## SHORT REPORT

# Conservation of *Anthracotherium magnum* fossils from Chiuppano, Italy

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Lignite deposits are characterized by a high probability of fossil preservation along with a high concentration of pyrite minerals. When fossils are discovered, exposure to the humidity and oxygen in the air begins a destabilization of the minerals and activation of chemical oxidation. In the last century, it was common practice to protect fossils by covering them with unspecified commercial varnish, but today it is clear this method is useless for long-term preservation. Moreover, varnish obliterates the precise features of teeth and bones, usually preventing researchers from correctly analysing and describing these specimens. In this paper, we describe the methodology applied for conserving fossils identified as *Anthracotherium magnum*, discovered in lignite deposits of Chiuppano (Vicenza, Italy) in the mid-twentieth century. We pre-prepared the specimens, removing varnish from the fossil surfaces, and we exposed them to an aerosol solution of PEG400 and concentrated ammonia. We discuss the colour shift of bones and the rediscovery of anatomical characteristics to underline the importance of prompt action in the preservation of fragile specimens for future exhibition.

Keywords: Pyrite decay; polyethylene glycol; ammonia; mammal fossils; Oligocene; Italy; Cetartiodactyla

### Introduction

The genus *Anthracotherium* was established by George Cuvier (1822) with a detailed description of fossils discovered at Cadibona (near Savona, Italy). Since this first description, several anthracotheres *sensu stricto* have been reported from Eocene and Oligocene sites in Asia and Europe, respectively (Tsubamoto et al. 2002). The first appearance of the genus in Europe is represented by *A. monsvialense* (de Zigno 1888), described from the early Oligocene of Monteviale. The complete fauna from this site has been recently reviewed by Pandolfi et al. (2017), and more than one hundred specimens of this small anthracothere have been described by Ghezzo & Giusberti (2016).

The fossils presented in this paper are from a small town located in the same province of Monteviale. Chiuppano is located 30 km north of the city of Vicenza at the base of the Asiago plateau (WGS84 latitude 45.762626, longitude 11.463182), and the surrounding areas are characterized by lignite beds. In this area, lignite deposits have been exploited since the early 19<sup>th</sup> century for fossil fuel extraction. From one of those lignite mines located near Contrada Marola, miners recovered several fossil specimens of a large mammal, at about 7–8 metres depth. The first researcher to encounter these fossils was F. Rando,

a teacher from Chiuppano, who delivered the materials to Professor P. Leonardi at the University of Ferrara in 1950 (Leonardi 1950). The entire collection of 22 bones was then described by B. Accordi (1951, tav. I-VI) as *Anthracotherium magnum* (De Blainville 1839–1864) and they are today housed in the Civic Library of Thiene (Vicenza). *A. magnum* evolved later than *A. monvialense*, during the middle Oligocene, and it is the largest anthracothere ever described in Europe and the last representative of the group before their extinction in the northern hemisphere at the end of this epoch.

# Pyrite in association with anthracothere fossils from Chiuppano

In 1951, Accordi first discussed the high quantity of pyrite in the fossils discovered at Chiuppano, as well as the associated difficulties related to their anatomical description and interpretation. Compositionally, lignite deposits include a large proportion of sulphides. Good preservation of fossils within such a protected environment contrasts with the relative richness of pyrite and marcasite (both with the chemical formula FeS<sub>2</sub>). The index of growth and accumulation of these minerals within the medullary cavities of bones is one of the most useful indicators of danger to fossil and mineral preservation throughout all museums of the world. In fact, pyrite and marcasite are commonly considered unstable minerals, as they are subject to natural oxidative processes through a chemical reaction known as pyrite decay (also known as pyrite disease or pyrite rot) (Larkin 2011). Such chemical processes

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mean that the minerals experience a phase change, transforming from sulphide to sulphate. The resulting crystals grow in volume, causing increased stresses within the fossil.

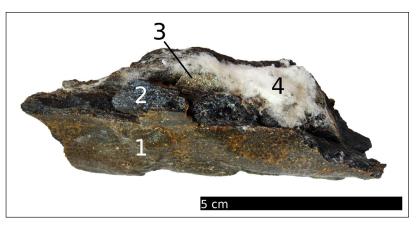
The causal factor of this oxidative reaction is related to the microcrystalline structure of pyrite, the presence of oxygen, and high relative humidity (RH) in the external environment (exceeding about 60 percent (Howie 1992)). The main product of pyrite decay is sulphuric acid ( $H_2SO_4$ ) and hydrated sulphates of iron (e.g.  $FeSO_4 \cdot H_2O$ ) (Waller 1987). Visibly, this consists of a yellow or grey dusty covering of the fossil surface, the formation of new fractures, breaks in the external cortical bone, even up to the complete destruction of bone surfaces (Howie 1992; Del Favero 2007) (**Figure 1**).

In the past, in an attempt to avoid pyrite decay and the consequent structural deterioration of fossils, each specimen was covered in a thick layer of unspecified varnish (**Figure 2**). This was common practice until the late 1970s, when palaeontologists and museum curators believed that pyrite decay could be prevented by excluding external air and humidity from direct contact with the unstable minerals (Howie 1978). However, this approach has proven counter-productive in the long-term for three main reasons. First, varnish in general is not moisture and gas impermeable. Moreover, the varnish used here was not homogeneously painted on the bone surfaces, and thus, it cannot guarantee an effective isolation of the fossil. Finally, the varnish layer turns from transparent to yellow and/or grey with age and masks all skeletal and tooth features, rendering the fossils unusable by researchers as it prevents an accurate description of anatomical characteristics of these extinct animals.

#### Methods

Before conservation, the original morphology of the bones was not clearly detectable, due to the presence of a thick layer of unspecified varnish, as well as disperse oxidized minerals. Moreover, the fossils were in general not well-preserved and remained extremely fragile throughout the entire analysis (**Figures 2** and **3**).

The first step of conservation consisted of removing the varnish with commercial water-free solvents to avoid exposing the minerals to additional humidity. This phase was concluded with a careful cleaning of the fossil surface using cotton swabs and acetone. Thus, the fossils



**Figure 1:** Fragment of the anthracothere from Chiuppano before conservation. It is possible to see different strata, from the external to the inner side of the bone: the layer of unspecified varnish (1), the cortical bone (2), stable crystals of pyrite (3), and the powdery white mineral results of oxidation (4).



**Figure 2:** Dental series of mandible ISN: 18 S242-10 teeth are completely covered by unspecified varnish. It is not possible to distinguish either external enamel or dentine, and the state of tooth wear is indistinguishable.

were prepared for the neutralization of sulphuric acids. Fossils were then placed on a grill tray to facilitate the complete circulation of gasses around the specimens (**Figure 4**), and were placed in an airtight polyethylene container with a mixture of polyethylene-glycol (PEG) 400 ( $C_{2n}H_{4n+2}O_{n+1}$ ) and concentrated ammonia (ammonium hydroxide solution – NH<sub>3</sub>), in a proportion of 5/1 (g/ml), respectively. Agents were mixed to create a volatile gas for the neutralization of sulphuric acids (Waller 1987; Howie 1992; Birker & Kaylor 1986; Rixon 1976, Larkin 2011). PEG400 guarantees a dry environment (for details about the process see Larkin 2011) where ammonia can operate, avoiding an increased RH inside the container.

The fossils were left in the sealed container with the ammonia vapour in a humidity-free environment for between 5 and 48 hours. The length of each reaction cycle was determined by the size of the fossils and the degree

of chemical decay. Ammonia gas guarantees the complete neutralization of sulphuric acid, with a predicted penetration of 1-2 millimetres per hour (Andrew 1999). At the end of this stage of the treatment, the minerals changed their colour from white and yellow to dark red and brown, resulting in a reddish hue on the surface of the entire collection.

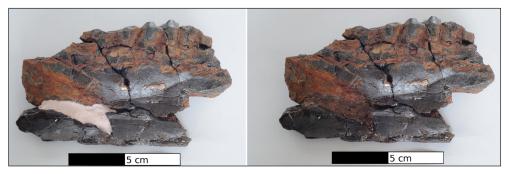
After exsiccation, crystal dusts were removed with micro-tools, acetone, and dry paper towels. Structural fractures were then filled with Balsite® W & K putty, a commercial epoxy resin developed by the Italian company CTS Srl. Balsite® is a cyclo-aliphatic polyamine mostly used for filling portions of wood in artistic and historical objects (Bailey et al. 2019). This neutral material was chosen because of its elasticity and lightness. It was applied, with positive results, for other palaeontological conservation treatments by the authors (Reggiani & Ghezzo 2015) (**Figure 5**).



**Figure 3:** Fossils in their original boxes, supported by a layer of cotton. Left: mandible ISN: 18 S242-10; Right: left proximal humerus ISN: 18 S242-22.



**Figure 4:** Mandible ISN: 18 S242-10 before the treatment (left) and prepared on the grill tray before the chemical reaction with PEG400 and ammonia (right).



**Figure 5:** Mandible ISN: 18 S242-10 after the chemical reaction. a) Fragments had been joined to each other and a portion of the horizontal branch had been filled with Balsite® (pink); b) final result.

To stabilize the inner structure of the fossils, each fragment was repeatedly immersed in a solution of Paraloid B72 (ethyl methacrylate and methyl acrylate copolymer) in acetone. Fissures were filled with a concentrated solution of the same stabilizer. Paraloid B72 is a thermoplastic resin commonly used in the conservation and restoration of cultural and palaeontological remains because of its stability (for both physical characteristics and transparency) and reversibility (Horie 1987; Borgioli and Cremonesi 2005). Fragments were adhered to each other using Mowital B60 HH, another reversible thermoplastic resin composed of polyvinyl butyral (PVB) (Borgioli and Cremonesi 2005) and known for its binding properties and versatility in solvents.

Finally, the fossils were arranged in new reagent-resistant boxes (cardboard). The interiors of the boxes were lined with polystyrene or polyurethane and the fossils were placed in their approximate anatomical position to reduce stress. On the top of each box, a label was added including an image of the fossil inside, the original specimen number, the accession number imposed by the Italian Superintendence (ISN), and any notes.

#### Health and Safety

Full-face masks and respiratory filters (type K) were used for protection from harmful solvent vapours. Body protection included the use of Tyvek clothing, and avoiding direct contact and penetration of liquids and solvents into the skin. Hands were protected with latex gloves.

While the chemical reactions were taking place, the fossils were closed with reagents in a hermetically sealed PVC box to reduce any risk of gas leaking. Ventilation control was not needed in this specific application because treatment took place in the Paleostudy laboratory, in an open environment outside the building where the access was restricted to people involved in the activity; however, it is highly recommended and should be mandatory when applying the same procedure indoors. Ammonia, polyethylene residuals, and other chemical products were post-processed as hazardous waste by a third party in compliance with Italian regulations for special waste.

#### Results

In 2007, the authors treated pyrite decay analogous to that discussed here in the Chiuppano fossils, which had affected the famous Eocene palm remains from the Bolca Lagerstätte. The fossilized logs had been displayed in the Sala delle Palme of the Museum of Geology and Palaeontology of the University of Padova (Del Favero et al. 2012). Because those unique fossilized palms were preserved on large and heavy slabs of matrix, it was not possible to move the entire collection to a laboratory. In that particular case, ethanol was preferred as solvent for the ethanolamine thioglycolate in situ, a method proposed by Cornish & Doyle (1984) and Cornish (1987). In the case of the Chiuppano fossils, however, the size of the specimens allowed us to transport them to the Paleostudy laboratory in Piove di Sacco (Padua) it was, therefore, possible to use a more invasive course for the penetration of the chemical agents. The mixture of PEG 400 and ammonia penetrates fossils more completely than ethanolamine thioglycolate and more efficiently prevents new oxidation. Moreover, this methodology improves the long-term stability of fragmentary fossils when it is associated with micro-climatic control in storage, avoiding dangers related to their possible collapse. Finally, the reaction can be easily controlled, and primer reagents are commonly available, limiting costs.

The primary observable change on the surfaces of the fossils after the conservation was a colour shift from a pale dusty appearance to a reddish and brown homogeneous colour, mostly due to the presence of iron within the minerals. This colour change must be properly recorded, particularly if there is a lack of evidence concerning the original location of the fossils. In this case, the location is known only broadly as the hamlet Contrada Marola. This consists of few square metres of a closed lignite mine with a homogeneous deposit. In an attempt to prevent the original appearance of the fossils from becoming completely disconnected from their context, a small portion of fragments that could not be re-joined were left unprocessed. Some parts of these still present microcrystals of stable, non-oxidized pyrite; they will be monitored for future reactions or decay. Despite these limitations, the fossils are now much more suitable for description, study, and analysis by researchers: the fossils are clean and their morphological features are legible.

As suggested by Howie (1992), the restored specimens should be maintained below 50 percent RH (30 percent RH is considered the best environment), and organic or water-free solvents should be used for future treatments. A final application of Paraloid B72 improves the resistance of the fossils to stresses and increases their rigidity.

The conservation treatment revealed the presence of portions of the original labels (**Figure 5**) on one mandible, ilium, and ulna (respectively ISN: 18 S242- 10, 24, and 28). The removal of the varnish also improved identification of a number of teeth and postcranial remains (distal epiphysis of a tibia – ISN: 18 S242-21 and 30-, femoral head – ISN: 18 S242-29 – and the occipital condyle – ISN: 18 S424-14-) (**Table 1**).

After more than fifty years without conservation, we now have the opportunity to verify the anatomical elements present in the original materials collected by Dr. Rando. Accordi (1951) published six tables representing the most important specimens. Our comparison reveals that several samples are now lost. Specifically, two upper molars (M2-M3) first described by Accordi (1951) and re-published in a local book by Rando (1958) were not found when the collection had been delivered to one of the authors (E.G.). Considering the good preservation of the fossils shown on figures in that original papers, it is plausible that they had been misplaced or removed by unknown individuals during the last 50 years, when fossil localities had been disputed between the institutions of Chiuppano and Thiene (their current repository). Such loss should be considered very serious, because the modern description and recognition of distinct species of anthracotheres is focused on the description of upper and lower teeth (Boisserie et al. 2010). Moreover, some features are today partially lost. We have not found the fragment of a crocodile skull, a

Original	From Accordi (1951) – 3 – List of				Notes (n. post-conservation)	ration)
numeration	remains of Anthracotherium	n. Repository (box)	Itali	an Superitende	Italian Superitendence Number (ISN)	Description
1	condilus occipitalis	confirmed (n. 12)	18	S242 – 1	14	reported as the astragalus in the historicai label
2	mandible with two horizons ramus with p3-m3	confi'med (n. 3–4)	18	S242 – 10	10, 11a–d	p3 has been completely lost
ε	fragment of a mandible with horizontal ramus and m2–m3 [rx]	confirmed (n. 2b)	18	S242 – 1.	12, 13	teeth are not recognisable because fragmented
4	fragmented mandible of a young Individual, and a portion of the last decidual molar	not found				
IJ	rostrum of a mandible with the synphisis and alveolus partially preserved	not found				
9	mandible of a very young animal (without the vertical ramus) and decidual teeth	not found				
7	incomplete tooth crown of an upper right canine of a male	confirmed (n. l with other dental remains)	18	S242 – 1!	15, 16, 17, 18	
8	complete tooth crown of an upper right canine of a young male					
6	complete tooth crown of a P3 [rx]					
10	complete tooth crown of a P4 [lx]	confirmed (n. 21)	18	S242 – 19	19	
11	fragment of a maxilla with complete M2–M3 [be]	not confirmed				
12	maxillar fragment with the last deciduous tooth (d3) and a portion of d2 [rx]	not confirmed				
13	epistropheus partially broken	confirmed (n. 20)	18	S242 – 25	5	the epistropheus tooth has been lost after Accordi (1951)
14	a probable lumbar vertebra (corpus)					
15	two joined caudal vertebra	not found				
16	Proximal epiphisis of a left humerus	confirmed (n. 17)	18	S242 – 2.	22, 34, 38, 40, 41, 42	articular head of a right humerus

**Table 1:** Comparison between the determinations made by Accordi (1951) and the description of bones after the restoring activity. Several specimens have been lost since 1950, and other fossils have been taxonomically reclassified.

(Contd.)

מ	From Accordi (1951) – 3 – List of			Notes (n. p	Notes (n. post-conservation)	vation)
numeration	remains of Anthracotherium	n. Repository (box)	Italia	Italian Superitendence Number (ISN)	er (ISN)	Description
17	distal epiphisis of a right humerus	confirmed (n. 11 and 10)	18	S242 – 23, 31, 32		distal epiphisis have been rot determined as Anthracotherium. The humerus head n. 17 was of a young animal
18	Proximal epiphisis of a right radius	confirmed (n. 14)	18	S242 – 28, 33, 38		determined as ulna and fragments
19	Proximal epiphisis of a right femur	confirmed (n. 19)	18	S242 29		right head of a femur
20	distal epiphisis of a right femur	confirmed (n. 6b)	18	S242 – 27		
21	fragment of a left last astragalus	not found				(see OSN = 18 S242-14)
22	distal end on a Ill (?) metacarpus	not found				
	Several fragments of long bones and teeth, not determinable	n. lb, 2, 15, 18	18	S242 – 20, 35, 36, 43, 44, 45a-b, 46a-c	3, 44, c	
		n. 5	18	S242 – 30		distal epiphysis of a tibia
		n. 6	18	S242 – 21		tibial dyaphisis
		n. 7	18	S242 – 24		fragment of an ileo, previously determined as an epiphysis of left humerus in the historical labels
	From the same deposit					
23	fragments of a crocodile skull	not found				
24	Small cylindrical coprolite	not found				
	Remains are hosted in the private collection of Mr. F. Rando in Chiuppano (Vicenza)	Today, they are displayed in the Palazzo Cornaggi, Thiene (Public Libiary)	the Palaz:	zo Cornaggi, Thiene (Public	: Libiary)	



**Figure 6:** From left to right: the epistropheus ISN: 18 S242-25 in 1951 (Accordi 1951) and today, before and after conservation (scale bar for the recent photo = 1 cm).

coprolite, and several other remains reported by Accordi, and the only epistropheus has lost its odontoid process, an important feature for species determination (**Figure 6**).

#### Conclusion

The technique described in this paper has allowed the authors to re-evaluate the morphology of the anthracothere (to be discussed in a forthcoming paper), which has been extinct for more than 30 million years. This methodology and the use of an ammonium hydroxide solution instead of ethanolamine thioglycolate in ethanol is more effective for the penetration of bone fissures. The technique has, hopefully, prolonged the preservation of this material for future study. It is important to underline here that there is no way to completely block the decay of pyrite without a controlled micro-climatic environment around the fossil. Such a conservation policy should be applied every time the presence of pyrite minerals is discovered; it is nowadays considered the only procedure able to control changes of relative humidity and decrease the risks of reactivation of the chemical reactions.

The same method and subsequent exhibition in a controlled environment can be applied to a diverse range of fossils that display the same mineral degradation, and it meets the requirement for a long-lasting solution to pyrite degradation of fossil collections, as well as fitting within a constrained budget. The treatment method thus described allows the local municipality to exhibit the fossils within the Public Library of Thiene (opening in June 2019) for the enjoyment of the community.

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#### **Competing Interests**

The described work was part of a project for the final exposition of the fossils with other archaeological artefacts, financially supported by the Municipality of Thiene. E.G. was paid for the conservation and for the design of the exposition. P.R. is the owner of the Paleostudy Lab. and he collaborated with the first author in the conservation activities. The authors didn't receive any support for the publication of this Short Report.

Both the authors has no competing interests, out of what has just been declared.

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