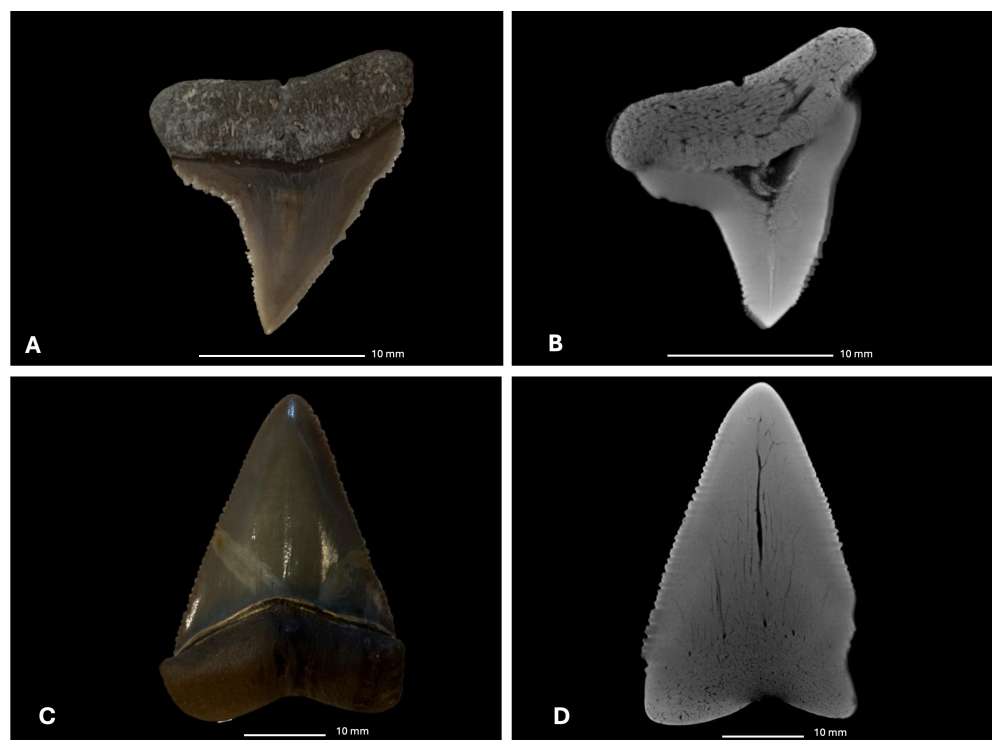


Figure 6. Tuscan Pliocene shark teeth and their internal structures as revealed by micro-CT imaging. (A) Tooth of *Carcharhinus plumbeus* from Castelfiorentino. (B) Micro-CT scan of the same tooth showing the open pulp cavity, the orthodontine layer in the crown, the osteodontine that forms the root, and the external enameloid layer (orthodont histotype). (C) Tooth of *Carcharodon carcharias*. (D) Micro-CT scan revealing a homogenous internal structure entirely composed of osteodontine, with no visible pulp cavity (osteodont histotype).

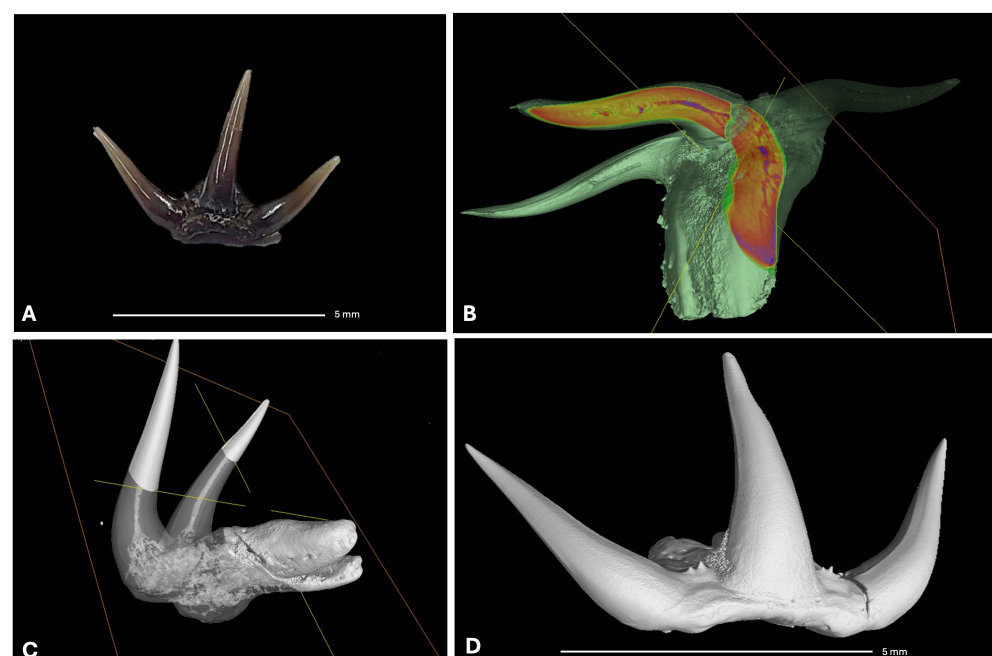


Figure 7. Tuscan Pliocene tooth of the extinct shark *Chlamydoselachus lawleyi* and its micro-CT-based reconstructions. (A) Tooth of *C. lawleyi* from Castelnuovo Berardenga. (B,C) Micro-CT visualizations showing the internal structure, including the open pulp cavity excavating the orthodontine layer, the external enameloid external layer, and the osteodontine root, consistent with the orthodont histotype. (D) 3D digital model generated from the CT dataset, illustrating the external morphology and spatial arrangement of the cusps.

Figure 7A displays a tooth of *Chlamydoselachus lawleyi*. As in modern representatives of *Chlamydoselachus*, this extinct frilled shark species exhibits an orthodont histotype, with the micro-CT imagery (Figure 7B,C) revealing a prominent pulp cavity enclosed by orthodentine, and a comparatively thin layer of enameloid at the crown surface. Several internal vascular channels are also visible, suggesting a well-developed pulp system. Overall, the observed histology is consistent with that of extant Chlamydoselachidae [56].

Additionally, several 3D reconstructions of fossils housed at the G.A.M.P.S. Museum, created with VGSTUDIO MAX, are shown in Figure 8. These reconstructions provide detailed virtual representations of the specimens, further showcasing the potential of micro-CT in paleontological research and museum curation.

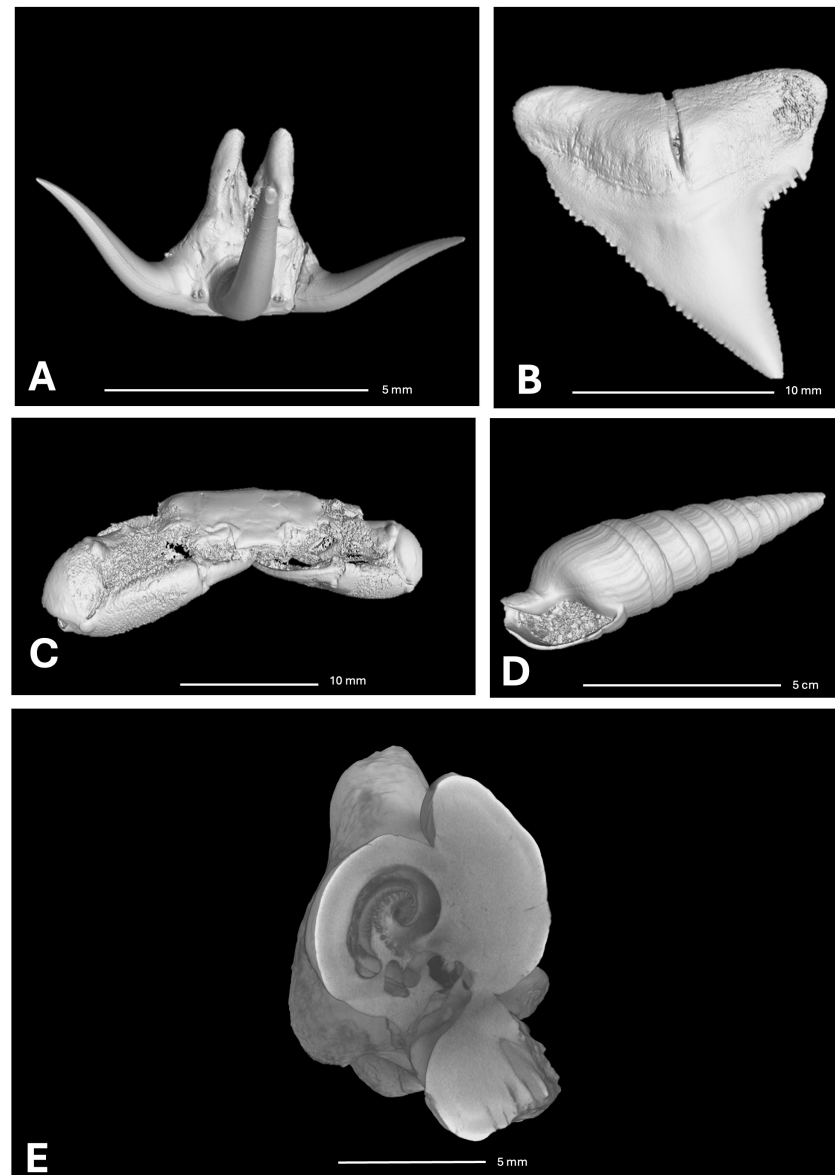


Figure 8. Three-dimensional models of some selected Tuscan Pliocene fossils housed in the G.A.M.P.S. permanent geo-paleontological exhibit (Scandicci). (A) Tooth of *Chlamydoselachus lawleyi* in apical view, (B) tooth of *Carcharhinus plumbeus* in lingual view, (C) fossilized crab (*Goneplax*) specimen in anterior view, (D) gastropod shell in apertural view, and (E) virtual cross-section through the periotic of the extinct dolphin *Etruridelphis giulii* (note the associated tympanic bulla in the background).

4. Discussion

The forthcoming discussion subsections will explore the diverse applications of micro-CT imaging in the fields of paleontology and conservation. By enabling non-destructive analysis, this technology significantly enhances the understanding of traces, body fossils and restoration techniques, thereby contributing to the advancement of scientific research and museum practices.

4.1. Trace Fossils: Challenges and Advantages of Micro-CT in Paleontology

The study of solitary bee species (*Eucera* sp.) within cells from Costa Vicentina, Portugal [41], highlights the significant potential of CT scanning in ichnology. This imaging technique allowed for non-destructive visualization of organisms within their cocoons, offering insights into the behavior and ecological roles of ancient eucerid species. Notably, the bee cells from Costa Vicentina are the first described in the Quaternary fossil record of mainland Europe and the first globally to include preserved progeny of the producers.

Micro-CT scanning proved invaluable due to the fragile nature of the bee remains, which would have been compromised by traditional extraction methods. The non-invasive nature of micro-CT scanning preserves scientific data and enables accurate identification of the producer. Combined with optical and SEM microscopy, micro-CT scanning facilitated the understanding that the cocoons served as breeding nests. These nests contained food provisions deposited by the female progenitor, with cell walls lined by a water-repellent membrane to maintain moisture and prevent fungi attacks. Radiocarbon dating of the organic lining indicates that the cocoons date back to the late Holocene, approximately 2975 years ago.

The preservation of *Eucera* sp. bees within their brood chambers, alongside monofloral pollen provisions, suggests these fossorial bees were impacted by adverse climatic conditions. Events such as spring storms, rapid sediment burial, or sudden temperature drops may have led to mass mortality through oxygen depletion or freezing. The presence of bee cells in carbonate palaeosols underscores the potential of high-resolution penetrative imaging to locate producing species, offering a unique opportunity to advance the understanding of bee nesting behaviors and their evolutionary history.

While micro-CT scanning has demonstrated significant potential in ichnology, particularly in the study of subfossil cocoons with solitary bees, it is not without limitations. Bioturbation structures, such as those created through the process of backfilling, present specific challenges. Organisms like echinoids create burrows by moving sediment from the front to the rear as they advance [37]. These backfilled tunnels, due to their composition of material identical to the surrounding matrix, typically exhibit minimal density contrast, rendering them nearly invisible to micro-CT scanning. Another notable limitation of micro-CT scanning in ichnology is related to the size of trace fossils. Ichnofossils, which are an integral part of the substrate, often exist within large rock blocks. Conventional micro-CT scanning is not suitable for such large samples as their volume and weight exceed the operational limits of micro-CT equipment.

Despite these limitations, micro-CT scanning remains a valuable tool in ichnology when appropriately applied. By addressing the challenges of density contrast and sample size, researchers can expand the utility of micro-CT scanning in studying trace fossils, thereby gaining deeper insights into ancient organism behaviors and interactions with their environments.

4.2. Menthol as a Restoration Material: Insights and Limitations

The use of menthol as a consolidant in fossil restoration is relatively new, requiring further research to fully understand its interactions with different substrates. In this

study, micro-CT played a crucial role in evaluating how menthol affected fossilized bone, particularly in two case studies: the skull of *Canis mosbachensis* (Figure 2) and a rib fragment from the Early Pleistocene site of Pirro Nord (Apricena, Foggia) (Figure 3). Without micro-CT, it would have been impossible to observe the internal effects of the treatment, making this technique indispensable for assessing both the structural impact of menthol and the preservation of the fossil's integrity.

Micro-CT scans revealed that menthol penetration into the bone's trabecular network was relatively superficial (Figure 4A,B), likely due to the irregularity of the trabeculae, which inhibited diffusion. This limited penetration proved beneficial as it reduced the risk of mechanical damage from tensile and contraction forces during sublimation. The comparison of pre- and post-treatment scans on the fragmented rib (Figure 4C–E) confirmed that menthol did not cause significant morphological alterations, preserving the fossil's structural integrity.

A notable challenge in the analysis was distinguishing menthol from the fossil material in the micro-CT scans. Due to its low radiopacity, menthol is difficult to detect; however, by employing dedicated image processing software, the image quality and contrast were enhanced. In this study, contrast optimization was achieved using VGStudio Max tools [47] through the adjustment of range parameters and setting air as the black reference point, allowing for the detection of even subtle radiopacity differences. This method provided a clearer view of the menthol distribution within the sample, aiding in the evaluation of its penetration and potential effects on bone structure.

Additionally, the behavior of menthol during application and sublimation was examined. Menthol is considered a particularly effective consolidant as it forms a stable reversible protective layer and sublimates without leaving any residue, making it especially suitable for temporary interventions in conservation.

When applied in a molten state, menthol undergoes phase transitions that can induce thermal and mechanical stress, with solidification causing volumetric shrinkage that could potentially deform fragile fossils. However, in the case of *Canis mosbachensis*, this was mitigated by applying menthol in layers and reinforcing it with a fiberglass sheet (Figure 2B,C). This structural support allowed for the careful micro-excavation of the skull from the limestone matrix and facilitated the removal of sediment covering the dorsal portion (Figure 2D,E). After sublimation, the fossil was stable but still required minimal infilling to support delicate areas and allow for safe handling and study (Figure 5A,B).

Sublimation, while a significant advantage of menthol, also affects its mechanical performance. In solvent-assisted solutions; the process occurs faster, but this results in reduced mechanical strength due to reverse migration—a phenomenon where menthol crystallizes near the surface, forming needle-like structures that weaken its consolidating properties. However, these crystals grow externally, meaning they do not cause internal damage to the fossil. Additionally, solvent-assisted menthol applications allow treatment at room temperature, eliminating the need for melting and making the process safer and more adaptable.

Micro-CT has proven essential in fossil conservation, offering insights that would otherwise be impossible to obtain. By allowing researchers to observe the internal structure of fossils before, during, and after restoration, this technique ensures that treatments do not compromise the integrity of specimens intended for study and museum exhibition. The findings of this study demonstrate that menthol is a safe and effective consolidant, particularly for fragile fossils that require temporary stabilization. Compared to traditional consolidants like cyclododecane, menthol offers several advantages: it is safer to handle, sublimates at room temperature, and allows longer processing times. Its limited penetration ensures that delicate structures remain unchanged, making it an excellent choice for

temporary fossil restoration in museum settings. Future research should focus on refining the application techniques and exploring solvent-based formulations to enhance menthol's performance across different fossil types.

4.3. Three-Dimensional Modeling and Digital Conservation: Implications for Museums

Micro-CT has transformed the study of paleontological specimens, offering a non-destructive method to analyze internal structures with unparalleled precision. Traditional fossil analysis often required invasive techniques that risked damaging specimens, limiting the ability to explore their internal composition. With the advent of high-resolution 3D imaging, researchers can now visualize fossils in extraordinary detail, gaining insights into their morphology, taxonomy, and conservation needs, while museums benefit from new methods of preservation and public engagement.

One of the most significant advantages of micro-CT in museum collections is its ability to classify and compare specimens by revealing internal anatomical features that would otherwise remain hidden. In our study, micro-CT scans of Pliocene shark tooth specimens from the G.A.M.P.S. Museum in Scandicci revealed distinct taxon-specific structural differences. The analysis included a *Carcharhinus plumbeus* tooth from Castelfiorentino (Florence) and a *Carcharodon carcharias* tooth from Volterra, highlighting key variations in their internal architecture (Figure 6). The *C. plumbeus* tooth (Figure 6A,B), a representative of the orthodont histotype that characterizes most Carcharhiniformes, features a well-defined pulp cavity surrounded by a layer of orthodentine and coated externally by a sharp enameloid layer, with a root composed of osteodentine, which is consistent with the taxonomic and functional features of the genus *Carcharhinus*. In contrast, the tooth of *Carcharodon carcharias*, a lamniform shark with an osteodont histotype (Figure 6D), is completely filled internally with osteodentine, with no visible pulp cavity or orthodentine layer being present. The enameloid layer is obvious, and the internal dentine structure appears more homogenous than in the aforementioned tooth of *C. plumbeus*. These histological differences underscore the evolutionary divergence between lamniform and carcharhiniform sharks and align with previous definitions of tooth histotypes based on internal tissue composition and development [57,59].

A third case study involved a fossilized tooth of *Chlamydoselachus lawleyi*, an extinct chlamydoselachid shark from the Pliocene of Castelnuovo Berardenga (Siena Province, Italy) (Figure 7A). Micro-CT imaging (Figure 7B,C) revealed a prominent pulp cavity and an evident layer of orthodentine surrounding it, which is consistent with the orthodont histotype as observed in the extant *Chlamydoselachus*. The scans also showed internal vascular channels and differences in mineral density between dentine and enameloid, thus offering insights into the tooth's complex morphology and microstructure. The external 3D reconstruction (Figure 7D) further highlights the distinctive multicuspoid crown. These histological differences reflect phylogenetic divergence among the studied taxa and correspond with their functional and developmental adaptations [57]. These findings demonstrate how micro-CT can significantly enhance the classification and comparative analysis of fossils, supporting both scientific research and museum exhibits through high-resolution visualizations of internal structures. Moreover, 3D reconstructions generated using VGSTUDIO MAX software [47] (Figure 7D) created interactive digital models, allowing both scientists and museum visitors to explore the fossil from multiple perspectives.

These examples highlight the multifunctional role of micro-CT in museums. First, it serves as a powerful digital archiving tool, allowing for detailed assessments of fossil stability and structural integrity, ensuring that delicate specimens remain undamaged during handling and research. Second, it enhances scientific research, providing in-depth visualization of internal structures that would otherwise be inaccessible. Finally, it supports

public engagement and education by enabling the creation of interactive exhibits, digital collections, and 3D-printed models, making fossils more accessible to both researchers and visitors.

The broad impact of digital paleontology [61] has become especially evident in recent years as museums seek ways to make their collections more accessible beyond physical exhibitions. The COVID-19 pandemic reinforced the importance of virtual archives, ensuring that scientific study could continue despite travel restrictions and museum closures [27,45,61]. Several institutions, including the British Museum, the Museum of Modern and Contemporary Art in Seoul, and the Van Gogh Museum, have already integrated virtual collections into their public offerings [62]. Similarly, the Natural History Museum of the University of Pisa has begun the digitization of vertebrate collections, enabling remote research and educational opportunities [27].

At the G.A.M.P.S. Museum, the digitization of its paleontological collection is ongoing, with the long-term goal of creating a virtual archive that enhances both scientific accessibility and public engagement. The application of micro-CT technology within this process is crucial as it ensures that detailed internal imaging of specimens is preserved for future generations. Micro-CT offers many benefits for research, preservation, and public engagement. However, access to this technology can sometimes be limited. Despite significant advancements in availability and functionality, the costs associated with equipment, software, and specialized training can pose challenges for adoption, particularly for smaller museums and institutions with limited budgets. Therefore, developing collaborative networks, sharing resources, and implementing open-access initiatives are essential to ensure that the advantages of digital paleontology are more widely accessible to all experts in the field. By integrating progressive 3D imaging, digital reconstruction, and interactive visualization, micro-CT is redefining how fossils are studied, conserved, and experienced, securing their scientific and cultural value for years to come.

5. Conclusions

Micro-CT has become a transformative tool in the preservation and analysis of cultural and natural heritage. Its high-resolution non-destructive imaging allows for detailed visualization of both external and internal structures, minimizing physical handling while maximizing scientific insight. In our study, we explored the application of micro-CT across various paleontological specimens to better understand their internal architectures and refine conservation methods.

Specifically, we implemented micro-CT to analyze the subsurface features of fossilized specimens, enabling us to observe internal structures that are otherwise inaccessible. This approach proved particularly valuable in identifying morphological details essential for species classification and evolutionary study. Our research also examined the use of menthol in fossil restoration, with micro-CT confirming its effectiveness as a non-invasive consolidant. The scans revealed that menthol stabilized delicate structures without altering or damaging the fossil material—a key finding for future restoration protocols.

Beyond analysis, micro-CT allowed us to generate high-fidelity 3D models of our specimens. These digital reconstructions not only preserve the physical details of the fossils but also serve as powerful tools for virtual curation, education, and remote research collaboration. The ability to share and study these models online has far-reaching implications for accessibility, especially in times of restricted physical access such as during the COVID-19 pandemic.

Our study reinforces the role of micro-CT in advancing virtual paleontology. By digitizing and interpreting fossil specimens in unprecedented detail, we contribute to a growing movement that combines traditional curation with innovative digital practices. Institutions

like the British Museum and the Geology and Paleontology Museum of Florence have already embraced such approaches, integrating 3D visualization and online archives into public exhibitions and academic research.

In summary, our work demonstrates how micro-CT not only enhances fossil analysis and restoration but also supports the broader mission of museums: to preserve, interpret, and share the story of life on Earth. As technology continues to evolve, micro-CT will remain a cornerstone in bridging science, paleontological conservation, and public engagement.

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