# Fossil pigments and pigment organelles – colouration in deep time

Bachelor's thesis

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# Contents



**Cover Picture:** Head of a fossil crane fly from the Fur Formation, Denmark.

# Fossil pigments and pigment organelles – colouration in deep

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**Abstract:** Pigmentation is important in all animals and plants, e.g., for protection against radiation, for signalling and as camouflage. The fossil record contains both colourful bird feathers and insect cuticles. The question is if original pigments and colours can be spared from diagenetic alteration through millions of years. This study uses SEM (scanning electron microscopy) and EDX (energy dispersive X-ray spectrometry) to examine the eyes of a fossil insect and a fossil feather, both from the Eocene Fur Formation, Denmark and two fossil feathers from the Eocene Green River Formation, USA. The EDX analysis showed elevated carbon intensities in the dark peripheral of the ommatidia and elevated calcium intensities towards the centre of each ommatidium. Elevated carbon and sulphur intensities were observed in all feathers relative to the surrounding matrix. The carbon in all four specimens is interpreted as originating from organic matter present in the fossils. The calcium in the insect eye is interpreted as the preserved crystalline cones of the ommatidia. It is possible that original pigments and pigments organelles are preserved; however this cannot be confirmed by the methods used in this study.

**Keywords:** Paleobiology, melanin, ommochrome, fossil, insect, feather, Fur Formation, Green River Formation.

**Supervisor:** Johan Lindgren

**Subject:** Lithosphere and Paleobiosphere Sciences

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# Fossila pigment och pigmentorganeller — färg genom djup tid

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**Sammanfattning:** Pigmentering är en viktiga hos alla djurgrupper; som skydd mot UV-strålning, för kommunikation mellan artfränder och som kamouflage. Det fossila arkivet har gett upphov till både fåglar och insekter med olika färgskiftningar. Men kan ursprungliga pigment och strukturella färger förbli intakta över miljontals år? Den här studien använder SEM (scanning electron microscopy) och EDX-analyser (energy dispersive X-ray spectrometry) för att undersöka ögonen hos ett insektsfossil och en fossil fjäder från Furformationen i Danmark och två fossila fjädrar från Green Riverformationen i USA. Vidare omfattar studien en genomgång av pigment och pigmentorganeller och av tidigare publicerade arbeten rörande fossila pigment och färger hos utdöda djur. Resultaten av EDX-analyserna visade höga värden av kol i de mörka delarna av ommatidierna och förhöjda värden av kalcium inuti varje enskilt ommatidium. Fjädrarna visade förhöja värden av kol och svavel jämfört med kringliggande matrix. Förekomsten av kol hos alla fyra fossil tolkas som rester av biologiska vävnader som fortfarande kan finnas kvar i fossilen. Kalciumet i de fossila insektsögonen tolkas som bevarade kristallina koner och/eller linser som utgör ett ommatidium. Det finns indikationer på att ursprungliga pigment och pigmentorganeller kan finnas bevarade. Detta kan dock inte styrkas med metoderna i den här studien.

**Nyckelord:** Paleobiologi, melanin, ommokrom, fossil, insekter, fjädrar, Furformationen, Green Riverformationen.

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# 1 Introduction

Insects and birds are two animal groups that are quite fascinating alive today and equally alluring in the fossil record. Both groups display a wide range of colours—from the brightest hues of the rainbow to the deepest black. What of these colours can be preserved through time for us to discover? How long do biological tissues and their components last once the organism has died, been scavenged upon and buried by sediments for millions of years? It seems as time itself is not the most important factor when it comes to preservation, but rather the circumstances surrounding the death and initial taphonomy of the organism.

Soft parts or non-biomineralized tissues (such as skin and feathers) are seldom preserved compared to biomineralized tissues (such as bones). Yet, in rare preservational environments soft parts occasionally occur (Orr et al., 2002). Rocks as old as Cambrian are known to house organically preserved cuticles of animals, such as insects (Stankiewicz and Briggs, 2007). A number of fossil feathers as well as insects has previously been analysed through different methods. Insect cuticles often have so called structural colours that display metallic hues. Structural colours are produced by the arrangement of layers with different refractive indices that scatter light (McNamara et al., 2011b). This is also observed in feathers (Fig. 1). Many fossil insects still retain a shimmering metallic colour and a few of these fossils have been analysed to determine if the original colours are preserved or if the original colours can be recreated (McNamara et al., 2011a, Stankiewicz et al., 1997, Parker and McKenzie, 2003, McNamara et al., 2011b). The preservation potential of insect pigments like ommochromes is unknown and so far none has been found in fossils. Most feathers are preserved as carbonaceous imprints and compression fossils, and in some cases microscopically small oval bodies, first interpreted as fossilised bacteria, can be found (e.g., Davis and Briggs, 1995). Subsequently, many of these fossil microstructures has been re-interpreted as the remains of pigment organelles (Vinther et al., 2010). However, the origin of the structures found in fossil feathers is difficult to determine based on their similarity with bacteria (e.g., Wogelius et al., 2011; Zhang et al., 2010; Lingham-Soliar, 2011).

*Fig.1*. A black feather with metallic blue and green hues. These metallic colours are produced by the pigments and their internal structure within the feather keratin. Species unknown.

## 1.1 Aim of the study

The purpose of this study is to describe biological pigments, such as melanin and ommochromes that occur in feathers and insect eyes respectively. Additionally, studies concerning alleged fossil pigmentation and pigment granules are reviewed. I have also analysed four fossil specimens and attempted to determine the origin of their colouration. The sampled material include one fossil insect with preserved eyes and a fossil feather from the Eocene Fur Formation of Denmark, two fossil feathers from the Eocene Green River Formation.

# 2 Geological settings

## 2.1 The Fur Formation

The Fur Formation crops out on different places around Limfjorden, north-western Jutland, Denmark (Fig. 2). The type locality is situated on the northern part of the Island of Fur at Knudeklint, from where the formation has got its name (Brooks, 2006). The age of the formation is Early Eocene, the Ypresian Stage, based on radiometric dating (Chambers et al., 2003, Lindow and Dyke, 2006). The formation consists of about 180 distinctive ash layers interbedded with approximately 60 m of marine diatomite (Pedersen et al., 2004, Brooks, 2006). Several horizons and isolated nodules of calcareous concretions are also present (Pedersen and Buchardt, 1996; Bertelli et al., 2010). The Fur Formation is subdivided into the upper Silstrup Member and the lower Knudeklint Member (Willumsen, 2004). The Fur

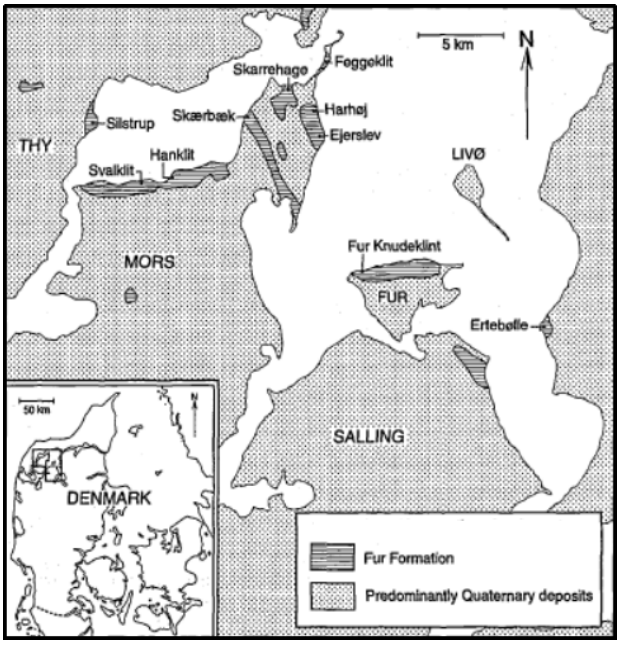


*Fig.2*. Location of the Fur Formation in north-western Denmark, where the fossil insect and one of the fossil feathers were collected. Modified from Pedersen & Buchardt (1996).

Formation overlays the Paleocene Ølst Formation and is stratigraphically followed by the Oligocene Røsnaes Formation (Brooks, 2006).

The volcanic ash layers are divided into a negative and a positive series (Brooks, 2006, Larsen et al., 2003). The lower negative series ranges from -39 to -1, and is an approximately 30 m thick diatomite with ash layers described as discreet, light-coloured bands that are set broadly apart. The upper positive series consists of around 25 meters of striped, lightly coloured diatomite. Here, the ash layers, range from +1 to +140, and they are basaltic, black in colour and set closely apart (Larsen et al., 2003). There is a general consensus that the sources of the ash layers with are volcanic eruptions associated with the formation of the North Atlantic Ocean (Brooks, 2006, Larsen et al., 2003).

The depositional environment is presumed to be in a subtropical climate characterized by upwelling, which enriched the surface waters with nutrients, causing tremendous blooms of diatoms and other planktonic organisms (Dyke and Lindow, 2009, Larsson, 1975). The degradation of these blooms depleted oxygen, causing anoxia (primarily in the lower and middle part of the formation) and somewhat acidic bottom conditions; this acidity dissolved the calcareous organisms (Bonde, 2008, Pedersen and Buchardt, 1996), which is shown by the lack of bioturbation by benthic organisms; only bacteria could thrive in these bottom conditions. Bottom conditions get slightly improved in the upper part of the formation as indicated by the presence of trace fossils and bioturbation (Dyke and Lindow, 2009). An offshore depositional environment about 50-100 km from the shore is inferred (Rust, 2000) with a water depth of several hundreds of metres (Bonde, 2008).

The preservation of the fossils are generally good to excellent with high resolution details thanks to the fine-grained quality of the diatomite (Brooks, 2006). There are generally two ways in which fossils are preserved; either as body fossils or as imprints. The body fossils can be found inside of the carbonate concretions preserved in three dimensions, while the imprints usually reside within the diatomite (Dyke and Lindow, 2009, Waterhouse et al., 2008). Fossils are just as common in these concretions as they are in the diatomite (Pedersen and Buchardt, 1996).

The most abundant fossils within the carbonate concretions are insects (Petrulevicius et al., 2008). The insects collected from the formation consists of more than 20.000 specimens (Kristoffersen, 2002) and the fauna is dominated by winged insects with poor flying skills, such as damselflies, grasshoppers, lacewings, crane flies, etc., indicating that the means of transportation from land primarily was by wind (Larsson, 1975). Fossils from other animal groups and plants can also be found in the formation; birds such as the tropical trogon (Kristoffersen, 2002) and parrots (Waterhouse et al., 2008), marine osteoglossomorph fishes, that today only exists as freshwater forms (Bonde, 2008), sea-turtles (Nielsen, 1959), and plant remains including angiosperm wood with internal microstructures, leaves of ginkgo, fruits, etc. (Sakala and Gyrc, 2011, Larsson, 1975).

## 2.2 The Green River Formation

The Green River Formation (GRF) is a complex series of foreland basins that were deposited during the early and middle Eocene. Strata of the formation crops out in the western United States and spans the three states, Wyoming, Colorado and Utah (Fig. 3) (Smith et al., 2008). The basins are separated from one another by several tectonic events; e.g. the Laramide orogeny that was active between the Cretaceous and Eocene from time to time. The GRF has a maximum thickness of 2 kilometres and lies as a lens of mostly lacustrine limestones inside the Wasatch Formation. Covering these formations is the middle Eocene Bridger Formation (Leggitt et al., 2007).

The deposition took place in a subtropical climate with an annual temperature of 15 to 21° C (Ferber and Wells, 1995). As indicated by the fossil fishes and invertebrates, this ancient lake system was a freshwater realm surrounded by subtropical woodlands, as indicated by fossil plants (Ksepka and Clarke, 2010). There were three major lakes within the system, each with great differences in chemistry; Lake Uinta, Lake Gosiute and Fossil Lake (Nesbitt et al., 2011, Smith et al., 2008). The basins were repeatedly drained an refilled, so that the lakes fluctuated both in volume and extent (Leggitt et al., 2007). Because of the orogeny, volcanism was extensive over the north western United States during the deposition of the formation, spreading both volcaniclastic material and tuffs. Besides the increase in sediment supply to the basins the volcanism also had an important effect on the deposition by increased rainfall

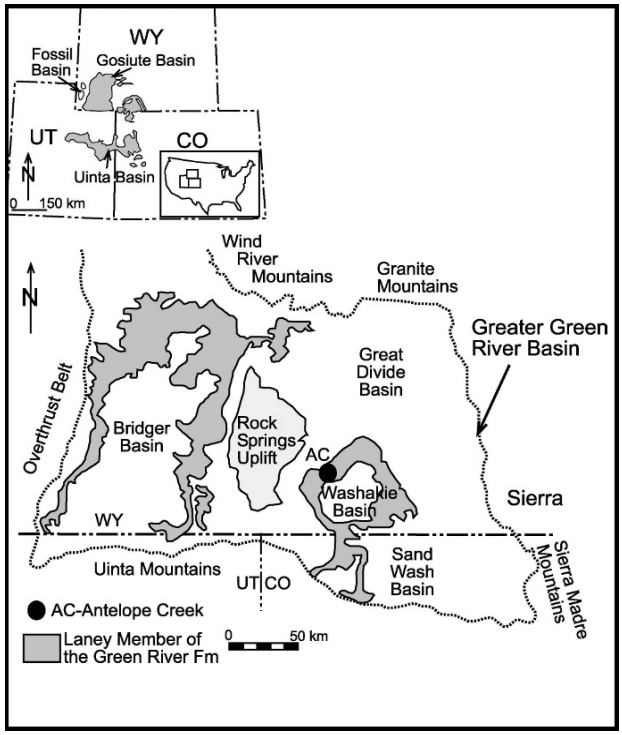


*Fig.3*. Location of the Green River Formation. The inset-map shows the extension of the three major basins crossing the three states Wyoming, Colorado and Utah. Modified from Ksepka & Clarke (2010).

and regional doming of the landscape (Smith et al., 2008).

The Green River Formation has been subdivided into three main facies associations based on lithologies and fossils; fluvial lacustrine, fluctuating profundal and evaporites. There is an abundance of molluscs in the fluvial lacustrine deposits that comprise sandstone, mudstone and limestone. The fluctuating profundal facies have occasional fish fossils and consists of organic-rich mudstone, oolites and stromatolites. The evaporites are correlated with the early Eocene climatic optimum and are thought to represent deposition in hyper saline lakes during periods of more arid climate (Smith et al., 2008).

With the formation being a Konservat Lagerstätte the fossil fauna is exceptionally preserved and best known for its plant and fish fossils (Ingalls and Park, 2010). Well over 300 000 fossil insect have been collected, representing well over 100 described families. Over 70 percent of these insects are exceptionally preserved and in some cases small structures, such as ears are preserved (Plotnick and Smith, 2012). The avian fauna of the GRF is mostly collected from Fossil Lake and the Fossil Butte Member, and in total 19 species have been described (Nesbitt et al., 2011). In addition to birds, such as parrots (Ksepka and Clarke, 2012) and frogmouths (Nesbitt et al., 2011), the GRF also includes, e.g., crocodiles, frogs and bats (Ferber and Wells, 1995).

# 3 Pigments and pigment organelles

The colour of an animal is a consequence of the absorption and reflection of certain wavelengths of visual light by some pigments and/or by tissue nanostructures that scatter light, that are perceived as different colours, so called structural colour (McNamara et al., 2011b). Colour and colour patterning is important for many organisms, for protection against UV-radiation and for intra- and interspecific communication, including camouflage, mimicry, and sexual signalling.

## 3.1 Melanin and melanogenesis

The most widespread form of colour in the animal kingdom is melanin pigmentation. There are two main forms of melanin; eumelanin and pheomelanin (McGraw et al., 2005). Where the former gives black, grey and brown colouration, the latter provides a lighter red-brown and brownish yellow colouration. Melanin in skin provides protection against UV-radiation by absorbing it and converting it into heat (Park et al., 2009).The chemical structure of melanin has previously been difficult to decipher owing to the fact that melanin basically is insoluble (Liu and Simon, 2003). Today, as research of new techniques for the characterization of melanin has developed further, and the melanosome and many of its proteins have been elucidated (Park et al., 2009).

Both eumelanin and pheomelanin are derived from the amino acid tyrosine and are produced within melanosomes in melanocytes (Fig. 4a). The melanocyte is a highly dendritic cell forming part of the epidermis. According to (Marks and Seabra, 2001) there are four stages of maturation for the melanosome (Fig. 4b); the first stage where pigments are absent, but in stage two and three intraluminal fibres develop that serve as templates for melanin. The melanin density increases until the melanosome has matured in stage four and no internal structures can be seen. During the maturation, structural proteins and enzymes are transported to the melanosome. The finished melanosome is transported from the

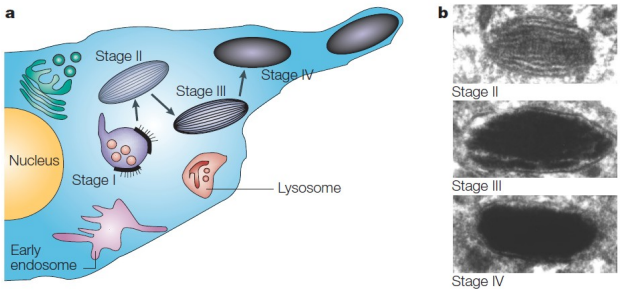


*Fig.4*. (A) A schematic diagram of the dendritic part of a melanocyte where the melanosomes form and mature and are later transported to the keratinocytes in skin and feathers. (B) Electron micrograph of the maturing stages two to four. From Marks and Seabra (2001).

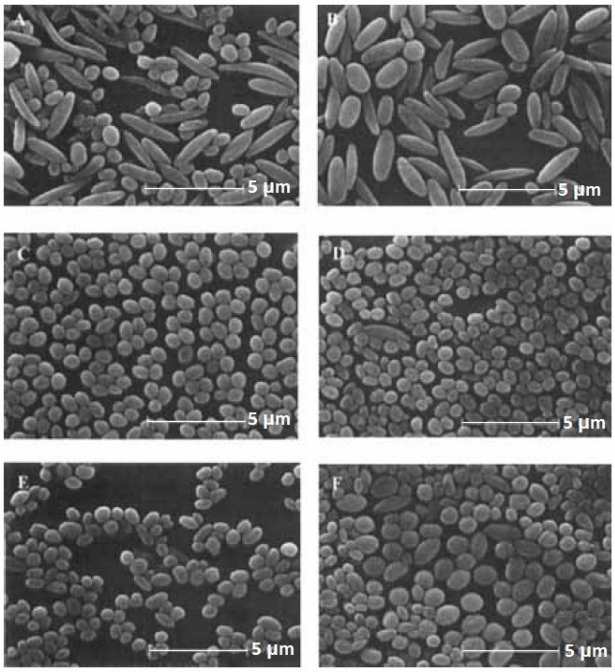


*Fig.5*. The different shapes of melanosomes in which all contain the pigment eumelanin. The melanosomes are extracted from different tissues in bovine eyes. Modified from Liu and Simon (2005).

melanocyte to the neighbouring keratinocytes where the melanosome is incorporated into skin and feathers (Marks and Seabra, 2001). Melanosomes are small, about 1 µm long, and can be either elongated or rounded (Fig. 5).

Eumelanin are high molecular weight polymers (Needham, 1978), that in insects are synthesized by the animal itself and then disperse it into their circulatory system (the hemolymph). (Needham, 1978), presumes that eumelanin in insects most likely always is black. Pheomelanin has not been detected in insects. Pheomelanin is different from eumelanin not only in colour but also in that pheomelanin incorporates the sulphur-containing amino acid cysteine and hence is a soluble polymer (Park et al., 2009, Menon et al., 1987). Pheomelanin is equally important as eumelanin in pigmentation of bird feathers (McGraw et al., 2005).
In feathers, the melanosomes are incorporated in keratin, a fibrous intricate helical structure derived from β-sheet proteins that constitute the feather. The melanosomal arrangement together with the keratin will scatter light and produce a certain colour. Melanin also makes the feather both thicker and harder which is efficient for resisting physical wear (Bonser, 1995). Melanin is also the most common pigment in the cuticle of insects apart from the sclerotization (hardening) of cuticles that produces a brown tint. Sometimes melanisation can be induced by physical injury and colour non-melanised cuticles (Richards, 1978).

## 3.2 Ommochromes

Ommochromes are a class of pigments, first found by Becker, 1941, that are located in the ommatidia (Fig. 7), the facets that comprise a compound eye (Exner, 1891, Needham, 1978, Stavenga, 1989). Ommochromes is derived from the amino acid with the highest molecular weight of them all; tryptophan. The content of tryptophan in animal proteins is extremely small (~1wt %) (Linzen, 1974). Ommochromes, together with pterins and carotenoids, constitute the so called screening pigments in insect eyes. Almost all types of eyes have screening pigments, which works as pupils to control the amount of incoming light to the photoreceptors by absorbing different wavelengths of light. The pigment granules can, in order to adapt to light conditions, undergo extensive movement towards and away from the lens. This can be seen from the outside by that the eye changes colour dramatically. If screening pigments were absent, large amounts of light from different directions would enter the compound eye, thus making the visual image unclear (Stavenga, 2002).

The screening pigment cells contain 0,5 µm sized, densely packed granules that are composed of the screening pigments bound to proteins (Stavenga, 2006). For insects there is a common classification of the pigment cells depending on their location in the ommatidia. The primary pigment cells (PPCs) are found enclosing the crystalline cone (Fig. 6) and the secondary pigment cells (SPCs) cross the retina from the cornea to a base-

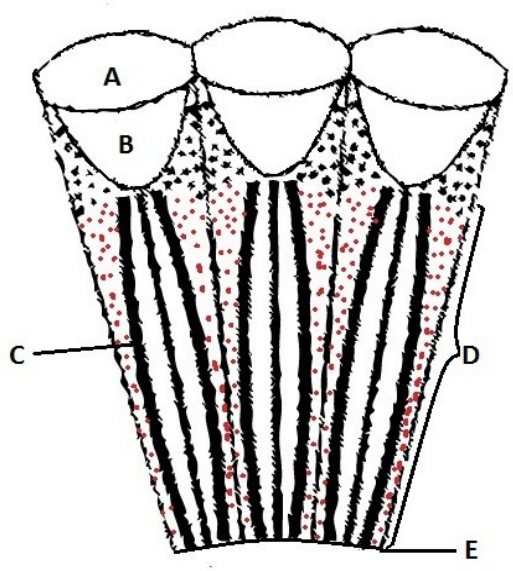


*Fig.6*. A schematic drawing showing three ommatidia and the positions of the screening pigments. The black dots represent the position of the primary pigment cells that enclose the crystalline cone. The red dots represent the position of the secondary pigment cells that cross the retina (D) down to the basal membrane (E). (A) The facet lens. (B) The crystalline cone or pseudocone. (C) The visual pigments that comprise the so called rhabdom (black long lines). Light enters straight through the lens and crystalline cone and passes through the opening in the middle between the rhabdomere. Modified from Stavenga (2006).

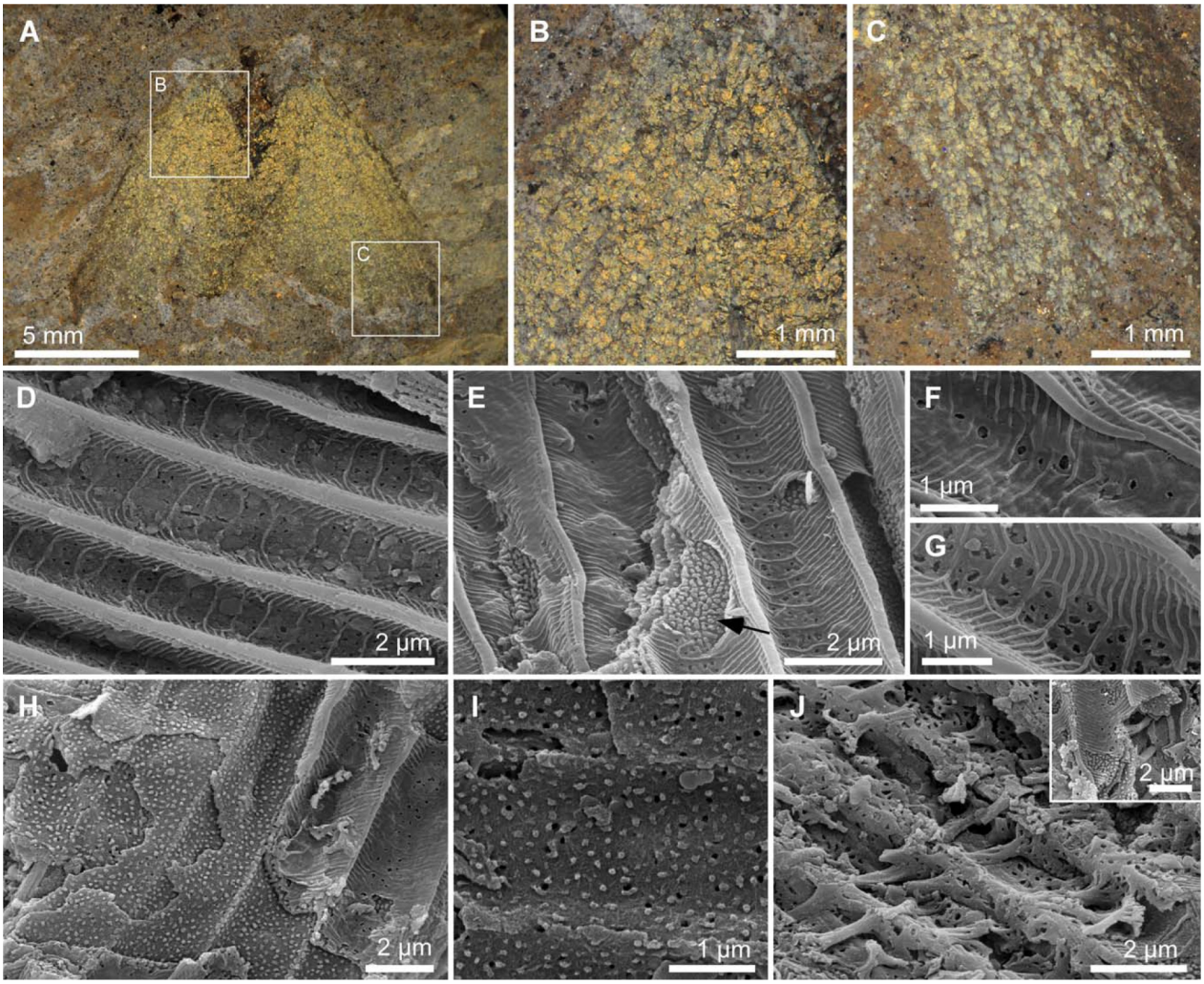


*Fig.7*. Structurally coloured lepidopterans from the Messel Formation. (A–C) Light micrographs of one of the specimens with details of marked areas (B, C). (D–J) Scanning electron micrographs of scales showing the different nanostructures that produce structured colour. Scale bars: (A) 5 mm; (B, C) 1 mm; (D, E, H, J) (including inset) 2 mm; (F, G, I) 1 mm. From McNamara et al. (2011b).

ment membrane (Stavenga, 1989). In many insect eyes the screening pigment is black to absorb all wavelengths in order to stray light from activating the photoreceptors but it may also come in brownish, bright red and dark purple colours. As in melanin, ommochrome has ion-exchange properties and takes part in anti-oxidation processes in the photoreceptors. Ommochromes can also work as a reservoir for calcium in insect eyes (Ukhanov, 1990; Stavenga, 1989).

# 4 Colour in the fossil record

## 4.1 Insects

One of the first indications of preserved original colours, found by (Stankiewicz et al., 1997), was the intact structures of the chitinous cuticles in 25 million year old fossil beetles from the Enspel Fossillagerstätte that matched those of its living counterparts. When conducting pyrolysis-gas chromatography- mass spectrometry, amino acids were found, i.e. tyrosine and tryptophan; precursors of melanin and ommochrome, respectively. In another study the complete setup of layers, alternating between high and low refractive index, causing iridescent colour were preserved in the elytra (chitinous forewing) of a fossil beetle. When drying the sample the iridescent colour disappeared, because water was the main component of the low refractive index layers (Parker and McKenzie, 2003).

A more recent study demonstrated the presence of a golden yellow metallic colour of the wing scales in fossil moths (Lepidoptera) (McNamara et al., 2011b) (Fig. 7A-C). The specimens were examined using techniques such as SEM, EDX and TEM (transmission electron microscopy). The electron dispersive X-ray analysis showed that the scales were organically preserved indicated by the dominance of carbon and lack of authigenic minerals. The measured reflectance peak on parts of the forewings generates blue colour ($\lambda max$= 473 nm) while the calculated reflectance peak generates a yellow-green colour ($\lambda max$= 565 nm); therefore this observed colour is an artifact resulting from diagenetic alteration of the biomolecules. Three-dimensional struc-

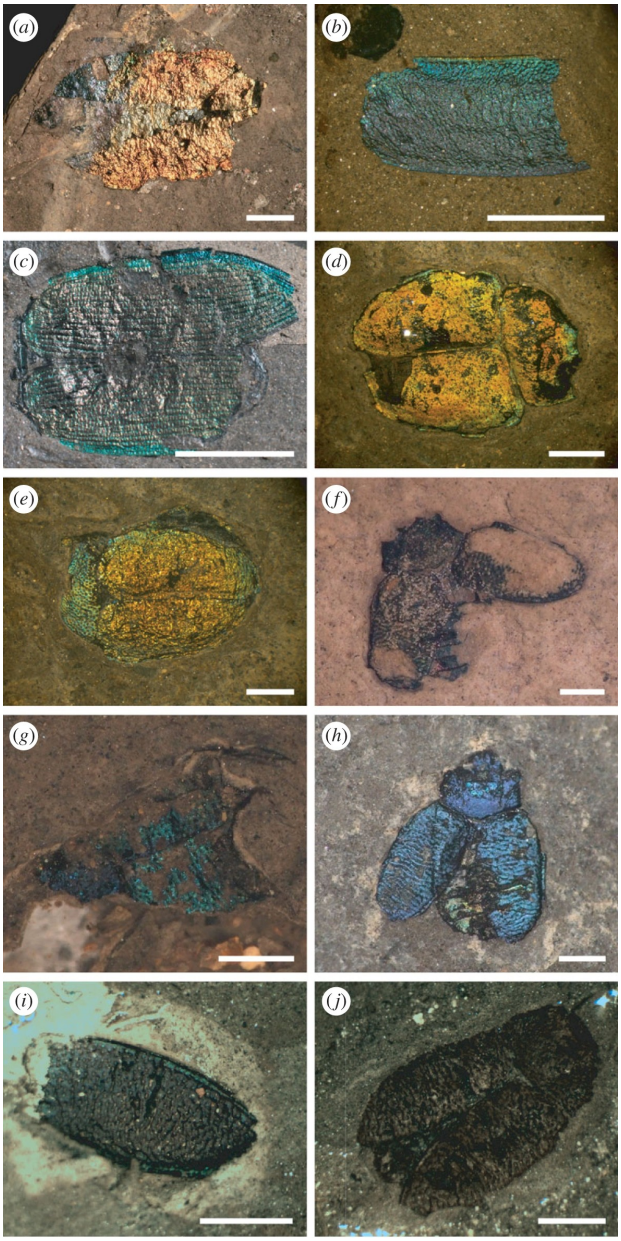


*Fig.8.*Cenozoic beetles examined by McNamara et al., (2011a) using SEM, TEM and reflectance microspectrophotometry. The colour producing nanostructures are intact, however the biomolecules have been altered (A–H) Well-preserved specimens with highly reflective and metallic cuticle colours. (I-J) Poorly preserved specimens with no or dull metallic colouration. Scale bars: (A,C) 5 mm; (B,D–J) 1 mm.

tures are sensitive to compaction during diagenesis; however, the ultrastructures showed no signs of compaction or desiccation cracks from dehydration during diagenesis (Fig. 7D-J). The original colours of the insect could be reproduced based on the unaltered nanostructures and their refraction index and reflectance peaks. It should be pointed out that three-dimensional structures are susceptible to compaction processes so these findings could be rare.

A similar study was conducted by McNamara et al. (2011a), who investigated iridescent colours of fossil beetles from five different lacustrine Fossil Lagerstätten. All of the beetles showed metallic colours (Fig.8), a diagnostic feature of ultrastructures in extant beetle cuticles. Here, the measured and predicted reflectance peaks were different to each other when compared to one another, to suggest that the original colours had been changed at the molecular level during diagenesis. However, the nanostructures were seemingly unaltered.

## 4.2 Birds

The preservation of feathers is not uncommon in the fossil record; there are findings in strata of Mesozoic age but they become more abundant in sediments of Cenozoic age. About 50 deposits are known to produce fossil feathers (Vinther et al., 2008). Until recently, it was generally believed that there were five ways in which feathers are preserved. 1. As carbonized traces where the carbon in the fossil has a higher percentage of organic carbon compared to the surrounding matrix. 2. Bacterial autolithification, when feather-degrading bacteria are mineralized, forming a pseudomorph of the feather. 3. Imprintation, a carbonized trace with lithified bacteria that produce both a cast and a mold of the decayed feather. 4. In amber, where feathers are presumed to be dehydrated but otherwise unaltered; and 5. As molds in coprolites (Davis and Briggs, 1995).

The first findings of micrometre sized rounded structures in fossil feathers were interpreted as lithified bacteria (Fig. 9) (Davis and Briggs, 1995). This is not strange since bacteria is the most successful organism on the planet and degrade organic matter, such as feathers (Liebig, 2007). The question is if all fossil feathers showing these structures could exclusively be the preservation of bacteria. Fossil bacteria are known from Precambrian age but are otherwise scares from the Phanerozoic. Bacteria mainly range from 0,5 µm and 2 µm in the long axis and they are either rod-shaped (bacilli) or spheroidal (cocci). Bacteria commonly live in colonies and these can build complex adhesive extracellular structures called glycocalyx that together with the bacteria create a biofilm. The glycocalyx help dissolve nutrients for the bacteria and protects them from bacteriophages. How bacteria fossilize is not yet entirely understood, but the attraction of metal ions seems to play a significant role (Liebig, 2007).

In recent years various fossilized microstructures have been re-interpreted as melanosomes, i.e. pigment organelles (Knight et al., 2011). In one study by Vinther et al. (2008), a feather from the Crato Formation was examined using SEM-EDX analysis and the microstructures found within the feather were compared to those of extant birds. The fossil feather has dark- and white-coloured bands (Fig. 10). In the dark bands microstructures were present, whereas t in the white bands comprised only sedimentary matrix. If these structures were indeed bacteria, then why were they present only in the dark bands? There is, however, more feather-degrading bacteria on living extant dark coloured bird feathers

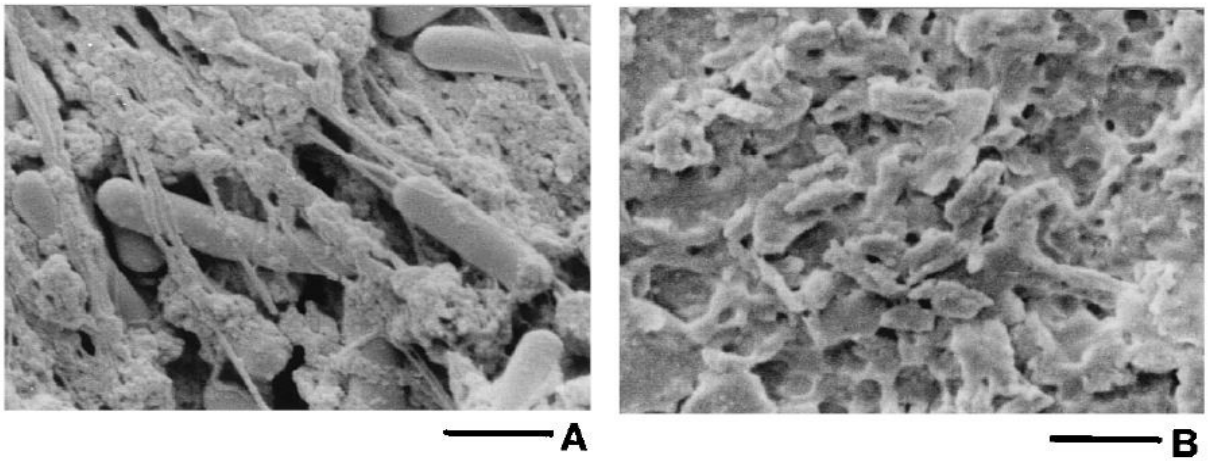


*Fig.9*. (A) Image of bacteria with glycocalyx on a feather from an extant bird, after decaying for one day in sub-tropical freshwater. (B) Showing fossil feather with honeycomb texture of inferred fossilized glycocalyx. Scale bar= 2µm. From Davis & Briggs (1995).

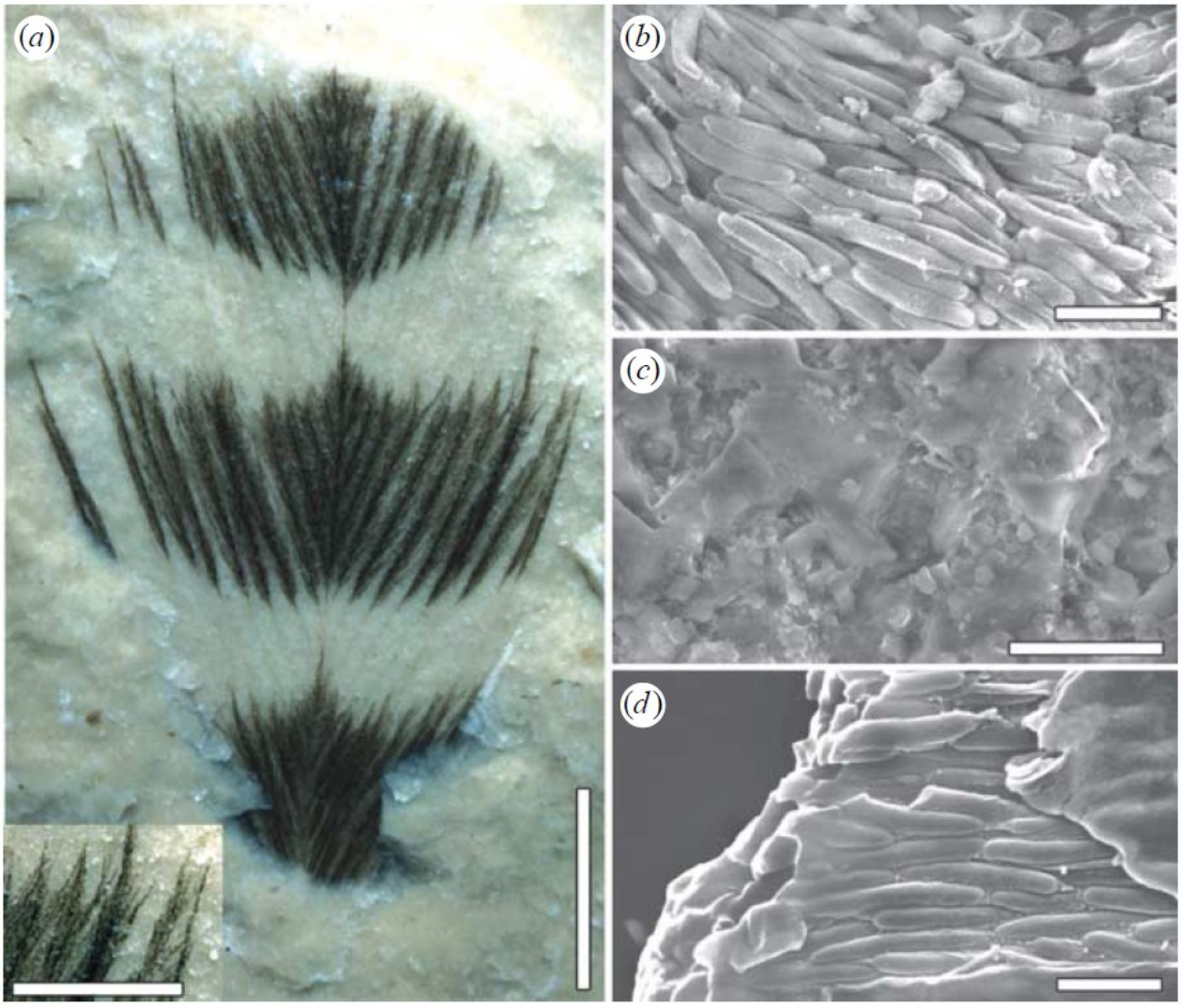


*Fig.10*. Ultrastructure of Cretaceous feather compared with that in a living bird. (A) Feather from the Early Cretaceous Crato Formation, Brazil, showing alternating dark-coloured bands with preserved barbules (inset). (B) The aligned eumelanosomes compose the dark bands whereas the light areas (C) reveal only the rock matrix. (D) A broken barbule from a modern Red-winged Blackbird (*Agelaius phoeniceus*) reveals eumelanosomes inside a matrix of β-keratin aligned along the barbule. Scale bars: (A) 3 mm, inset 1 mm; (B) 1 mm; (C) 10 mm; (D) 1 mm. From Vinther et al. (2008).

(Burtt and Ichida, 2004). The reason for this is that birds with dark melanin-rich feathers are most abundant in humid and warm climates where the pressure of bacteria is higher, rather than, that bacteria prefer melanin. This would indicate that melanin was evolved in feathers to protect the feathers from degradation by bacteria (Burtt and Ichida, 2004). Feather degrading bacteria like Bacillus licheniformis do feed on keratin, but there is no reports that they feed on melanosomes. Further, white feathers also do degrade much faster than dark feathers (Gunderson et al., 2008).

Based on the similarity with extant feathers the micro-bodies and their pattern found on the feather from the Crato Formation, are interpreted as fossilized eumelanosomes (Vinther et al., 2008). The feather also contained carbon like most fossil feathers, which likely is derived from the melanosomes, according to the authors. In another study by Vinther et al., (2010), brownish-red and green coloured feathers were examined. This colour is presumed to be a result of diagenetic alteration. The feathers are mainly carbonaceous and the alleged melanosomes cover the entire surface of the feathers. According to Vinther et al. (2010) the rod-shaped melanosomes are aligned side by side inside degraded keratin and mainly display two kinds of arrangement. These arrangements, in accordance with extant feathers would produce colours of iridescent blue, green or copper. However, since the keratin has degraded, its thickness cannot be determined, something that affects the exactness of the predicted colour (Vinther et al., 2010). A similar study (Barden et al., 2011) also found carboxylic acid functional groups that presumably belonged to eumelanin in the darker regions of fossil feathers. Further studies by Zhang et al. (2010) and Carney et al. (2012) examined bird feathers and dinosaur feathers, respectively. Both studies concluded that both eumelanosomes and pheomelanosomes were present but not authigenic bacteria. They argued that the bodies they found inside the inferred feather keratin were melanosomes, since the melanosomes are incorporated inside keratin in extant feathers, and that no authigenic minerals, such as pyrite were present.

Another way to determine fossil melanosomes would be to perform detailed chemical analyses as done by Wogelius et al. (2011) on two fossil birds. The distribution of biologically important metal ions, like Ca, Cu, Co and Zn, also give an indication of the melanin distribution. In extant birds, melanic keratin can sequester available copper and can be correlated with pigment density. The analyses showed that Cu was enriched within both the feathers and the inferred fossil melanosomes. In the second fossil no melanosomes were preserved, however the Cu concentrations formed discrete patches consistent in size with melanosome dimensions. In this way, pigments can be identified even after the melanosomes have been destroyed.

A newly published article by Lindgren et al. (2012), investigate the possibility to examine the chemical properties of melanosome-like structures in the eye of a fossil fish from the Fur Formation (Fig. 11A). The main

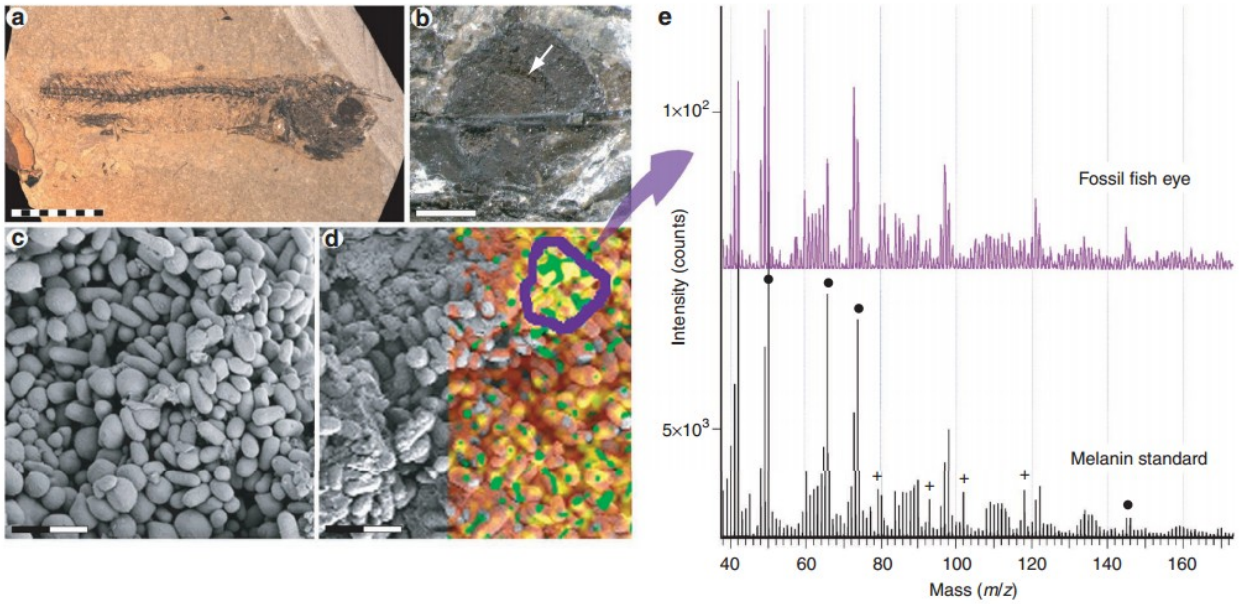


*Fig.11*. Negative ion ToF-SIMS spectra of melanin and microbodies from the fish fossil of the Fur Formation, Denmark. (A) Optical photograph of the specimen. Scale bar: 10 mm. (B) Close-up of the brownish matter located in the eye; position of the area analysed with SEM, TEM, ToF-SIMS and IR microspectroscopy indicated by an arrow. Scale bar: 1mm. (C) A detailed SEM image of the eye, showing preserved fossil melanosome-like bodies. Scale bar: 2 µm. (D) A semi-transparent ion image showing the distribution of melanin-derived ions belonging to synthetic and natural melanin spectra; the purple circle marks the area from which the mass spectrum presented in (E) was collected. Scale bar: 2 µm. (E) Negative ToF-SIMS spectra of the eye of the fossil and a natural melanin standard below. Note the nearly identical appearance of the two spectra. From Lindgren et al. (2012).

method of this investigation was time-of-flight secondary ion mass spectrometry (ToF-SIMS). This method is an excellent tool for mapping the chemical structures of biological samples with high resolution since all chemical compounds including melanin has a unique signature. The study also combined infrared microspectroscopy, TEM and SEM. The results of the ToF-SIMS on the fossil eye revealed mass spectra with peak positions and intensities matching those of nitrogen-rich melanin (Fig. 11E).

# 5 Material and methods

## 5.1 Fossil specimen

A nearly complete fossil insect from the diatomite of the Fur Formation, taxonomically ascribed to the order Diptera (flies) and the family Ptychopteridae (phantom crane flies) by Freiwald and Rainer (1992) (Fig. 12A). The eyes of the crane fly were of particular interest because these structures contain ommochromes in extant insects. The two compound eyes are approximately 2 mm in diametre.

One small feather from an unknown bird collected from the diatomite of the Fur Formation. The fossil specimen is ca 19 mm in length and 7 mm at its widest part. The feather is borrowed from the collection of Museum Salling-Fur Museum and has the catalogue number FUM 1980 (Fig. 12B)

Two bird feathers of unknown taxonomy collected from the Eocene Green River Formation. The first feather (GRF1) measures ca 14 mm in length and 2 mm in maximum width (Fig. 12C). The second feather (GRF2) is 12 mm long and about 2 mm wide (Fig. 12D).

## 5.1 SEM and EDX

The total of five samples were analysed with scanning electron microscopy (SEM) and energy dispersive x-ray spectrometry (EDX or EDS). A small area was chosen on each fossil and enhanced to get a good view of potential microstructures. For the EDX analyses a larger section of each fossil was chosen in which both the fossil and the surrounding matrix were in view. This was

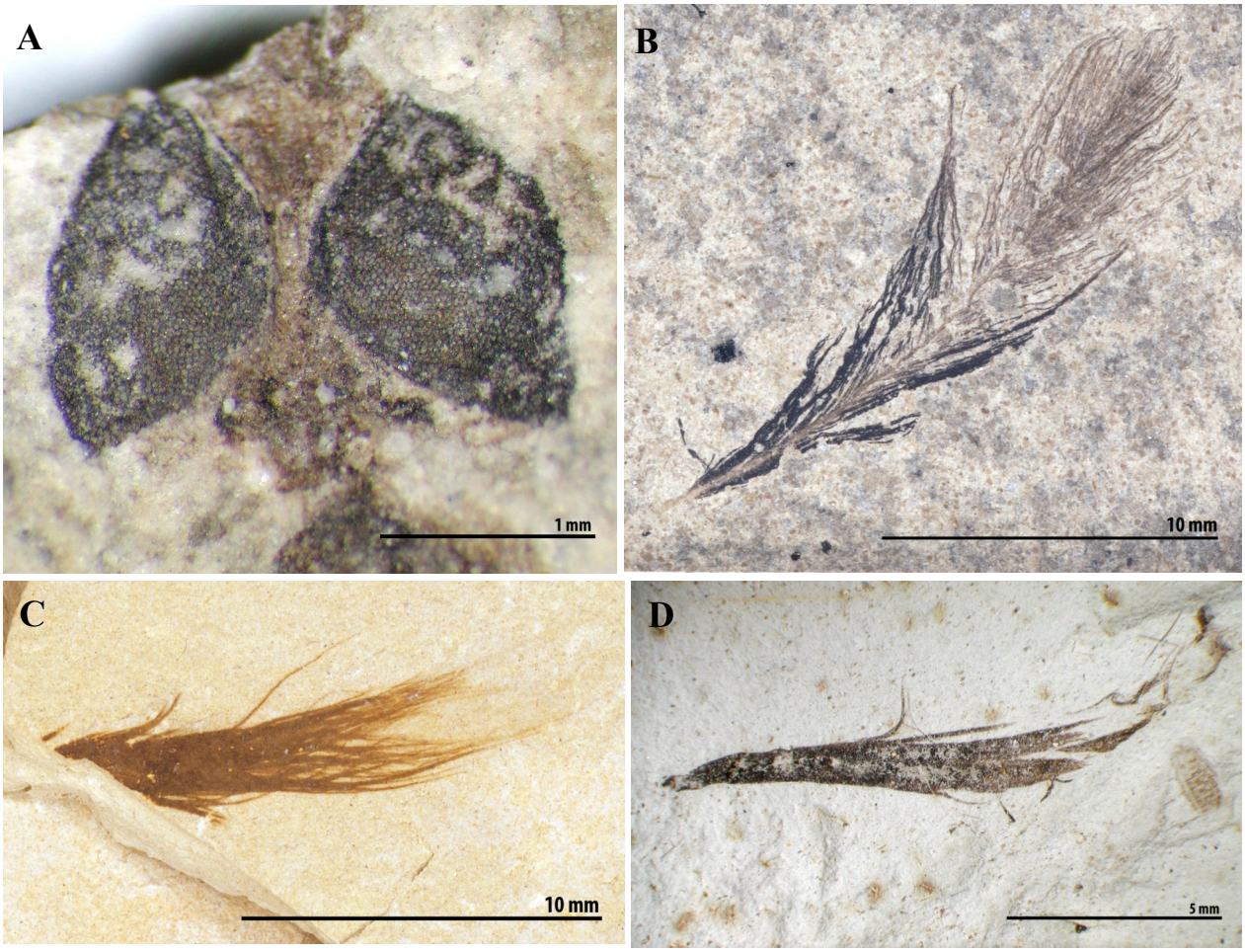


*Fig.12*. (A) The head of the crane fly from the Fur Formation. Note the small dot-like features, each representing a fossilized facet len of the compound eye.Scale bar: 1mm. (B) Photograph of the fossil feather FUM 1980, from the Fur Formation, Denmark. Scale bar: 10mm. (C) Photograph of a fossil feather (GRF1) from the Green River Formation, USA. Scale bar: 10 mm. (D) Photograph of a second fossil feather (GRF2) from the Green River Formation, USA. Scale bar: 5mm.

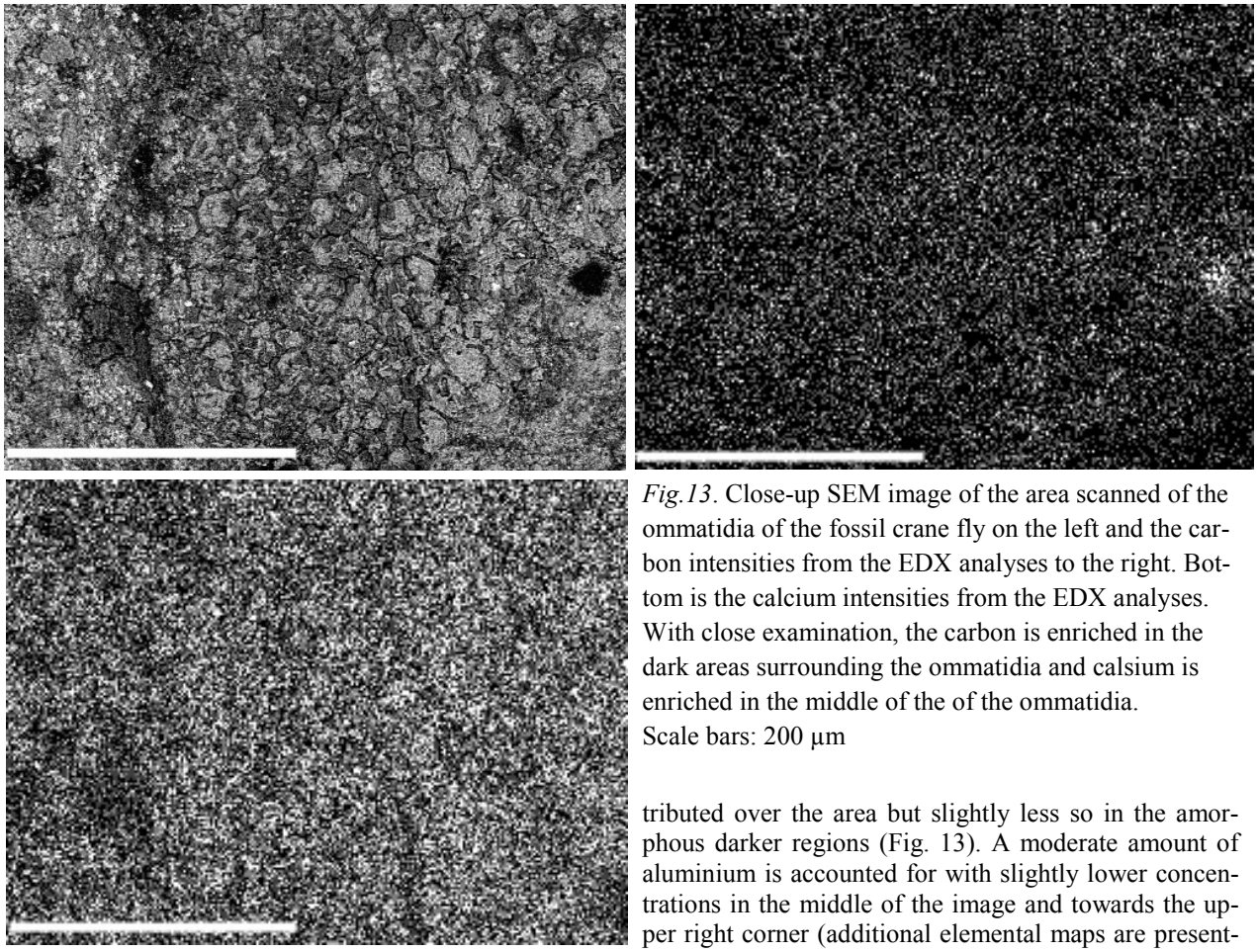

*Fig.13*. Close-up SEM image of the area scanned of the ommatidia of the fossil crane fly on the left and the carbon intensities from the EDX analyses to the right. Bottom is the calcium intensities from the EDX analyses. With close examination, the carbon is enriched in the dark areas surrounding the ommatidia and calsium is enriched in the middle of the of the ommatidia.
Scale bars: 200 µm

done in order to compare the chemical compositions of the fossils with their surrounding matrices. Each specimen was handled carefully with gloves to prevent contamination. The samples were attached to glass thin sections with carbon tape, measured and inserted into the sample-container in the SEM.

EDX-analyses show X-ray spectra of the sample, where different peaks in the spectra are specific for any given element. When running the sample through the EDX it sends out a high energy beam that excites the atoms in the sample. Each time an atom is hit by the beam it emits an x-ray which is counted; this is represented by a white dot in the EDX picture. One image per element was created and the running time for each fossil scan varied around 5 minutes.

# 6 Results from SEM and EDX

## 6.1 Crane fly

Energy dispersive X-ray of the left eye of the crane fly shows a slightly elevated carbon signal in the darker regions of the ommatidia and in the dark region in the middle right and in several amorphous spots in the left part of the scanned surface (Fig. 13). There is an abundance of oxygen across the entire area with slightly elevated intensities at the lower right side and towards the left side. Calcium is also high and ubiquitously distributed over the area but slightly less so in the amorphous darker regions (Fig. 13). A moderate amount of aluminium is accounted for with slightly lower concentrations in the middle of the image and towards the upper right corner (additional elemental maps are presented in Appendix figure 1). Sparse amounts of both nitrogen and sodium are evenly spread over the entire area. The distribution pattern of silica is somewhat comparable to that of aluminium; apart from there being more silica. Moderate amounts of both magnesium and iron are evenly scattered over the surface with slightly higher intensities of iron. The sulphur content is also moderate without any particular pattern .

## 6.2 FUM 1980

Carbon occurs in moderate amounts in the matrix and large amounts in the feather (Fig. 14). Large amounts of calcium occur over the entire surface but with slightly higher intensities in the matrix compared to the feather (additional elemental maps are presented in Appendix figure 2). There seems to be a slightly smaller amount of aluminium in the feather compared to the matrix. Both chlorine and iron occur in moderate amounts, as do manganese and nitrogen. Magnesium exists in moderate amounts in a fairly even distribution, but with a possibly higher amount in the matrix. Oxygen and silica occur in large amounts and is concentrated to the matrix. Sulphur is moderately abundant in the feather. Additionally, from a previous study of the feather a high-resolution SEM image of small elongate bodies around 1.0-1.5 µm in length can be seen (Fig.14).

## 6.3 GRF1

The concentration of carbon is higher in the feather than

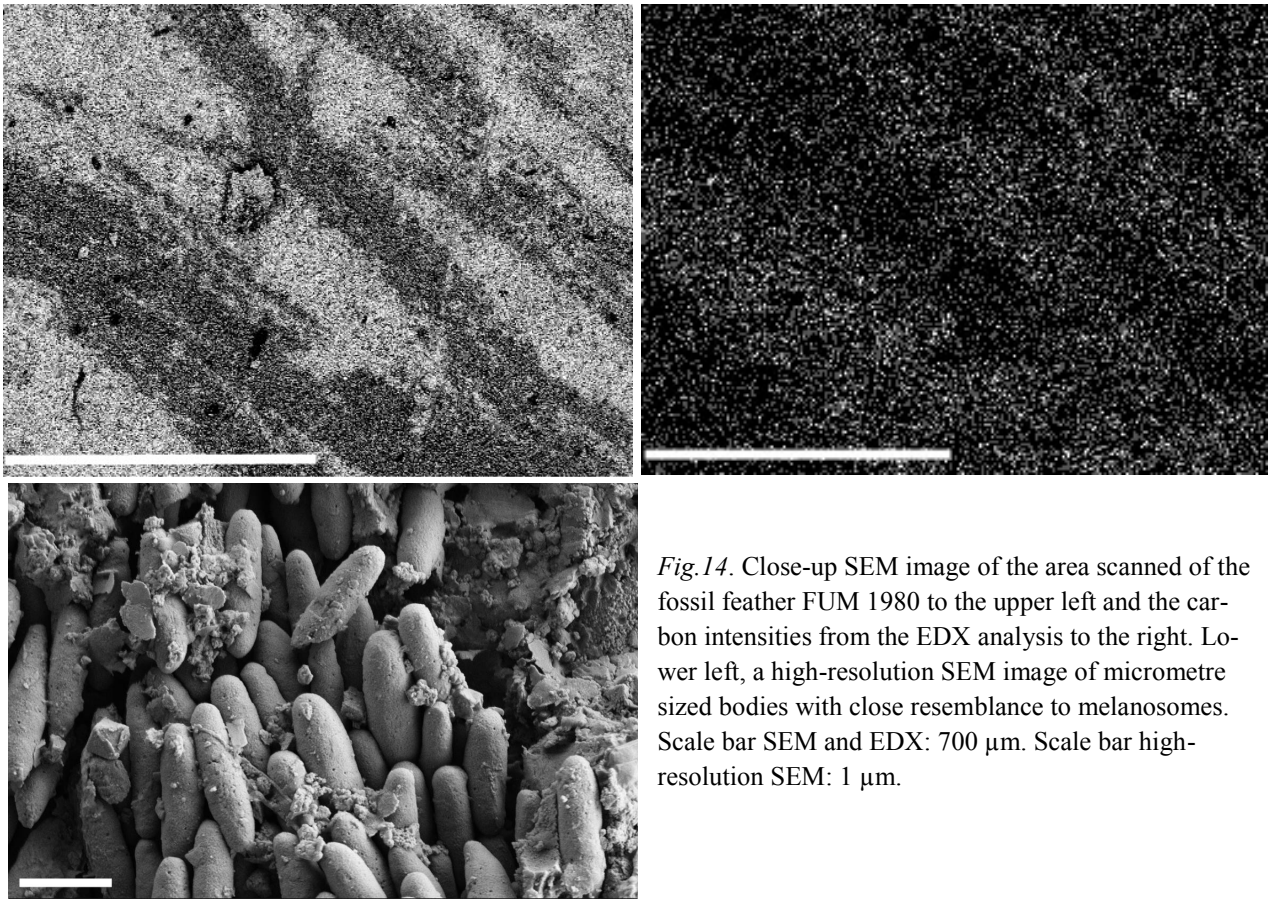

*Fig.14*. Close-up SEM image of the area scanned of the fossil feather FUM 1980 to the upper left and the carbon intensities from the EDX analysis to the right. Lower left, a high-resolution SEM image of micrometre sized bodies with close resemblance to melanosomes. Scale bar SEM and EDX: 700 µm. Scale bar high-resolution SEM: 1 µm.

in the matrix (Fig. 15). Calcium (additional elemental maps are presented in Appendix figure 3), fluor and iron are also present in the feather to a greater extent than in the matrix. Aluminium is abundant and has a higher affinity for the matrix, as do potassium and sodium which occur in moderate amounts. The intensity of magnesium is moderate and seems to be concentrated to the matrix. Nitrogen is moderate with no specific distribution pattern. A moderate amount of sulphur exists primarily in the feather. There are high concentrations of silica in the matrix. Oxygen is abundant and pervasive in both the feather and in the matrix.

## 6.4 GRF2

This feather consists mainly of carbon (Fig. 16). Aluminium (additional elemental maps are presented in Appendix figure 4) and calcium exists in large amounts but are a bit more concentrated in the matrix. The intensities of iron and nitrogen are low to moderate and ubiquitously distributed. Potassium and magnesium are abundant with slightly higher intensities in the matrix. Abundant amounts of oxygen and silica both with a higher concentration in the matrix.

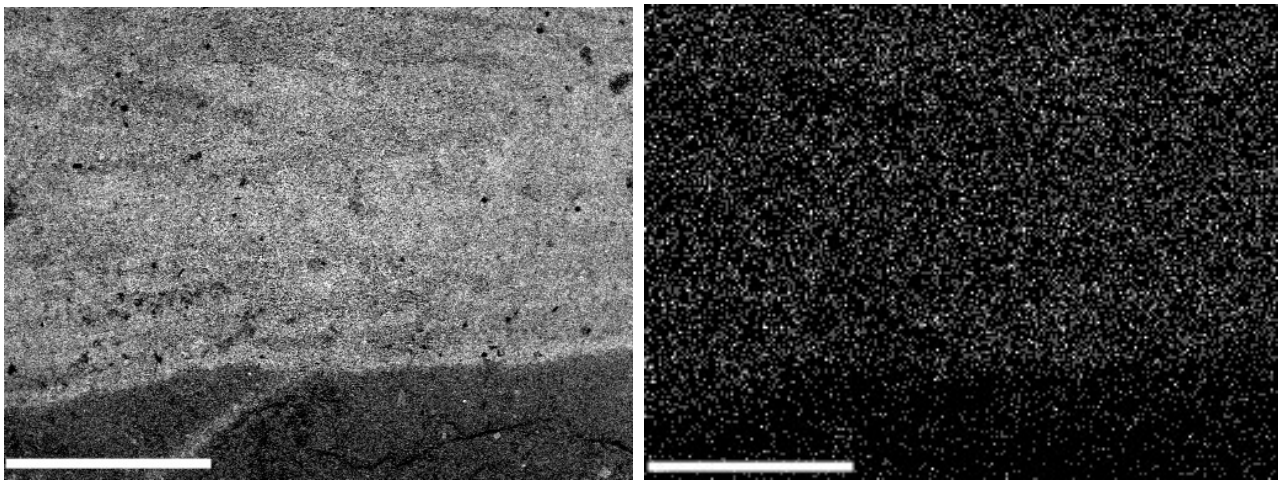

*Fig.15*. On the left, a close-up SEM image of the area scanned of the fossil feather GRF1 from the Green River Formation, USA. On the right is the carbon intensities from the EDX analysis. Scale bars: 1 mm.

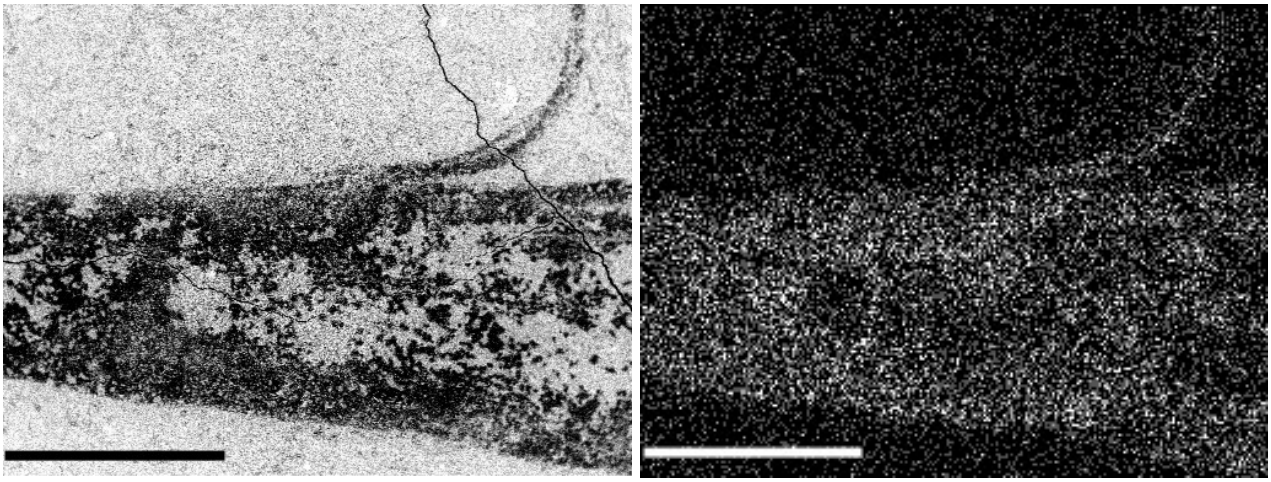

*Fig.16.* To the left, a close-up image of the fossil feather GRF2 from the Green River Formation, USA. To the right are carbon intensities from my EDX analysis. Scale bars: 1 mm.

# 7 Discussion

The carbon signal seems to be elevated in the darker regions surrounding the visible ommatidia in the preserved compound eyes of the crane fly. This could indicate that the pigment granules surrounding the crystalline cone are still present in the specimen; however, the resolution of the EDX image is not high enough for a confident determination. The crystalline cone and the lens are made up of pure CaCO3 crystals and the calcium signal is indeed higher in these areas compared to the matrix. This could be evidence of a crystalline cone and lens being unaltered during diagenesis. The surrounding matrix is mostly made up of silica from the diatom frustules, but there is a small amount of calcium present in the diatomite. The signal of calcium in the sediment is, however, much lower than in the specimen. Ommochrome also seem to work as a calcium reservoir (Ukhanov, 1990) which could perhaps contribute to the amount of calcium seen in the specimen.

In all of the feathers the carbon was evidently enriched compared to the surrounding matrix. These carbonized imprints as they are sometimes referred as (see, e.g., (Davis and Briggs, 1995)), are with all likelihood, not as the words imply altered into pure carbon or graphite during diagenesis. This carbon probably originates from biological tissues as my analyses indicate (Davis and Briggs, 1995). A bacterial origin is also possible; however, the lithification process where minerals replace tissue takes time and a bacterium would readily be dissolved after death by autolytic processes. These processes can in turn be slowed or seize if the bacterium accumulates metal ions (Liebig, 2007).

The oblong shaped bodies in the FUM 1980 (Fig. 14), were aligned “head to toe” in an organized matter. Authors like Vinther et al. (2010) argues that bacteria do not arrange themselves in this way. However, the shape of the bodies is not enough to determine whether they were bacteria or melanosomes since they are similar in both appearance and size (Barden et al., 2011). Another problem with shape is that eumelanosomes can be both rod-shaped and spherical, and in that regard similar to pheomelanosomes and can thus easily be mistaken for one another (Liu et al., 2005). However, the solubility of pheomelanin could affect its preservation potential negatively.

The fossil feathers are also enriched with sulphur; this could possibly be a residue of the sulphur component in pheomelanin. Around 10wt% sulphur is incorporated in pheomelanin (Menon et al., 1987). Still, there is no way of knowing if pheomelanin is present without further investigations. Copper as well as zinc exists only in very small quantities and only in the feather from Anchiornis huxleyi. Trace metals, such as copper and zinc, can be a diagnostic for melanin; however, this cannot prove a melanin content in this case without additional methods.

# 8 Conclusions and summary

Concerning the preservation of the ommatidia in the fossil crane fly, there are indications that the ommatidial lens and or the crystalline cone are preserved to some extent. There are also enhanced intensities of carbon in the dark areas surrounding each ommatidium that indicate a preservation of the screening pigments, preferentially dark-coloured ommochrome. No sign of any bacterial biofilms or bacterium.

The carbon intensities in the feathers suggest there is organic matter preserved, and the elongate microstructures in FUM 1980 indicate that the source of the carbon signal could be that of preserved melanosomes. With the insolubility and abrasion resistance of eumelanin it is possible that it could be preserved in favourable conditions.

With the accumulated knowledge of melanosomes and their morphology combined with the chemical properties and structure of melanin the provenance of the fossil micro-bodies can be evaluated properly. The preservation of natural melanin in million-year-old fossils can give us additional understanding of the evolution of pigment and colouration. Further investigation of

the fossil eyes and the four feathers are needed to be certain of any existence of pigments. One way to investigate melanosomes is to analyse fossil samples with infrared spectroscopy which give information on the molecule bonds which are specific for i.e. melanin and other pigments. As done in studies of fossil insects, the absorption peak is suitable for detecting number of double bonds, solvents, substituents, etc. in pigments since the peak positions are shifted systematically depending on these factors (Needham, 1978).

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# 10 References

Barden, H. E., Wogelius, R. A., Li, D. Q., Manning, P. L., Edwards, N. P. & Van Dongen, B. E. 2011: Morphological and geochemical evidence of eumelanin preservation in the feathers of the Early Cretaceous bird, *Gansus yumenensis*. *Plos One*, 6, 1-9

Bertelli, S., Lindow, B. E. K., Dyke, G. J. & Chiappe, L. M. 2010: A well-preserved 'Charadriiform-like' fossil bird from the early Eocene Fur Formation of Denmark. *Palaeontology*, 53, 507–531.

Bonde, N. 2008: Osteoglossomorphs of the marine Lower Eocene of Denmark—with remarks on other Eocene taxa and their importance for palaeobiogeography. *Geological Society, London, Special Publications*, 295, 253-310.

Bonser, R. H. C. 1995: Melanin and the abrasion resistance of feathers. *University of California Press*, 97, 590-591.

Brooks, K. 2006: When the Sun died over northern Europe: the unique geology of Denmark's inland islands. *Geology today*, 22, 180-186.

Burtt, E. H. & Ichida, J. M. 2004: Gloger's rule, feather-degrading bacteria, and color-variation among song sparrows. *Condor*, 106, 681-686.

Carney, R. M., Vinther, J., Shawkey, M. D., d'Alba, L. & Ackermann, J. 2012: New evidence on the colour and nature of the isolated Archaeopteryx feather. *Nature Communications*, 3, 1-6

Chambers, L. M., Pringle, M., Fitton, G., Larsen, L. M., Pedersen, A. K. & Parrish, R. 2003: Recalibration of the Paleocene-Eocene boundary (P-E) using high precision U-Pb and Ar-Ar isotopic dating. *EGS-AGU-EUG Joint Assembly Nice*. Abstract #9681

Davis, P. G. & Briggs, D. E. G. 1995: Fossilization of feathers. *Geology*, 23, 783-786.

Dyke, G. & Lindow, B. 2009: Taphonomy and abundance of birds from the Lower Eocene Fur Formation of Denmark. *Geological Journal*, 44, 365-373.

Exner, S. 1891: The physiology of the compound eyes of insects and crustaceans: a study. Germany, 177.

Ferber, C. T. & Wells, N. A. 1995: Paleolimnology and taphonomy of some fish deposits in "Fossil" and "Uinta" Lakes of the Eocene Green River Formation, Utah and Wyoming. *Palaeogeography Palaeoclimatology Palaeoecology*, 117, 185-210.

Freiwald, A. & Rainer, W. 1992: Insekten aus der Fur Formation von Dänemark (Moler, ob. Paleozän / unt. Eozän?). 7. Ptychopteridae (Diptera). *Meyniana*, 44, 179-187.

Gunderson, A. R., Frame, A. M., Swaddle, J. P. & Forsyth, M. H. 2008: Resistance of melanized feathers to bacterial degradation: is it really so black and white? *Journal of Avian Biology*, 39, 539-545.

Ingalls, B. R. & Park, L. E. 2010: Biotic and taphonomic response to lake-level fluctuations in the Greater Green River Basin (Eocene), Wyoming. *Palaios*, 25, 287-298.

Knight, T. K., Bingham, P. S., Lewis, R. D. & Savrda, C. E. 2011: Feathers of the Ingersoll Shale, Eutaw Formation (Upper Cretaceaous), Eastern Alabama: The largest collection of feathers from North American mesozoic rocks. *Palaios*, 26, 364-376.

Kristoffersen, A. V. 2002: An Early Paleogene Trogon (Aves: Trogoniformes) from the Fur Formation, Denmark. *Journal of Vertebrate Paleontology*, 22, 661-666.

Ksepka, D. T. & Clarke, J. A. 2010: New fossil mouse bird (Aves: Coliiformes) with feather preservation provides insight into the ecological diversity of an Eocene North American avifauna. *Zoological Journal of the Linnean Society*, 160, 685-706.

Ksepka, D. T. & Clarke, J. A. 2012: A new stem parrot from the Green River Formation and the complex evolution of the grasping foot in Pan-Psittaciformes. *Journal of Vertebrate Paleontology*, 32, 395-406.

Larsen, L. M., Fittonc, J. G. & Pedersen, A. K. 2003: Paleogene volcanic ash layers in the Danish Basin: compositions and source areas in the North Atlantic Igneous Province. *Lithos*, 71, 47-80.

Larsson, S. G. 1975: Palaeobiology and mode of burial of the insects of the Lower Eocene Mo-clay of Denmark. Bulletin of the Geological Society of Denmark, 24, 193-209.

Leggitt, V. L., Biaggi, R. E. & Buchheim, H. P. 2007: Palaeoenvironments associated with caddisfly-dominated microbial-carbonate mounds from the Tipton Shale Member of the Green River Formation: Eocene Lake Gosiute. *Sedimentology*, 54, 661-699.

Liebig, K. 2007. Bacteria. In: Briggs, D. E. G. & Crowther, P. R. (eds.) Palaeobiology 2. United Kingdom: *Blackwell Publishing*, 253-256.

Lindgren, J., Uvdal, P., Sjövall, P., Nilsson, D. E., Engdahl, A., Schultz, B. P. & Thiel, V. 2012: Molecular preservation of the pigment melanin in fossil melanosomes. *Nature Communications*, 3, 1-7.

Lindow, B. E. K. & Dyke, G. J. 2006: Bird evolution in the Eocene: climate change in Europe and a Danish fossil fauna. *Biological Reviews*, 81, 483-499.

Lingham-Soliar, T. 2011: The evolution of the feather: *Sinosauropteryx*, a colourful tail. *Journal of Ornithology*, 152, 567-577.

Linzen, B. 1974: The tryptophan-ommochrome path way in insects. In: Treherne, J. E. (ed.) *Advances in insect physiology*. Academic Press Inc, 10, 118-225.

Liu, Y., Hong, L., Wakamatsu, K., Lto, S., Adhyaru, B. B., Cheng, C. Y., Bowers, C. R. & Simon, J. D. 2005: Comparisons of the structural and chemical properties of melanosomes isolated from retinal pigment epithetlium, iris and choroid of newborn and mature bovine eyes. *Photochemistry and Photobiology*, 81, 510-516.

Liu, Y. & Simon, J. D. 2003: Isolation and biophysical studies of natural eumelanins: applications of imaging technologies and ultrafast spectroscopy. *Pigment Cell Research*, 16, 606-618.

Marks, M. S. & Seabra, M. C. 2001: The melanosome: membrane dynamics in black and white. *Molecular Cell Biology*, 2, 1-11.

Mcgraw, K. J., Safran, R. J. & Wakamatsu, K. 2005: How feather colour reflects its melanin content. *Functional Ecology*, 19, 816-821.

McNamara, M. E., Briggs, D. E. G., Orr, P. J., Noh, H. & Cao, H. 2011a: The original colours of fossil beetles. *Proceedings of the Royal Society B, Biological Sciences*, 279, 1114-1121.

McNamara, M. E., Briggs, D. E. G., Orr, P. J., Wedmann, S., Noh, H. & Cao, H. 2011b: Fossilized biophotonic nanostructures reveal the original colors of 47-million-year-old moths. *Plos Biology*, 9, 1-8.

Menon, I. A., Basu, P. K., Persad, S., Avaria, M., Felix, C. C. & Kalyanaraman, B. 1987: Is there any difference in the photobiological properties of melanins isolated from human blue and brown eyes? *British Journal of Ophthalmology*, 71, 549-552.

Needham, A. E. 1978: Insect biochromes: Their chemistry and role. In: Rockstein, M. (ed.) Biochemistry of insects. *Academic Press, inc.*, 233-305

Nesbitt, S. J., Ksepka, D. T. & Clarke, J. A. 2011: Podargiform affinities of the enigmatic *Fluvioviridavis platyrhamphus* and the early diversification of Strisores ("Caprimulgiformes" plus Apodiformes). *Plos One*, 6, 1-14.

Nielsen, E. 1959: Eocene Turtles from Denmark. *Bulletin of the Geological Society of Denmark*, 14, 96-114.

Orr, P. J., Kearns, S. L. & Briggs, D. E. G. 2002: Back scattered electron imaging of fossils exceptionally preserved as organic compressions. *Palaios*, 17, 110-117.

Park, H. Y., Kosmadaki, M., Yaar, M. & Gilchrest, B. A. 2009: Cellular mechanisms regulating human melanogenesis. *Cellular and Molecular Life Sciences*, 66, 1493-1506.

Parker, A. R. & Mckenzie, D. R. 2003: The cause of 5 0 million-year-old colour. *Proceedings of the Royal Society of London Series B, Biological Sciences*, 270, 151-153.

Pedersen, G. K. & Buchardt, B. 1996: The calcareous concretions (cementsten) in the Fur Formation (Paleogene, Denmark): isotopic evidence of early diagenetic growth. *Bulletin of the Geological Society of Denmark*, 43, 78-86.

Pedersen, G. K., Pedersen, S. a. S., Steffensen, J. & Pedersen, C. S. 2004: Clay content of a clayey diatomite, the Early Eocene Fur Formation, Denmark. *Bulletin of the Geological Society of Denmark*, 51, 159-177.

Petrulevicius, J. F., Wappler, T., Wedmann, S., Rust, J. & Nel, A. 2008: New megapodagrionid damsel flies (Odonata: Zygoptera) from the Paleogene of Europe. *Journal of Paleontology*, 82, 1173-1181.

Plotnick, R. E. & Smith, D. M. 2012: Exceptionally preserved fossil insect ears from the Eocene Green River Formation of Colorado. *Journal of Paleontology*, 86, 19-24.

Richards, A. G. 1978: The chemistry of insect cuticle. In: Rockstein, M. (ed.) *Biochemistry of insects*. 205-232

Rust, J. 2000: Fossil record of mass moth migration. *Nature*, 405, 530-531.

Sakala, J. & Gyrc, V. 2011: A new species of Rhysocaryoxylon (Juglandaceae) from the Lower Eocene Fur Formation of Mors Island (northwest Jutland, Denmark). *Bulletin of the Geological Society of Denmark*, 59, 44-48.

Smith, M. E., Carroll, A. R. & Singer, B. S. 2008: Synoptic reconstruction of a major ancient lake system: Eocene Green River Formation, western United States. *Geological Society of America Bulletin*, 120, 54-84.

Stankiewicz, B. A. & Briggs, D. E. G. 2007: Animal cuticles. In: Briggs, D. E. G. & Crowther, P. R. (eds.) *Palaeobiology II*. Blackwell publishing. 259-261

Stankiewicz, B. A., Briggs, D. E. G., Evershed, R. P., Flannery, M. B. & Wuttke, M. 1997: Preservation of chitin in 25-million-year-old fossils. *Science*, 276, 1541-1543.

Stavenga, D. G. 1989: Pigments in compound eyes. In: Stavenga, D. G. & Hardie, R. C. (eds.) *Facets of vision.* Germany: Springer-Verlag. 152-172.

Stavenga, D. G. 2002: Colour in the eyes of insects. *Journal of comparative physiology A, neuroethology, sensory, neural, and behavioral physiology*, 188, 337-348.

Stavenga, D. G. 2006: Invertebrate screening pigments. In: Warrant, E. & Nilsson, D. E. (eds.) *Invertebrate vision*. United States of America: Cambridge University Press. 10-13.

Ukhanov, K. Y. 1991: Ommochrome pigment granules: a calcium reservoir in the dipteran eyes. *Comparative Biochemistry and Physiology*, 98, 10-16.

Waterhouse, D. M., Lindow, B. E. K., Zelenkov, N. V. & Dyke, G. J. 2008: Two new parrots (psittaciformes) from the Lower Eocene Fur Formation of Denmark. *Palaeontology*, 51, 575-582.

Willumsen, P. S. 2004: Palynology of the Lower Eocene deposits of northwest Jutland, Denmark. *Bulletin of the Geological Society of Denmark*, 52, 141-157.

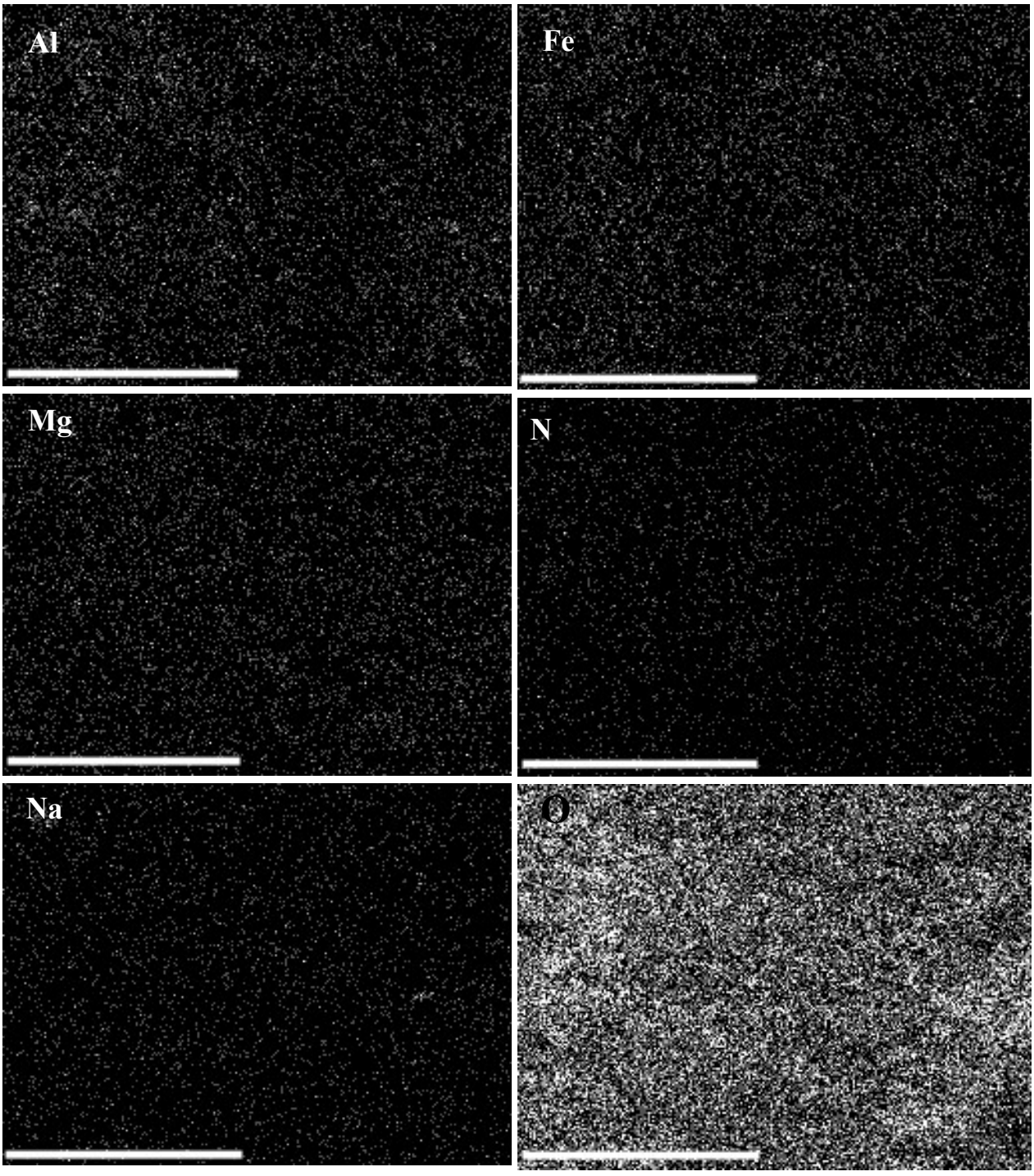
Vinther, J., Briggs, D. E. G., Clarke, J., Mayr, G. & Prum, R. O. 2010: Structural coloration in a fossil feather. Biology Letters, 6, 128-131.

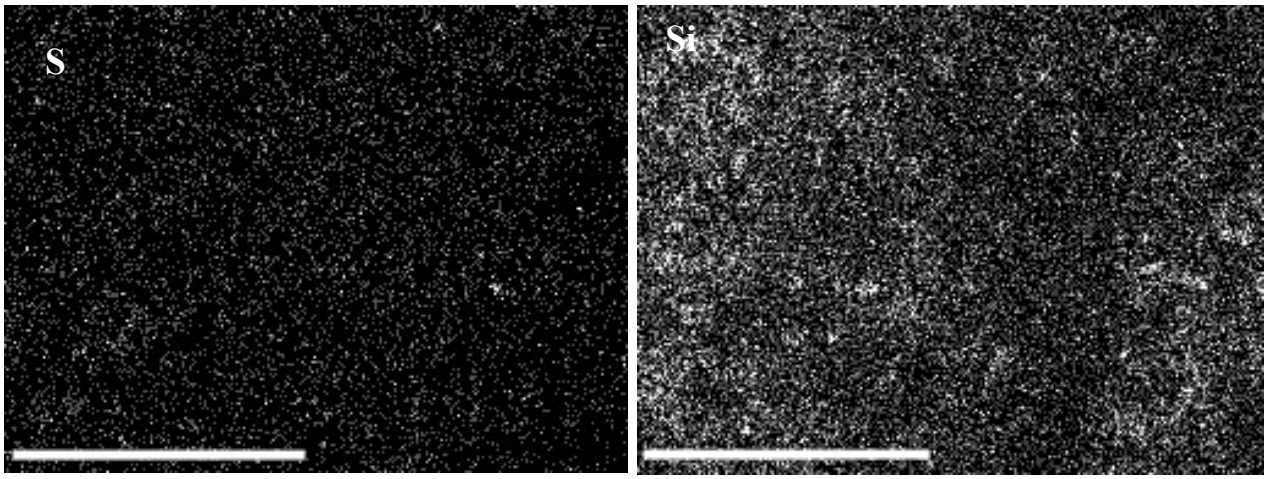
Vinther, J., Briggs, D. E. G., Prum, R. O. & Saranathan, V. 2008: The colour of fossil feathers. *Biology Letters*, 4, 522-525.

Wogelius, R. A., Manning, P. L., Barden, H. E., Edwards, N. P., Webb, S. M., Sellers, W. I., Taylor, K. G., Larson, P. L., Dodson, P., You, H., Da-Qing, L. & Bergmann, U. 2011: Trace Metals as Biomarkers for eumelanin pigment in the fossil record. *Science*, 333, 1622-1626.

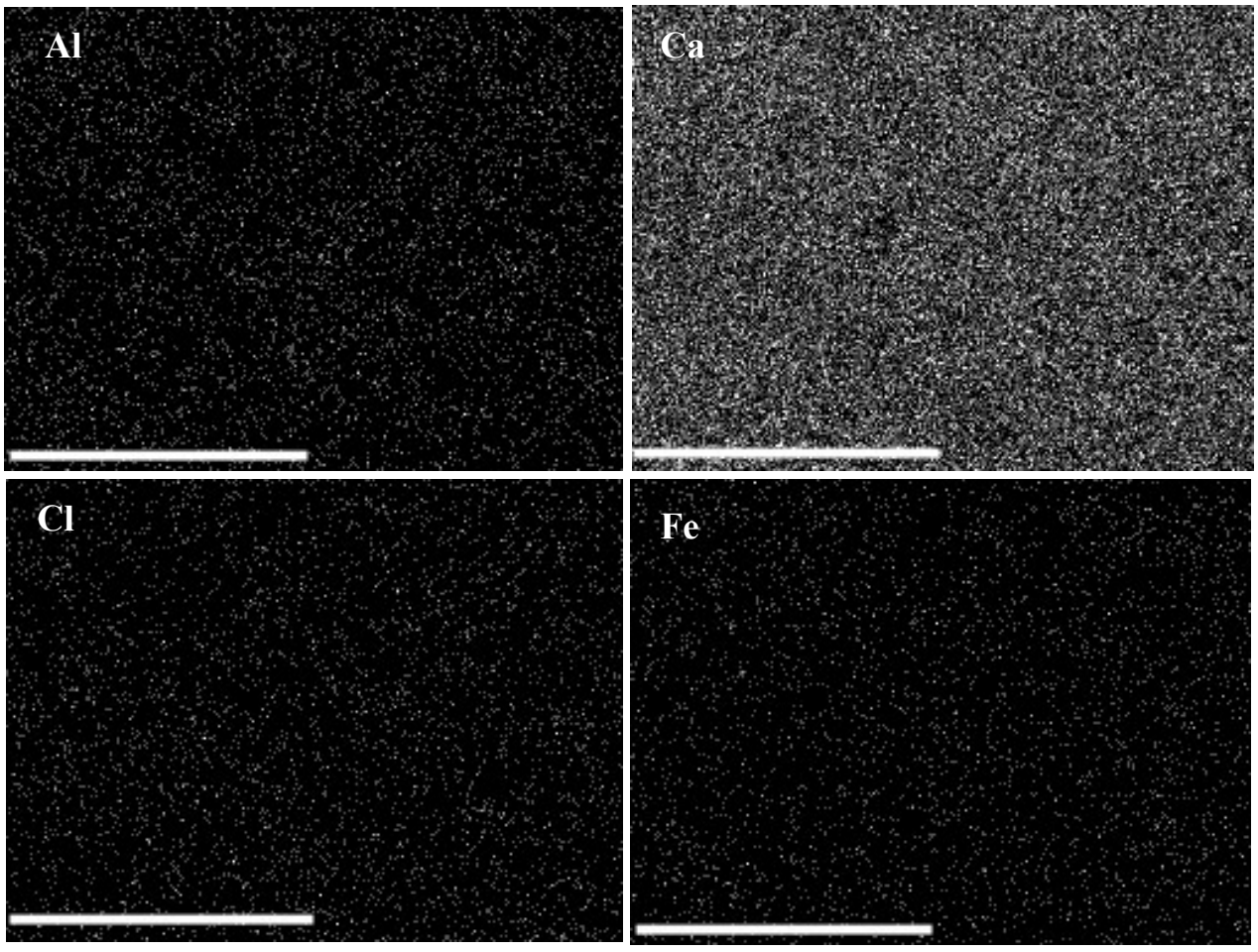
Zhang, F. C., Kearns, S. L., Orr, P. J., Benton, M. J., Zhou, Z. H., Johnson, D., Xu, X. & Wang, X. L. 2010: Fossilized melanosomes and the colour of Cretaceous dinosaurs and birds. *Nature*, 463, 1075-1078.

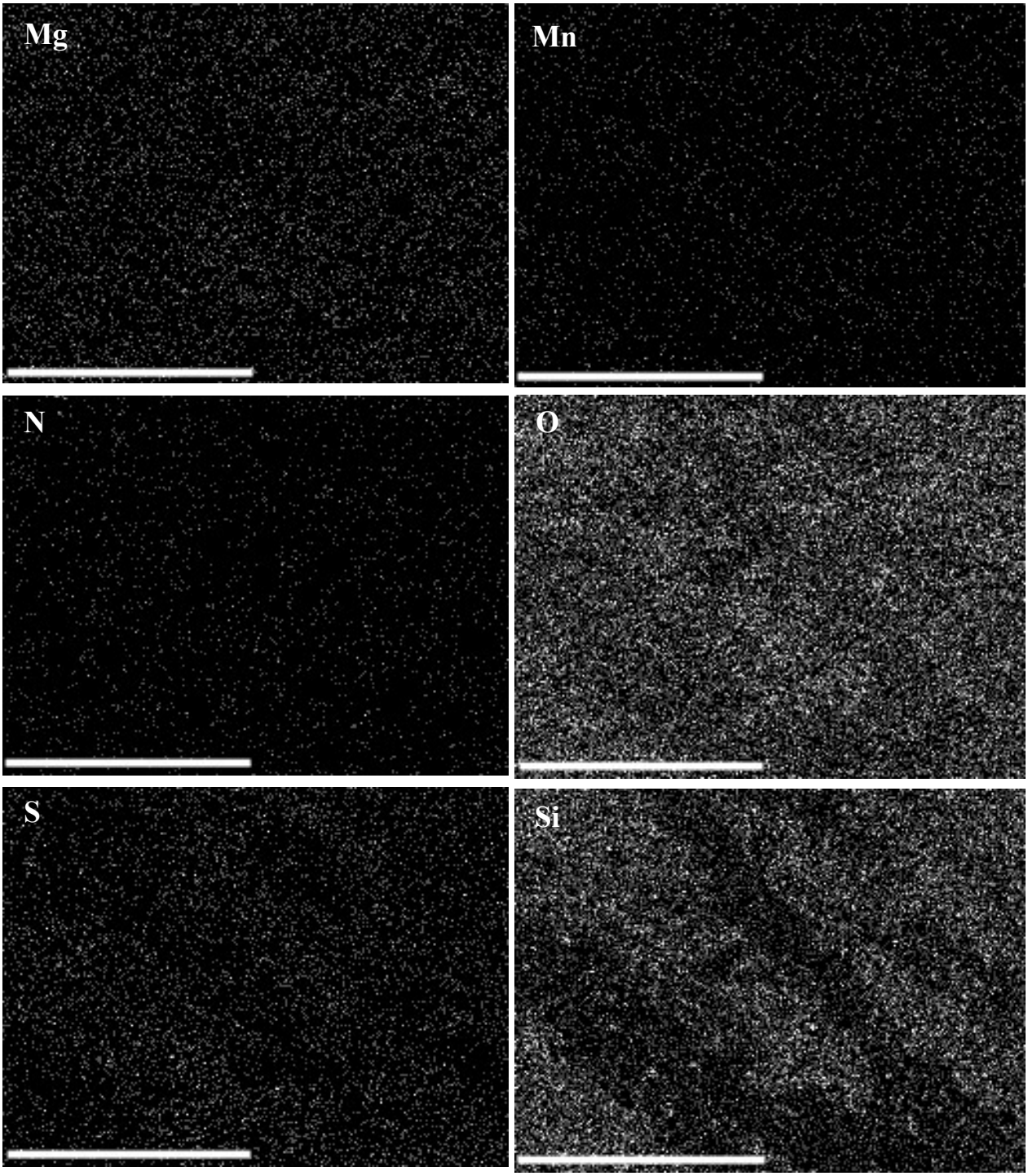
# 11 Appendix



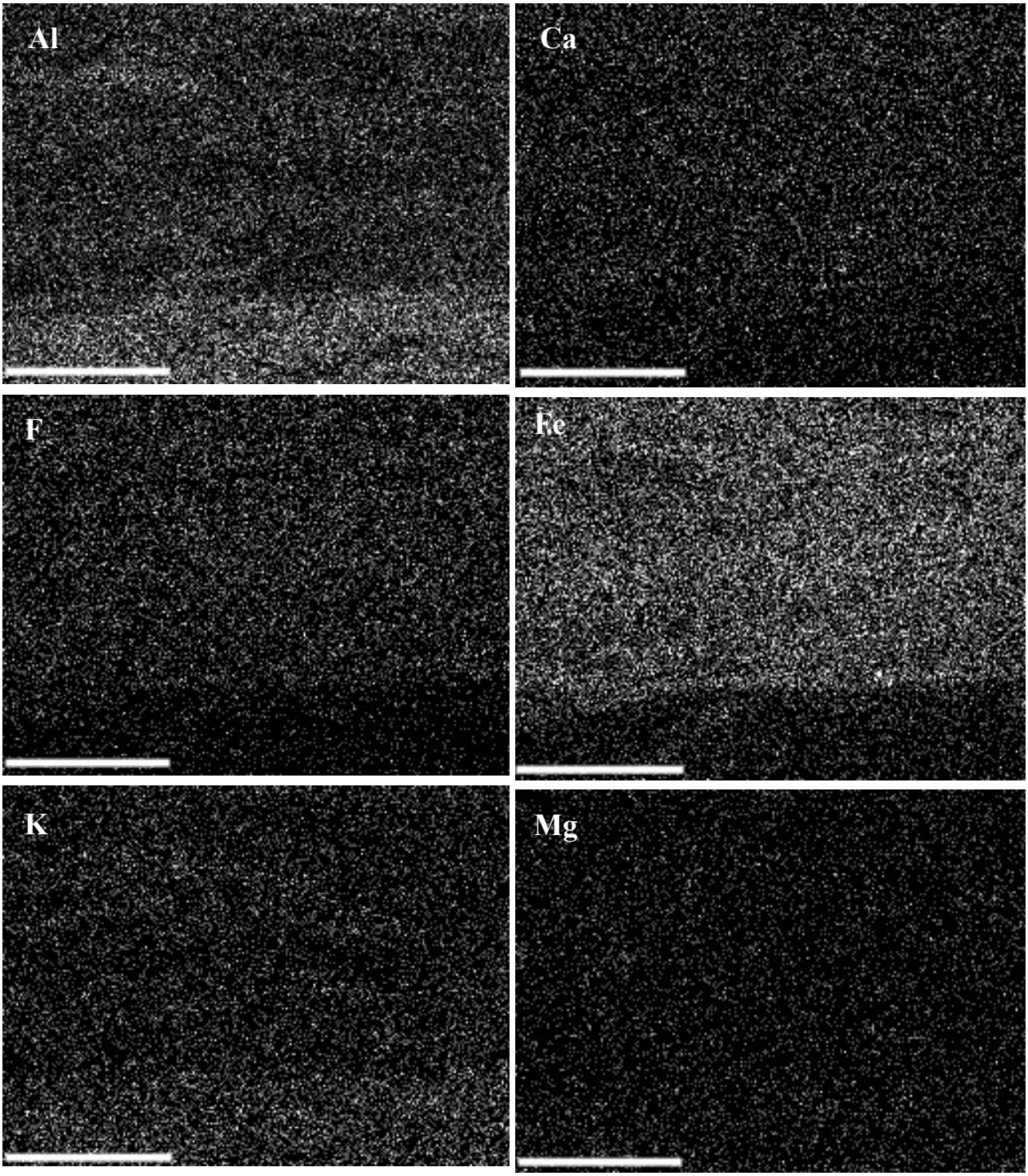



*Appendix figure 1*. Elemental maps of the crane fly. Scale bars: 200 µm






*Appendix figure 2*. Elemental maps of the fossil feather FUM 1980. Scale bars: 700 µm.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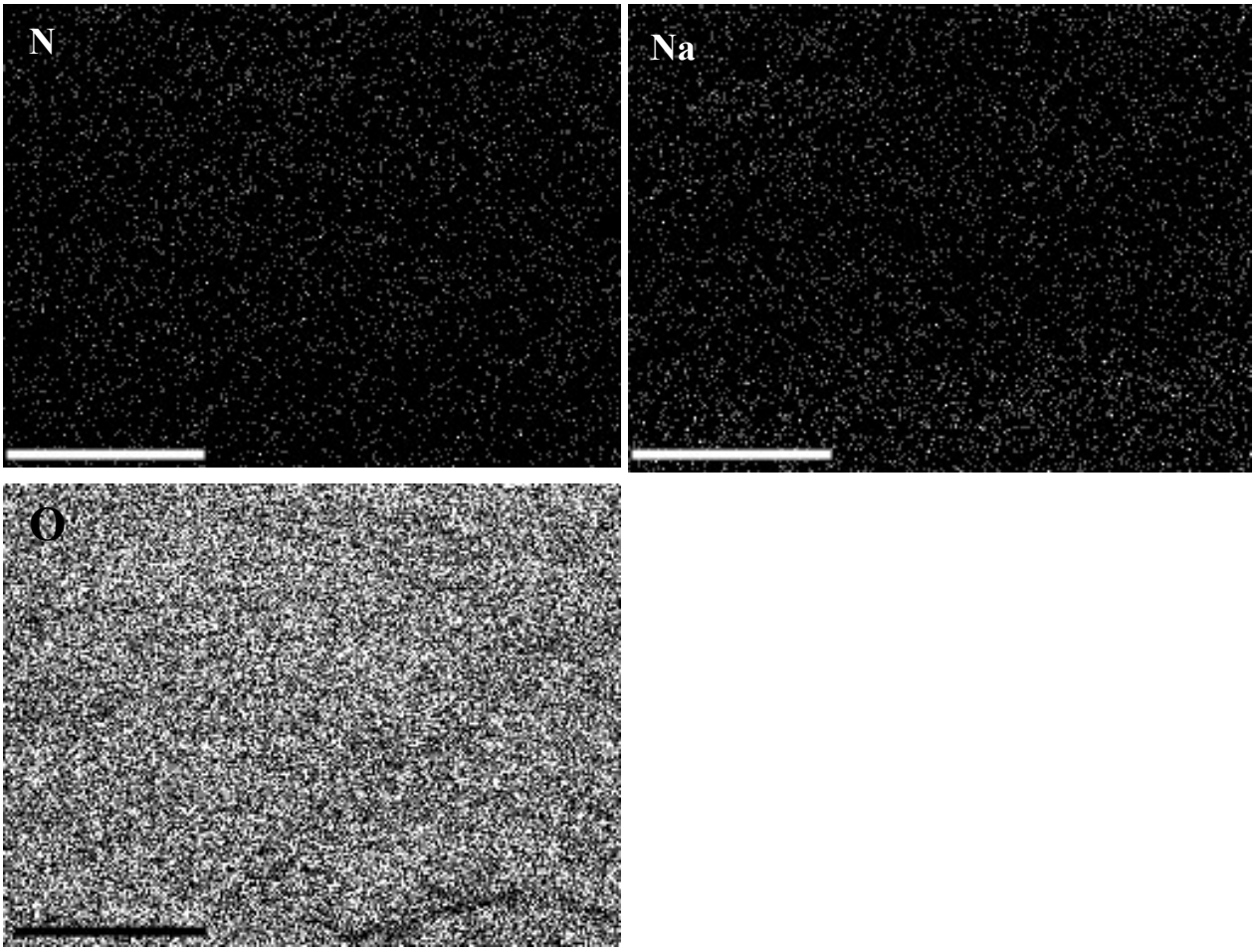
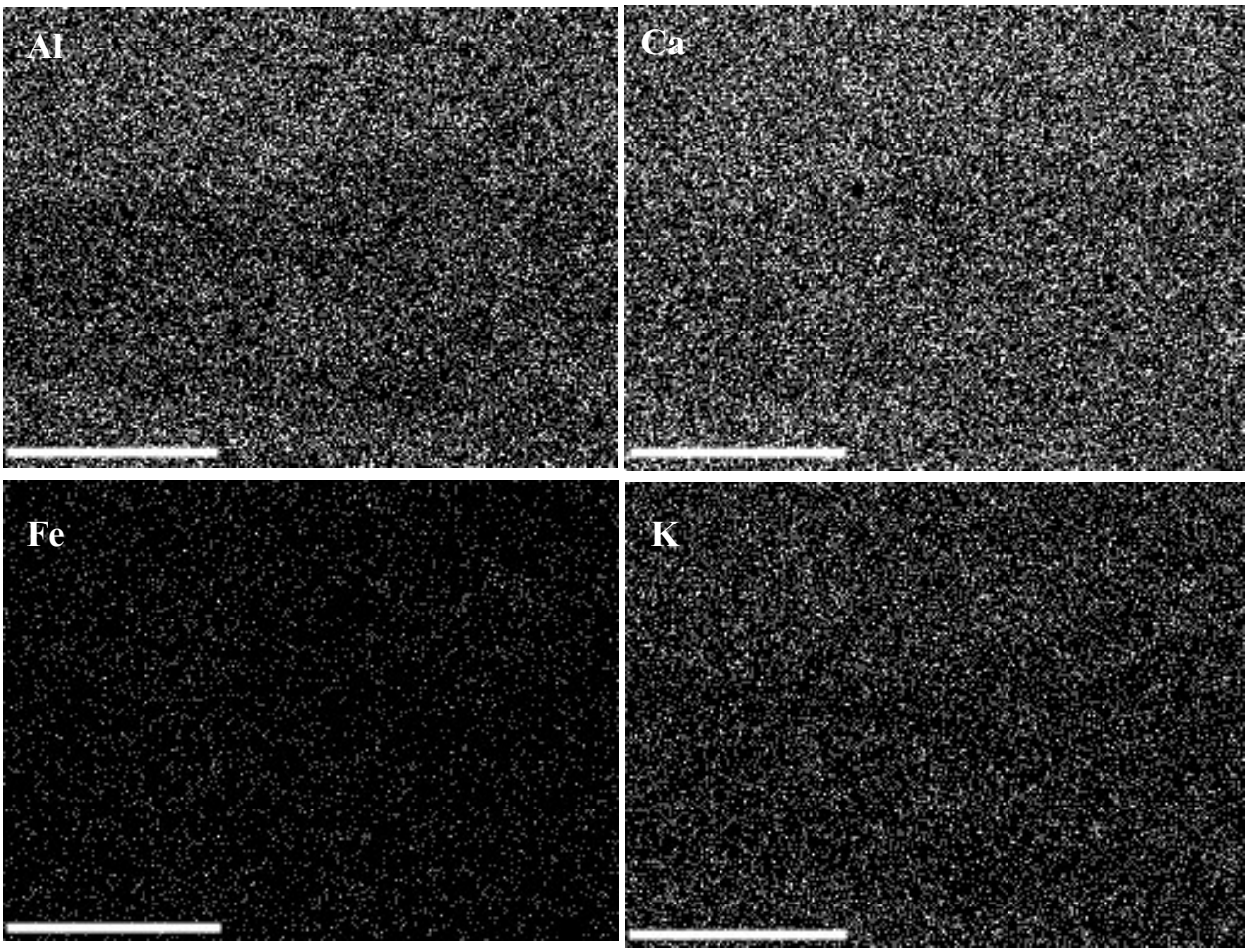
F
Fe
K
Mg




*Appendix figure 3*. Elemental maps of the fossil feather GRF1. Scale bars: 1 mm.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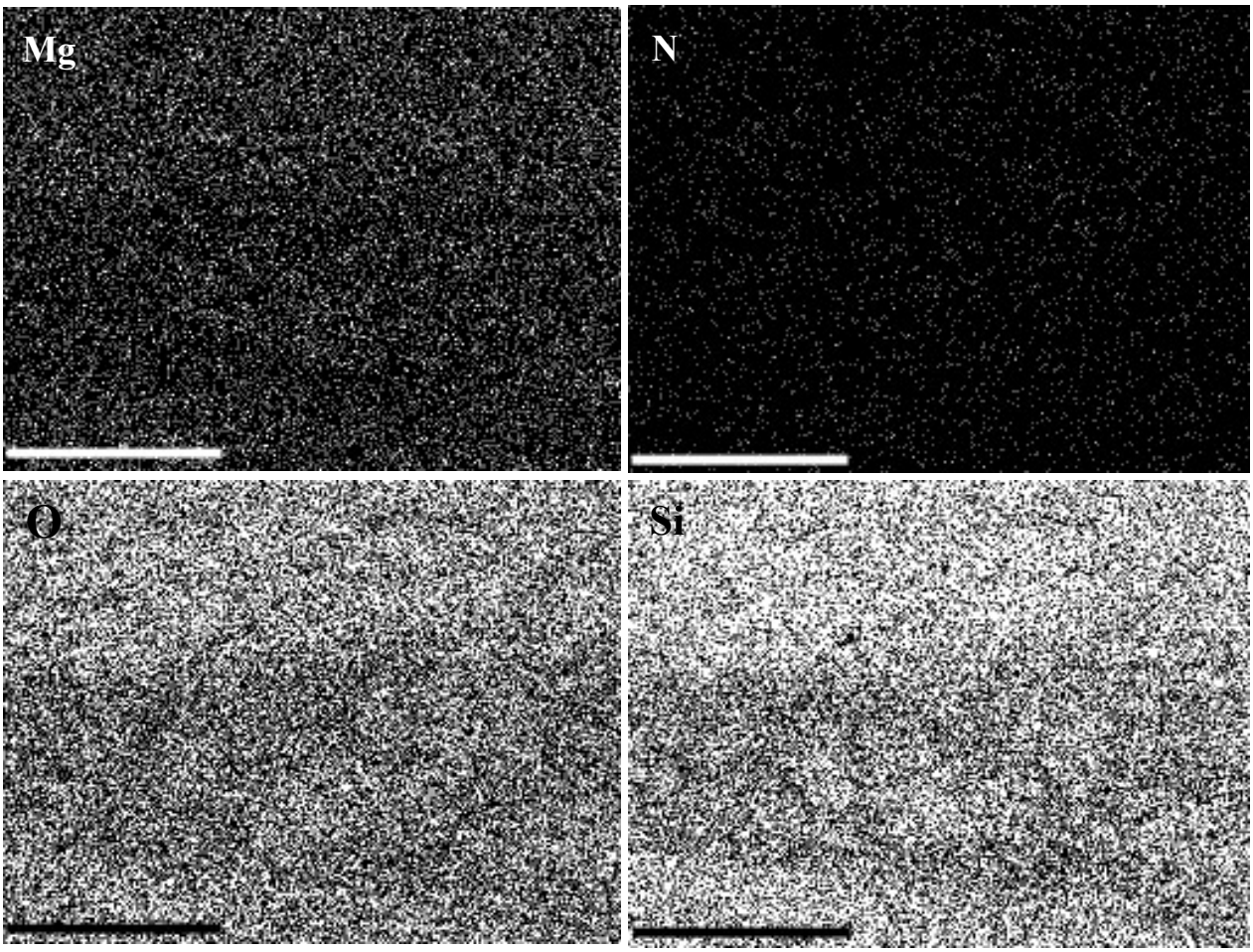
K




*Appendix figure 4*. Elemental maps of the fossil feather GRF2. Scale bars: 1 mm.