PRESERVED PIGMENTATIOIN IN A PENNSYLVANIAN-AGED ‘PALAEONISCOID’ FISH

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ABSTRACT

The fairly recent discovery of melanosomes, preserved microscopic organelles that produce melanin, and their unique morphology that is indicative of color hue has led to the rapid growth of Paleocolor, a field that had produced many pigment reconstructions of fossilized birds, mammals, and insects. Understanding fossilized pigment patterns provides strong evidence for ecological relationships and habitat characteristics, such as countershading and disrupted coloration, when compared to the pigments of today.

Despite the field's growth, little research has been conducted on the melanosomes of fish. This study aimed to document melanosome-based pigment patterns on a Carboniferous-aged fossil fish specimen with visible dark stripes. These dark stripes were found to have melanosomes when compared to non-pigmented sections of the fish, which were located and photographed using an Environmental Electron Scanning Microscope. The distribution of these melanosomes within the visible pigmented stripes is therefore evidence of both disruptive coloration and countershading, which indicates the fish lived in a nearshore environment with a complex background.

This thesis is dedicated to my parents, Kurt and Katie, my two little sisters, Adeline and Clara, and my grandparents, Sassy and Eddie. Thank you all for your unconditional support and for always believing in me.

ACKNOWLEDGEMENTS

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INTRODUCTION

The field of paleocolor has yielded exciting insights not just into the coloration and patterns of extinct fauna, but into behaviors, environments, and ecological niches with renewed clarity. The study of paleocolor is rooted in the fossilization and preservation of a pigment, specifically melanin, and of the microstructures that produced this pigment in life. Melanin is a polymer consisting of indoles and other products created by the oxidation of the amino acid tyrosine by the enzyme tyrosinase (Riley, 1997). Melanin is common throughout the plant and animal kingdoms, but in vertebrate species, is found only in retinal pigment and epithelial tissue. (Riley, 1997) Melanosomes are organelles responsible for melanin synthesis. Like melanin (Riley, 1997), they have been found in the eyes, internal organs, and integument of exceptionally preserved fossil vertebrates (Vinther, 2020). The timeframe of their original evolution is not fully known, with one study suggesting evidence for melanin-based color patterns in early trilobites, which would extend melanosome evolution into the Paleozoic and possibly as far back as the Cambrian (Kobluk and Mapes, 1989). Other researchers maintain that melanin extends back to the very early development of life on Earth, suggesting that melanin was early life's main form of defense against harmful UV rays produced by the sun (Galván and Solano, 2016). Melanin can also be responsible for complex pattern creation; an example being 98% of all birds with complex plumage patterns relying on melanin as it is directly controlled by cells and therefore more readily available when compared to other pigment sources such as carotenoids (Galván et al, 2017). Complex pattern creation, UV protection, specifically through thermoregulation due to its dark coloration being more likely to absorb heat, as well as possessing antimicrobial properties, are all key functions of melanin that can directly benefit pigmented organisms

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(Mackintosh, 2001). These functions are specific to melanosomes located within the integument, and such melanosomes will be the focus of this study.

There are two types of melanin seen in both living and fossil vertebrates. The one seen most frequently is eumelanin, which produces black, dark grey, and dark brown pigments. The second is phaeomelanin, which was not documented in fossil vertebrates until sometime after the initial discovery of eumelanin (Colleary et al, 2015). Phaeomelanin produces lighter tones than Eumelanin, specifically light brown, yellow, and reddish hues. The two melanins have distinct chemistries and different pathways through which they are synthesized (Vinther, 2020), and it is the varying structure and morphology of eumelanin-bearing melanosomes versus phaeomelanin-bearing melanosomes that allow them to be readily distinguished from one another. Phaeomelanosomes found in fossilized cephalopod ink have an average diameter of ~0.2μm and are considered small, while eumelanosomes, most commonly found in vertebrates, are larger at 0.4μm to 2μm (Vinther, 2016). This is partly due to their shape, which corresponds strongly with the type of melanin produced. The lighter toned phaeomelanin is produced in phaeomelanosomes that are typically ovoid with similar length axes. These are often referred to as meatball-shaped. The darker toned eumelanin comes from eumelanosomes that are elongated along one axis, producing a cylindrical shape that has been referred to as sausage shaped (Vinther, 2016). Variations in melanosome morphology can further alter pigmentation, and while beyond the scope of this study, it should be noted that traits such as iridescence or the matte black color seen on penguins- known as penguin-black- have different shaped melanosomes. For example, iridescent melanosomes are cylindrical similar to typical eumelanosomes, but are flattened or hollow as well as more elongate (Vinther, 2016).

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In 2004, researchers examining Jurassic and Early Cretaceous fossil teuthid (squid) ink under an electron scanning microscope found spherical grains identical to modern cephalopod ink (Doguzhaeva, Mapes, and Mutvei, 2004) which are comprised of eumelanin produced by eumelanosomes "meatballs". The fossil ink sac was three-dimensionally preserved while the rest of the specimen was not, suggesting that melanin was degradation resistant, and the fossil ink was relatively unchanged for nearly 200 million years. (Vinther, 2020).

Before the fossilized ink was fully analyzed and identified as such, the melanosome microstructures were believed to be preserved bacteria (Roy, 2020). Since that time many in-depth studies have been conducted to support the paradigm shift from bacteria to melanosomes (Colleary et al, 2015); (Gabbott et al, 2016); (Moyer et al, 2014); (Roy et al, 2020). Perhaps the strongest evidence comes from melanosome distribution compared to localized pigment in similar modern specimens. Vinther (2008) analyzed the alternating dark and light bands of a fossil feather from the Early Cretaceous Crato Formation of Brazil for melanosome distribution and found eumelanosomes present in the dark bands and not the light bands. There is no documented reason for bacteria to only be preserved where there is pigmentation, as well as there being no reason for melanosomes to exist where there is no melanin present such as the light banding of the feather (Vinther 2008). Multiple instances of this have been found in ensuing years, with preserved melanosome distribution in the eyes of Carboniferous cyclostomes aligning with modern cyclostomes (Gabbott et al, 2016), supporting the presence of melanosomes instead of fossilized bacteria, and contributing to a better understanding of the evolution of the vertebrate eye due to the basal phylogenetic position of cyclostomes (Gabbot et al, 2016). Another study involved experimental maturation of modern melanin samples, and found it aligned with melanin

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seen in fossils that are hypothesized to have undergone diagenetic chemical alteration (Colleary, 2015).

A further example of melanosome preservation pertains to the iconic proto-avian *Archaeopteryx lithographica*. Found in the 150-million-year-old Solnhofen Lagerstatte in Bavaria in 1861, *Archaeopteryx* provides essential data on the origin of birds, but its color had been unknown until relatively recently. In 2011, a study was published describing the melanosome distribution and morphology of an *Archaeopteryx* feather (Carney, 2011). The study revealed the feathers to have been black in color, but more interestingly, this black pigmentation was seen in feathers that were obscured by overlapping covert feathers, sparking questions regarding melanin's purpose in these areas as these feathers would not have been visible during life. Both thermoregulation and protection against feather-degrading bacteria may be involved (Carney, 2011); (Carney et al, 2020).

Another study looked at a chondrenchelyid chondrichthyan, *Harpagofututor volsellorhinur*, from the Upper Mississippian, and found evidence of pigment in the reproductive tracts of mature specimens. The correlation between body length and melanin pigment present allows for confidence in determining relative age based on whether the specimen was sexually mature and possessed the melanin in the reproductive tract (Grogan and Lund, 1997). Further species with paleocolor reconstruction include pterosaurs (Cincotta et all, 2022), microraptors (Quanguo et al, 2012), *Eoconfuciornis* (Yanhong et al, 2016), and even the armored dinosaur *Borealopelta* (Brown et al, 2017)

The study of paleocolor to date has largely focused on reconstructing pigment in feathers and has therefore largely been restricted to birds and feathered theropods such as *Sinosauropteryx* (Fiann et al, 2017). However, some work has been done on well-preserved skin

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of mammals and on some non-feathered dinosaurs, such as the ornithischian *Psittacosaurus* (Vinther et al, 2016). Other non-feathered species with paleocolor reconstructions include mosasaurs, ichthyosaurs, and leatherback turtles (Lindgren et al, 2014). As seen through the chondrenchelyid *Harpagofututor volsellorhinus* reproductive tract pigment in sexually mature adults, melanosome distribution can also be a tool for describing ontogeny, sex, and possible sexual dimorphism (Grogan and Lund, 1997). For well-studied characters such as eyes in vertebrates, melanin can be used for further phylogenetic classification, such as when the eye pigment of the Tully monster or *Tullimonstrum* helped determine that it was a vertebrate and not an invertebrate (Clements et al, 2016). Another study focused on a fossil moonfish specimen *Mene rhombea* that has visible countershading and disruptive coloration through striping that suggest it lived in open waters but swam close to reefs (Rossi et al, 2022). When compared to modern moonfish, the melanosome-based patterns differ from the fossil specimen, and modern moonfish instead have evolved to live in shallow coastal settings due to the hypothesis that moonfish geographic distribution and ecology have changed over the last 48 million years. This offers a chance for further study of phenotypic comparison between the fossil fish and extant relatives to better understand molecular signalling mechanisms for controlling pattern evolution (Rossi et al, 2022). The focus of this study is a late Paleozoic paleoniscoid-grade actinopterygian fish collected from the fine-grained muddy limestone of the Calhoun Shale Formation at the Hamilton Quarry site in Greenwood County, southeastern Kansas (Gottfried, 1989), which preserves an apparent color pattern. The specimen is a subadult based on its small size, near-absence of scale cover, narrow body depth, large orbit, poorly ossified snout region, and lack of ornament on the dermal skill bones. Streaky mottlings along the dorsal one-third of the body and

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two longitudinal mid-body lateral stripes can be seen on the specimen and were interpreted as preserved color pattern in an earlier study (Gottfried, 1989).

Melanosomes offer a unique insight into paleoecology and environmental interactions through cryptic coloration. The pattern on the fossil actinopterygian fish from Kansas indicates both countershading and disruptive coloration (Gottfried, 1989). Countershading is when an organism has a darker surface on the dorsal side that typically faces a light source and a lighter surface on the opposing ventral side. It is hypothesized to have multiple benefits, including compensation for one's own shadow, simultaneously blending in with two different backgrounds in two different directions, altering the animal's 3D appearance, and UV protection (Stevens and Merilaita, 2008). This is most often seen in open water species (Vinther, 2015). Disruptive coloration breaks up the animal's outline, further obscuring its presence and making detection more difficult (Stevens and Merilaita, 2008). This happens through a pattern of contrasting coloration that reach the edge of the body outline. Disruptive coloration is more likely to evolve in prey species of fish that live in environments with high habitat diversity and/or environments with a strong presence of visual hunters (Duarte and Gawryszewski, 2019).

Mapping the distribution and morphology of melanosomes on the lower Paleozoic actinopterygian fish from Hamilton, Kansas can offer insight into the preservation of melanin patterns in ancient fish; an understudied group in the field of paleocolor.

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GEOLOGIC SETTING

The fish specimen was collected from a muddy limestone unit of the Calhoun Shale formation at the Hamilton Quarry site in Greenwood County, southeastern Kansas. During the time of deposition (Upper Carboniferous), the Hamilton Quarry site was a paleochannel that was subjected to megacyclothems related to Milankovitch cycles, with an obvious pattern of shale and limestone caused by periodic transgression and regression of the nearby epicontinental sea (Bridge, 1988). This pattern could also have been affected by tectonic activity in the seafloor (Bridge, 1988). Such changes in sea level make the overall salinity of the Hamilton Quarry site uncertain, but the environment likely ranged from nearshore marine to brackish tidal flats and possible lagoons (French et al. 1988).

A large range of other fossil specimens have come from the Hamilton Quarry site, including invertebrates and plants, as well as vertebrates. Invertebrates include bivalves (Maples and Mapes, 1988), Eurypterids (Kues, 1988), Ostracoda (Kaesler, 1988), Arachnida (Hanson, et al, 1988), and even terrestrial insects (Durden, 1988). Hamilton Quarry's most prolific vertebrate are acanthodians, of which hundreds have been discovered ranging widely in length from 54 to 410mm, which has led to studies of acanthodian growth (Schultze, and Chorn, 1989). A wide variety of other fishes, including osteolepidids, crossopterygians (Schultze, 1988), dipnoans, actinopterygians, and chondrichthyans (Zidek, 1988) are also present, along with dissorophid amphibians (Daly, 1988) and diapsid and pelycosaur 'reptiles' (Reisz 1988). Eighteen paleoniscoid specimens have been recovered, including the subject of this report. The palaeoniscoids also show a range of sizes, further supporting the Hamilton Quarry site as a low-energy environment either representing or located near to hatching or spawning sites utilized by these fish (Gottfried, 1988).

SPECIMEN DESCRIPTION

The pigmented fish specimen (University of Kansas Museum of Natural History Vertebrate Paleontology [KUVP] 96910) is preserved along an irregular bedding plan in part and counterpart [initially although only the part is currently available for study]. The specimen is 30mm long and includes well-preserved fins, the head section, and the body section. The caudal fin is not as well-preserved, and during life, the fish is estimated to have been just over 40mm in length (Gottfried, 1989). It possesses multiple subadult traits beyond its small size, including narrow body depth, a proportionally large eye orbit, an unossified or poorly ossified snout, little scale cover, and lack of dermal skull bone ornament (Gottfried, 1989). It's shape and position of the dorsal and anal fins and its size suggest assignment to the likely paraphyletic genus '*Elonichthys'* (Gottfried, 1989). This paraphyletic taxon is relatively common in Carboniferous and Permian deposits (Schultze and Bardack, 1987). It is considered more basal than actinopterygians that are phylogenetically closer to extant neopterygians but is more crownward than the basal actinopterygian *Cheirolepis* (Mickle, 2012). Two visible lines of pigment, one on the upper body in the dorsal region, and one opposite on the lower side of the body, run from the head region to the caudal fin. Whether or not the upper line crosses the eye is unclear. Generally, the dorsal region of the specimen appears darker with some streaky mottlings, though the focus of this study will be the two lateral stripes.

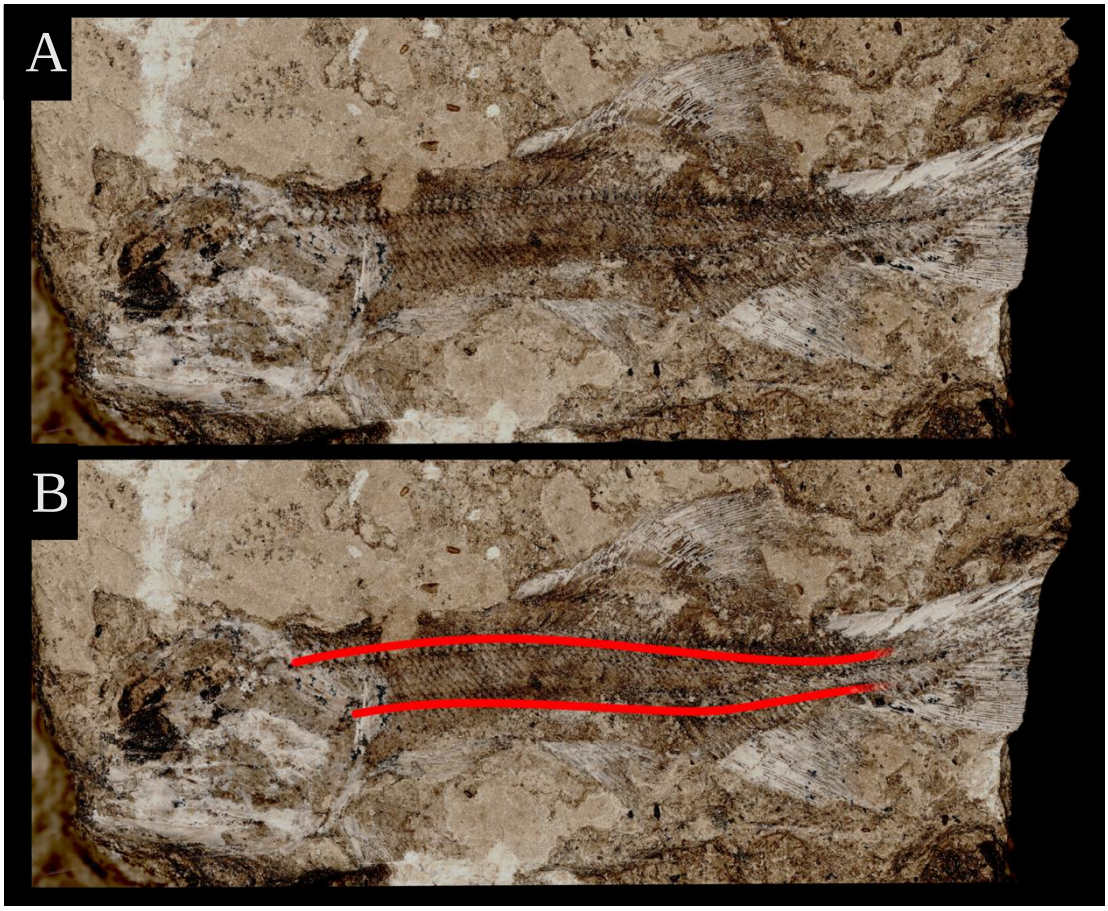

Figure 1. Altered mosaic image of fish specimen taken by a Keyence Light Microscope

(A) The image has lower saturation, higher contrast, and a warmer temperature to highlight the pigmented stripes. **(B)** Identical image with two red lines tracing the stripes.

METHODS

The fish specimen was originally collected from the Hamilton Quarry, Kansas, in 1988. The first step in mapping melanosomes was to create a detailed photograph mosaic of the entire specimen using a Keyence VHX-6000 light microscope. The key microscope features used were large depth-of-field using focus stacking, as well as 3D automatic stitching. To collect melanosome images, the Thermofisher Quattro ESEM [Environmental Scanning Electron Microscope] from the Center for Advanced Microscopy at Ohio State University was employed. It was operated at 5.00kV, low vacuum at 32pA, with LVD. 60 images that represent the full specimen were stitched together by hand in Microsoft Excel. Their respective coordinates were retrieved through the image file metadata and used to correlate the area locations on the mosaic map. *ImageJ* was then used to measure the diameter of five randomly selected melanosomes at the four best areas.

RESULTS

Specific locations on the specimen were selected for ESEM imaging based on the likelihood of melanosomes being present. Ten sites were chosen in total, with seven of them encompassing the top, middle, and bottom of the specimen's body in two roughly straight lines so any change in melanosomes distribution would be seen. Each body site had between 2 and 4 images taken at different scales. They eye, as it is already known to contain melanosomes (Gabbott et al, 2016) was also imaged for correlation.

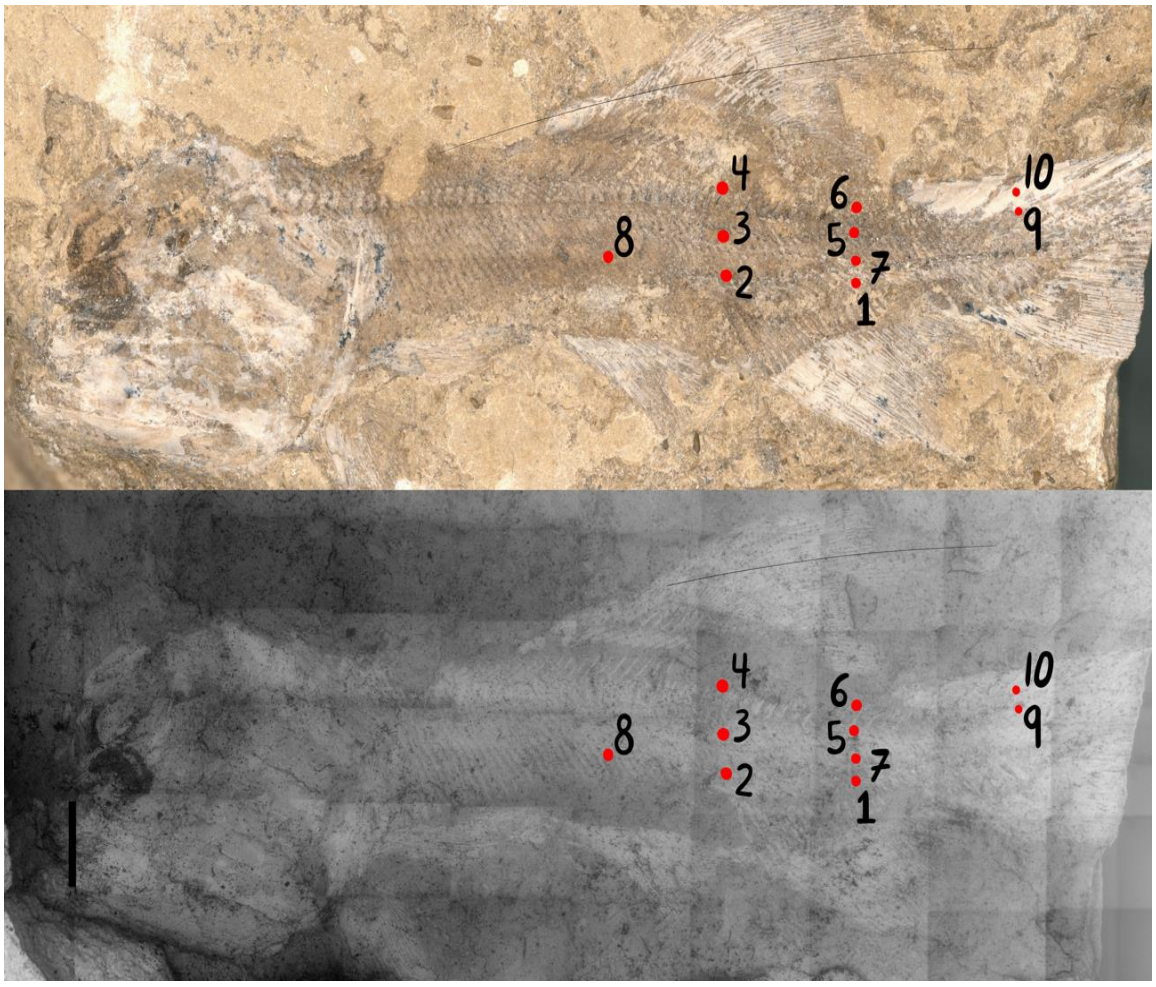

Figure 2. Original mosaic image (unaltered) and ESEM mosaic image of specimen with areas labeled

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Areas 1, 2, 4, and 6 were selected because of their placement on the pigmented line. Areas 5 and 8 are near the line, but not directly on it. Areas 9 and 10 are on the base of the tailfin, and areas 7 and 3 are located along the central line of the specimen, which is where we predict melanosomes to be absent.

After evaluating the images, areas 1, 2, 4, and 6 appear to show the best-defined melanosomal microstructures. These microstructures appear very similar to those seen in the eye area. Areas 7, 8, 9, and 10 possessed few potential microstructures that were not as clearly seen prior area images. Areas 5 and 3 held virtually no melanosome candidates, instead displaying a fuzzy, 'cauliflower'-like texture that was too small in scale to be properly assessed.

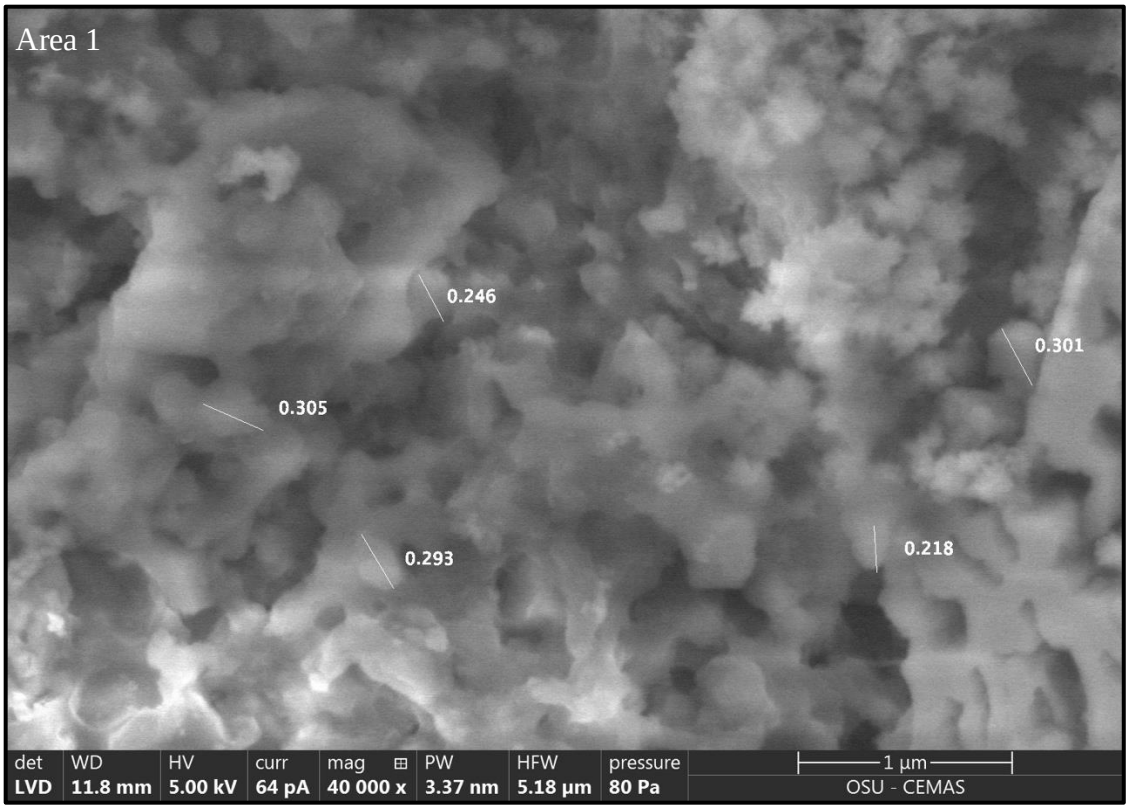

Figure 3. ESEM images of Areas 1, 2, 4, and 6 with diameter measurements (µm) of five randomly selected melanosomes

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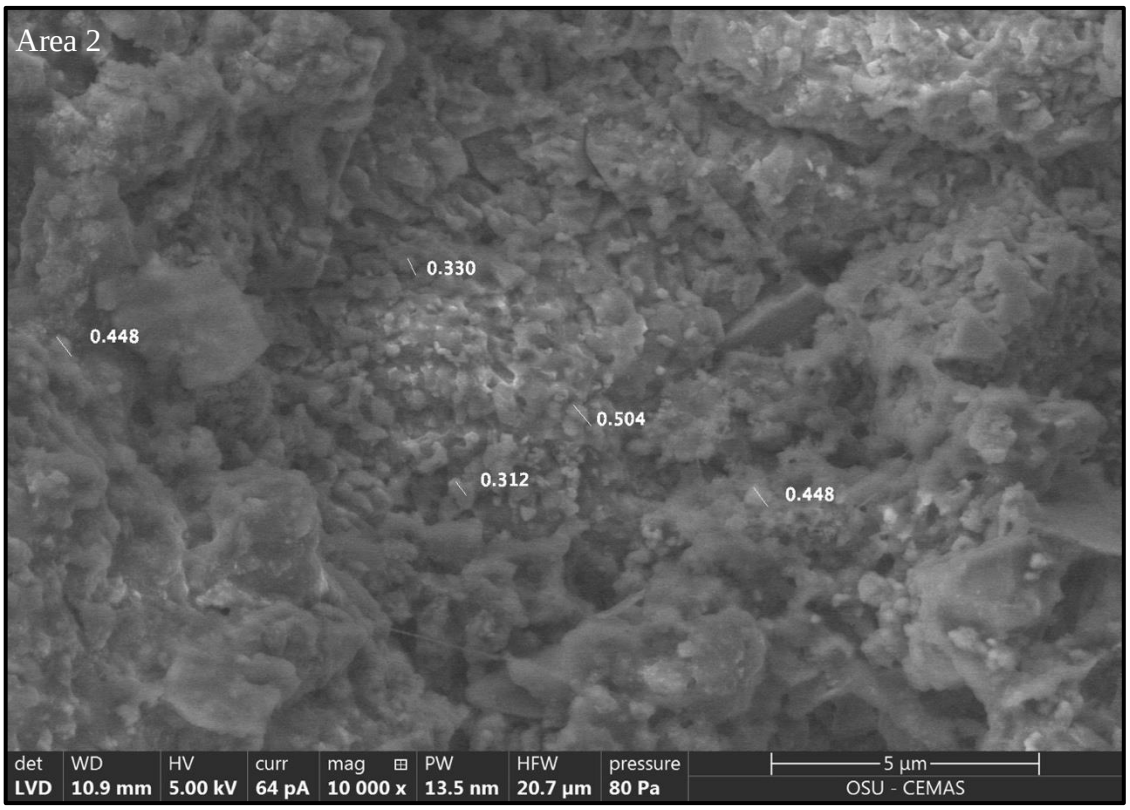

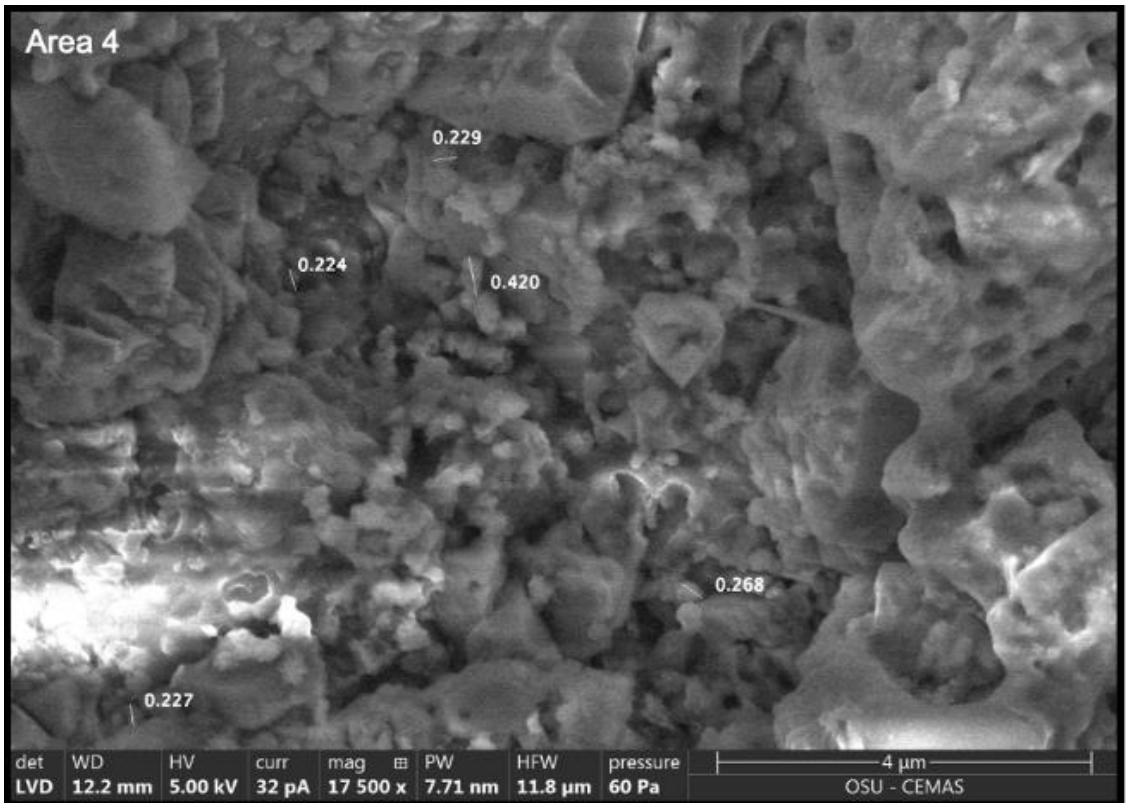

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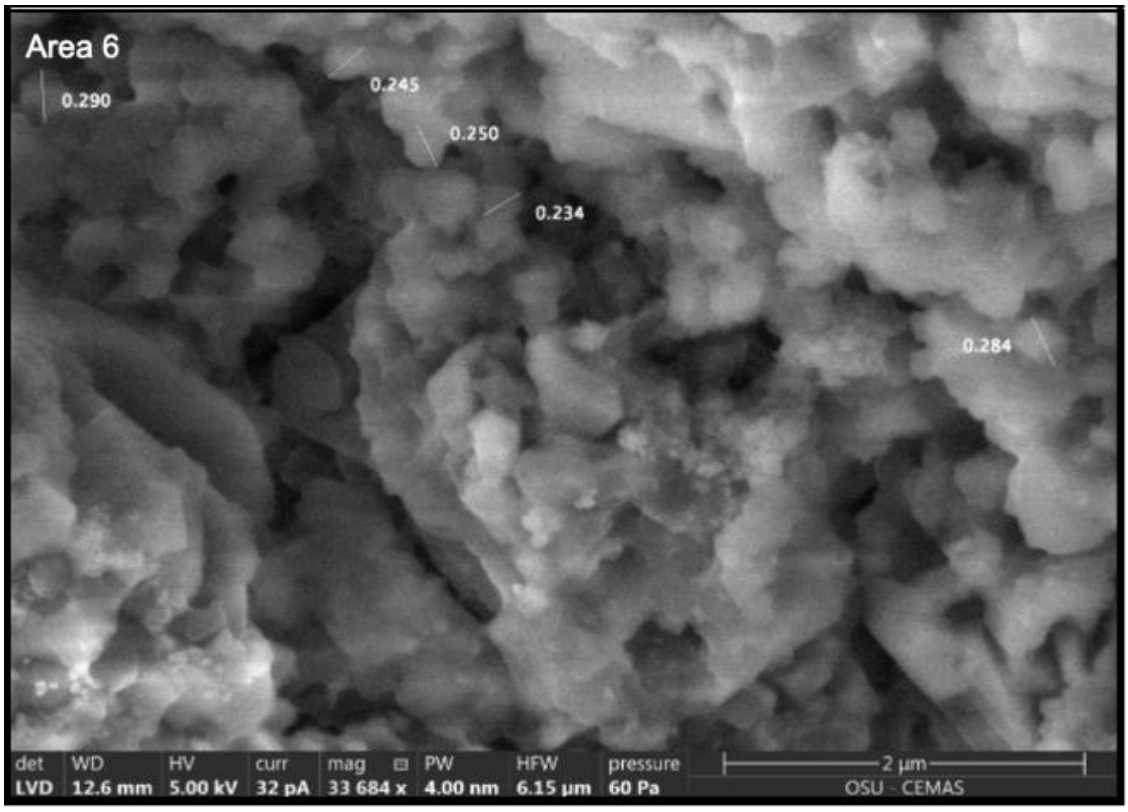

For each site with the high-quality melanosome imaging, random sampling was done to determine the average diameter of the melanosomes present. The averages of five random melanosome diameters on each image were 0.273 µm (Area 1), 0.408 µm (Area 2), 0.278 µm (Area 4), and 0.261 µm (Area 6). The eye area was also measured for correlation and had a diameter of 0.403µm.

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Length (µm)				
	Area 1	Area 2	Area 4	Area 6
1	0.246	0.448	0.420	0.250
2	0.305	0.330	0.268	0.284
3	0.301	0.312	0.229	0.290
4	0.293	0.504	0.227	0.234
5	0.218	0.448	0.244	0.245
Average	0.273	0.408	0.278	0.261

Table 1. Diameter measurements and averages for Areas 1, 2, 4, and 6

An EDS analysis was also run at areas 4 and 8 (many melanosomes and few melanosomes respectively) to highlight areas of carbon to make spotting melanosomes easier as they are largely comprised of carbon. With images taken at higher magnification, the carbon map did not yield significant differences in concentrations. With lower magnification over the eyespot, an area already known to have high melanosome concentrations, the carbon map did show a clear delineation between the matrix and more carbon-rich eyespot. Despite this, using carbon mapping as a technique to find melanosome concentrations did not prove to be very successful. We hypothesize this could be caused by weathering of the surface and long-term exposure to contaminants such as dust. The element that showed the most delineation was phosphorous; its maps showing significant concentrations in the ossified parts of the specimen, highlighting the 'bone' structure in a way we had hoped carbon would highlight melanosome concentrations.

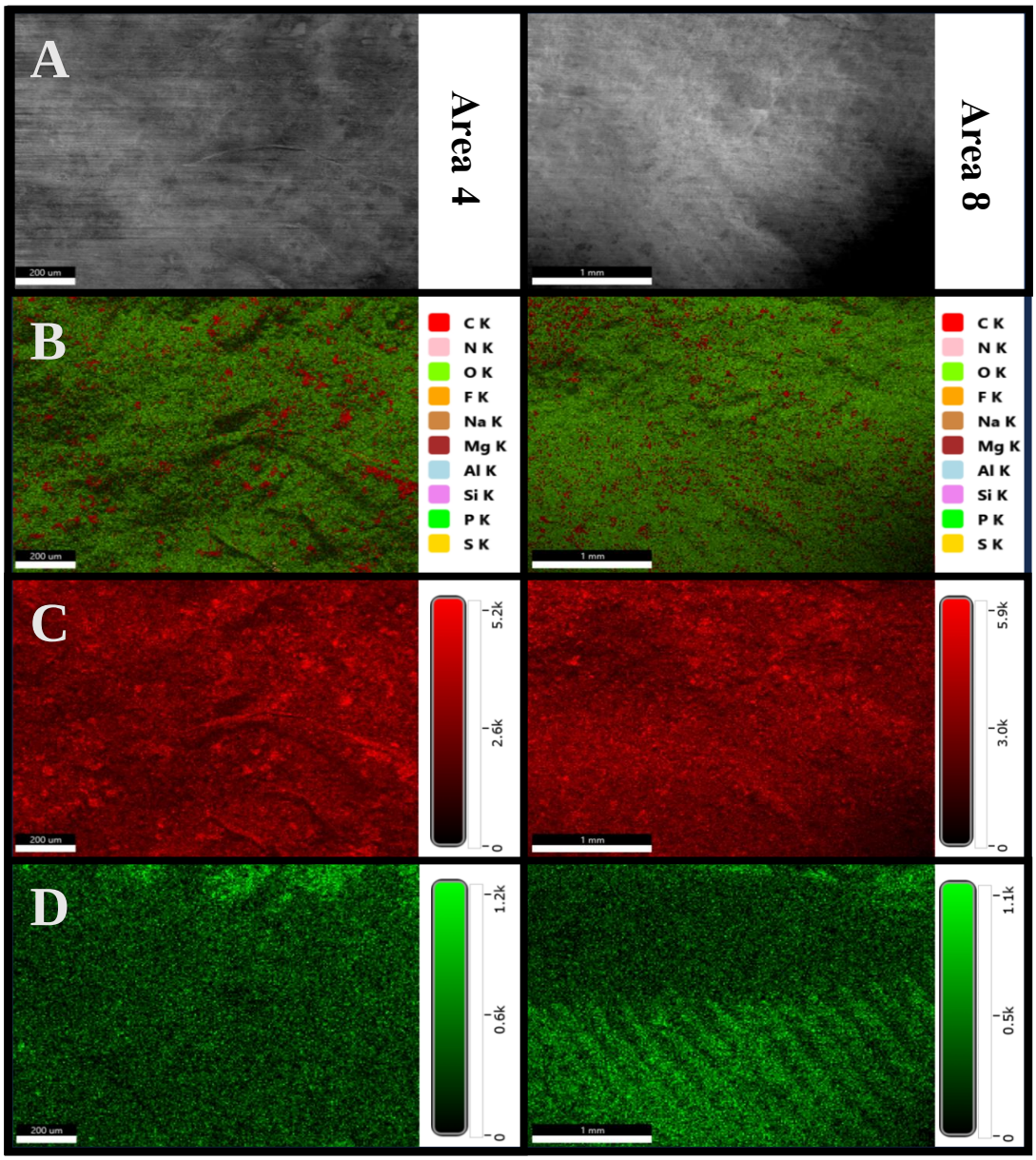

Figure 4. Results of EDS mapping

(A) ESEM image of Area 4 (left) and Area 8 (right) before element detection. (B) Element amount and frequency as colored pixels over the Area 4 and Area 8 sampling locations. Represented elements include Carbon (C), Nitrogen (N), Oxygen (O), Fluorine (F), Sodium (Na), Magnesium (Mg), Aluminum (Al), Silicon (Si), Phosphorus (P), and Sulfur (S). (C) Area 4 and Area 8 EDS with detected carbon visible. (D) Area 4 and Area 8 EDS with detected phosphorous visible.

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DISCUSSION

Compiling ESEM images from different areas where pigment was hypothesized to be present on the specimen was a success. During the search, it became obvious that two different 'landscapes' dominated the surface. One was coined as 'barren' due to its lack of obvious structures and overall plain appearance save for one recurring characteristic that was predominantly unique to the barren areas. These structures were too small to be accurately analyzed and had a fuzzy, cauliflower-like appearance. The smallest melanosomes are believed to have a diameter of ~0.2 µm (Vinther, 2016) and this structure was far smaller, removing the possibility of melanosomes at the barren areas (Areas 5 and 3).

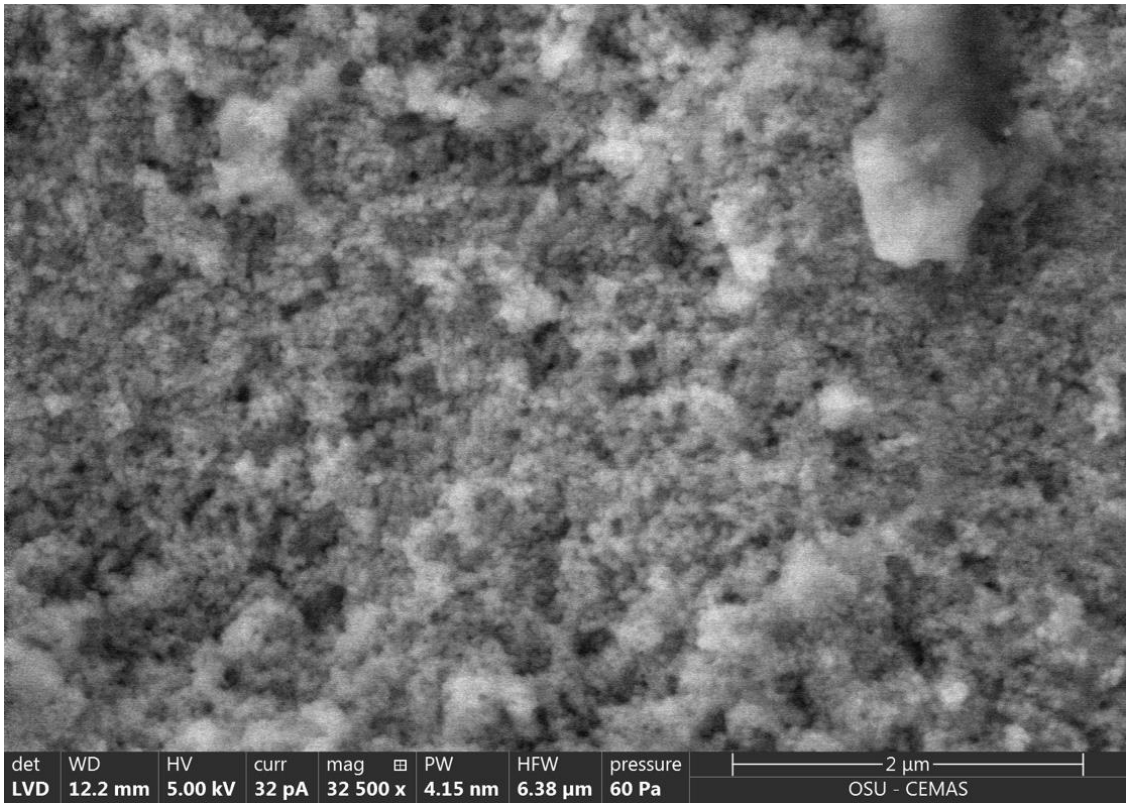

Figure 6: "Cauliflower" texture at Area 3 and Area 5 seen when melanosomes are generally absent

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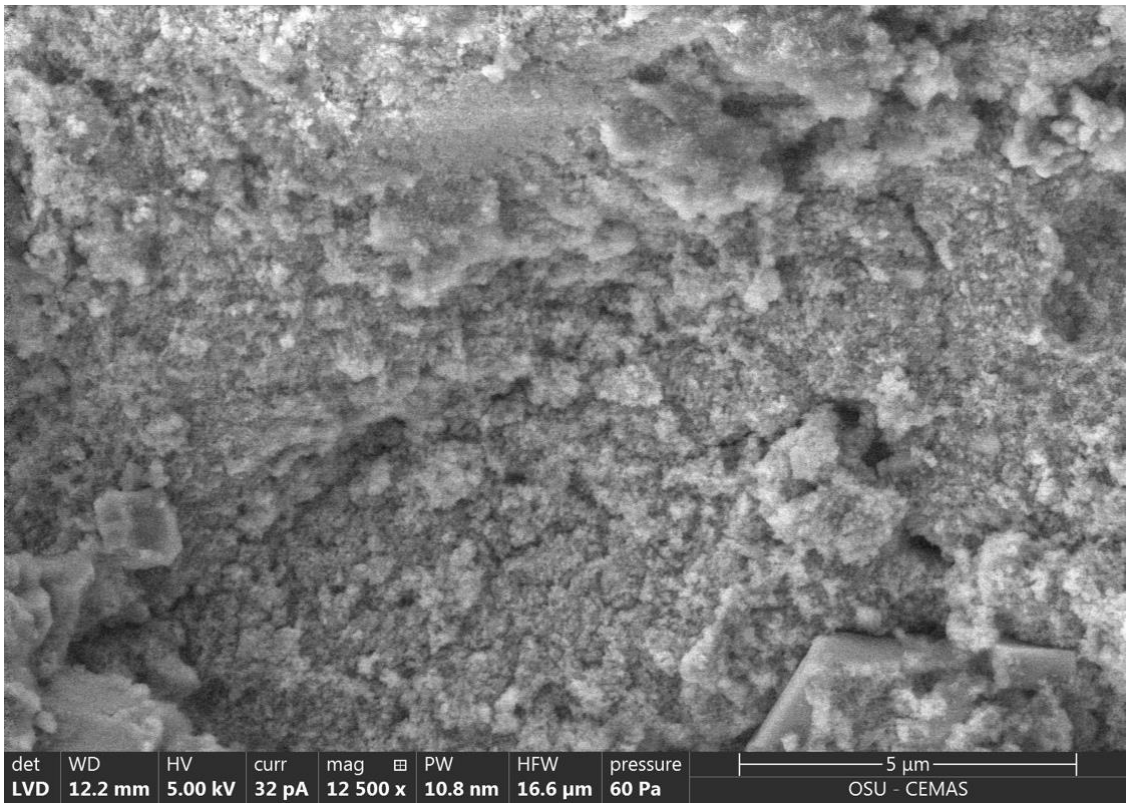

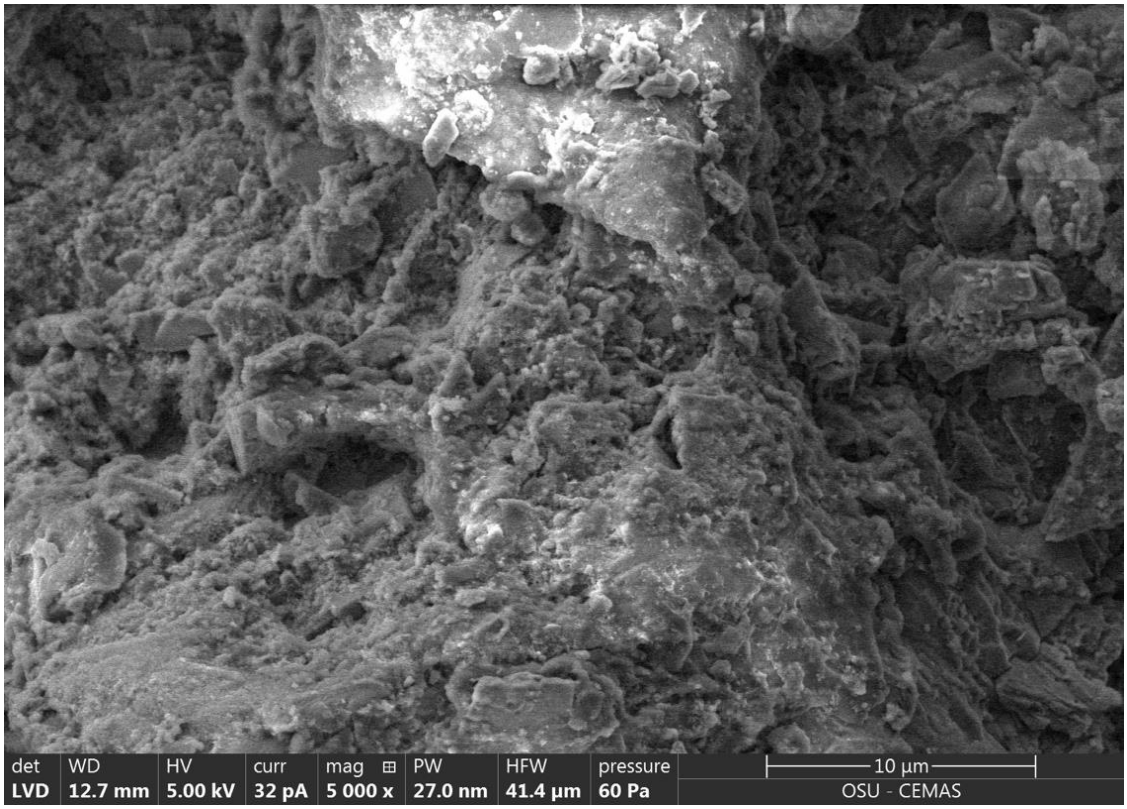

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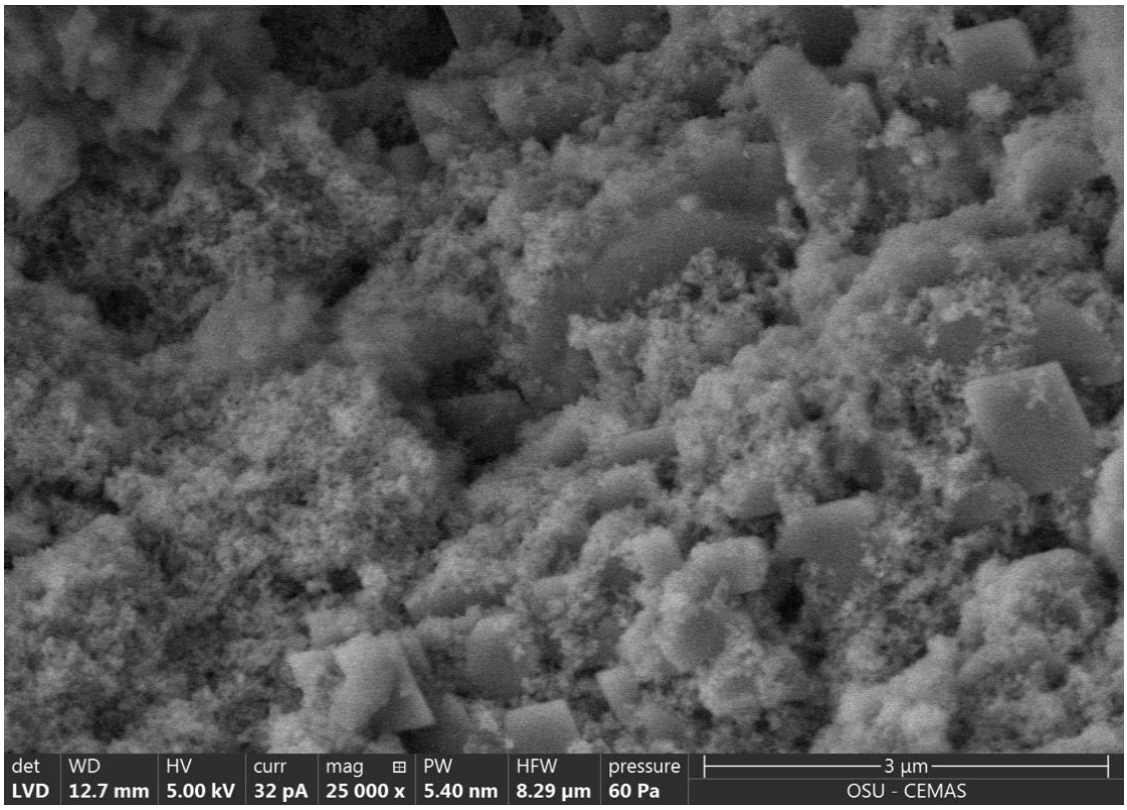

The second landscape encompassed the areas with the best-defined melanosomes, which were all located on the pigmented stripes. These areas demonstrated clusters of sphere-like structures that could be isolated or in a matrix of other structures. Between all areas, the average diameter of the melanosomes was 0.289µm. This measurement, along with the very low aspect ratio due to being near-spherical, indicates the probable presence of phaeomelanosomes instead of eumelanosomes. This would also suggest that the color of the specimen's stripes was most likely a rufous brown in life. There is some debate on whether phaeomelanosomes can be found outside of birds and mammals (D'Alba and Shawkey, 2019). This approach negates melanosome morphology in reptiles, fish, etc., and instead suggests that any melanosome found is a eumelanosome despite the seemingly phaemelanosome characteristics. However, some researchers believe phaemelanin has been discovered in fossil dinosaurs (Brown et al, 2017) and

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other animals. The fossil fish specimen shows a strong correlation between melanosomes being present only where the pigment has apparently been preserved, and this debate could change whether the fish had stripes of rufous brown or grey to black during its life but not the presence of preserved pigment.

The remaining areas (7, 8, 9, and 10) were a mix of the two landscapes and included few melanosomes as well as some cauliflower-like structures. Each of these areas was on the border of a pigmented stripe and can therefore reasonably be expected to hold some pigment from melanosomes while also having barren areas devoid of melanosomes.

Multiple other fossil specimens of different species have had their melanosomes imaged and analyzed. A fossilized moonfish was preserved with an assortment of different melanosomes, each type representing a different area of the body (Rossi et al, 2022). Organs had smaller, more elongate melanosomes, while the integument had larger melanosomes that preserved in a well-defined pattern (Rossi et al, 2022). The specimen in this study displays integumentary melanosomes in a well-defined pattern as well, but these melanosomes are close to the smallest size possible, raising the question of how large any organ-based melanosomes that might have preserved could be.

Quite different from the Eocene moonfish, an early Cretaceous specimen of the dinosaur *Psittacosaurus* was also found to have possessed integumentary melanosomes, though instead of finding ovoid or elongate shapes, ovoid impressions were reported where melanosomes were

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plucked out during the fossil's history (Vinther et al, 2016). This can possibly be seen on the fossil fish in area 4.

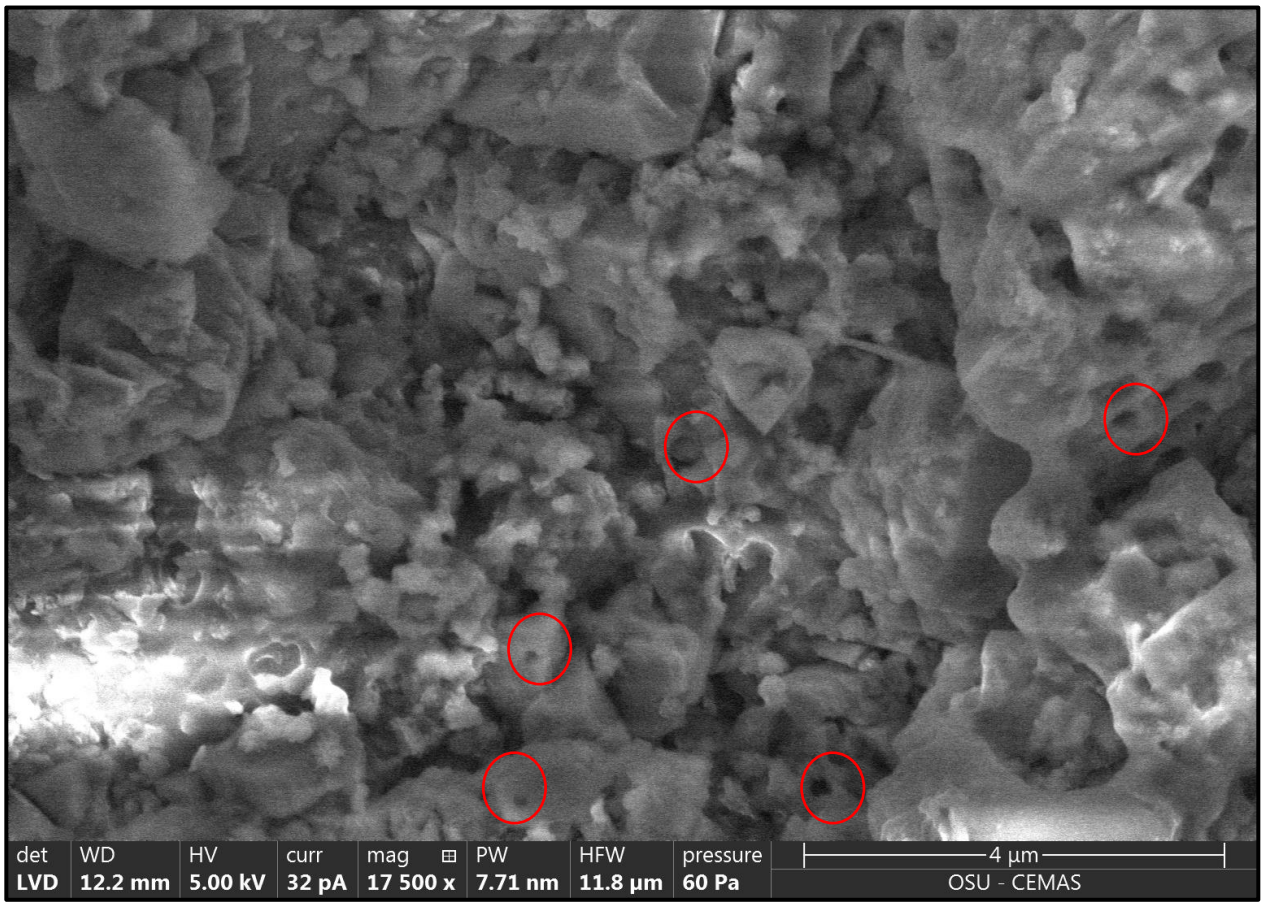

Figure 7. Area 4 with possible melanosome impressions circled in red

As the impressions were near-spherical, the coloration on the *Psittacosaurus* is interpreted as rufous brown despite being a reptile (Vinther et al, 2016). The *Archeopteryx* also had melanosome impressions alongside actual melanosomes, and these impressions followed the barbules of a feather just like actual melanosomes (Carney et al, 2011). According to another dinosaur study that focused on a well-preserved *Borealopelta*, no melanosomes could be found through SEM imaging. However, the specimen itself is a rich reddish-brown color and perfectly captures a phaemelanin shade. It is suggested that this is the result of phaeomelanosomes being less stable during autoclave experiments (Brown et al, 2017), which could be an explanation for

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the original belief that all organisms besides mammals and bird do not possess phaeomelanosomes. Lindgren et al (2014) focused on color adapted for underwater environments through analyzing melanosomes found in an ichthyosaur, mosasaur, and fossil leatherback turtle. Small amounts of phaemelanin were detected, though the skin primarily consisted of eumelanin. This further supports the existence of phaemelanin outside of mammals and birds (Lindgren et al, 2023)

Understanding melanosome type and distribution has led to inferences pertaining to the ecological niches of extinct species, including those already mentioned. The moonfish lived in a different environment than modern moonfish (Rossi et al, 2022). This is inferred based on the type of countershading and horizontal stripes that support a more open marine habitat near peri-reefal systems. The horizontal stripes were also likely used as a confusion technique while they swam in large schools to disorient predators. Modern moonfish possess rows of spots instead of horizontal striping, which is theorized to be due to ecologic restructuring of marine systems during the Cenozoic (Rossi et al, 2022).

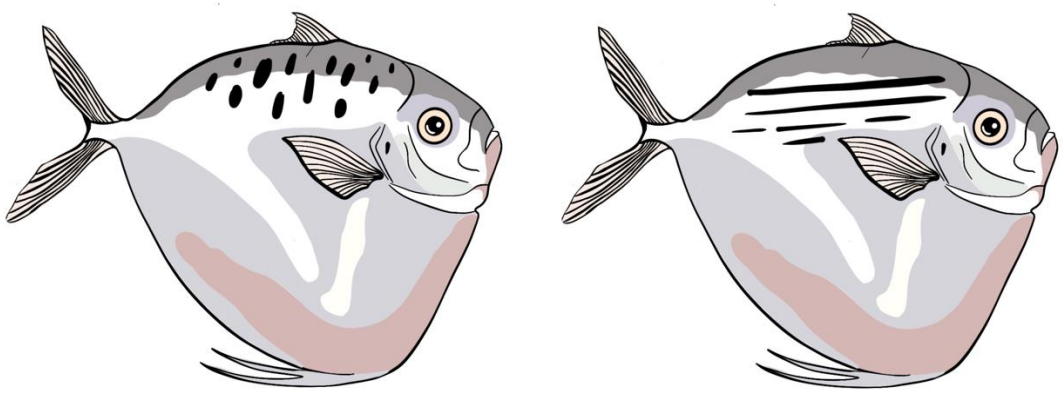

Figure 8. Illustration showing the difference in patterns of a modern moonfish (left) and fossil moonfish (right) (After Rossi et al, 2022)

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Another marine example, the ichthyosaur, had uniform dark pigmentation (Lindgren et al, 2023), which could have acted as background matching in the low light environments ichthyosaurs would reach during a deep dive. Mosasaurs, while not having an obvious pigment pattern, are thought to have been dull and non-reflective. Both marine reptiles possessed dark pigment for crypsis and suggested thermoregulation, with the latter indicating habitation of cold environments (Lindgren et al, 2023).

Terrestrial specimen examples possess similar qualities. The well-preserved *Borealopelta* has strong evidence for countershading despite its large size exceeding any modern terrestrial specimen with counter shading. Modern large herbivores do not display counter shading due to general low predation as their size makes them a difficult target for most predators (Brown et al, 2017). The same is true for modern apex predators, as well as smaller herbivores with protective body parts, such as horns or the dermal plating of the ankylosaurids. Therefore, a *Borealopelta* utilizing this method of crypsis raises questions as to what predators could have preyed upon an organism that, by modern expectations, would be too formidable to act as easy prey (Brown et al, 2017). The *Psittacosaurus* example led to the reconstruction of its environment through diffuse illumination tests to see which best distorted its body-shape due to its pigment pattern. It is now known that they lived in closed light environments, such as a forest with a canopy (Vinther et al, 2016). A *Sinosauropteryx* displayed countershaded crypsis to break up its body-shape both to hide it from predators, as well as prevent its own prey from detecting it. It inhabited large open areas where hiding in the cover of foliage was limited, which aligns with its heavily pigmented pattern relative to its small size (Fiann et al, 2017).

Discovering information about the environments inhabited by these specimens due to their various crypsis techniques based on preserved melanosome distribution allows for more in-

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depth descriptions of other fossil specimen niches. Counter-shading plays on shadows and light angles and intensities to disrupt the 3D perception of the specimen. Disruptive coloration tries to match the background environment, thus obscuring its body shape, and lowering its recognizability (Vinther, 2020). In a study focusing on the survivability of modern perch with disruptive coloration in different environments, an environment with a 'busy' background of different colors and shadows was greatly increased when compared to a background that is uniform in appearance (Phillips, et al. 2017). The overall darker dorsal section on the Hamilton Quarry fish specimen with some pigmented streaks that are near perpendicular to the pigmented lines would act as counter shading, with the darker tone disrupting shadows that would otherwise highlight the body shape of the fish. The stripes fall under disruptive coloration. Like the perch in the study from Phillips, et al., it is likely the Hamilton Quarry fish specimen lived in an environment with a complex background, such as within or near to a reef. This supports the interpreted depositional environment of the Hamilton quarry as a tidal inlet with fresh-to-brackish water where the fish likely hatched in a nearshore hatching ground (French et al, 1988). These stripes would then break up the body shape and make it harder to differentiate from the background and therefore harder for predators to discern.

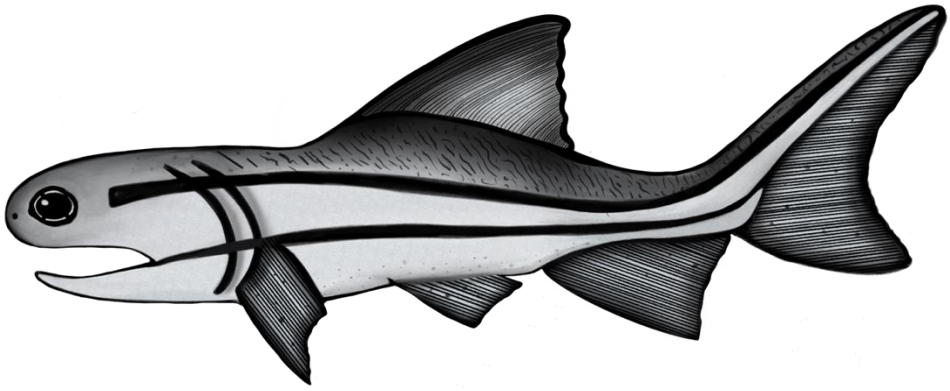

Figure 9. Reconstruction of what the specimen could have looked like in life

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CONCLUSION

The preservation and mapping of melanosome distribution offers insights into past ecological relationships and environments. The fish specimen of this study showed a strong positive correlation between presence of melanosomes at visibly pigmented locations, further supporting the interpretation of two lateral stripes and a likely darker dorsal section. These lateral stripes act as disruptive coloration, while the dorsal pigment acts as countershading. Therefore, the fish specimen likely lived in an environment with a complex background, which aligns with the perceived inlet environment of the Hamilton Quarry location. Further research should include more detailed mapping of fossilized fish melanosomes to better understand both paleoecology and how pigment patterns have evolved through time.

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