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Skin patterning and internal anatomy in a fossil moonfish from the Eocene Bolca Lagerstätte illuminate the ecology of ancient reef fish communities

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Abstract: Colour patterning in extant animals can be used as a reliable indicator of their biology and, in extant fish, can inform on feeding strategy. Fossil fish with preserved colour patterns may thus illuminate the evolution of fish behaviour and community structure, but are understudied. Here we report preserved melanin-based integumentary colour patterning and internal anatomy of the fossil moonfish *Mene rhombea* (Menidae) from the Bolca Lagerstätte (Eocene (Ypresian), north-east Italy). The melanosome-based longitudinal stripes of *M. rhombea* differ from the dorsal rows of black spots in its extant relative *M. maculata*, suggesting that the ecology of moonfish has changed during the Cenozoic. Extant moonfish are coastal schooling fish that feed on benthic invertebrates, but the longitudinal stripes and stomach contents with fish remains in *M. rhombea* suggest unstructured open marine ecologies and a piscivorous diet. The localized distribution of extant moonfish species in the Indo-Pacific Ocean may reflect, at least in part, tectonically-driven reorganization of global oceanographic patterns during the Cenozoic. It is likely that shifts in habitat and colour patterning genes promoted colour pattern evolution in the menid lineage.

Key words: colour patterning, Bolca Lagerstätte, soft tissue preservation, melanosome, internal anatomy.

THE Bolca Lagerstätte (48 Ma, Verona, Italy) provides a remarkable window into an ancient hotspot of biodiversity in reef-associated marine ecosystems and adjacent terrestrial areas in the Middle Eocene. The fish fauna includes thousands of specimens (representing at least 200 taxa) that preserve evidence of colour patterning such as spots, bars and stripes (Blot 1969; Friedman & Carnevale 2018). The study of colour patterns in extinct teleosts has the potential to shed light on ontogeny (Shoji *et al.* 2003; Price *et al.* 2008), sexual dimorphism (Shoji *et al.* 2003), signalling (Negro *et al.* 2020), and even within-lineage evolution of colour patterns (Salis *et al.* 2019), but has not been the focus of previous work. This may reflect (at least in part) uncertainty in the phylogenetic placement of fossil taxa, even where phylogenies are well constrained and include extant relatives; this is exacerbated for fossils attributed to poorly resolved teleost groups such as percomorphs. Further, the evolution of colour patterning in specific lineages remains particularly elusive due to pervasive bias against preservation of fossil soft tissues (but see Gabbott *et al.* 2016).

The Bolca fish fauna has been studied extensively, with particular focus on its diversity (Friedman & Carnevale 2018), taxonomy (Carnevale *et al.* 2014) and palaeoenvironment (Marramà *et al.* 2016). However, the soft tissue taphonomy of the fish fauna, especially evidence of colour patterns, has received little attention (but see Wilby & Briggs 1997). The ecological significance of the colour patterns is therefore unknown.

Here we report a new specimen of the moonfish MCSNV 18–19/2020 (*Mene rhombea* Volta; Menidae) collected in 2019 from the Pesciara site at Bolca (48 Ma, Verona, Italy) (Fig. 1A; Fig. S1). This site represents a peri-reefal marine setting influenced by both the coast and the open ocean (Papazzoni *et al.* 2014; Marramà *et al.* 2021); *M. rhombea* is an iconic taxon for the Bolca ichthyofauna and is represented by abundant material (the exact number of specimens is not available). MCSNV

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18–19/2020 is complete and fully articulated with extensive soft tissue preservation, similar to other specimens from the same locality (Carnevale *et al.* 2014). The specimen shows striking colour patterning, which provides an ideal opportunity to explore the evolution of patterning and its potential ecological significance within the moonfish lineage.

GEOLOGICAL SETTING

The geology and sedimentology of the Pesciara site have been revised recently (Papazzoni & Trevisani 2006; Marramà *et al.* 2016). In brief, the Pesciara site is located in the eastern part of the Lessini Mountains (Southern Alps), 25 km north-east of the village of Bolca (Verona, Italy). The site includes a *c.* 20 m-thick succession of limestone that is associated with volcaniclastic rocks; the stratigraphic relationship between the two lithologies is not clear. The limestones comprise cyclic alternations of finely laminated micritic limestones and coarse-grained biocalcarenite both with a marine benthic fauna (Marramà *et al.* 2016). The micritic limestones contain abundant fossil fish, plants and invertebrates, most of which show evidence of soft tissues. The micritic limestone has been referred to the *Alveolina dainelli* zone (late Ypresian, *c.* 48 Ma; Papazzoni & Trevisani 2006) based on benthic foraminifera.

METHOD

Fossil samples

The specimen comprises part (MCSNV 18/2020) and counterpart (MCSNV 19/2020). Samples of fossil soft tissues (2–4 mm^2) and associated sediment were dissected from MCSNV 18/2020 using sterile scalpels and placed on carbon tape on aluminium stubs for further analysis via scanning electron microscopy (SEM) and Raman spectroscopy.

Institutional abbreviation. MCNSV, Museo Civico di Storia Naturale di Verona, Italy.

Modern melanosomes

Tissues of extant moonfish *M. maculata* were not available for study (this taxon is not usually commercially available in Europe). For this reason, skin, liver, heart, spleen, kidney and eyes were dissected from a specimen of the European sea bass (*Dicentrarchus labrax*) purchased locally. Tissue samples (each *c.* 100 mg) were dissected with sterile tools and stored at −80°C prior to enzymatic melanosome extraction using the protocol in Rossi *et al.* (2019; see Appendix S1).

Scanning electron microscopy

Fossil tissue samples and melanosome extracts were placed on carbon tape on top of aluminium stubs, sputter coated with Au/Pd and examined using a JEOL IT100 VP-SEM at an accelerating voltage of 20 kV and working distance of 8–10 mm. For each fossil sample and melanosome extract, long and short axes of 50 melanosomes orientated perpendicular to line of sight and in focus were measured from digital images using ImageJ (Rueden *et al.* 2017).

Raman spectroscopy

Samples on stubs were analysed using a Renishaw inVia Qontor microscope with a 532 nm laser. Three points of interest were analysed to account for potential variability within the sample. Raman spectra were imported into the software Renishaw WiRE 5.3 and processed as follows: spikes were removed and the range 1100–1700 cm^{-1} was selected. Finally, the three spectra were smoothed and merged.

Statistical analyses

Differences in the geometry of melanosomes among all extracts were tested using ANOVA and Tukey post hoc tests in R (RStudio Team 2016). Normality and homoscedasticity were assessed using residual v fitted plots in R.

RESULTS

Ultrastructure and chemistry of the preserved soft tissues of the fossil moonfish

The preserved soft tissues in MCSNV 18–19/2020 comprise films of dark-coloured material in the epaxial region of the body (herein termed the dorsum), the eyespot and the anterior portion of the abdomen. In addition, a near-homogeneous beige-coloured film is evident immediately ventral to the vertebral column; ventral of this, the specimen does not preserve soft tissues (Fig. 1A; Fig. S1). The dorsum is dark in colour close to the dorsal body margin but also shows three prominent, wide, dark longitudinal stripes (*c.* 1.5–2 mm thick, *c.* 120 mm long); these dark stripes are separated by light-toned interstripes

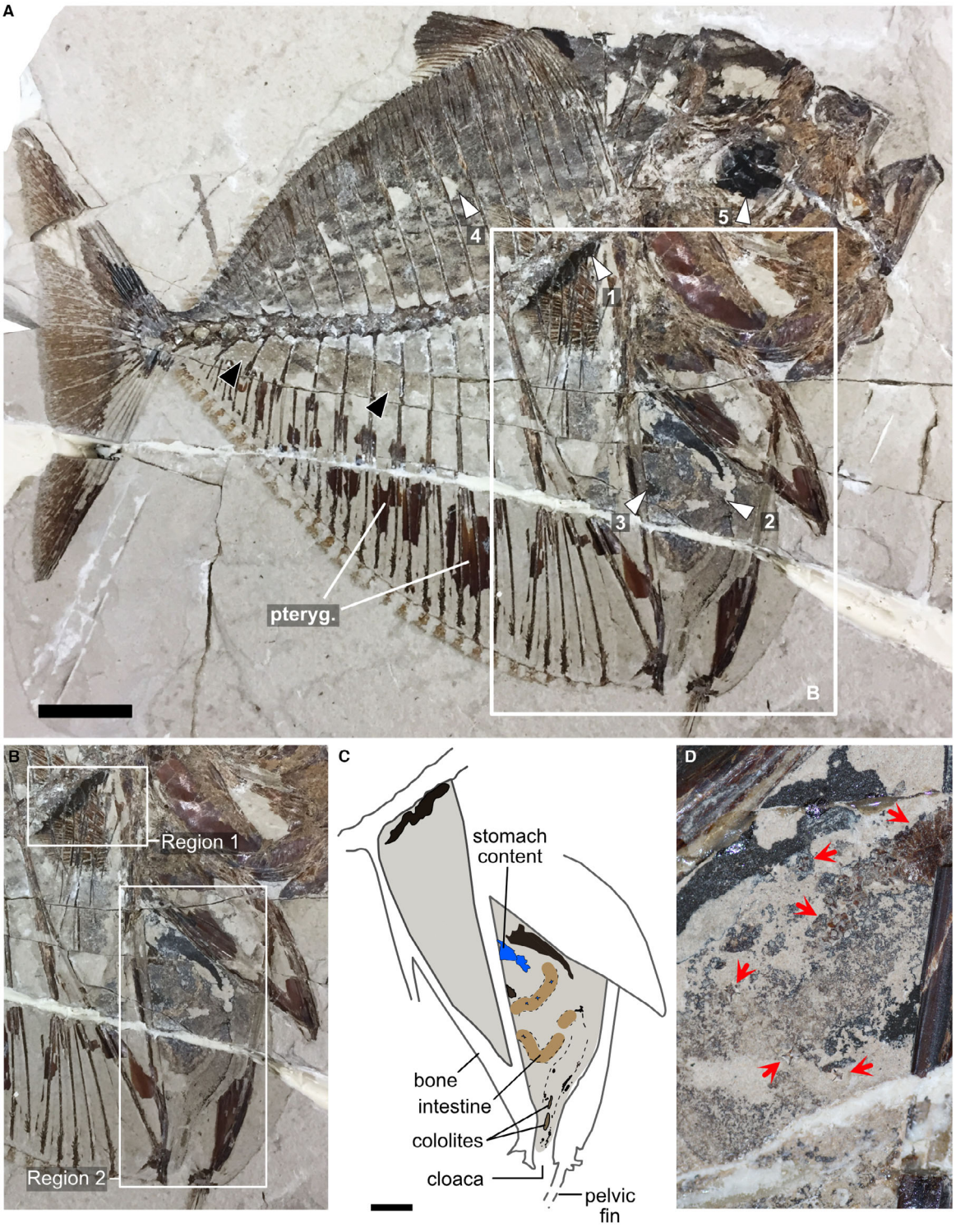

FIG. 1. Soft tissues and stomach content of the fossil moonfish. A, MCSNV 18/2020 (part) *Mene rhombea* (Menidae); the brown-coloured material present ventrally corresponds to the remains of the anal fin bones (pterygiophores; pteryg.); white triangles and associated numbers indicate sampling locations; black triangles indicate the most ventral evidence of the integument. B–C, detail of the abdomen showing stomach contents, cololites, and preservation of the gut as carbonaceous material; C, schematic representation with soft tissue interpretation. D, detail of the abdomen of the counterpart MCSNV 19/2020 showing the upper part of Region 2, illustrating the stomach and intestine (see Fig. S1); red arrows indicate the vertebrae and other ingested bones of clupeid fish. Scale bars represent: 20 mm (A); 10 mm (B, C).

(*c.* 2.5 mm thick). Ventral to these stripes, four thin (<1 mm thick, *c.* 60 mm long) and somewhat discontinuous dark stripes are separated by *c.* 0.5 mm-thick interstripes. In the abdomen, dark-coloured soft tissues occur in two regions ventral to the anterior vertebral column, one immediately adjacent to the vertebrae (Region 1; Fig. 1B) and the other between the coracoid, postcleithrum and basipterygium (Region 2; Fig. 1B). Region 2 also includes stomach contents (small vertebrae and scales, probably belonging to a sardine (Clupeidae)), buff-coloured soft tissues arranged in a continuous and undulate shape (interpreted as the remains of the intestine) and two grey cololites (Fig. 1C, D). In addition, three small clupeid vertebrae occur within the intestine (Fig. 1D).

Our SEM results reveal that all samples of the fossil soft tissue films comprise densely packed, three dimensional microbodies (Fig. 2). The microbody film is *c.* 2–4 μm thick in the dorsum, eyespot and Region 2, and *c.* 300 μm thick in Region 1.

Raman spectra of the microbody film show two prominent broad bands in the region *c.* 1100–1700 cm^{-1} that are centred at *c.* 1360 and 1590 cm^{-1} (Fig. 3). These bands correspond to those reported from previous studies of both extant (Huang *et al.* 2004) and fossil eumelanin (Pinheiro *et al.* 2019). The band centred at *c.* 1360 cm^{-1} (often termed the kerogen D (disordered) band) is associated with structural defects in eumelanin and contributions from methyl and/or methylene C–H (Huang *et al.* 2004). The band at *c.* 1590 cm^{-1} (the kerogen G (graphite) band) reflects aromatic C–C stretching. These data are consistent with the interpretation that the three dimensionally preserved microbodies are fossilized melanosomes.

The moonfish melanosomes derive from several body regions (Data S1; Table S1). The eyespot shows both

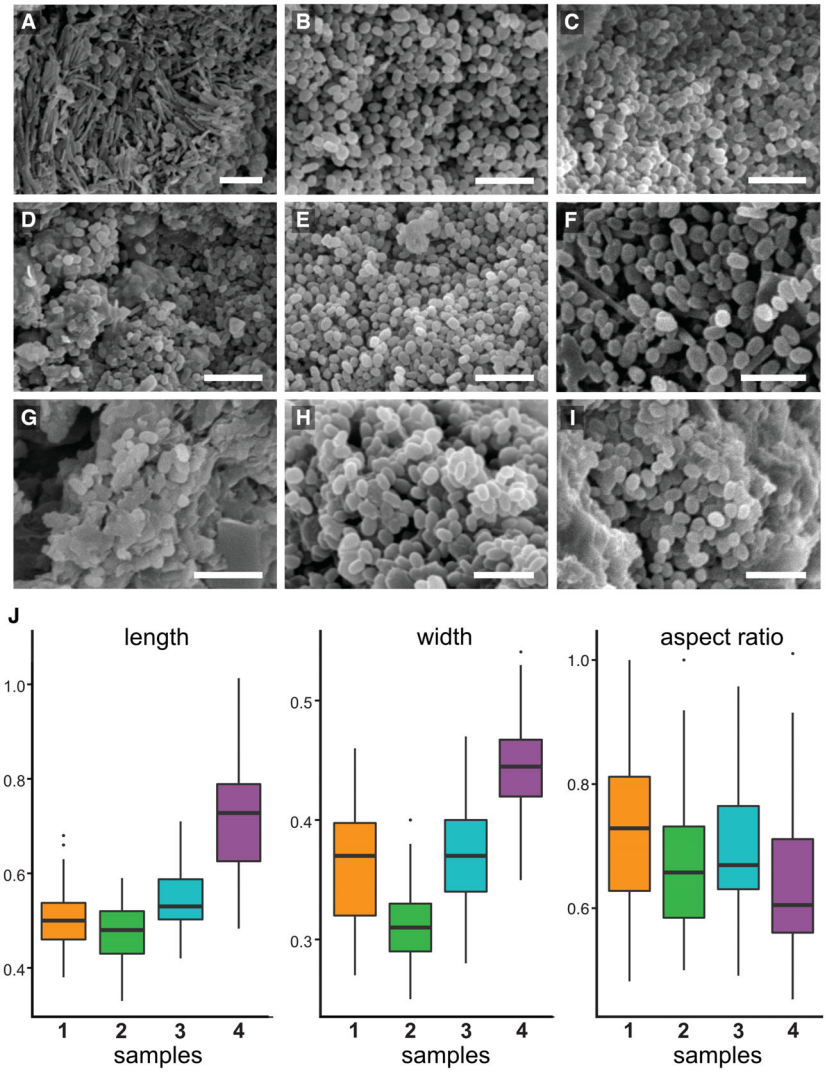

FIG. 2. Fossil moonfish melanosome geometry. A–E, SEM micrographs of fossil melanosomes from *Mene rhombea*: A, eyespot sample 5; B, Region 1 sample 1; C, Region 2 sample 2; D, Region 2 sample 3; E, dorsum sample 4. F–I, SEM micrographs of melanosomes extracted from the eye (F), heart (G), skin (H) and spleen (I) of a European sea bass (*Dicentrarchus labrax* (Perciformes, Moronidae)) for comparison. J, box plots showing fossil melanosome length (μm), width (μm) and aspect ratio. All scale bars represent 2 μm.

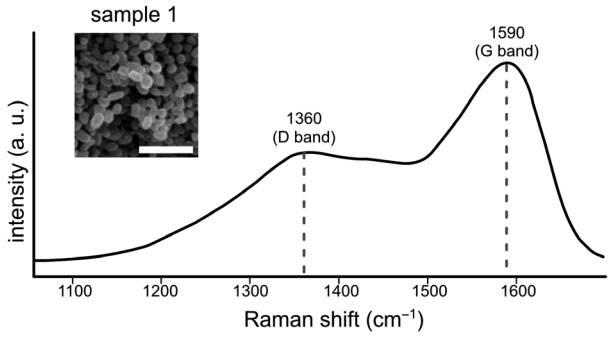

FIG. 3. Raman spectroscopy of the fossil soft tissues. Merged spectrum of three measurements of sample 1. Raman peaks at *c.* 1360 and 1590 cm^{-1} represent kerogen D and G bands, respectively. SEM micrograph on the top left corner is sample 1, Region 1. Scale bar represents 2 µm.

elongate (length: 1260 nm ± 305 nm; width: 254 nm ± 28 nm; Fig. 2A) and ovoid (720 nm ± 58 nm; 558 nm ± 74 nm) forms. In the abdomen, the smallest melanosomes occur in the most anterior part of Region 2 (476 nm ± 63 nm; 314 nm ± 33 nm; sample 2 in Fig. 2C, J), with intermediate sized melanosomes in Region 1 (i.e. sample 1 in Fig. 2B: 502 nm ± 68 nm; 362 nm ± 51 nm) and large melanosomes in the posterior part of Region 2 (i.e. sample 3 in Fig. 2D: 545 nm ± 67 nm; 374 nm ± 42 nm). Differences in geometry are statistically significant for length and width for all of these melanosome populations (except for those in Region 1 and the posterior part of Region 2; Table S2). Melanosomes from the dorsum (708 nm ± 107 nm; 446 nm ± 47 nm; sample 4) are larger than those in any region of the abdomen (Fig. 2J). These melanosomes differ to those of Region 1 and Region 2. All measured melanosomes plot within the known morphospace for vertebrate melanosomes (Huang *et al.* 2004; Li *et al.* 2014), specifically, within the morphospace region corresponding to extant ectotherms (Fig. S2).

SEM analyses of melanin extracts prepared from fresh tissues of the extant European sea bass (*D. labrax*) reveal ovoid melanosomes in the skin, heart and spleen (Fig. 2G–I). Melanosomes from the spleen differ significantly in geometry to those from the skin and heart (Fig. S3; Table S3).

DISCUSSION

Anatomical origins of the fossil melanosomes

Our data on melanosomes in extant sea bass are consistent with previous studies (McNamara *et al.* 2018; Rogers *et al.* 2019; Rossi *et al.* 2019) that reported tissue-specific melanosome geometries in extant amphibians, reptiles, birds and mammals. This supports the hypothesis that tissue-based partitioning of melanosome geometry is pervasive in vertebrates (McNamara *et al.* 2021), including fish, and that the geometry of preserved melanosomes can discriminate between melanosomes from different tissues (McNamara *et al.* 2018; Rossi *et al.* 2019). In MCSNV 18–19/2020, ovoid melanosomes vary in geometry in different body regions and show a Raman signal consistent with fossil eumelanin.

The spatial distribution and geometry of fossilized melanosomes from different soft tissue samples allow interpretation of the internal anatomy of the fossil moonfish (Fig. 4). Elongate melanosomes in the eyespot are interpreted as evidence of the retinal pigmented epithelium (RPE) as in other vertebrates (Vinther *et al.* 2008; Rogers *et al.* 2019); ovoid melanosomes in the eye cannot be assigned to any specific ocular tissue but could potentially derive from the RPE, iris, choroid or sclera (Rogers *et al.* 2019). Based on their position in the body, fossil melanosomes from Region 1 in the abdomen correspond to those of the head kidney; those in the anterior part of Region 2 may derive from the heart or the frontal lobe of the liver. Melanosomes in the posterior part of Region 2 may derive from the peritoneum (Steinel & Bolnick 2017) and/or the alimentary canal (Fishelson *et al.* 1997). These observations expand the existing dataset on the distribution and geometry of melanosomes in internal organs of vertebrates (McNamara *et al.* 2018; Rossi *et al.* 2019; Heingård *et al.* 2021).

Dorsal melanosomes are interpreted as integumentary melanosomes. In the dorsum, melanosomes preserve well-defined patterns comprising a dark dorsum, pale venter and horizontal stripes (Fig. 4); internal organs are absent. Integumentary melanosomes are larger than internal melanosomes from the abdomen.

Interpretation of melanosome-based colour pattern in the fossil moonfish

The extant moonfish *Mene maculata* displays countershading defined by a dark coloured, metallic blue dorsum with longitudinal rows of black irregular spots and a contrasting silver-white ventrum (Fig. 5A). These patterns and colours are produced by pigment cells (chromatophores) organized into the layered structure termed the dermal chromatophore unit (DCU) (Schartl *et al.* 2016) within the upper dermis. Based on current understanding of the DCU in extant teleosts (Salis *et al.* 2019), dark skin regions in the dorsum of *M. maculata* are almost certainly generated by melanin pigments stored within melanosomes, located inside the melanophores in the basal part of the DCU and in epidermal melanocytes. The metallic blue optical effect is typically produced by a layer of iridophores (that contain crystals of guanine that

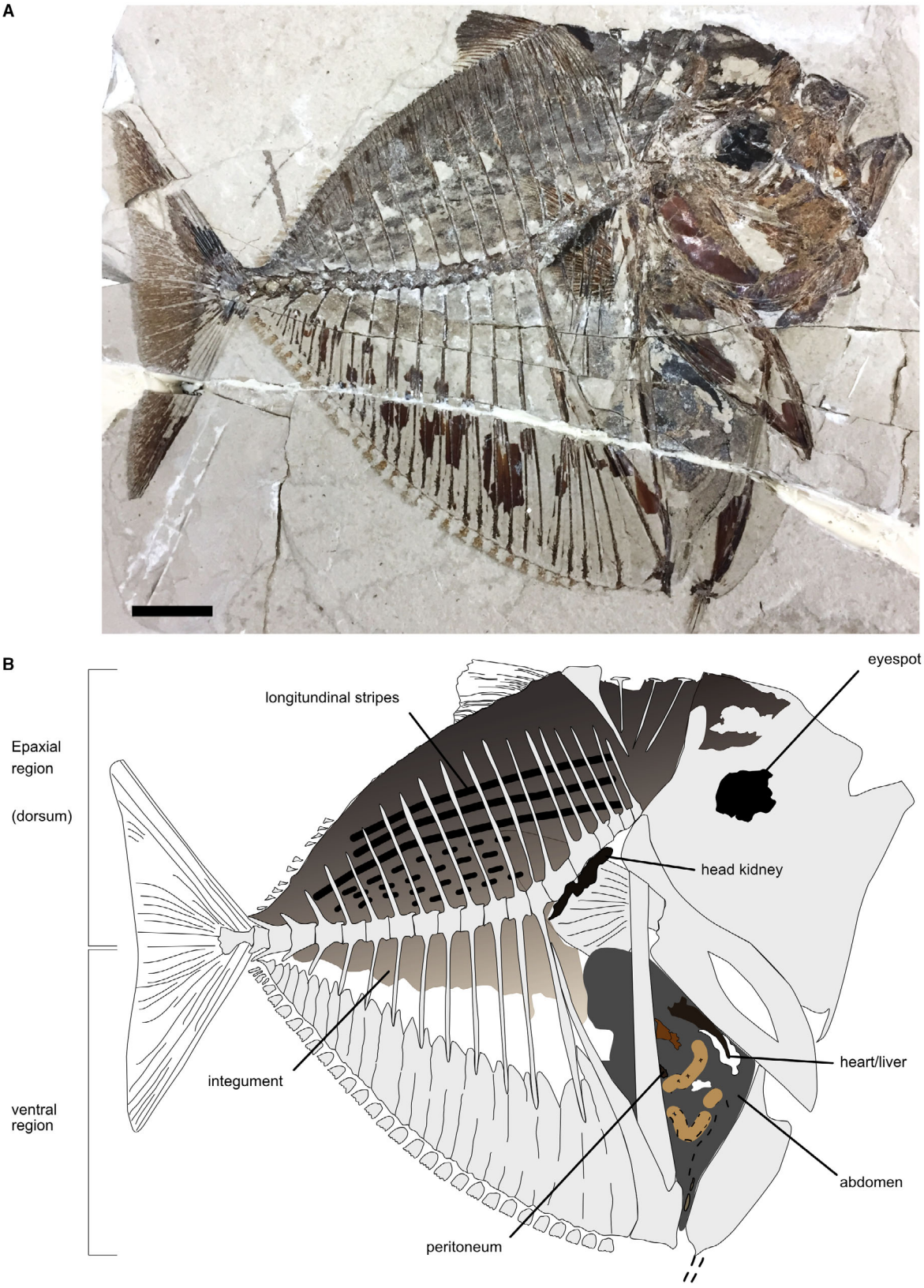

FIG. 4. Interpretation of the soft tissue anatomy of MCSNV 18–19/2020. A, MCSNV 18/2020 (part) *Mene rhombea* (Menidae). B, schematic representation of interpreted soft tissues.

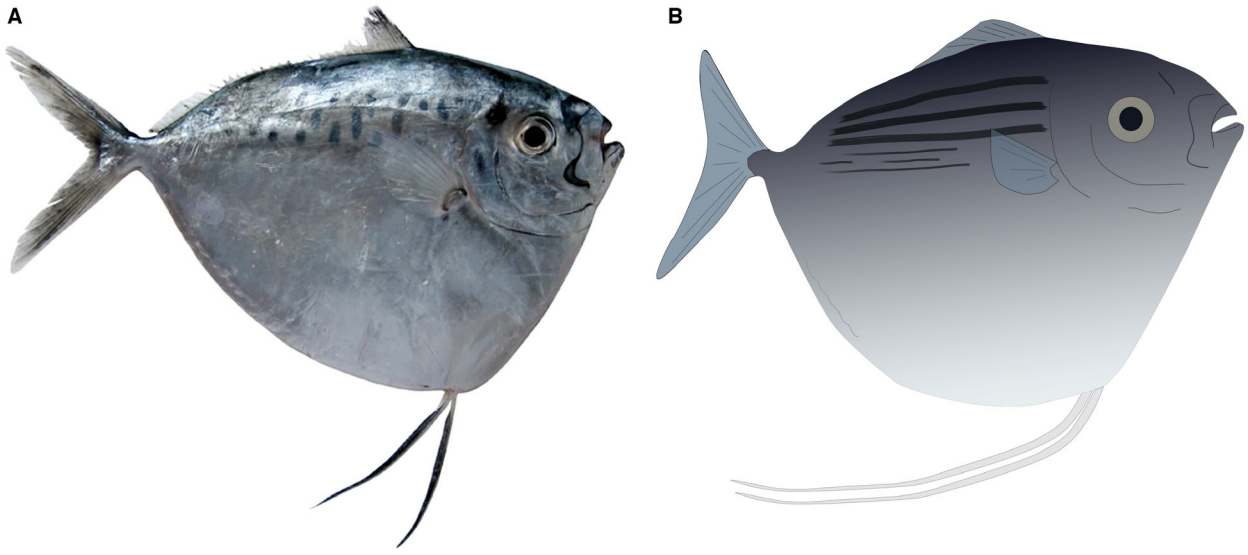

FIG. 5. Evolution of menid colour patterns. A, the extant moonfish *Mene maculata*. B, schematic reconstruction of the Eocene *Mene rhombea* based on evidence for countershading and longitudinal dorsal stripes. The length of the anal fins is estimated based on data available for fossil and extant taxa.

scatter light) that overlay the melanophores. The silver-white colouration of the ventrum is produced by leucophores and iridophores; melanophores are usually absent. The presence/absence and abundance of different pigment cells overlying the melanophores can substantially impact visible skin colour (Salis *et al.* 2019). Accurate interpretation of integumentary colouration in fossil vertebrates is therefore possible only where all chromatophore types are preserved. Fossil evidence of chromatophores, however, usually occurs as carbonaceous films, comprising largely or exclusively melanosomes, associated with the integument (Vinther 2020) and/or internal organs (Rossi *et al.* 2019). Entire melanophores (bearing melanosomes) have been reported only in the mineralized skin of a 10 myr-old fossil snake (that also preserves entire xanthophores and iridophores (McNamara *et al.* 2016)) and a 180 myr-old ichthyosaur (Lindgren *et al.* 2018). The absence of non-melanin-bearing chromatophores in most vertebrate fossils reflects loss during decay and/or maturation. During decay, melanosomes can disperse within the integument (Nedza *et al.* 2019) and mix within the abdomen (McNamara *et al.* 2018), potentially altering, and even obliterating, original colour patterns. Most interpretations of the original colouration of fossil vertebrates have been based on spatial distributions and geometries of preserved melanosomes (Vinther 2020) and/or chemical evidence of melanin (Lindgren *et al.* 2018) and are thus melanin-based colour reconstructions.

Specimen MCSNV 18–19/2020 preserves melanosome-rich tissues, stomach contents, cololites and a fully complete and articulated skeleton; collectively these observations suggest incomplete decay and/or limited external disturbance (e.g. via current activity or scavenging) prior to burial. The retention of spatially localized populations of melanosomes with distinct geometries indicate that melanosome mixing did not occur among different tissues of the abdomen and between these and the dorsum. Longitudinal stripes have been observed in other specimens of *M. rhombea* (Carnevale *et al.* 2014) (and in the sympatric *M. oblonga*; Blot 1969) and thus the patterning observed in MCSNV 18–19/2020 is interpreted as preserved original skin patterning.

The melanin-based component of the colour pattern of *M. rhombea* can be interpreted based on the preservation of melanosomes and the variation in visible tone of the integument (Fig. 5B). The fossil moonfish was countershaded (i.e. with a dark dorsum and light ventrum); superimposed on this background pattern were at least three prominent, dark, dorsal longitudinal stripes; minor discontinuous stripes occur between the prominent stripes and the vertebral column.

The ecological significance of melanosome-based colour patterning in M. rhombea

Colour patterning can shed light on various aspects of the biology of extant animals (Negro *et al.* 2020). In extant fish, certain colour patterns are related to ontogeny, gender and behaviour, and are associated with certain habitat and feeding strategy. Current understanding of the biological significance of colour patterns in extant teleosts derives from direct observation and experimental studies on model species (e.g. zebrafish (Price *et al.* 2008) and various

African cichlids (Seehausen *et al.* 1999)). These insights can reasonably be applied to other members of the group.

Extant fish inhabiting epipelagic and mesopelagic zones (0–1000 m deep) are usually countershaded, irrespective of body size (Ruxton *et al.* 2004). These zones include unstructured habitats that lack visual reference points and vegetation, rocks and/or caves that provide protection from predators. Fish in these habitats often exhibit horizontal stripes (Seehausen *et al.* 1999) and schooling behaviour, whereby individuals of the same species swim together using the colour patterns of neighbours as landmarks for orientation (Pavlov & Kasumyan 2000). Schooling behaviour is considered to have evolved in pelagic fish in the Triassic (Eibl-Eibesfeldt 1962), plausibly facilitating foraging, reproduction, migration and protection against predators (Kasumyan & Pavlov 2018). In extant schooling fish, protection against predators is provided by the number of individuals forming the school and the spacing among them within the group. In particular, melanin-based horizontal stripes in schooling fishes produce a confusion effect, whereby predators are unable to focus on individual prey (Price *et al.* 2008). The longitudinal dark and light-toned stripes in extant schooling fish is linked to piscivore feeding strategies (Seehausen *et al.* 1999; Miguez & Munuzuri 2006).

Extant moonfish typically live in schools in shallow coastal waters (50–200 m deep), feed on small epibenthic invertebrates (Woodland 2001) and, in turn, are the prey of larger pelagic fish such as marlin and sailfish (Pangalia *et al.* 2014). Given its similar skeletal anatomy to extant moonfish, *M. rhombea* was previously assumed (Blot 1969) to have a similar diet and ecology.

Our findings suggest differently. The preservation of sardine remains in the stomach contents of MCSNV 18–19/2020, plus longitudinal stripes, indicate a piscivorous diet. Clupeids are abundant at the Pesciara site and, as with extant analogues, are considered to represent diurnal schooling fish that were the preferred prey of various larger teleosts (Marramà & Carnevale 2015; Marramà *et al.* 2016), including moonfish. Countershading and horizontal stripes also suggest that *M. rhombea* lived in unstructured open marine habitats, but swam close to the peri-reefal system of the Pesciara to feed. As in extant schooling fish, it is likely that the horizontal stripes and their associated confusion effect functioned as a deterrent to large pelagic predators (Price *et al.* 2008); candidates at Pesciara include barracudas and large scombroids and carangoids (Carnevale *et al.* 2014).

Broader implications for moonfish evolution

The fossil record of menids includes several species (usually preserved as skeletal remains lacking soft tissues; reviewed in Friedman & Johnson 2005) from the Paleocene to the Miocene with a circumglobal distribution and broad environmental tolerance (Friedman & Johnson 2005). The melanosome-based stripes in *M. rhombea* differ from the colour patterns in their extant relatives. The fossil patterns are consistent with open marine habitats and thus with the hypothesis (Friedman & Johnson 2005) that the ecology and geographical distribution of moonfish have changed over the last 48 million years: from open marine and globally distributed, to shallow coastal settings in the Indo-Pacific Ocean. The shift in geographical distribution echoes that in other Cenozoic teleosts (e.g. Chanidae (milkfishes) and Siganidae (rabbitfishes); Bannikov & Parin 1997) all of which are today confined to the Indo-Pacific Ocean despite broader geographical distributions in the past (see Friedman & Johnson 2005). This, plus the progressive ecological restructuring of marine systems during the Cenozoic (Norris *et al.* 2013; Marramà *et al.* 2016) may have driven changes in colour patterning in moonfish from longitudinal stripes to rows of spots.

These phenotypical changes may have been accompanied by shifts in the genetic controls involved in colour pattern formation within the menid lineage. Pigmentation genes are often pleiotropic (especially those relating to melanization; Urabe *et al.* 1993) but innovation in colour pattern is not necessarily linked to the evolution of other anatomical characters. For instance, stripe integrity in zebrafish is linked to Edn3b endothelin signalling pathways (Spiewak *et al.* 2018) whereby mutation of Edn3b generates rows of spots or blotches but not stripes (Irion & Nusslein-Volhard 2019); other anatomical characters are not affected. Mutation of Edn3b therefore represents a plausible genetic pathway to explain the shift in colour patterning from stripes to spots in Menidae since the Eocene, without associated alteration of skeletal anatomy. Colour pattern formation can involve several other genes that are conserved across vertebrates (Bertolesi *et al.* 2019), for example, stripes are linked to TYR (Burgon *et al.* 2020), KIT (Mallarino *et al.* 2016) and MIFT (Salis *et al.* 2019), but how these genes impact patterning is not fully understood (Funk & Taylor 2019). In extant teleosts, skin patterning is linked to various genetic controls; for example, *agouti* controls dorso-ventral patterning (Irion & Nusslein-Volhard 2019), and *ltk* (Fadeev *et al.* 2016) and *sox5* (Nagao *et al.* 2014) are required for iridophore and xanthophore development, respectively (Salis *et al.* 2019). The orientation of stripes is controlled during development by physical features of the body (e.g. the lateral line, scales and muscles (Parichy 2003)) and by the reaction-diffusion gradient among chromatophores (Shoji *et al.* 2003). As discussed above, colour pattern formation in fish can also be affected by other biological (e.g. ontogeny, sexual maturity, signalling) and environmental

(e.g. light levels, habitat structure, predation pressure) controls.

As recently proposed for other vertebrates (McNamara *et al.* 2021), our study shows how phenotypic comparison of fossil fish and a close extant relative, placed in a genetic context, can facilitate exploration of the molecular signalling mechanisms controlling pattern evolution within the lineage. This in turn informs on the evolution of ecological strategies in marine fish communities on geological timescales. Application of this approach to other fossil vertebrates with preserved patterning will provide additional novel insights into genomic aspects of the evolution of melanization and fossil behaviour.

CONCLUSION

Fossil moonfish specimen MCSNV 18–19/2020 exhibits melanin-based colour patterning (stripes and countershading) and melanin-based evidence of internal organs and stomach contents. Melanosomes are identified using data on their geometry, chemistry and anatomical distribution. Melanosomes preserved in different body regions differ in geometry, facilitating discrimination of the skin, peritoneum, head kidney and heart and/or liver. The preserved colour pattern and the remains of fish in the stomach suggest that *M. rhombea* was piscivorous and inhabited coastal to open marine environments, unlike its extant relative *M. maculata*, which feeds on small epibenthic invertebrates and lives exclusively in coastal environments. Differences in integumentary patterning in the fossil moonfish and its extant relative *M. maculata* probably reflect an ecological shift in habitat and possibly changes in the genes controlling colour pattern formation over the last 48 million years.

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Author contributions. VR, GC, MM designed the project; RZ discovered and prepared the specimen; VR sampled the fossil specimen and performed melanin extractions, SEM and statistical analyses; RU and VR performed Raman spectroscopic analysis; VR, GC and MM wrote the paper with input from all coauthors.

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SUPPORTING INFORMATION

Additional Supporting Information can be found online (https://doi.org/10.1111/pala.12600):

Appendix S1. Includes supplementary methods, Figures S1–S3 and Tables S1–S3.

Figure S1. Photograph of the specimen MCSNV 19/2020 (counterpart).

Figure S2. Vertebrate melanosome morphospace.

Figure S3. Geometry of melanosomes from different tissues of the European sea bass (*D. labrax*).

Table S1. Mean and standard deviation of fossil melanosome length and width.

Table S2. ANOVA and Tukey post-hoc tests of fossil melanosome geometry.

Table S3. ANOVA and Tukey post-hoc tests of modern melanosome geometry.

Data S1. Dataset of melanosome measurements.

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