CRYPSIS IN THE PELAGIC REALM: EVIDENCE FROM EXCEPTIONALLY PRESERVED FOSSIL FISH LARVAE FROM THE EOCENE STOLLEKLINT CLAY OF DENMARK

by MIRIAM HEINGÅRD1, PETER SJÖVALL2, RENÉ L. SYLVESTERSEN3, BO P. SCHULTZ3 and JOHAN LINDGREN1

1Department of Geology, Lund University, Lund, Sweden; miriam.heingard@geol.lu.se, johan.lindgren@geol.lu.se
2Materials & Production, RISE Research Institutes of Sweden, Borås, Sweden; peter.sjovall@ri.se
3Museum Salling, Fur Museum, Fur, Denmark; rlsy@museumsalling.dk, bosc@museumsalling.dk

Typescript received 2 November 2020; accepted in revised form 3 July 2021

Abstract: Marine deposits of earliest Eocene age in northern Jutland, Denmark, are renowned for yielding diverse teleost assemblages that have proved central for enhancing our understanding of the early evolution of many extant actinopterygian clades. In this study, we investigate diminutive larval fish fossils from the Stolleklint Clay, Ølst Formation, that retain multiple soft-tissue features preserved as distinct dark-coloured stains. To examine the elemental and molecular composition of these soft parts, we employed a combination of time-of-flight secondary ion mass spectrometry (ToF-SIMS), scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDS). Our analyses revealed that the preserved structures contain chemically identifiable eumelanin intimately associated with densely aggregated microbodies that are morphologically consistent with melanosome organelles. Thus, we conclude that the carbonaceous structures represent traces of originally melanized body parts, including the eyes and peritoneum. Comparable pigmentation patterns are seen in many extant teleost larvae that use semi-transparency as a means of camouflage in pelagic environments, to suggest a similar visual appearance of the Stolleklint Clay fish fossils. This in turn suggests that adaptations for concealment and UV-protection had already evolved by the beginning of the Eocene, notably during a time interval characterized by an extreme greenhouse climate, when the global fish fauna become increasingly modern in composition.

Key words: melanin, melanosome, pigmentation, camouflage, Teleostei, Ølst Formation.
stage fish fossils from the Stolleklint Clay of the Ølst Formation. These specimens display structures composed of dark matter that were subjected to high-resolution imaging, elemental and molecular analyses. Our investigation shows that these remnant soft parts are consistent with originally eumelanin-containing internal and integumentary tissues of extant marine fish larvae.

GEOLOGICAL SETTING

The fossils analysed in this study were buried in fine-grained detrital sediments belonging to the Stolleklint Clay, an informal rock unit that crops out locally in the Limfjord region of north-western Jutland, Denmark (Heilmann-Clausen 1995). The Stolleklint Clay is of earliest Ypresian (earliest Eocene) age and constitutes the lowermost part of the Ølst Formation (Heilmann-Clausen 1995), a sedimentary succession that occurs sporadically across the Danish Basin, from northern Jutland (Heilmann-Clausen et al. 1985) to Femern Bælt (Sheldon et al. 2012). In the Limfjord area, the Ølst Formation is represented only by the Stolleklint Clay (Heilmann-Clausen et al. 1985; Heilmann-Clausen 1995). The sediments are thought to have accumulated beneath deep, oxygen-depleted waters within an enclosed marine basin during the PETM (Heilmann-Clausen et al. 1985; Bonde 1997; Bonde et al. 2008) forming grey laminated clays rich in organic matter, with occasional interbedded ash layers that derive from volcanism associated with the opening of the North Atlantic (Heilmann-Clausen 1995; Larsen et al. 2003).

MATERIAL AND METHOD

We selected five fossil fish larvae (Fig. 1; FUM-N-12779, FUM-N-12781, FUM-N-13476, FUM-N-16240 and NHMD 625460) that were mechanically prepared without the addition of either preservatives or consolidants. Four specimens (FUM-N-12781, FUM-N-13476, FUM-N-16240 and NHMD 625460) were split along their long axis to produce opposing part and counterpart samples, whereas one individual (FUM-N-12779) is represented only by a part section. All fossils were examined and photographed using an OlympusSZX10 stereomicroscope equipped with an Olympus SC30 digital camera. In addition, the dark matter in NHMD 625460 (Fig. 1A) was analysed by field-emission scanning electron microscopy (FEG-SEM), energy-dispersive x-ray spectroscopy (EDS), and time-of-flight secondary ion mass spectroscopy (ToF-SIMS).

ToF-SIMS

In ToF-SIMS, a focused beam of high-energy (primary) ions bombards the sample surface, causing the emission of secondary ions, which carry detailed information about molecular compounds and structures on the sample surface (Thiel & Sjövall 2015). By scanning the primary ion beam over a selected analysis area on the sample surface and tracking the yield of secondary ions at different positions, spatially resolved molecular data is obtained and can be presented either as ion images (displaying the signal intensity of selected secondary ions over the analysis area) or as mass spectra from selected regions of interest (ROIs) within the analysis area. Identification of eumelanin by ToF-SIMS is based on the close mass spectral agreement between a sample and well-defined eumelanin standards (e.g. Lindgren et al. 2012, 2014). Although none of the peaks in eumelanin reference spectra by themselves are specific to this pigment, the detection of all major eumelanin-related ions at a relative intensity distribution similar to that of the reference spectra has been found to provide reliable evidence for the presence of eumelanin in fossil samples (Lindgren et al. 2012, 2014, 2015b, 2019).

ToF-SIMS analyses were conducted in a TOFSIMS IV instrument (IONTOF GmbH, Germany) using 25 keV Bi6+ primary ions and low-energy electron flooding for charge compensation. Positive and negative ion data were acquired under static conditions (maximum primary ion dose density < 1012 cm⁻²) with the instrument optimized for high mass resolution (m/Δm ≈ 3000–5000). Calibration of the mass scale was done using the C−, C2−, C3− and C4− ions in negative-ion mode, and CH4+, C2H5+, C3H7+ and C4H9+ ions in positive-ion mode. As a comparative dataset, ToF-SIMS analyses were undertaken on synthetic and natural variants of eumelanin, pheomelanin, several porphyrins and three microbial mats (see details
in Lindgren et al. 2012, 2014, 2015a). We also examined a pyomelanin chemically derived via auto-oxidation of homogentisic acid (HGA; see Turick et al. 2002 for methodology), as well as isolated melanosomes from the eyes of a teleost fish, *Astatotilapia latifasciata* (Lindgren et al. 2015a, b).

**RESULTS**

**General description**

The fish larvae from Stolleklint Clay measure between 8 and 14 mm in standard length (i.e. length from the tip of
the snout to the distal end of the last vertebra) and are preserved as flattened, yet largely articulated skeletons that are accessible either in oblique lateral or ventrolateral view (Fig. 1). With the exception of FUM-N-16240 (which can only be assigned to Actinopterygii), all fossils belong to the clade Perciformes. In addition, FUM-N-12779 probably represents a scombrid-like fish based on the presence of finlets. Associated with the bones are dispersed (Figs S1, S2). In both anatomical features, some tissue residues are absent.

Ultrastructural and elemental analyses

Initial macroscopic examination showed well-defined brownish–blackish matter that was distinct from the adjacent bones and sedimentary matrix in both texture and colour (Fig. 1D, G). Subsequent SEM imaging of the orbital stain in NHMD 625460 revealed a dense mass of morphologically heterogeneous microbodies (measuring about 0.7–2.5 µm in maximum dimension), ranging from sub-spherical to highly elongate (Fig. 2A). Although the different morphotypes occurred close to one another, mixing was limited. In stark contrast to this morphological disparity, the abdominal structure contained only sub-spherical to highly elongate (Fig. 2A). SEM-EDS analysis showed that both the orbital and abdominal structure were enriched in carbon relative to the surrounding sediment, where silicon, oxygen and aluminium instead dominated (Figs S1, S2). In both anatomical features, some microbodies occurred partially embedded within a mineral precipitate (Fig. 2B; Fig. S3).

ToF-SIMS

ToF-SIMS data obtained from the orbital and abdominal pigmentation provided evidence for remnant eumelanin in both structures. Negative-ion spectra acquired from the carbonaceous matter faithfully reproduced all major peaks in a reference spectrum of a natural (Sepia) eumelanin standard, both with regard to precise peak positions (mass-to-charge ratio (m/z) values at high mass resolution; Table S1; Heingärd et al. 2021) and their relative intensity distribution (Fig. 2G). Notably, distinct peaks at m/z 50, 66, 74, 90, 98 and 122 represent key nitrogen-bearing ions derived from the eumelanin molecular structure (Lindgren et al. 2012). These peaks were equally prominent (comparable to the other eumelanin-related peaks) in spectra obtained from the fossil structures as they were in the eumelanin reference spectrum, to provide strong evidence for the identification of this pigment in the fossil sample. Additional peaks in the spectra from the fossil originated from sediment-related ions, for example, SiO$_4^{2-}$ (m/z 60), SiO$_3^{2-}$ (m/z 76), FeO$_4^{2-}$ (m/z 88), FeO$_5^{2-}$ (m/z 104), AlSiO$_4^{2-}$ (m/z 119) and Si$_2$O$_7^{2-}$ (m/z 136) (Table S2). Moreover, peaks at m/z 80, 81 and 97 correspond to sulfate-related ions (SO$_4^{2-}$, HSO$_3^-$ and HSO$_4^-$, respectively). These were localized primarily to the eumelanin-rich (pigmented) areas.

Positive-ion ToF-SIMS data from the pigmented structures revealed relatively weak but significant spectral features characteristic of proteinaceous materials (Samuel et al. 2001; Wagner & Castner 2001), including peaks corresponding to CH$_4$N$^+$ (m/z 30), C$_2$H$_4$N$^+$ (m/z 44), C$_3$H$_7$N$^+$ (m/z 70), C$_4$H$_9$N$^+$ (m/z 72), C$_5$H$_{12}$N$^+$ (m/z 86), and C$_6$H$_{10}$N$^+$ (m/z 120) (Fig. S4; Table S3). Furthermore, ion images showed co-localization of all of these N-containing organic fragment ions, suggesting a common origin (Fig. S5). Their spatial distribution was also distinct from those of other components, including polyaromatic and aliphatic compounds, as well as the surrounding sedimentary matrix (Fig. 3A–D). However, the relative signal intensity distributions of these N-bearing fragment ions in the spectra from the fossil were noticeably different from those of modern protein reference samples of collagen, keratin and haemoglobin, showing considerably higher relative intensities from the smaller protein fragment ions (CH$_2$N$^+$ and C$_3$H$_7$N$^+$) and lower intensities from C$_3$H$_7$N$^+$, for example (Fig. 3E).

DISCUSSION

Ultrastructural and chemical preservation

Multiple observations favour an interpretation of the fossil microbodies as remnant melanosomes: (1) the presence of eumelanin residues; (2) their morphology and size, which are within the known ranges of melanosomes in extant vertebrates (e.g. Liu et al. 2005; Sweet et al. 2012); and (3) the marked difference in shape between
the microbodies in the orbital and abdominal stains. The morphological disparity of the remnant melanosomes in the orbital pigmentation is similar to that seen in modern vertebrate eyes (e.g. Liu et al. 2005), as well as previously described fossil fish eye residues (e.g. Lindgren et al. 2012, 2015a), suggesting that the orbital stain in NHMD 625460 likewise represents the fossilized remains of an eye. In addition, the different morphologies are located within distinct taphonomic layers. Several melanin-bearing tissues exist in vertebrate eyes (Liu et al. 2005). Some of these, for example the iris and choroid, contain melanosomes that are relatively homogenous in form (Liu et al. 2005). The retinal pigment epithelium (RPE), on the other hand, contains a variety of melanosome morphologies (Glickman et al. 2001; Liu et al. 2005; Lindgren et al. 2012) reflecting functional differences related to position in the RPE and proximity to apical processes rather than melanin content (Kim & Choi 1998; Liu et al. 2005; Burgoyne et al. 2015). Post mortem decomposition may cause tissue collapse, which in turn could concentrate the more decay-resistant melanosomes from functionally different parts of the eye into densely stacked layers (Clements et al. 2016; McNamara et al. 2018). The observed morphological disparity is thus likely to reflect the original melanosome diversity of the larval fish eyes, where the individual layers represent the various

**FIG. 2.** FEG-SEM and negative-ion ToF-SIMS data collected from the remnant eye and posterior part of the peritoneal structure in NHMD 625460. A–B, FEG-SEM micrographs of the eye (A) and peritoneal (B) residues; note the differences in shape and size of the microbodies, some of which remain partially embedded in a mineral precipitation, between the two structures. C, E, negative-ion ToF-SIMS images (200 × 200 µm²) of ions characteristic of eumelanin (green: C₆H⁺ + C₃N⁻ + C₆H⁺ + C₃N⁻) and sediment (red: SiO₂⁻ + SiO₃⁻ + SiHO₃⁻) in the eye (C) and peritoneal (E) residues. D, F, total ion images with regions of interest (ROIs) representing eumelanin-rich areas in the ‘eye’ and ‘peritoneum’ (D (blue outline) and F (green outline), respectively) and sediment (F (red outline)). G, negative-ion ToF-SIMS spectra of the ROIs indicated in (D) and (F), together with a reference spectrum of a natural eumelanin standard (from Sepia officinalis; stars denote sulfate ions (SO₃⁻, HSO₃⁻ and HSO₄⁻). Scale bars represent 2 µm (A, B). Peak assignments and observed m/z values are provided in Table S1 (eumelanin) and Table S2 (sediment and sulfate) and in Heingård et al. (2021).
functional parts of the RPE, as well as other melanosome-bearing tissues. Conversely, the microbodies of the abdominal pigmentation are exclusively sub-spherical in shape, consistent with internal melanosomes observed in extant fish (Goda & Fujii 1996, fig. 5), and without a tendency to secondary layering. This obvious difference and close agreement to melanosomes in modern tissues, suggest that the pigmentations derive from anatomically distinct structures.

In addition to eumelanin, our ToF-SIMS analyses detected a number of positive ions characteristic of proteins, which could indicate the presence of endogenous proteinaceous moieties in the otherwise largely melanized structures. However, the relative intensities of the nitrogen-containing peaks differed from those of our modern reference samples (Fig. 3E), which might be explained by partial degradation of the proteinaceous matter in the fossil. Regardless, without further in-depth analyses, it is not possible to confidently identify the source(s) of these ions.

Sulfate ions were identified in both melanized residues, as well as in the adjacent sedimentary matrix (albeit at lower signal intensities; Fig. 2G). Sulfur is a natural component of pheomelanin; however, none of the peaks that represent S-bearing ions in ToF-SIMS spectra from pheomelansins (see Lindgren et al. 2014) were prominent in the fossil samples. In addition, there was no indication of melanin leakage into the surrounding sediments. Instead, it is more likely that the presence of sulfate in the pigmented structures indicates secondary incorporation of sulfur into the eumelanin molecular structure (see McNamara et al. 2016).

Rationale for assignment of ontogenetic stage

All stages of the ontogenetic series of teleosts have been documented in the fossil record (Cloutier 2010), however, the early developmental history is usually considerably less known than later stages, a condition that probably reflects the rather poor fossilization potential of fish larvae. Furthermore, although fossils are known (Thomson et al. 2003; Mücklich et al. 2009; Mizumoto et al. 2019; Carnevale & Bannikov 2020), detailed records of specific larval characters, such as pigmentation patterns, are rare. In fact, we are only aware of a single publication describing fossil larval pigmentations (Marramaù & Carnevale 2015, fig. 9A), but without the level of details shown herein.

In addition to their small size, the Stolleklint Clay fish fossils display several features consistent with relatively early ontogenetic stages. An upward caudal inclination of the notochord can be seen in all five fish fossils, indicating that the notochord flexion was ongoing and perhaps even completed at the time of death. This 45° dorsal bending is completed during the final stage of the larval phase, termed the post-flexion stage (Kendall et al. 1984). Additionally, both the fin rays and caudal fin appear to be well developed; these body parts normally grow rapidly during the flexion stage to become fully formed during the juvenile phase (Kendall et al. 1984). Furthermore, the

**FIG. 3.** Positive-ion ToF-SIMS data from pigmented structures in NHMD 625460. A–C, ion images of the posterior part of the peritoneal structure (same area as in Fig. 2E, F) representing: A, sediment; Al⁺ (m/z 27), Si⁺ (m/z 28) and Fe⁺ (m/z 56); B, N-containing organics; CH₄N⁺ (m/z 30), C₂H₂N⁺ (m/z 44) and C₄H₄N⁺ (m/z 70); C, polyaromatics; PAH; C₆H₅⁺ (m/z 77), C₇H₇⁺ (m/z 91), C₈H₈⁺ (m/z 115), C₁₀H₁₀⁺ (m/z 128) and C₁₃H₁₃⁺ (m/z 165) (Stephan et al. 2003; Lindgren et al. 2018). D, overlay image of A–C, displaying sediment in red, N-containing organics in green, and PAH in blue; note distinct spatial distribution of N-containing organic fragment ions relative to ions derived from the sediment and PAHs, as well as from eumelanin (Fig. 2E). E, signal intensity distribution of characteristic ‘protein’ fragment ions in ToF-SIMS spectra from the pigmented fossil structures (‘peritoneum’ (posterior and anterior) and ‘eye’) and three modern protein reference samples (collagen type I, alpha-keratin and haemoglobin). Peak assignments and observed m/z values of the N-containing fragment ions are provided in Table S3.
fossils seemingly lack scales and a complete body pigmentation (Fig. 1). This is typical for marine fish larvae (Kendall et al. 1984; Urho 2002), and unlike the condition of adult teleosts from the same formation (Fig. 4). Instead, the fossil fish larvae display pigmentation patterns that are entirely consistent with melanin depositions in integumentary and internal tissues of extant teleost larvae (Fig. 1H; Moser et al. 1984; Ré & Meneses 2008; Mwalamu et al. 2014; Rodríguez et al. 2017). Although the distribution of colour-producing agents can be unique to a species (Ahlstrom & Moser 1976; Baldwin 2013), recurrent melanin pigmentation patterns exist that have presumably evolved independently in different lineages, and are found in nearly all extant teleost groups during their larval phase (Moser 1981; Moser et al. 1984; Ré & Meneses 2008). This includes distinct melanophores on the top of the head, along the dorsal and ventral body margins, as well as in the dorsal part of the peritoneum (Fig. 1H). These same features are preserved in great detail in the Stolleklint Clay fish larvae, potentially down to individual cells (Fig. 1D), strongly suggesting a larval origin. For instance, the elongate abdominal structure (indicated by arrowheads in Fig. 1G) corresponds perfectly with the distribution of peritoneal melanophores associated with the gas bladder and/or gut in modern fish larvae (Fig. 1H), an observation that is independently corroborated by the melanosome and eumelanin pigment residues detected in our analyses. In modern fishes, these internal melanophores are often visible through the otherwise semi- to fully transparent larvae before being lost or hidden by muscles and integumentary pigments during later ontogenetic stages (e.g. Cunningham 1891; Ahlstrom & Ball 1954; Clarke et al. 1997; Leis et al. 2002; Parichy et al. 2009; Çoban et al. 2012; Kondo et al. 2013; Park et al. 2017; Roux et al. 2019).

Palaeobiological implications

A pelagic larval stage is a common life history trait among extant marine fishes (Sars 1879; Kendall et al. 1984). In these homogenous open water environments, small animals, such as fish larvae, commonly use transparency as a means of camouflage (Breder 1962; Meyer-Rochow 1974; McFall-Ngai 1990; Johnsen 2001, 2014). For tissues to become transparent, absorbance and scattering of light need to be minimized (Johnsen 2001). Modifications to achieve this condition include adjustments of the size and packing of organelles (as well as other subcellular components; Johnsen & Widder 1999), a high water content in the tissues (e.g. mesoglea in cnidarians; Chapman 1976), and clearing agents to change the refractive index of extracellular fluids and/or cytoplasm (Johnsen 2014). Transparency in animals with complex tissues, such as fish larvae, is relatively poorly understood (Johnsen 2001). However, studies on eel larvae have shown that these fishes use a hydrated extracellular matrix in their bodies, which consists of transparent energy-storing glycosaminoglycans (Pfeiler 1999; Pfeiler et al. 2002). Moreover, they have flattened bodies that function to minimize the optical path through the tissues, thereby reducing scattering (Meyer-Rochow 1974; Miller 2009). Although many of these biochemical and ultrastructural alterations have low preservation potential, transparency in the fossil larvae described herein can nonetheless be inferred from their overall lack of body pigmentation and squamation, in combination with the highly specific pigmentation patterns that are identical to those observed in extant transparent pelagic fish larvae. Given that a full body pigmentation is preserved in adult teleosts from the Stolleklint Clay (Fig. 4), these features strongly suggest that the fossil fish larvae likewise were at least semi-transparent in life, presumably as a means of crypsis in open-water settings.

Few small pelagic animals are fully transparent but instead exhibit rudimentary colour patterns despite the apparent risk of detection by visually hunting predators, to suggest important functions of these pigmentations (for instance, melanin in vertebrate eyes is essential for visual capacity; Strauss 2005). Nonetheless, the functional aspects of specific pigmentation patterns in otherwise transparent fish larvae (as well as other zooplankton) are generally poorly understood (Moser 1981; Kendall et al. 1984). The peritoneal melanophores associated with the gut have been hypothesized to help conceal the larvae from predators by masking light refracted from gut content and/or screening bioluminescent prey in the

FIG. 4. Examples of non-larval teleost fish fossils from the earliest Eocene Stolleklint Clay. A, FUM-N-11870 (Scombridae). B, FUM-N-14772 (Carangidae). Note potential countershading. Scale bars represent 5 mm.
alimentary canal (Herring 1967; Moser 1981; Eastman & DeVries 1997). Similarly, the shield of melanophores above the gas bladder could potentially reduce light refraction in this area (Moser 1981). It has also been suggested that specific pigmentation patterns might play a role in intraspecific recognition among fish larvae (Moser 1981). Furthermore, the transparent nature of many larvae means that vital parts, such as the developing internal organs, may be exposed to harmful ultraviolet solar radiation (UVR). UVR has been shown to be detrimental to fish, especially during their early life stages (Hunter et al. 1979; Kouwenberg et al. 1999; Browman et al. 2000; Lesser et al. 2001; Steeger et al. 2001) and it has been suggested that the primary function of pigments in young fish is protection against harmful UVR rather than camouflage (Mueller & Neuhaus 2014). Moreover, pigmentation in crustacean zooplankton, inhabiting similar niches to many ichthyoplankton, have been shown to reduce UVR-induced damage (Morgan & Christy 1996; Bashevkin et al. 2019). Thus, the taxonomically widespread development of melanophores arranged in certain patterns in fish larvae, shown here to be traceable at least back to the early Eocene, might also indicate an adaptive photo-protective function for these eumelanin deposits (Breder 1962; Moser 1981). Indeed, a recent study (Kapp et al. 2018) found that all teleosts develop melanophores that cover the haematopoietic niche housing stem cells during their larval phase. The stem cells received measurable DNA damage when the protective pigmentation was experimentally removed (Kapp et al. 2018).

CONCLUSION

The fossil fish larvae described in this study exhibit residues of both internal and integumentary tissues in the form of dark organic stains, shown here to be dominated by traces of the pigment eumelanin. These remnant soft parts correspond in size, composition and anatomical position to eyes, peritoneum and integumental melanophores, respectively, forming pigmentation patterns that are virtually identical to those of extant pelagic teleost larvae.

Modern fish larvae are known to use semi-transparency as a means of crypsis in featureless open water environments. Consequently, the close resemblance between our fossils and extant teleosts indicates that the Eocene fish larvae used a similar strategy to camouflage themselves. Thus, the preserved pigmentation patterns are likely to represent means for concealment and UV protection, but also requirements for visual capacity.

Acknowledgements. The photograph of the modern sea bream larva shown in Figure 1H is credited to Bernd Ueberschär. Niels Bonde provided taxonomic information on FUM-N-12779, FUM-N-12781, FUM-N-13476, FUM-N-16240, NHMD 625460 and FUM-N-14772. We thank Annie Marie Kaargaard for the discovery of NHMD 625460. Financial support for this project was provided by a Grant for Distinguished Young Researchers (Award No 642-2014-3773; Swedish Research Council) to Johan Lindgren, as well as a project grant (Award No 2019-03731; Swedish Research Council) to Peter Sjövall. Rana N. S. Sodhi and an anonymous referee commented on an earlier draft of this manuscript.

DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.pShqBzkP6

Editor. Lionel Cavin

SUPPORTING INFORMATION

Additional Supporting Information can be found online (https://doi.org/10.1111/pala.12574):

- Fig. S1. Energy-dispersive x-ray spectroscopic maps.
- Fig. S2. Energy-dispersive x-ray spectroscopic line scan.
- Fig. S3. Selected spectra from energy-dispersive x-ray spectroscopy.
- Fig. S4. Positive TOF-SIMS spectra.
- Fig. S5. Positive ToF-SIMS ion images.
- Table S1. Eumelanin-related ions in the negative-ion spectra (ToF-SIMS).
- Table S2. Sediment- and sulfate-related ions in the negative-ion spectra (ToF-SIMS).
- Table S3. Protein fragment ions in the positive-ion spectra (ToF-SIMS).

REFERENCES

AHLSTROM, E. H. and BALL, O. P. 1954. Description of eggs and larvae of jack mackerel (Trachurus symmetricus) and distribution and abundance of larvae in 1950 and 1951. Fishery Bulletin, 56, 209–245.

AHLSTROM, E. H. and MOSER, H. G. 1976. Eggs and larvae of fishes and their role in systematic investigations and in fisheries. Revue des Travaux de l’Institut des Pêches Maritimes, 40, 379–398.

BALDWIN, C. C. 2013. The phylogenetic significance of colour patterns in marine teleost larvae. Zoological Journal of the Linnean Society, 168, 496–563.

BASHEVKIN, S. M., CHRISTY, J. H. and MORGAN, S. G. 2019. Photoprotective benefits of pigmentation in the transparent plankton community: a comparative species experimental test. Ecology, 100, e02680.

BONDE, N. 1987. Moler og fossiler – især fisk. Skamol, 52 pp.
BONDE, N. 1997. A distinctive fish fauna in the basal ash-series of the Fur/Ølsted Formation (U. Paleocene, Denmark). 33–48. In THOMSEN, E. and PEDERSEN, S. A. S. (eds), Geology and palaeontology of the Mo-clay. Aarhus Geoscience, 6, 68 pp.

BONDE, N., ANDERSEN, S., HALD, N. and JAKOBSEN, S. L. 2008. Danekræ: Danmarks bedste fossiler. Gyldendal, Copenhagen, 225 pp.

BREDER, C. M. 1962. On the significance of transparency in osteichthid fish eggs and larvae. Copeia, 1962, 561–567.

BROWMAN, H. I., ALONSO RODRIGUEZ, C., BÉLAND, F., CULLEN, J. J., DAVIS, R. F., KOUWENBERG, J. H. M., KUHN, P. S., McARTHUR, B., RUNGE, J. A., ST-PIERRE, J.-F. and VETTER, R. D. 2000. Impact of ultraviolet radiation on marine crustacean zooplankton and ichthyoplankton: a synthesis of results from the estuary and Gulf of St. Lawrence, Canada. Marine Ecology Progress Series, 199, 293–311.

BURGOYNE, T., O’CONNOR, M. N., SEABRA, M. C., CUTLER, D. F. and FUTTER, C. E. 2015. Regulation of melanosome number, shape and movement in the zebrafish retinal pigment epithelium by OA1 and PMEL. Cell & Development Biology, 128, 1400–1407.

CARNÉVALE, G. and BANNIKOV, A. F. 2020. A tholichthys-like larva (Teleostei, Percomorpha) from the Eocene of northern Caucasus, Russia. Lethaia, 54, 204–210.

CHAPMAN, G. 1976. Reflections on transparency. 491–499. In MACKIE, G. O. (ed.), Coelenterate ecology and behavior. Springer.

CLARKE, M. E., DOMEIER, M. L. and LAROCHE, W. A. 1997. Development of larvae and juveniles of the mutton snapper (Lutjanus analis), lane snapper (Lutjanus synagris) and yellowtail snapper (Lutjanus chrysourus). Bulletin of Marine Science, 61, 511–537.

CLEMENTS, T., DOLOCAN, A., MARTIN, P., PURNELL, M. A., VINTHER, J. and GABBOTT, S. E. 2016. The eyes of Tullimonstrum reveal a vertebrate affinity. Nature, 532, 500–503.

CLOUTIER, R. 2010. The fossil record of fish ontogenies: insights into developmental patterns and processes. Seminars in Cell & Developmental Biology, 21, 400–413.

ÇOBAN, D., SUZER, C., YILDIRIM, Ş., SAKA, Ş. and FIRAT, K. 2012. Morphological development and allometric growth of sharpnose seabream (Diplodus puntazzo) larvae. Turkish Journal of Fisheries & Aquatic Sciences, 12, 883–891.

CUNNINGHAM, J. T. 1891. On some larval stages of fishes. Journal of the Marine Biological Association of the United Kingdom, 2, 68–74.

EASTMAN, J. T. and DEVRIES, A. L. 1997. Morphology of the digestive system of Antarctic nototheniid fishes. Polar Biology, 17, 1–13.

GLICKMAN, R. D., JACQUES, S. L., HALL, R. T. and KUMAR, N. 2001. Revisiting the internal absorption coefficient of the retinal pigment epithelium melanosome. Proceedings of SPIE, 4257, 134–141.

GOJA, M. and FUJII, R. 1996. Biology of the chromatophores of the ice goby, Leucopsetron petrii. Zoological Science, 13, 783–793.

GRENS, J. A., SJÖVALL, P., ERIKSSON, M. E., SYLVESTERSSEN, R. L., MARONE, F., SIGFRIÐSDÓNN CLAUSS, K. G. V., TAYLOR, G. J., CARLSON, S., UVDAL, P. and LINDGREN, J. 2017. Molecular and microstructural inventory of an isolated fossil bird feather from the Eocene Fur Formation of Denmark. Palaeontology, 60, 73–90.

HEILMANN-CLAUSEN, C. 1995. Palaeogene allejringer over danskekalken. 70–114. In NIELSEN, O. B. (ed.), Danmarks geologi fra Kridt til i dag. Aarhus Geokompender, 1. Geologisk Institut, Aarhus Universitet, 290 pp.

HEILMANN-CLAUSEN, C., NIELSEN, O. B. and GERSNER, F. 1985. Lithostratigraphy and depositional environment in the upper Paleocene and Eocene of Denmark. Bulletin of the Geological Society of Denmark, 33, 287–323.

HEINGÅRD, M., SJÖVALL, P., SYLVESTERSSEN, R. L., SCHULTZ, B. P. and JOHAN LINDGREN, J. 2021. Data from: Crypsis in the pelagic realm: evidence from exceptionally preserved fossil fish larvae from the Eocene Stollekild Clay of Denmark. Dryad Digital Repository. https://doi.org/10.5061/dryad.p5bhpkp6

HERRING, P. J. 1967. The pigments of plankton at the sea surface. Symposia of the Zoological Society of London, 19, 215–235.

HUNTER, J. R., TAYLOR, J. H. and MOSER, H. G. 1979. Effect of ultraviolet irradiation on eggs and larvae of the northern anchovy, Engraulis mordax, and the Pacific mackerel, Scomber japonicas, during the embryonic stage. Photochemistry & Photobiology, 29, 325–338.

JOHNSEN, S. 2001. Hidden in plain sight: the ecology and physiology of organismal transparency. The Biological Bulletin, 201, 301–318.

JOHNSEN, S. 2014. Hide and seek in the open sea: pelagic camouglage and visual countermeasures. The Annual Review of Marine Science, 6, 369–392.

JOHNSEN, S. and WIDDER, E. A. 1999. The physical basis of transparency in biological tissue: ultrastructure and the minimization of light scattering. Journal of Theoretical Biology, 199, 181–198.

KAPP, F. G., PERLIN, J. R., HAGEDORN, E. J., GANSNER, J. M., SCHWARZ, D. E., O’CONNELL, L. A., JOHNSON, N. S., AMEMIYA, C., FISHER, D. E., WÖLFE, U., TROMPOUKI, E., NIEMEYER, C. M., DRIEVER, W. and ZON, L. I. 2018. Protection from UV light is an evolutionarily conserved feature of the haematopoietic niche. Nature, 558, 445–448.

KENDALL, A. W. Jr, AHLSTROM, E. H. and MOSES, H. G. 1984. Early life history stages of fishes and their characters. 11–22. In MOSES, H. G., RICHARDS, W. J., COHEN, D. M., FAHAY, M. P., KENDALL, A. W. and RICHARDSON, S. L. (eds), Ontogeny and systematics of fishes. Allen Press, 760 pp.

KIM, T. and CHOI, J. B. 1998. Melanosomes of retinal pigment epithelium – distribution, shape, and acid phosphatase activity. Korean Journal of Ophthalmology, 12, 85–91.

KONGO, M., MAEDA, K., HIRASHIMA, K. and TACHIHARA, K. 2013. Comparative larval development of three amphidromous Rhinogobius species, making reference to
their habitat preferences and migration biology. Marine & Freshwater Research, 64, 249–266.

KOUWENBERG, J. H. M., BROWMAN, H. L., CULLEN, J. J., DAVIS, R. F., ST-PIERRE, J.-F. and RUNGE, J. A. 1999. Biological weighting of ultraviolet (280–400 nm) induced mortality in marine zooplankton and fish. I. Atlantic cod (Gadus morhua) eggs. Marine Biology, 134, 269–284.

LARSEN, L. M., FITTON, J. G. and 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.

LEIS, J. M., TRNSKI, T. and BECKLEY, L. E. 2002. Larval development of Pagellus natalensis and what larval morphology indicates about relationships in the perciform fish family Sparidae (Teleostei). Marine & Freshwater Research, 53, 367–376.

LESSER, M. P., FARRELL, J. H. and WALKER, C. W. 2001. Oxidative stress, DNA damage and p53 expression in the larvae of Atlantic cod (Gadus morhua) exposed to ultraviolet (290–400 nm) radiation. Journal of Experimental Biology, 204, 157–164.

LINDGREN, J., UVDAL, P., SJÖVALL, P., NILSSON, D. E., ENGDAL, A., SCHULTZ, B. P. and THIEL, V. 2012. Molecular preservation of the pigment melanin in fossil melanosomes. Nature Communications, 3, 824–831.

LINDGREN, J., SJÖVALL, P., CARNEY, R. M., UVDAL, P., GREN, J. A., DYKE, G., SCHULTZ, B. P., SHAWKEY, M. D., BARNES, K. R. and POLCYN, M. J. 2014. Skin pigmentation provides evidence of convergent melanism in extinct marine reptiles. Nature, 506, 484–488.

LINDGREN, J., MOYER, A., SCHWEITZER, M. H., SJÖVALL, P., UVDAL, P., NILSSON, D. E., HEIMDAL, J., ENGDAL, A., GREN, J. A., SCHULTZ, B. P. and KEAR, B. P. 2015a. Interpreting melanin-based colouration through deep time: a critical review. Proceedings of the Royal Society B, 282, 20150614.

LINDGREN, J., SJÖVALL, P., CARNEY, R. M., CINCOTTA, A., UVDAL, P., HUTCHESON, S. W., GUSTAFSSON, O., LEFEVRE, U., ESCUILLÉ, F., HEIMDAL, J., ENGDAL, A., GREN, J. A., KEAR, B. P., WAKAMATSU, K., YANS, J. and GODEFROIT, P. 2015b. Molecular composition and ultrastructure of Jurassic paravian feathers. Scientific Reports, 5, 13520.

LINDGREN, J., KURIYAMA, T., MADSEN, H., SJÖVALL, P., ZHENG, W., UVDAL, P., ENGDAL, A., MOYER, A. E., GREN, J. A., KAMEZAKI, N., UENO, S. and SCHWEITZER, M. H. 2017. Biochemistry and adaptive colouration of an exceptionally preserved juvenile fossil sea turtle. Scientific Reports, 7, 13324.

LINDGREN, J., SJÖVALL, P., THIEL, V., ZHENG, W., ITO, S., WAKAMATSU, K., HAUFF, R., KEAR, B. P., ENGDAL, A., ALWMARK, C., ERIKKSSON, M. E., JARENMARK, M., SACHS, S., AHLBERG, P. E., MARONE, P., KURIYAMA, T., GUSTAFSSON, O., MAMBBERG, P., THOMEN, A., RODRÍGUEZ-MEIZOSO, I., UVDAL, P., OJIMA, M. and SCHWEITZER, M. H. 2018. Soft-tissue evidence for homeothermy and crypsis in a Jurassic ichthyosaur. Nature, 564, 359–365.

LINDGREN, J., NILSSON, D. E., SJÖVALL, P., JARENMARK, M., ITO, S., WAKAMATSU, K., KEAR, B. P., SCHULTZ, B. P., SYLVESTERSEN, R. L., MADSEN, H., LAFOUNTAIN, J. R. Jr, ALWMARK, C., ERIKKSSON, M. E., HALL, S. A., LINDGREN, P., RODRÍGUEZ-MEIZOSO, I. and AHLBERG, P. 2019. Fossil insect eyes shed light on trilobite optics and the arthropod pigment screen. Nature, 573, 122–125.

LIU, Y., HONG, L., WAKAMATSU, K., ITO, S., ADHYARU, B. B., CHENG, C. Y., BOWERS, C. R. and SIMON, J. D. 2005. Comparisons of the structural and chemical properties of melanosomes isolated from retinal pigment epithelium, iris and choroid of newborn and mature bovine eyes. Photochemistry & Photobiology, 81, 510–516.

MARRAMÁ, G. and CARNEVALE, G. 2015. The Eocene sardine Bolcaichthys catopygopterus (Woodward, 1901) from Monte Bolca, Italy: osteology, taxonomy, and paleobiology. Journal of Vertebrate Paleontology, 35, e1014490-2.

McFALL-NGAI, M. J. 1990. Crypsis in the pelagic environment. American Zoologist, 30, 175–188.

MCNAMARA, M. E., VAN DONGEN, B. E., LOCKYER, N. P., BULL, I. D. and ORR, P. J. 2016. Fossilization of melanosomes via sulfurization. Palaeontology, 59, 337–350.

MCNAMARA, M. E., KAYE, J. S., BENTON, M. J., ORR, P. J., ROSSI, V., ITO, S. and WAKAMATSU, K. 2018. Non-integumentary melanosome bias reconstructions of the colours of fossil vertebrate skin. Nature Communications, 9, 2878.

MEYER-ROCHOW, V. B. 1974. Leptocephali and other transparent fish larvae from the South-Eastern Atlantic Ocean. Zoologischer Anzeiger, 192, 240–251.

MICKLICH, N. R., TYLER, J. C., JOHNSON, G. D., SWIDNICKA, E. and BANNIKOV, A. F. 2009. First fossil records of the tholichthys larval stage of butterfly fishes (Perciformes, Chaetodontidae), from the Oligocene of Europe. Paläontologische Zeitschrift, 83, 479–497.

MILLER, M. J. 2009. Ecology of Anguilliform Leptocephali: remarkable transparent fish larvae of the ocean surface layer. Aqua-BioScience Monographs, 2, 1–94.

MIZUMOTO, N., MIYATA, S. and PRATT, S. C. 2019. Inferring collective behaviour from a fossilized fish shoal. Proceedings of the Royal Society B, 286, 20190891.

MORGAN, S. G. and CHRISTY, J. H. 1996. Survival of marine larvae under the countervailing selective pressures of photodamage and predation. Limnology & Oceanography, 41, 498–504.

MOUSER, H. G. 1981. Morphological and functional aspects of marine fish larvae. 89–126. In LASKER, R. (ed.) Marine fish larvae: Morphology, ecology, and relation to fisheries. University of Washington Press, 131 pp.

MOUSER, H. G., RICHARDS, W. J., COHEN, D. M., FAHAY, M. P., KENDALL, A. W. and RICHARDSON, S. L. 1984. Ontogeny and systematics of fishes. Allen Press, 760 pp.

MUELLER, K. P. and NEUHAUSS, S. C. F. 2014. Sunscreen for fish: co-option of UV light protection for camouflage. PLoS One, 9, e87372.
MWALUMA, J. M., KAUNDA-ARARA, B. and STRYDOM, N. A. 2014. A guide to commonly occurring larval stages of fishes in Kenyan coastal waters. Western Indian Ocean Marine Science Association Book Series, 15, Mombasa, 73 pp.

PARICHY, D. M., ELIZONDO, M. R., MILLS, M. G., GORDON, T. N. and ENGESZER, R. E. 2009. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. Developmental Dynamics, 238, 2975–3015.

PARK, J.-M., HAN, K.-H., KANG, S.-W. and LEE, J.-T. 2017. External morphological development of post-larvae and juveniles of red seabream, Pagrus major. Development & Reproduction, 21, 63–69.

PEDERSEN, G. K., PEDERSEN, S. A. S., BONDE, N., HEILMANN-CLAUSEN, C., LARSEN, L. M., LINDOW, B., MADSEN, H., PEDERSEN, A. K., RUST, J., SCHULTZ, B. P., STOREY, M. and WILLUMSEN, P. S. 2011. Molerområdets geologi – sedimenter, fossiler, askelag og glacialteknik. Dansk Geologisk Forening, Copenhagen, 135 pp.

PEFEILER, E. 1999. Developmental physiology of elopomorph leptocephali. Comparative Biochemistry & Physiology A, 123, 113–128.

PEFEILER, E., TOYODA, H., WILLIAMS, M. D. and NIEMAN, R. A. 2002. Identification, structural analysis and function of hyaluronan in developing fish larvae (leptocephali). Comparative Biochemistry & Physiology B, 132, 443–451.

RASMUSSEN, J. A., MADSEN, H., SCHULTZ, B. P., SYLVESTERSSEN, R. L. and BONDE, N. 2016. The lowermost Eocene deposits and biota of the western Limfjord region, Denmark – Field Trip Guidebook, 2nd International Mo-clay Meeting, 2–4 Nov. 2016 at Museum, Skive and the Fossil and Mo-clay Museum, Museum Mors, Nykøbing Mors, Denmark, 35 pp.

RE, P. and MENESES, I. 2008. Early stages of marine fishes occurring in the Iberian Peninsula. IPIMAR/IMAR, Lisboa, 282 pp.

RODRIGUEZ, J. M., ALEMANY, F. and GARCIA, A. 2017. A guide to the eggs and larvae of 100 common Western Mediterranean Sea bony fish species. Food & Agriculture Organization of the UN, Rome, Italy, 256 pp.

ROUX, N., SALIS, P., LAMBERT, A., LOGUEUX, V., SOUALT, O., ROMANS, P., FRÉDÉRICH, B., LECCHINI, D. and LAUDET, V. 2019. Staging and normal table of postembryonic development of the clownfish (Amphiprion ocellaris). Developmental Dynamics, 248, 545–568.

SAMUEL, N. T., WAGNER, M. S., DORNFELD, K. D. and CASTNER, D. G. 2001. Analysis of poly(amino acids) by static time-of-flight secondary ion mass spectrometry (TOF-SIMS). Surface Science Spectra, 8, 163–184.

SARS, G. O. 1879. Indberetninger til Departementet for det Indre fra Professor, Dr. G.O. Sars om de afham i aarene 1864–78 anstillete undersøgelser angaende saltvandsfiskeriene. Berg & Ellessens Bogtrykkeri, Christiania, 221 pp.

SHELDON, E., GRAVESEN, P. and NØHR-HANSEN, H. 2012. Geology of the Femern Bælt area between Denmark and Germany. Geological Survey of Denmark & Greenland Bulletin, 26, 13–16.

STEGER, H.-U., FREITAG, J. F., MICHL, S., WIEMER, M. and PAUL, R. J. 2001. Effects of UV-B radiation on embryonic, larval and juvenile stages of North Sea plaice (Pleuronectes platessa) under simulated ozone-hole conditions. Helgoland Marine Research, 55, 56–66.

STEPHAN, T., JESSBERGER, E. K., HEISS, C. H. and ROST, D. 2003. TOF-SIMS analysis of polycyclic aromatic hydrocarbons in Allan Hills 84001. Meteoritics & Planetary Science, 38, 109–116.

STRAUSS, O. 2005. The retinal pigment epithelium in visual function. Physiological Reviews, 85, 845–881.

SWEET, M., KIRKHAM, N., BENDALL, M., CURREY, L., BUTHELL, J. and HEUPEL, M. 2012. Evidence of melanoma in wild marine fish populations. PLoS One, 7, e41989.

THIEL, V. and SJÖVALL, P. 2015. Time-of-flight secondary ion mass spectrometry (TOF-SIMS): principles and practice in the biogosciences. 122–170. In GRICE, K. (ed.) Principles and practice of analytical techniques in geosciences. Royal Society of Chemistry, 393 pp.

THOMSON, K. S., SUTTON, M. and THOMAS, B. 2003. A larval Devonian lungfish. Nature, 426, 833–834.

TURICK, C. E., TISA, L. S. and CACCAVO, F. Jr 2002. Melanin production and use as a soluble electron shuttle for Fe(III) oxide reduction and as a terminal electron acceptor by Shewanella algae BrY. Applied & Environmental Microbiology, 68, 2436–2444.

URHØ, L. 2002. Characters of larvae – what are they? Folia Zoologica, 51, 161–186.

VINCENTH, J., BRIGGS, D. E. G., PRUM, R. O. and SARANATHAN, V. 2008. The colour of fossil feathers. Biology Letters, 4, 522–525.

WAGNER, M. S. and CASTNER, D. G. 2001. Characterization of adsorbed protein films by time-of-flight secondary ion mass spectrometry with principal component analysis. Langmuir, 17, 4649–4660.

WILLUMSEN, P. S. 2004. Palynology of the lower Eocene deposits of northwest Jutland, Denmark. Bulletin of the Geological Society of Denmark, 52, 141–157.