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Recent advances in amniote palaeocolour reconstruction and a framework for future research

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ABSTRACT

Preserved melanin pigments have been discovered in fossilised integumentary appendages of several amniote lineages (fishes, frogs, snakes, marine reptiles, non-avialan dinosaurs, birds, and mammals) excavated from lagerstätten across the globe. Melanisation is a leading factor in organic integument preservation in these fossils. Melanin in extant vertebrates is typically stored in rod- to sphere-shaped, lysosome-derived, membrane-bound vesicles called melanosomes. Black, dark brown, and grey colours are produced by eumelanin, and reddish-brown colours are produced by phaeomelanin. Specific morphotypes and nanostructural arrangements of melanosomes and their relation to the keratin matrix in integumentary appendages create the so-called 'structural colours'. Reconstruction of colour patterns in ancient animals has opened an exciting new avenue for studying their life, behaviour and ecology. Modern relationships between the shape, arrangement, and size of avian melanosomes, melanin chemistry, and feather colour have been applied to reconstruct the hues and colour patterns of isolated feathers and plumages of the dinosaurs *Anchiornis*, *Sinosauropteryx*, and *Microraptor* in seminal papers that initiated the field of palaeocolour reconstruction. Since then, further research has identified countershading camouflage patterns, and informed subsequent predictions on the ecology and behaviour of these extinct animals. However, palaeocolour reconstruction remains a nascent field, and current approaches have considerable potential for further refinement, standardisation, and expansion. This includes detailed study of non-melanic pigments that might be preserved in fossilised integuments. A common issue among existing palaeocolour studies is the lack of contextualisation of different lines of evidence and the wide variety of techniques currently employed. To that end, this review focused on fossil amniotes: (*i*) produces an overarching framework that appropriately reconstructs palaeocolour by accounting for the chemical signatures of various pigments, morphology and local arrangement of pigment-bearing vesicles, pigment concentration, macroscopic colour patterns, and taphonomy; (*ii*) provides background context for the evolution of colour-producing mechanisms; and (*iii*) encourages future efforts in palaeocolour reconstructions particularly of less-studied groups such as non-dinosaur archosaurs and non-archosaur amniotes.

Key words: palaeocolour, melanin, melanosomes, exceptional preservation, amniotes, taphonomy.

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I. INTRODUCTION

Colours and their macroscopic patterns are critical to understanding the life and behaviour of an animal (Burley, Krantzberg & Radman, 1982; Butcher & Rohwer, 1989). Animals routinely employ colouration for aposematism, crypsis, sociosexual selection, and physiological purposes (Cuthill *et al.*, 2017). The age, sex, and species of extant animals are often identified based on colour patterns (Peterson, 1999). Integumentary colour-producing mechanisms contribute towards functions such as thermoregulation, resistance of the integument to mechanical abrasion, and protection from stressful environmental conditions such as ultraviolet (UV) radiation, humidity, pathogens, and long-term climate change (Roulin, 2014). Given the diverse range of biological information provided by colouration it is important that descriptions of fossil taxa include palaeocolour reconstructions. For the purposes of this review, we distinguish between colour and colour patterns, with the former representing a general hue and the latter being a complex macroscale feature created by localised concentrations of pigments and structural mechanisms.

The colour palette of extant animals is fashioned from complex permutations and combinations of pigments (e.g. carotenoids, porphyrins, psittacofulvins and melanins) and structural components (e.g. keratin and collagen) (Fig. 1A). Naturally occurring pigment molecules (i.e. biochromes) produce colour by preferentially absorbing certain wavelengths of light while permitting others to be reflected (Hill & McGraw, 2006*b*) (Fig. 1B). It is these wavelengths of reflected light that impart the observable colour, whereas 'structural colours' are produced when light is scattered at the interfaces of layered nanoscale arrangements of reflective tissue constituents (e.g. arrays of different morphotypes of melanosomes in keratinous matrices of vertebrate integumentary structures, chitins in arthropods) that vary in refractive indices (Prum, 1999; Hill & McGraw, 2006*b*) (Fig. 1C, D). Additionally, colour changes through rapid spatial dispersal of pigment molecules, pigment-containing vesicles, and reflective structures in ectothermic animals (e.g. crustaceans, cephalopods, fishes, amphibians, and non-avialan/non-dinosaurian reptiles) are controlled by neuroendocrine stimulation of cellular assemblies. These cellular assemblies termed chromatophores are categorised into classes according to the hues imparted (xanthophores: yellow; erythrophores: red; melanophores: black/brown; leucophores: white; cyanophores: blue; iridophores: reflective/iridescent) with the process causing these rapid colour changes termed metachrosis (Boyer & Swierk, 2017).

Melanin is of particular interest in fossilised organisms due to its resilience to diagenesis (i.e. thermal stability) (Glass *et al.*, 2012, 2013; Colleary *et al.*, 2015) since it is argued that long-term thermal stability through diagenesis is the ultimate factor conferring organic fossilisation potential to biomolecules (Parry *et al.*, 2018; Saitta, Kaye & Vinther, 2018*d*). The earliest known preserved fossil melanin dates to the late Carboniferous, ~307 million years ago (Gabbott *et al.*, 2016). The ability of melanin and melanin-bearing membrane-bound vesicles called melanosomes to be preserved within fossilised integumentary structures such as skin and feathers in non-avialan dinosaurs, early birds, non-dinosaurian reptiles and mammals has brought forth a unique opportunity to infer the actual colour patterns of these extinct animals (Vinther *et al.*, 2008; Lindgren *et al.*, 2014, 2015, 2018; Colleary *et al.*, 2015; Vinther, 2015; Manning *et al.*, 2019; Yang *et al.*, 2019), enabling a range of novel hypotheses to be articulated (e.g. predator–prey interactions, aposematism, crypsis, sexual selection, behaviour, and habitat choice) (Vinther *et al.*, 2008, 2016; Lindgren *et al.*, 2010; Zhang *et al.*, 2010; Li *et al.*, 2012; Smithwick *et al.*, 2017; Saitta *et al.*, 2018*c*). Thus far, different studies have used different methods to infer palaeocolour and there is no overarching framework to maximise the repeatability and accuracy of reconstructions. Here, we review the different procedures currently available and propose a holistic protocol for palaeocolour reconstruction focussed on amniotes that accounts for taphonomic loss of information, pigment types and chemistry, morphology and arrangement

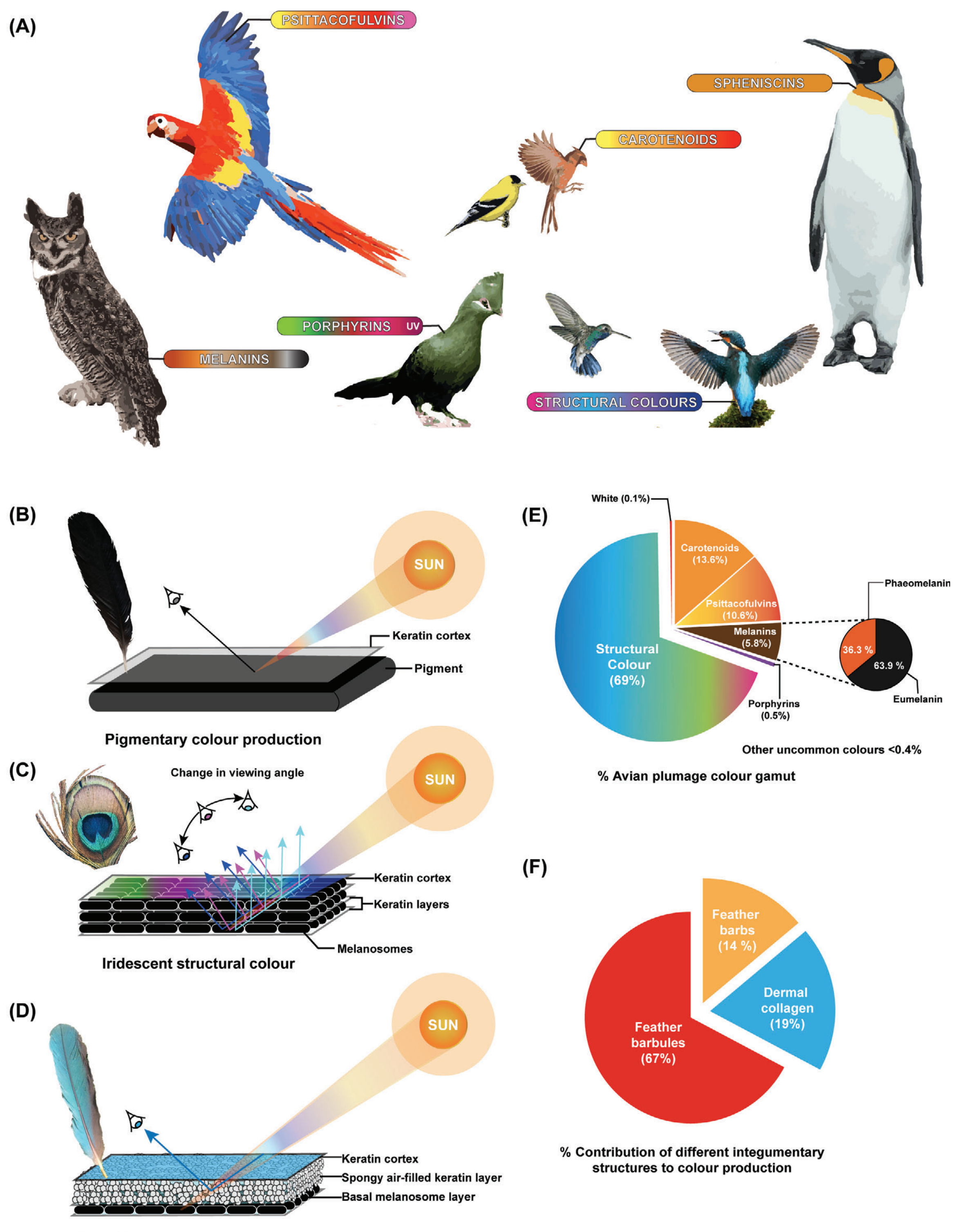

Fig. 1. (A) Different colour-production mechanisms in birds and the ranges of colours produced. (B–D) Optical mechanisms of colour production in bird feathers: pigmentary (B), iridescent structural (C), and non-iridescent structural (D). (E) Approximate percentage contribution of different colour-producing mechanisms to the avian plumage gamut based on data in Stoddard & Prum (2008, 2011). (F) Contribution of different integumentary structures to the production of colours based on data in Hill & McGraw (2006*b*). Artwork in A created using reference photographs from Wikipedia licenced under the Creative Commons attribution 4.0.

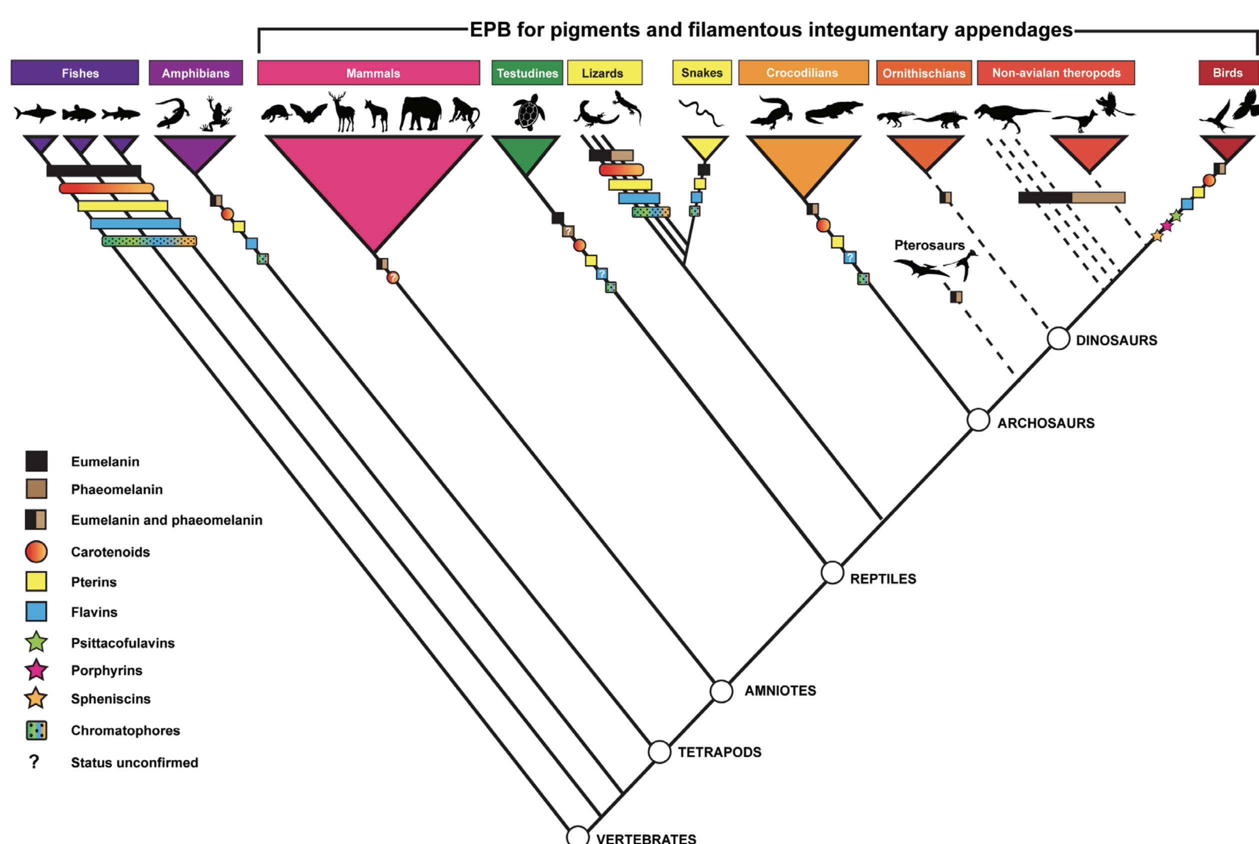

Fig. 2. Simplified cladogram showing the distribution of different types of colour-producing pigments in vertebrates: fishes (Courts, 1960; Johnson & Fuller, 2014; Sefc, Brown & Clotfelter, 2014; Kottler, Künstner & Schartl, 2015; Cal *et al.*, 2017), amphibians (Obika & Negishi, 1972; Czeczuga, 1980; Thorsteinsdottir & Frost, 1986; Ichikawa, Ohtani & Miura, 1998; Thibaudeau & Altig, 2012), mammals (Ito & Wakamatsu, 2003; Galván *et al.*, 2016*b*), testudines (Gopalakrishnakone, 1986; Roulin, Mafli & Wakamatsu, 2013; Steffen *et al.*, 2015; Brejcha & Kleisner, 2016), lizards (Taylor & Hadley, 1970; Fitze *et al.*, 2009; Cuervo, Belliure & Negro, 2016; Boyer & Swierk, 2017; Megía-Palma, Jorge & Reguera, 2018), snakes (Blair & Graham, 1954; Kikuchi, Seymoure & Pfennig, 2014), crocodilians (Alibardi, 2011), extinct archosaurs (Li *et al.*, 2014) and birds (Stoddard & Prum, 2011; Cuthill *et al.*, 2017). Dotted lines indicate stem groups; bold lines indicate crown groups. Note that this cladogram shows the distribution of colour systems confirmed by prior published research but does not comment on ancestral states or the mode of evolution of colour-producing mechanisms. Iridescence is not included on this cladogram because the mechanisms producing iridescence differ between birds and non-avian vertebrates. Silhouettes for cyprinid fish (illustration by Ellen Edmonson, vectorised by Timothy J. Bartley), batrachid (illustration by Nobu Tamura, vectorised by T. Michael Keesey), rodent (vectorised by Michael B.H.), canid (illustration by Sam Fraser-Smith, vectorised by T. Michael Keesey), elephant (T. Michael Keesey), lizard (illustration by Nobu Tamura, vectorised by T. Michael Keesey), *Psittacosaurus* (vectorised by Pete Bucholz), *Tyrannosaurus rex* (vectorised by Scott Hartman), *Velociraptor* (vectorised by Emily Willoughby), and troodontid dinosaur (vectorised by Emily Willoughby) were downloaded from www.phylopic.org. Remaining silhouettes, free of copyright, were also downloaded from www.phylopic.org. All silhouettes used are subject to CC Public Domain Dedication 1.0 licence.

of pigment-bearing vesicles, products of pigment diagenesis, and preserved macroscopic colour patterns.

II. PIGMENTARY MECHANISMS OF COLOUR PRODUCTION IN AMNIOTES

Much work on fossil colour reconstruction has focused on filamentous integuments of non-avialan dinosaurs, birds and closely allied species (Vinther *et al.*, 2008; Lindgren *et al.*, 2010; Li *et al.*, 2012; Vinther, 2015). Extant birds, the only living dinosaurs, and mammals can act as a useful extant phylogenetic bracket (Witmer, 1995) for studying pigmentation and colours in fossilised filamentous integuments, a key adaptation in the evolution of bird-line archosaurs parallel to mammalian hair (Fig. 2). The rationale for this choice is: (*i*) the relatively close morphological, developmental, and molecular similarities of their various epidermal integumentary outgrowths (e.g. scales, filaments, feathers and hairs) (Dhouailly, 2009; Dhouailly *et al.*, 2019) and (*ii*) their shared pigmentary and structural colour-producing mechanisms (Hofreiter & Schöneberg, 2010; Schartl *et al.*, 2016).

The vast repertoire of colours and patterns in vertebrates are generated by 378 known genetic loci (Montoliu, Oetting

& Bennett, 2011). Pigmentation in the pelage of several mammals including mice (Robbins *et al.*, 1993), rabbits (Fontanesi *et al.*, 2006), sheep (Våge *et al.*, 1999), dogs (Newton *et al.*, 2000), big cats (Eizirik *et al.*, 2003), cows (Klungland *et al.*, 1995), horses (Marklund *et al.*, 1996) and humans (Ito & Wakamatsu, 2003) have been analysed thus far, and there is consensus that mammalian integuments are dominated by two variants of melanin pigments: eumelanin and phaeomelanin (Ito & Wakamatsu, 2003). Two proteins, melanocortin 1 receptor (MC1R) and agouti signalling protein (ASIP), play critical roles in vertebrates in determining which variant of melanin is expressed within hair and in regulating the spatial distribution of these pigments, resulting in complex patterning (Hofreiter & Schöneberg, 2010). Polymorphism in the colouration of mammalian pelage in the wild and in domesticated species also occurs due to mutations in the MC1R loci (Switonski, Mankowska & Salamon, 2013). Loss-of-function mutations usually lead to phaeomelanic, paler, reddish/yellowish colours (Ha *et al.*, 2003; Rees, 2003), whereas gain-of-function mutations lead to eumelanic black/darker-brown colours (Kijas *et al.*, 1998; Våge *et al.*, 1999). No other form of pigmentation in mammalian skin or hair is currently known, except the carotenoid pigmentation in the skin and hairs of bats (Bolívar-Cimé, Clarke & Racey, 2012; Galván *et al.*, 2016*b*) and the porphyrin-mediated UV fluorescence in the pelage of New World flying squirrels (*Glaucomys* spp.) (Kohler *et al.*, 2019). Blue structural colours in mammals have been studied in the rump and facial skin of mandrills (*Mandrillus sphinx*), the scrotum of the vervet monkey (*Cercopithecus aethiops*), mouse opossum (*Marmosa mexicana*) and woolly opossum (*Caluromys derbianus*) and are produced by light scattering in dermal collagen arrays (Prum & Torres, 2004).

In homeotherms, dead keratinous epidermal tissues undergo melanisation. By contrast, ectotherms like fishes, lissamphibians, and reptiles (e.g. turtles, tortoises, crocodilians, lizards and snakes) have pigmentation placed under neuronal and hormonal control within specialised chromatophore assemblies of the metabolically active dermis (Fujii, 1993; Holman, 2003; Mathger *et al.*, 2003; Kerney, 2011). The integumentary ultrastructure, histology, distribution of pigments, and mechanisms producing colour patterns in crocodilians and testudines have only recently been investigated in detail (Alibardi, 2011; Rowe *et al.*, 2013). In general, modern crocodilians have adopted dull skin colours rather than brighter hues, likely given their restricted ecological range (i.e. semi-aquatic). Dull and pale cryptic patterns on their scaly skin (e.g. dark stripes, spots, splotches) have been shown to function in camouflage (Webb, Manolis & Whitehead, 1987). While the darker colours in crocodilians are generated by melanosome-bearing chromatophores (melanophores), the diffuse lighter colours are generated using pterin- and carotenoid-containing chromatophores (xanthophores) and guanine-crystal-bearing chromatophores (iridophores). Colours of the paler regions vary from grey or white (in Alligatoridae), through to pale brown (e.g. *Crocodylus niloticus*), and orange-yellow (e.g. *Crocodylus porosus*). The only detailed histological study of pigment systems in crocodilians suggests that eumelanin is the sole type of melanin found in epidermal melanocytes and dermal melanophores (Alibardi, 2011). The localisation, concentration, and distribution of melanocytes in different layers of skin and scales control the intensity of the darker colours as well as macroscopic colour patterning (Alibardi, 2011). Rapid and reversible changes in colours of reptile skin are caused by the translocation of pigments under neuronal or hormonal control within the chromatophores in the epidermis and dermis, in response to changes in the environment (Taylor & Hadley, 1970; Sherbrooke & Frost, 1989; Kuriyama *et al.*, 2006; Merchant *et al.*, 2018). However, in crocodilians and testudines, the temporal onset of this colour change is much more gradual (i.e. days to weeks) compared to that of other reptiles, such as anole lizards (i.e. minutes to hours) (Rowe *et al.*, 2013; Merchant *et al.*, 2018; Staniewicz, Youngprapakorn & Jones, 2018).

Scaly integuments have been suggested to be the ancestral condition in non-avialan dinosaurs since filamentous integuments are currently unknown in most ornithischians, all sauropodomorphs and some early theropod lineages such as ceratosaurids, abelisaurids, and allosauroids, with many of these groups instead preserving extensive, well-developed scale impressions (Bonaparte, Novas & Coria, 1990; Coria & Currie, 2006; Coria & Chiappe, 2007). Feather-like epidermal structures have been suggested to be the derived condition in the common ancestor of all coelurosaurian dinosaurs (Barrett, Evans & Campione, 2015). However, homoplastic loss of filaments in the scaly integuments of coelurosaurian tyrannosaurids has also been suggested (Bell *et al.*, 2017), although others argue that this might be influenced by taphonomic bias against organic feather preservation (Saitta *et al.*, 2018*b*) in North American tyrannosaurids. Among ornithischians, scales in *Kulindadromeus* have been suggested to be secondarily derived from feathers based on hypotheses relating to evolutionary development (Dhouailly, 2009; Godefroit *et al.*, 2014; Dhouailly *et al.*, 2019). Additionally, the presence of branched filamentous pycnofibres in pterosaurs, similar to primitive feathers in dinosaurs, also hints at the possibility that the ancestral state of all avemetatarsalians was filamentous (Yang *et al.*, 2019). If this is the case, then the evolution of morphologically disparate integumentary filaments may show complex patterns of multiple independent filament losses across the archosaur phylogeny. A key factor that has the potential to bias these clade-wide studies of integumentary structures is taphonomy, given that preservation of integumentary structures varies widely across different fossiliferous sites and deep time (Davis & Briggs, 1995; Wilson *et al.*, 2016), particularly in Triassic localities in which we find the earliest representatives of dinosaurs and their close relatives (Clarke, 2013).

The integumentary structures of dinosaur and pterosaur fossils share close morphological and, presumably, partial functional homology with modern bird feathers (Xu, Zhou & Prum, 2001; Barrett *et al.*, 2015; Mayr *et al.*, 2016; Yang *et al.*, 2019) and given the avian-like physiology of

dinosaurs (O'Connor & Claessens, 2005; Schachner, Lyson & Dodson, 2009; Schachner *et al.*, 2011), the similarity of fossilised melanosomes in exceptionally preserved fossil dinosaur feathers to those of modern birds (Li *et al.*, 2010, 2012; Eliason, Shawkey & Clarke, 2016), and the ability of melanosome morphology and melanin chemistry to elucidate palaeocolour in dinosaurs, a discussion of colour mechanisms in modern birds is warranted.

The myriad colours in bird feathers are fashioned by a complex interplay of pigments and structural mechanisms (Fig. 1E, F)(Shawkey, Morehouse & Vukusic, 2009). The major pigments contributing to colour production in the avian integument include carotenoids, melanins, porphyrins, and psittacofulvins (Hill & McGraw, 2006*a*,*b*; Cuthill *et al.*, 2017). Other pigments such as pterins and flavins play a relatively minor role in producing feather colours. Additionally, spheniscins are specialised pigments that are limited to penguins. Key research by Stoddard & Prum (2008) on avian visual sensitivities and later work (Stoddard & Prum, 2011) using 965 feather samples examined from 111 avian species led to the quantification of the approximate percentage of colours contributing to avian plumage colour gamut. While the largest and smallest proportions of the avian colour gamut are occupied by structural colours (∼69%) and non-pigmented white (∼0.1%), respectively, simple pigmentary colour prevalence is as follows: carotenoids (∼13.6%), psittacofulvins (∼10.6%), melanins (∼5.8%), and porphyrins (∼0.5%) (Fig. 1E). Avian pigments are briefly discussed below.

(1) Carotenoids

Carotenoids are linear, conjugated 40-C tetraterpenoid molecules divided into two major categories based on different functional groups (Matsuno, 1989). Non-substituted and non-polar carotenoids with only carbon and hydrogen atoms are referred to as carotenes (Fig. 3C) whereas substituted, polar carotenoids with oxygen-containing functional groups are collectively designated as xanthophylls (Fig. 3F) (Lu & Li, 2008). These pigments comprise over 1100 distinct chemical entities (Yabuzaki, 2017). Carotenoids are synthesised *de novo* by various organisms (e.g. bacteria, plants, algae, and fungi) and acquire their yellow/orange to red colours from the 'chromophore centre'. Chromophore centres (not to be confused with dermal chromatophores) are molecules or functional groups containing alternating single/double/triple bonds (i.e. conjugation) directly responsible for absorption of light and imparting colour (McHale, 2017). Greater degrees of conjugation within the chromophore centre result in greater absorption of short wavelengths in the violet to green region of the visible spectrum (400–550 nm) (Hill & McGraw, 2006*b*). A key difference between carotenoids and other pigments is that *de novo* biosynthesis of carotenoids does not occur in animals due to absence of the enzyme phytoene synthase. Thus, carotenoid-based colouration in animals is accomplished through dietary uptake (Brockmann & Völker, 1934; Matsuno, 1989; Goodwin, 1992; Sefc *et al.*, 2014) or through symbiotic association (e.g. marine filter-feeders) (Maoka, 2011). Although carotenoids are not synthesised by animals, the metabolic framework to process them after uptake does exist, allowing for conversion into non-dietary forms widely prevalent in avian feathers, beaks, and skin. After ingestion and metabolic processing, these pigments enter the bloodstream through both lipid-dependent and lipid-independent mechanisms and are eventually deposited in keratinous dead tissue through passive diffusion (Parker, 1996). It has been shown that only yellow carotenoids are assimilated by animals and can be bio-converted to make red ones (Goodwin, 1986). Blends of different carotenoid types can then create intermediate hues. Not all types of ingested carotenoids become incorporated into keratinised tissue (Hill & McGraw, 2006*b*). The process of carotenoid incorporation is governed by multiple selective factors such as diet (Rock *et al.*, 1992; Williams, Boileau & Erdman, 1998), intestinal endoparasites (Ruff & Fuller, 1975), toxins (Osborne *et al.*, 1982), diffusion thresholds (Parker, 1996), and unequal binding affinity for different lipoprotein types (Hill & McGraw, 2006*b*). Hence, the intensity of carotenoid-based colours can provide honest signals of diet and health for sexual selection to act upon (Weaver *et al.*, 2018).

(2) Psittacofulvins

Psittacofulvins are a class of highly colourful pigments restricted to the plumage of parrots (Psittaciformes). Initially mistaken for carotenoids, this unique class of lipid-soluble, red-yellow pigments was first named by Krukenberg (1882). Further research (Völker, 1936, 1937) highlighted that these pigments were independent of dietary uptake, unlike carotenoids. The detailed chemistry of psittacofulvins has only recently been revealed by high-performance liquid chromatography (HPLC) coupled with ultraviolet–visible (UV/VIS) spectroscopy and mass spectrometry (MS) (Stradi, Pini & Celentano, 2001) (Fig. 3G). This new approach identified at least four different variants of psittacofulvins and suggested a linear polyenal (i.e. long-chain-conjugated aldehyde) structure for each, differing only in the number of C=C conjugations. Based on this structure they also postulated two putative metabolic pathways by which psittacofulvins could be synthesised: through a polyketide pathway derived from acetyl-CoA or through enzymatic desaturation of fatty acids. Further work by McGraw & Nogare (2004) discovered a fifth psittacofulvin variant in parrots and demonstrated that these pigments are exclusively limited in distribution to feathers and do not impart colouration to any other tissue of the body. Mundy (2018) confirmed that red colours in parrot feathers are produced by long-chain-conjugated polyenals whereas yellows are produced by polyunsaturated 14-, 16-, or 18-C fatty acids. Additionally, Mundy (2018) identified a point mutation in the gene *MuPKS*, a member of the polyketide synthase gene family implicated in fatty acid biosynthesis, which causes green budgerigars (a result of structural blue combined with yellow psittacofulvins) to develop blue colouration. Therefore, *MuPKS* is likely responsible for generating

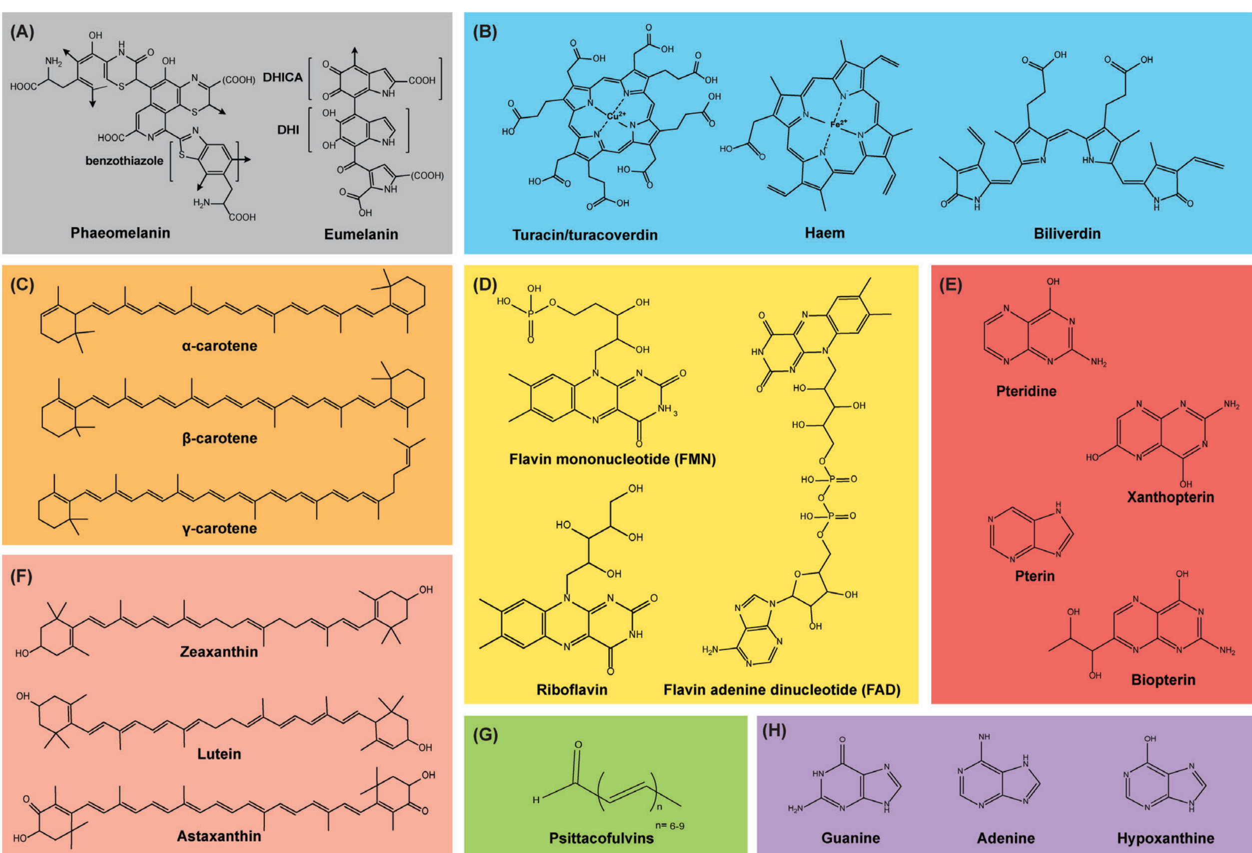

Fig. 3. Molecular structures of some common examples of pigments in the animal kingdom: melanins (A), porphyrins (B), carotenes (C), flavins (D), pterins (E), xanthophylls (F), psittacofulvins (generalised molecular structure) (G) and purines (H).

psittacofulvins. Polyketide synthases can cyclically add 2-C moieties to produce yellow psittacofulvins, which can then generate red psittacofulvins with further downstream processing that mirrors the synthesis of carotenoid pigments (Mundy, 2018). The reflectance properties of psittacofulvins have been investigated by various researchers (Krukenberg, 1882; Hudon & Brush, 1992; McGraw & Nogare, 2005). Characteristic absorption peaks at wavelengths shorter than those of carotenoids (Völker, 1936) and small reflectance peaks in the UV region (Pearn, Bennett & Cuthill, 2001; McGraw & Nogare, 2005) have been identified. Parrots can also blend colours by combining psittacofulvins with structural mechanisms. As in the budgerigar example, structural blue with yellow psittacofulvins produces green (for detailed mechanisms, see Section III).

(3) Melanin

The term melanin, derived from the ancient Greek *μελανος* ('melanos') meaning 'dark', was first coined by the Swedish chemist Berzelius (1840) to designate a dark-brown pigment isolated from retinal membranes. Several classification schemes have been proposed for melanin pigments during the last 50 years (Nickerson, 1946; Nicolaus, Piattelli & Narni, 1959; Nicolaus, 1968; Riley, 1992, 1997; Pezzella *et al.*, 1997; Ito & Wakamatsu, 1998; Wakamatsu & Ito, 2002; Borovansky & Riley, 2011; Prota, 2012; d'Ischia *et al.*, 2013), but no clear-cut, all-encompassing definition has emerged that sufficiently explains all of the properties of this diverse group of pigments (Prota, 1988, 2012; d'Ischia *et al.*, 2013). Melanin is frequently found in integumentary structures like mammalian hair (Goding, 2007) and avian feathers (Stoddard & Prum, 2011) as well as vertebrate skin (Morales-Guerrero *et al.*, 2017; Parolini *et al.*, 2018; Pshennikova & Voronina, 2018). It can also be found localised in other vertebrate organs, for instance, the retina of the eye, cochlea of the inner ear, and in certain regions of the brain and liver (Borovansky & Riley, 2011; Rossi, McNamara, Webbet *et al.*, 2019). At the chemical level, current consensus has broadly defined melanins as groups of highly heterogenous molecules derived from the oxidation of phenolic compounds and downstream polymerisation of the resulting intermediates (Solano, 2014). Melanin pigments produce colours ranging from black, grey or dark-brown, to reddish-brown by near-uniformly increasing broadband reflectance of visible wavelengths in the 300–700 nm range as well as wavelengths of the UV spectrum invisible to the human eye (Hill & McGraw, 2006*b*). Variation in the hues imparted by melanin is due to differences in the chemical units that make up these polymeric molecules (Riley, 1997).

Animal melanins (Fig. 3A) consist of eumelanin and phaeomelanin. Eumelanin is formed by the

oxidative polymerization of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) (Ito & Wakamatsu, 1998). Phaeomelanin is formed by the spontaneous combination of dopaquinone (an aromatic derivative of L-dihydroxyphenylalanine, an intermediate of the tyrosine catabolic pathway) with the amino acid cysteine to generate cysteinyl-dopa which undergoes further oxidation to become phaeomelanin (Greco *et al.*, 2011). Eumelanin principally produces hues ranging from darker shades of brown to black, while phaeomelanin produces rufous/reddish-brown colours. The presence of a higher number of carbonyl groups (C=O) in eumelanin causes strong absorption in the red region of the visible spectrum which makes it appear dark brown/black, whereas fewer carbonyl groups in the benzothiazole-rich (sulphur-containing) phaeomelanin produces paler brown to buff colours (Nickerson, 1946). Functionally, melanins can be further categorised in terms of their source (i.e. animal, plant, fungal, and synthetic) (Bell & Wheeler, 1986; Riley, 1997; Solano, 2014; Xiao *et al.*, 2015), but alternative terminology (i.e. eumelanin, phaeomelanin, neuromelanin, sepiamelanin, allomelanin, and pyomelanin) also appears frequently in the literature (Pezzella *et al.*, 1997; Schmaler-Ripcke *et al.*, 2009; Solano, 2014; Varga *et al.*, 2016). Neuromelanin is thought to be made up of mixtures of eumelanin and phaeomelanin and is found in the catecholaminergic neurons of substantia nigra (SN) of the brain (Graham, 1979; Carstam *et al.*, 1991; Zecca *et al.*, 2001; Solano, 2014), whereas eumelanin in cephalopod ink has been characterised as sepiomelanin (Pezzella *et al.*, 1997; Palumbo, 2003). Allomelanin and pyomelanin are plant, fungal, or bacterial in nature (Varga *et al.*, 2016). The enormous heterogeneity shown by these polymeric molecules can be attributed to differences in phenolic and quinone intermediates produced during oxidation steps as well as the types of intermediates eventually ending up as monomers that undergo polymerisation into the final product. Thus, the use of ambiguous terminology has reduced 'melanin' to something of a wastebasket term for any black/dark-brown pigment from any source, irrespective of chemical characterisation, although similarities exist.

Animal melanins are typically stored in microscopic, lipid membrane-bound, sub-cellular vesicles called melanosomes (Schraermeyer, 1996; Marks & Seabra, 2001). Melanosomes vary in morphology and distribution in a tissue- and taxon-specific way (Hong *et al.*, 2006; Borovansky & Riley, 2011; Eliason *et al.*, 2016). In bird feathers and mammalian hair, eumelanin has been noted to be stored in rod-shaped eumelanosomes, whereas phaeomelanin has been reported to be found in spherical-shaped phaeomelanosomes (Vinther *et al.*, 2008). In some tissues, such as the vertebrate retinal pigment epithelia, two distinct melanosome morphologies can be observed: ovoid and rods arranged basoapically (Kim & Choi, 1998). In amphibians, integumentary melanosome morphology is transitional between rod and spherical shapes, resembling a laterally condensed ellipsoid (Colleary *et al.*, 2015). For many years, the binary categorisation of animal melanins into eumelanin and phaeomelanin prompted authors to classify melanin-based colours discretely in birds, with eumelanin pigment localising exclusively to rod-shaped 'eumelanosomes' and phaeomelanin to spherical 'phaeomelanosomes' (Trinkaus, 1948; Somes & Smyth, 1965). However, it is now thought that both pigments can co-occur within melanised feathers (McGraw *et al.*, 2004). The relative proportion of phaeomelanin and eumelanin is key to the final shade imparted to the feathers. For example, the total melanin concentration is roughly the same in the pale reddish-buff feathers of red-winged blackbirds (*Agelaius phoeniceus*) and the black feathers of zebra finches (*Taeniopygia guttata*), but due to differences in the relative proportions of the two pigments (83% phaeomelanin in the former and 92% eumelanin in the latter) they show markedly different colour shades (McGraw *et al.*, 2004). This raises the question of co-localisation of eumelanin and phaeomelanin within a single type of melanosomes in feathers, as suggested by the 'casing model' of neuromelanin whereby phaeomelanin granules form a core and eumelanin molecules aggregate onto the surface of that core (Ito, 2006). Additionally, the correlation between melanosome shape and colour does not hold in retinal pigment epithelium, within which eumelanin is stored in both shapes of melanosomes (Wang, Dillon & Gaillard, 2006). Therefore, the melanosome shape–colour correlation in feathers would be better explained by characterising rod-shaped melanosomes as eumelanin-dominant and spherical melanosomes as phaeomelanin-dominant. Liu *et al.* (2014) tested the hypothesis that different colours of feathers correspond to relative proportions of eumelanin and phaeomelanin using laser desorption synchrotron post-ionisation (synchrotron-LDPI)-MS. Peak probability contrast from MS data showed that black feather colours can be attributed to oxidised forms of DHICA/DHI units of eumelanin and are most clearly distinguishable from other colours. Brown is dominated by higher proportions of phaeomelanin composed of oxidised versions of benzothiazine, benzothiazole, and isoquinolines. Grey colour is derived from phaeomelanin with nominal amounts of eumelanin and isoquinoline derivatives. Galván & Wakamatsu (2016) largely corroborated the conclusions of Liu *et al.* (2014) using an expanded colour gamut from birds and mammals but suggested that carboxylated DHICA and benzothiazole unit concentrations could act as correlates of the intensity of blacks and reddish-brown colours, respectively. Additionally, they also noted that colour phenotypes are not produced by DHICA or benzothiazoles in isolation but are composed of varying proportions of both. The chemical methods of Liu *et al.* (2014) and Galván & Wakamatsu (2016) could potentially be useful in reconstructing palaeocolour from preserved melanin signatures in fossils.

(4) Porphyrins

Porphyrins are a class of pigments characterised by square-planar, macrocyclic, and nitrogen-containing tetrapyrrole rings connected *via* methine (=CH–) bridges (Finar, 1956). These molecules, apart from being integumentary colourants, also play important physiological roles in non-

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integumentary tissues ranging from the oxygen-bearing haem co-factor in the respiratory metalloprotein haemoglobin in animals to the key light-absorbing component of chlorophyll in plants (Rimington, 1957). In birds, porphyrins (Fig. 3B) can be classified into three groups: (*i*) natural porphyrins (e.g. uroporphyrin, coproporphyrin, and protoporphyrin) limited to brown eggshells and rusty-hued feathers in owls (Strigiformes), bustards (Otididae), and nightjars (Caprimulgidae), (*ii*) metalloporphyrins in blood (i.e. haem), (*iii*) red and green colour-imparting porphyrins (i.e. turacin and turacoverdin) in turacos (Musophagiformes), and (*iv*) bilins in blue egg shells (e.g. biliverdin) (Derrien & Turchini, 1925; Völker, 1938; Ponka, 1999; Weidensaul *et al.*, 2011; Galván *et al.*, 2016*a*). Natural porphyrins are derived from succinyl CoA (an intermediate of the Krebs cycle), and the amino acid glycine (Needham, 2012). Metalloporphyrins are produced by enzymatic addition of metal ions such as iron, as in haem (Ponka, 1999), or copper, as in turacins (Rimington, 1939). Bilins are formed further downstream by oxidative degradation of haem in the liver but have also been found transported to more peripheral tissues like oviducts for deposition in egg shells (Poole, 1965, 1966). The spectral absorption of porphyrin, like other pigments, owes its origin to conjugated double-bond chromophore centres within the molecules, but porphyrins are unique in their ability to fluoresce red under UV light (Derrien & Turchini, 1925). Relatively large, polymerised porphyrins in brown egg shells show broad-spectral absorbance of wavelengths similar to melanins (Hill & McGraw, 2006*b*), but the aromatic double-bonded structure that confers wide spectral absorbance also makes them highly photolabile (Moan, 1988; Arakane *et al.*, 1996; Rotomskis, Bagdonas & Streckyte, 1996), causing them to fade over time. Additionally, the linking of these pigments to proteins and metal ions impacts not only the final colour produced but quenches the intense red fluorescence. Notably, although the fluorescent properties of porphyrin-containing feathers are not conspicuous under normal illumination, transient salmon-pink fluorescence in hidden, basal barbs of belly feathers has been observed in Otididae, which is proposed to function in short-duration sexual displays (Galván *et al.*, 2016*a*).

(5) Spheniscins

Spheniscins are a newly characterised category of endogenously synthesised, yellow-orange pigments exclusively limited to penguins (Sphenisciformes) and are suggested to have evolved once in fossil stem penguins (Thomas *et al.*, 2013). Chromatography and elemental composition (CHN) analysis highlighted their similarity to yellow and red pterin pigments (see Section II.6), although their solubility indicates crucial differences with other yellow-red pigments (McGraw *et al.*, 2007). Comparison of Raman and mid-infrared spectra of spheniscins with other avian pigments reveals 17 unique spectral bands in spheniscins in the wave number (ν) range 300–1700 cm^{-1} with the five most intense bands at 1577 cm^{-1} (vs, very small), 1285 cm^{-1} (s, small), 683 cm^{-1} (m, medium), 1469 cm^{-1} (m, medium) and 1351 cm^{-1} (m, medium). Raman spectra also predict the putative chemical structure of sphensicins to contain aromatically bonded heterocyclic rings with the possibility of tautomeric rearrangement under low pH conditions. However, further work is required to clarify the chemical properties of spheniscins (Thomas *et al.*, 2013).

(6) Other uncommon pigments

Pterins and related pigments (e.g. certain purines and flavins) are by-products derived from catabolism of the purine nucleotides – adenosine triphosphate and/or guanosine triphosphate (Fig. 3H) (Hill & McGraw, 2006*b*; Nelson, Lehninger & Cox, 2008). Pterins (Fig. 3E) are nitrogenous heterocyclic compounds with two-pyrimidine-ring skeletons which differ in their linear or cyclic substituents and impart characteristic red-orange-yellow colours, although colourless variants have also been reported (Fox, 1976). The compounds are frequently encountered in insect wings and eyes (Pfleiderer, 1994). Among vertebrates, pterins have been found in the skin and eyes of some fishes (Grether, Hudon & Endler, 2001), amphibians (Thorsteinsdottir & Frost, 1986), and reptiles (Steffen & McGraw, 2007). In avian species, they have been reported only as ocular colourants and do not occur in integumentary structures (Oliphant & Hudon, 1993). While both purines and pterins absorb very short wavelengths of light (guanine, $\lambda_{max} = 246$ and 273 nm; hypoxanthine, $\lambda_{max} = 249$ nm), pterins additionally absorb light >300 nm (Needham, 2012).

Flavins (Fig. 3D) are heterocyclic compounds with the basic structure of 7,8-dimethyl-10-alkylisoalloxazine (Rivlin, 2012; Edwards, 2014). The key dietary source of these pigments is vitamin B_2 (riboflavin) and its derivatives, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Rivlin, 2012). Riboflavin has been described to impart yellow colouration to skin patches in fishes (Courts, 1960) and amphibians (Obika & Negishi, 1972). Riboflavin is sometimes co-deposited with melanin to produce olive or dark-green hues in snakes (Blair & Graham, 1954; Villela & Thein, 1967). Pure riboflavin in aqueous solution shows four distinct absorbance peaks (λ_{max}) at 220, 266, 375, and 475 nm but is highly photolabile even under white-light illumination. This photolability is a result of its fluorescence at longer wavelengths (∼530 nm), which causes the colour to bleach due to breakdown of the chromophore centre even under low energy/long wavelength illumination (Hill & McGraw, 2006*b*). Riboflavin has also been postulated to influence the colour of bird egg yolks (Gliszczyńska-Świgło & Koziołowa, 2000), but additional studies are needed.

III. COMBINED STRUCTURAL AND PIGMENTARY MECHANISMS

Structural colour-producing mechanisms greatly expand the range of vertebrate integumentary colours in isolation as

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well as in combination with pigmentary colours. In relation to the use of an extant phylogenetic bracket approach to uncovering possible structural colours in dinosaurs, there is ample evidence of structural colour mechanisms in modern birds (Fox, 1976; Prum, 1999; Vinther *et al.*, 2010; McCoy *et al.*, 2018) but little is known in crocodilian scales except for a minor role played by guanine-crystal-containing iridophores, which act as reflective platelets to produce extremely weak iridescence. This is due to the fact that reflective platelets occur at low spatial frequencies in the epidermis and dermis and are not arranged in highly ordered vertical arrays, a prerequisite for strong iridescence (Alibardi, 2011). Blue, white, and iridescent shimmering effects in bird feathers are produced by two types of light scattering (coherent and incoherent) caused by nanoscale architecture of the material making up the feathers. Incoherent scattering occurs when light is scattered in different directions by materials lacking an ordered assembly, with wavelengths of different scattered light waves out of phase with each other (Hulst & van de Hulst, 1981; Bohren & Huffman, 2008). Coherent scattering occurs when light waves are scattered by materials with a highly ordered internal structure, and emerge in phase and reinforce each other (Prum *et al.*, 1999; Prum, Andersson & Torres, 2003; Prum & Torres, 2003*b*). The most common classes of colour-producing structures in feathers are (*i*) non-pigmented keratin with randomly oriented vacuoles, which produce a white colour through incoherent light scattering, (*ii*) feather barbules with nanostructural arrays of pigment and β-keratin, which typically produce iridescence through coherent scattering with colour depending on the viewing angle, and (*iii*) the spongy medullary layer of the barb rami or main feather rachis, which produces non-iridescent blues, violet, green, and UV colours (Hill & McGraw, 2006*b*). Recently, a fourth mechanism was discovered whereby 'super-black' feathers in birds-of-paradise (Paradisaeidae) achieve their colour from highly flattened bottlebrush-like barbules that are devoid of pigments and are extremely effective at trapping light through multiple instances of internal scattering, resulting in directionally mediated structural absorption (McCoy *et al.*, 2018).

Iridescence-producing melanosome–keratin arrays recognised in 14 out of 32 bird orders can be assembled into either flat laminar nanostructures, crystal-like lattices with a square or hexagonal unit cell, or irregular to quasi-ordered arrays (Hill & McGraw, 2006*b*). The morphology of the melanosomes is closely related to the type of ordering in these repeating arrays. Peafowls possess solid square unit cells of rod-shaped melanosomes (Zi *et al.*, 2003), whereas trogons possess closely packed hollow melanosomes with hexagonal unit cells (Durrer, 1986). In some species like the scrub euphonia (*Euphonia affinis*), hollow melanosomes are arranged into uneven quasi-ordered structures (Hill & McGraw, 2006*b*). Rod-shaped melanosomes (solid or hollow) can be organised into structures ranging from exclusively laminar to three-dimensional lattices to produce iridescent structural colour. However, platelet-like melanosomes found in hummingbirds can only be packed into laminar sheets to produce iridescence (Greenewalt, Brandt & Friel, 1960). Likewise, the structure of air spaces within these arrangements can range from single cavities to multiple small compartments interspersed between the melanosomes. Thus, laminar and crystal-like lattices produce strong iridescence, whereas quasi-ordered structures impart weak or non-iridescent colours (Hill & McGraw, 2006*b*).

Non-iridescent structural colours are usually created in two ways: through modification of the internal structure of feather barbules (Dyck, 1987) or by three-dimensional quasi-ordered arrays of box-shaped medullary cells in feather barbs and rami, filled with a spongy matrix of β-keratin and air (Dyck, 1971; Prum *et al.*, 1999). In the first category, iridescence is offset through equivalent backscattering at all viewing angles by arranging keratin–melanosome layers along curvatures in specialised rounded ridges on the barbule surface. The architecture of the second category is like Swiss cheese, with larger volumes of air compared to keratin that permit coherent light scattering (Hill & McGraw, 2006*a*,*b*). Melanosomes in the basal pigmentary layer of non-iridescent feathers although ellipsoidal, are morphologically distinct from those producing black, brown and iridescent colours but overlap significantly with grey-colour-producing melanosomes (Babarović *et al.*, 2019).

Structural colour can also occur in avian integumentary tissues other than feathers. Non-iridescent structurally coloured skin, bill sheaths, caruncles and scales are present in at least 250 bird species. This was first investigated in 19 avian families from 11 orders by Auber (1974) under the assumption that all blue or green colours are structural as opposed to being pigmentary due to the rarity of blue or green pigments in avian integument (Fox, 1976). The anatomy and mechanism of colour production in structurally coloured skin, beaks, and scales was later explored in 31 species of birds (from 17 families and 10 orders ranging from passerines to palaeognaths) (Prum & Torres, 2003*a*). This study illustrated coherent scattering of light from arrays of parallel collagen fibres in the dermis and that variations in colour can be produced by altering the size and spacing of these fibres. The arrangement of these collagen fibres functionally resembles the rounded quasi-ordered nanostructures in feather barbules and scatters light waves equivalently in all directions perpendicular to the collagen fibres.

Pigmentary and structural mechanisms imparting integumentary colours in animals have traditionally been studied as separate components. However, mounting evidence indicates that many colours are rendered through combined mechanisms and are difficult to produce by either mechanism in isolation (Dyck, 1971; Shawkey *et al.*, 2009; Stoddard & Prum, 2011; Shawkey & D'Alba, 2017). The complex interplay of pigmentary systems and optical and/or material properties of the integumentary structures are discussed in the following sections.

(1) Pigments and ordered nanostructures

Colour production through the combination of pigments and ordered nanostructures is frequently observed in birds (Noh

et al., 2010). Pigmentary green colours are comparatively rare except for turacoverdin, which is limited exclusively to Musophagidae. Since turacoverdin-based green colours are dependent on large quantities of Cu^{2+} ions, a trace dietary mineral (Dyck, 1976), shades of green colours in most birds are produced by an interaction of structural and pigmentary mechanisms in one of three ways: (*a*) coherent scattering in the feather barbules at the interface of highly ordered melanosome–keratin arrays yielding iridescent green (Hill & McGraw, 2006*b*), (*b*) interaction between carotenoid pigments in the barbs and melanin-bearing melanosome layers in the barbules yielding olive-green (Dyck, 1976), and (*c*) quasi-ordered spongy keratin layers containing carotenoid or psittacofulvin yielding non-iridescent green (Dyck, 1976; Stavenga *et al.*, 2011; Stoddard & Prum, 2011; D'Alba, Kieffer & Shawkey, 2012). Previous hypotheses suggested that blue-colour-producing spongy keratin nanostructures combine with yellow pigments to generate the green colours (Hill & McGraw, 2006*b*). However, new evidence suggests that the keratin layer is also predisposed to producing peak reflectance in the green spectral region by itself, but that the spectral reflectance curve is wide enough to include blue wavelengths (D'Alba *et al.*, 2012; Shawkey & D'Alba, 2017). The pigment molecules absorb these blue wavelengths and significantly augment the saturation of the green colours (Shawkey & D'Alba, 2017). New research also posits that the production of non-iridescent blue colours has a substantial contribution from the ordered melanosome arrays lying basal to the spongy keratin layer (Parnell *et al.*, 2015). The basal melanosome layer plays a critical role of siphoning away the incoherently scattered white wavelengths (owing to the broad-based spectral absorption of melanin) which would otherwise greatly diminish the blue hue to impart a more whitish colour (Shawkey & Hill, 2006; Zhang *et al.*, 2015).

(2) Pigments and disordered nanostructures

Pigment molecules, by definition, produce colours by absorbing only certain wavelengths of light and reflecting all others. However, optical/material properties of sheathing biopolymers (e.g. feather keratin, arthropod chitin, or plant cellulose) also impact the wavelengths absorbed/reflected and influence the final colour imparted. Texture and thickness of the encasing material can regulate brightness – a thicker array of keratin with randomly oriented vacuoles will promote greater incoherent scattering, and when combined with deposited pigments (carotenoid/psittacofulvin), can produce a bright yellow/green colour. By contrast, thin arrays of disordered keratinous material combined with pigments produce duller colours. Whether pigments or structural biopolymers have the greatest effect in imparting the final colour is a matter of considerable debate (Shawkey *et al.*, 2006; Jacot *et al.*, 2010; Evans & Sheldon, 2011; Shawkey & D'Alba, 2017).

(3) Dermal chromatophore assemblies

Colour changes also occur through rapid spatial dispersal of pigment molecules, pigment-containing vesicles, and reflective structures in ectothermic animals (e.g. crustaceans, cephalopods, fishes, amphibians and reptiles). These are controlled by neuroendocrine stimulation of cellular assemblies (Bagnara & Hadley, 1973). Diverse colours and dynamic colour changes result from the interaction between three-dimensionally organised layers of dermal cells including various types of pigments and reflective nanostructures which are organised into 'dermal chromatophore units' (Bagnara, Taylor & Hadley, 1968). The chromatophore units are composed of three layers of cells. These layers have been recognised in ectothermic vertebrates such as fishes (Fujii, 1993), amphibians (Hadley & Bagnara, 1969; Ichikawa *et al.*, 1998), and reptiles (Taylor & Hadley, 1970; Sherbrooke & Frost, 1989). The most superficial pigmented layer, lying immediately below the epidermis and basal lamella, consists of pterin- and/or carotenoid-bearing xanthophores (yellow-orange) and erythrophores (red). Lying below the xanthophores and erythrophores is a layer made up of nanoscale reflective platelets (guanine/purine crystals) embedded within iridophores and leucophores (Kuriyama *et al.*, 2006; Boyer & Swierk, 2017). The shape, orientation, and three-dimensional organisation of the platelets influence the production of structural colours ranging from white to violet *via* incoherent scattering, coherent scattering, thin-film interference, and/or diffraction. The innermost layer, called the melanophore layer, contains melanin pigments and produces black/brown colours. The fine-scale and rapid colour change (i.e. metachrosis), which can yield wide variation in macroscale integumentary patterns, is facilitated by the fine-tuned co-localisation and dynamic regulation of the ratios of different pigment-bearing chromatophore types (Boyer & Swierk, 2017; Shawkey & D'Alba, 2017). Metachrosis is a key mechanism behind predator-evasion behaviours like camouflage (Cacciali *et al.*, 2018).

IV. DIAGENETIC TRANSFORMATIONS OF PIGMENTS AND STRUCTURAL COLOURS IN FOSSILS

The taphonomy of pigments other than melanin, carotenoids and porphyrin have not yet been studied in detail within fossils. The known diagenetic pathways of pigments and structural colour are discussed below.

(1) Melanins

Most of what is known about the chemistry of eu- and phaeomelanin is through alkaline peroxide degradation and hydrolysis (Ito *et al.*, 2011). HPLC and MS have been used to characterise the resulting melanin degradation products from this treatment (Ito & Wakamatsu, 1998). These products have since been used as diagnostic markers for eumelanin and phaeomelanin in modern samples treated with alkaline peroxide degradation and hydrolysis. Pyrrole-2,3,5-tricarboxylic acid

(PTCA), pyrrole-2,3,4-tricarboxylic acid (iso-PTCA), pyrrole-2,3,4,5-tetracarboxylic acid (PTeCA), and pyrrole-2,3-dicarboxylic acid (PDCA) are markers for eumelanin. Thiazole-4,5-dicarboxylic acid (TDCA), 4-amino-3-hydroxyphenylalanine (4-AHP), and thiazole-2,4, 5-tricarboxylic acid (TTCA) have been suggested as markers for phaeomelanin (Pezzella *et al.*, 1997; Ito & Wakamatsu, 1998; Ward *et al.*, 2008; Ito *et al.*, 2011). These markers have also been recovered as products of pyrolytic gas chromatography of fossil samples (Glass *et al.*, 2012). Glass *et al.* (2013) further suggested that diagenesis and prolonged thermal maturation cause eumelanin subunits to crosslink together, granting exceptional stability through deep time (Fig. 4A). Although the precise diagenetic pathway for phaeomelanin currently remains unknown, Glass *et al.* (2012) noted that the pyrolysis gas chromatography mass spectra for fossil tissues known to contain eumelanin sometimes additionally indicated the presence of sulphur-containing molecular fragments (e.g. thiophenes, alkylthiophenes, etc.). These sulphur-bearing molecular fragments were attributed to the incorporation of sulphur into eumelanin by early diagenetic processes occurring chiefly in euxinic marine palaeoenvironments, resulting in organic preservation through a vulcanisation-like process of this biopolymer whose natural thermal stability already allows for organic fossilization (McNamara *et al.*, 2016*b*). McNamara *et al.* (2016*b*) showed that pyrite-rich freshwater palaeoenvironments (e.g. oil shales from Messel, Germany and Libros, Spain) can yield similar sulphur signals in mass spectra of fossil eumelanin. One example of this type of euxinic depositional environment is in the preservation of the nodosaur *Borealopelta markmitchelli*, which has been suggested to preserve fossil phaeomelanin (Brown *et al.*, 2017). This raises an important question of whether the inference of fossil phaeomelanin chemical markers from euxinic environments could potentially be erroneous (i.e. derived from taphonomic incorporation of environmental sulphur rather than from endogenous phaeomelanin). Colleary *et al.* (2015), McNamara *et al.* (2016*a*) and Brown *et al.* (2017) however, agree that there is a low chance of conflation of eumelanin and phaeomelanin in fossils from mass spectra data. The potentially environmental thiophene and its derivatives differ chemically from the endogenous nitrogen-containing markers in phaeomelanin (e.g. 4-AHP for benzothiazine moieties). Furthermore, melanosome morphology, tissue type, and phylogenetic placement of the specimen could potentially provide independent lines of evidence to distinguish fossil phaeomelanin from fossil eumelanin.

Differentiating integumentary melanosomes from those of visceral organs in two-dimensionally flattened fossils has been suggested to be complicated. Recent work posits that weak post-burial hydrodynamic disturbances in quiet or stagnated lakebeds can cause visceral organ melanosomes to be redistributed to the integumentary surface (McNamara *et al.*, 2018*a*). However, two-dimensional flattening of carcasses has been suggested to have minimal effect on at least the lateral expansion or distortion of non-biomineralised fossil tissues (Briggs & Williams, 1981), and preliminary experimental taphonomy has suggested this to hold true in the case of avian plumage/carcasses (Saitta, Clapham & Vinther, 2018*a*) as well as lizard carcasses and beetle exoskeletons (Saitta *et al.*, 2018*d*). Furthermore, organic preservation of melanin-containing organs such as the retina and liver can show discrete localisation patterns in fossils without evidence of lateral distortion (Sallan & Coates, 2014; Smithwick *et al.*, 2017). The fact that three-dimensional ultrastructural melanosome alignment in feathers (Vinther *et al.*, 2010; Li *et al.*, 2012; Vitek *et al.*, 2013) and macroscopic colour patterns (Vinther *et al.*, 2008; Smithwick *et al.*, 2017) can be preserved unaltered in fossils suggests that taphonomic disturbance of melanosome distribution does not occur to any significant degree during carbonaceous fossilisation and compression. Therefore, it is unclear whether melanosome redistribution within a carcass would be a taphonomic factor of great concern. Regardless, sampling from regions of the fossil clearly attributable to integument or integumentary structures would be a simple solution.

(2) Carotenoids

Bacteria, halophilic archaea, photosynthetic eukaryotes, and a host of non-photosynthetic organisms have been reported to produce carotenoid pigments (Britton, 1995). Fossil carotenoids have been detected in sediments as far back as the Precambrian using gas chromatography-tandem mass spectrometry (GC–MS–MS) (Marshall & Marshall, 2010; French *et al.*, 2015). Most carotenoids, however, do not survive long-term diagenesis in forms that are readily linkable to their biological precursors and exact taxonomic origin. During early sedimentary diagenesis, different types of carotenoids vary in stability. Those containing 5,6-epoxide linkages (i.e. three-carbon ring with an oxygen atom) rapidly degrade in early diagenesis within anoxic and stagnant lakebed sediments and are converted to loliolide, isololiolide, and other derivatives in aqueous phase (Fig. 4B) (Repeta, 1989). However, there is evidence that some aromatic carotenoids (e.g. β-carotenes, lutein, zeaxanthin) retain their chemical backbone structures in the form of saturated perhydro derivatives (e.g. carotanes, lycopane) (Fig. 4C). These carotenoids are degraded primarily though microbial oxidation in a much more gradual manner compared to those containing 5,6-epoxide linkages, as noted from recent anoxic environments (Repeta, 1989). Such carotenoids can potentially become molecular fossils. β-carotenes can also undergo internal aromatisation reactions to generate several novel products that have also been noted as potential fossil biomarkers (Fig. 4D) (Koopmans, De Leeuw & Damsté, 1997). Recently some advances have been made detecting the molecular remnants of perhydro derivatives using Raman spectroscopy (Marshall & Marshall, 2010). Raman spectroscopy combined with a near-infrared laser is capable of detecting distinctive absorption bands for unaltered carotenoids in modern feathers placed behind an amber matrix but failed to do so in a fossil feather sample preserved

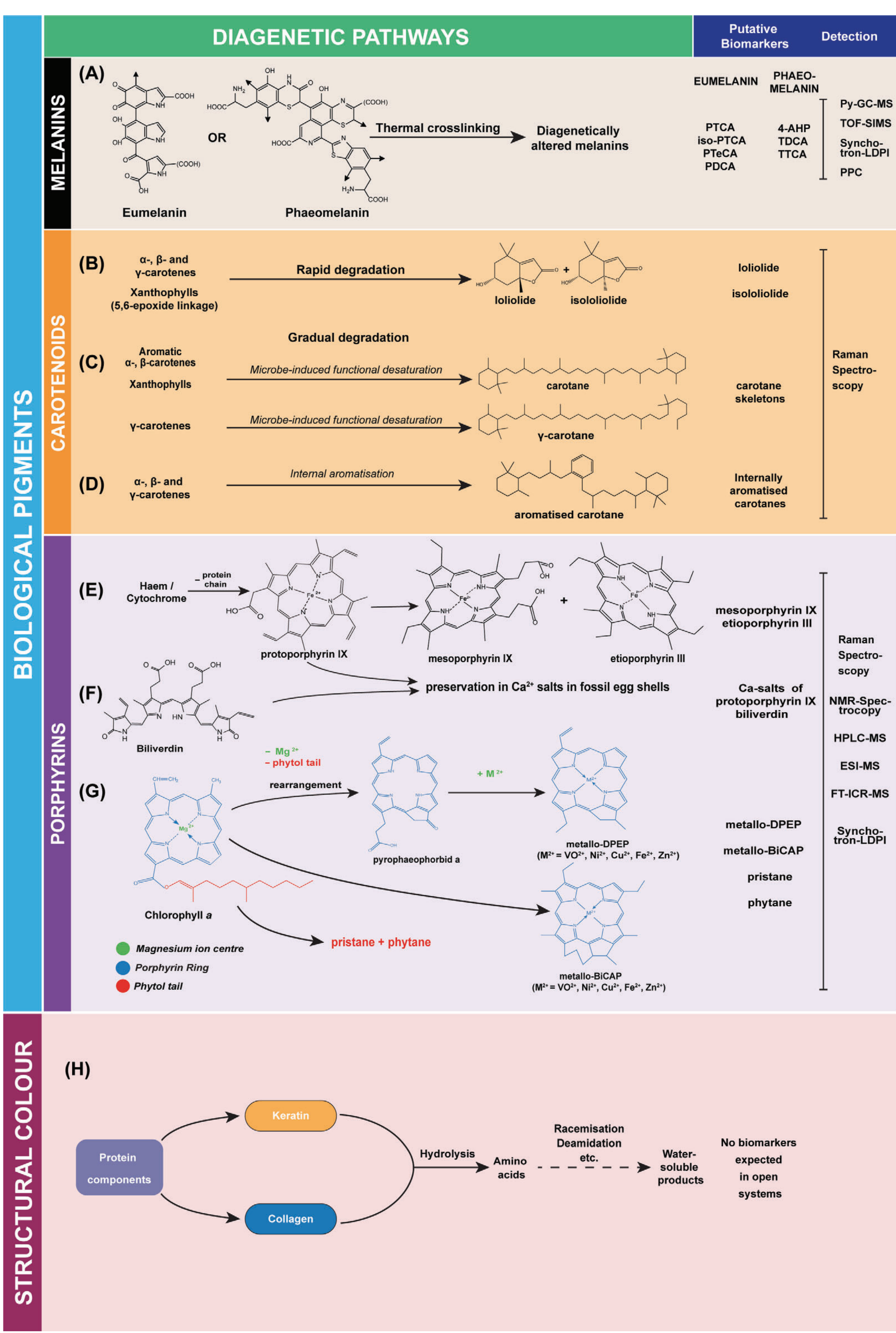

Fig. 4. Diagenetic pathways for biological pigments and structural colour-producing mechanisms along with their potential biomarkers. 4-AHP, 4-amino-3-hydroxyphenylalanine; ESI-MS, electrospray ionisation mass spectrometry; FT-ICR-MS, Fourier-transform ion cyclotron resonance mass spectrometry; HPLC-MS, high performance liquid chromatography; iso-PTCA, pyrrole-2,3,4-tricarboxylic acid; NMR, nuclear magnetic resonance; PDCA, pyrrole-2,3-dicarboxylic acid; PPC, peak-probability contrast; PTCA, pyrrole-2,3,5-tricarboxylic acid; PTeCA, pyrrole-2,3,4,5-tetracarboxylic acid; Py-GC-MS, pyrolysis-gas chromatography-mass spectroscopy; synchotron-LDPI, laser desorption-ionisation; TDCA, thiazole-4,5-dicarboxylic acid; TOF-SIMS, time of flight secondary-ion mass spectroscopy; TTCA, thiazole-2,4,5-tricarboxylic acid.

as a carbonaceous compression as well as fossil feather amber inclusions (Thomas *et al.*, 2014). A further test of the efficacy of this technique would be to repeat the analysis with a larger sample size of fossil feathers since the fossils examined could have lacked carotenoids *in vivo*.

It should be noted that carotenoids are generally acquired through microbial/plant-based diet in birds and detection of carotenoids in fossils whose matrices contain large amounts of organic microbial/plant matter could potentially lead to erroneous colour prediction, a serious issue when organic melanin preservation often coincides with lagerstätten representing lake or lagoonal palaeoenvironments. Therefore, comparison of the fossil with the surrounding matrix is vital to exclude false positive carotenoid detections. Irrespective of the challenges of detecting fossil carotenoids that are demonstrably endogenous to a fossil rather than environmental, predicting their original hue (i.e. red, orange, or yellow) could be challenging due to changes in conjugation through diagenesis which alter the produced hue. Morphological interpretation of xanthophores (i.e. chromatophores containing carotenoid and/or pterins) has been suggested in a 11.2–8.7-million-year-old fossil snake (McNamara *et al.*, 2016*a*) but the inorganic, phosphatic preservation of these structures in this specimen is unusual given that such pigments might be expected to preserve organically, warranting further investigation. Experimental demonstration of xanthophore morphology preserved *via* authigenic mineralization as phosphate would provide strong evidence for such a claim.

(3) Porphyrins

Geoporphyrins (i.e. diagenetically altered porphyrins from various sources) are abundant in sediments, coal, and oil both across the globe and in deep time (Van Berkel, Quirke & Filby, 1989; Huseby & Ocampo, 1997; Junium, Freeman & Arthur, 2015). Two categories of about a hundred geoporphyrins have been described with the first sourced from the diagenesis of haems, cytochromes, and natural tetrapyrroles and the second from the diagenesis of the chlorophylls of phototrophs (Montforts & Glasenapp-Breiling, 2002). The typical first step in haem/cytochrome diagenesis is the hydrolytic detachment of the porphyrin ring from the protein components followed by a series of reactions leading to the formation of a wide variety of porphyrin derivatives that are either free or complexed with metal ions, including mesoporphyrin IX and etioporphyrin III (Killops & Killops, 2013) (Fig. 4E).

Biological pigments produced *in vivo* by catabolism of haems, biliverdin, and protoporphyrin, all of which impart colouration to modern bird eggs, have also been detected in fossil eggs ranging from the Miocene-Holocene subfossil blue-green eggshells of the upland Moa (*Megalapteryx didinus*) and pale brown eggs of North Island Moa (*Euryapteryx curtus*) (Igic *et al.*, 2010) to the blue-green Late Cretaceous macrooolithiid eggshells attributed to the oviraptorid *Heyuannia huangi* (Wiemann *et al.*, 2017). Wiemann, Yang & Norell (2018) examined a larger sample of fossilised non-avialan dinosaur and early bird eggshells and suggested that the evolution of coloured eggshells with macroscale patterns (speckles and spots) can potentially be traced to a single origin at the base of paravians. While protoporphyrin is relatively unreactive due to resonance stabilisation of conjugated double bonds, biliverdin is much more labile due to its linear, oxidised nature (Gorchein, Lim & Cassey, 2009). It has been suggested that formation of calcium salts and entrapment in the eggshell matrix facilitates the preservation of biliverdin (Fig. 4F) and protoporphyrin in these fossils (Wiemann *et al.*, 2017, 2018).

Although not produced in vertebrates, it is of interest to discuss chlorophyll diagenesis as it relates to challenges in studying fossil vertebrate porphyrins due to complications from environmental sources of geoporphyrins. In plants, chlorophyll *a* ($C_{55}H_{72}MgN_4O_5$) quickly undergoes *post-mortem* chemical transformation in aquatic sediments and loses its green colour (Fig. 4G). Of the three functional groups of the porphyrin ring that characterise chlorophyll molecules and influence colour production, carboxylic acid and a centrally coordinated metal ion are lost, while the phytol chain can be preserved (albeit hydrolytically cleaved from the porphyrin ring). Post-separation from the molecule, the phytol chain undergoes reactions of various types in the sediment and forms unsaturated carbon skeletons of the diastereomers phytane and pristane (Killops & Killops, 2013). The porphyrin ring can be preserved through one of two different diagenetic pathways (Fig. 4E–G): (*i*) a product of one early diagenetic pathway of chlorophyll *a* is pyrophaeophaeophorhbid a, which ultimately leads to metallodeoxyphylloerythrethioporphyrin (metallo-DPEP) (Killops & Killops, 2013). The type of metal ion in metallo-DPEP (e.g., VO^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+}) inserted into the coordination centre is dependent on the pH, redox potential (Eh), metal availability, and thermal maturity of the sediments (Junium *et al.*, 2015). (*ii*) Alternatively, phaeophytin (i.e. chlorophyll lacking the Mg^{2+} ion) undergoes a ring cleavage followed by cyclisation to form bicycloalkanoporphyrines (BiCAPs) with or without metal ions (e.g. VO^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, Zn^{2+}). A major challenge in studying geoporphyrins is the occurrence of highly altered fossil porphyrins in matured kerogen pools that do not always unambiguously indicate their original biological source (Killops & Killops, 2013), thus contamination from surrounding sediments could be difficult to rule out in some fossils suspected to contain integumentary porphyrins.

(4) Structural colour

While the structural colour-producing mechanisms in feathers have been studied in detail in modern birds (Hill & McGraw, 2006*b*) and in a limited manner in the feathers of fossil paravians (Vinther *et al.*, 2010; Li *et al.*, 2012; Vitek *et al.*, 2013; Hu *et al.*, 2018), reports on such mechanisms in the skin of non-avialan dinosaurs or early birds do not exist, leaving crucial gaps in our reconstruction of palaeocolour. This is due to the fact that proteins like

keratin and dermal collagen are prone to denaturation, hydrolysis, deamidation, racemisation, thermal degradation and dissolution of certain constituent amino acids through diagenesis (Ortiz *et al.*, 2018) and do not appear readily to fossilise organically (Briggs & Summons, 2014; Saitta *et al.*, 2017; Smithwick *et al.*, 2017) (Fig. 4H). Therefore, inferences regarding structural colouration derived from keratin frameworks and dermal collagen in the fossil record are likely to be difficult. Since keratin does not appear to fossilise organically (Armstrong *et al.*, 1983; Saitta *et al.*, 2017, 2018*b*), only the pigment components/layers of structural colour-imparting tissue arrays will be available to identify structural colouration in fossils, as is the case for the few known examples of iridescence in fossils of originally keratinous structures (Vinther *et al.*, 2010; Li *et al.*, 2012; Vitek *et al.*, 2013; Hu *et al.*, 2018).

It has been suggested that keratin protein can preserve phosphatically (McNamara *et al.*, 2018*b*). However, Saitta *et al.* (2018*b*) note that most fossil keratinous structures are easily explained *via* preservation of endogenous calcium phosphate without needing to invoke taphonomically induced phosphatization. For example, most phosphatically preserved keratinous structures include feather rachises and claw, beak, and osteoderm sheaths (Murphy, Trexler & Thompson, 2006; Christiansen & Tschopp, 2010) – structures known to be calcified *in vivo* in order to increase the hardness of the keratinous structure (Blakey, Earland & Stell, 1963; Blakey & Lockwood, 1968). Experimental decay experiments capable of inducing microbially mediated phosphatisation of keratin would provide strong support for the idea that keratin protein can be authigenically mineralised and would thereby provide the opportunity to investigate fossil structural colour that involves keratin nanostructural components. However, such support is currently lacking, and it appears that keratin protein loss during fossilisation is the most conservative explanation.

V. EXISTING PALAEOCOLOUR RESEARCH

Colour patterns and organic staining from fossil pigments across broad geographical and temporal ranges have long been recognised in the fossil record by the naked eye (Williams, 1930; Carpenter, 1970; Kříž & Lukeš, 1974; Pan *et al.*, 2013). Pigments have been discovered in phylogenetically diverse fossils preserved as dark carbonaceous remnants, including fossil algae (Wolkenstein, Gross & Falk, 2010), leaves (Rieseberg & Soltis, 1987), cephalopods (Glass *et al.*, 2012; Williams, 2017), trilobites (McRoberts *et al.*, 2013), crinoids, eurypterids, graptolites (Vinther, 2015), insects (McNamara *et al.*, 2013*b*), stem lampreys like *Tullimonstrum* (Clements *et al.*, 2016; McCoy *et al.*, 2016), fishes (Gabbott *et al.*, 2016), frogs (Colleary *et al.*, 2015), snakes (McNamara *et al.*, 2016*a*), marine reptiles (Whitear, 1956; Lindgren *et al.*, 2014), non-avialan dinosaurs (Li *et al.*, 2010, 2012; Field *et al.*, 2013; Vinther *et al.*, 2016; Peteya *et al.*, 2017; Hu *et al.*, 2018), birds (Vinther *et al.*, 2008, 2010; Gren *et al.*, 2017; Peteya *et al.*, 2017) and mammals (Colleary *et al.*, 2015; Manning *et al.* 2019). The earliest chemical study on palaeocolour noted the similarity between infrared (IR) spectra of melanin in fossilised and modern cephalopod ink (Beyermann & Hasenmaier, 1973). However, it was the application of electron microscopy to fossils that provided the catalyst for the development of palaeocolour reconstruction by allowing nanometre- to micron-scale objects to be imaged for the first time. Electron micrographs of fossil feathers and hairs from the middle Eocene Messel lagerstätten (Wuttke, 1983) showed large fabrics of micron-sized rod-shaped and spherical to sub-spherical structures, which were originally interpreted as preserved microbial biofilms associated with decay of the original tissue. This microbial interpretation was supported in later work (Davis & Briggs, 1995; Martill & Frey, 1995) until these microbodies were alternatively identified decades later as melanin-bearing organelles called melanosomes (Vinther *et al.*, 2008; Zhang *et al.*, 2010).

Subsequent research linked colour patterns in isolated fossil feathers to melanosome localisation whereby melanosomes were present in the darker, carbonaceous regions of the fossil but were lacking in intervening non-stained areas (Vinther *et al.*, 2010; Vitek *et al.*, 2013), paving the way for reconstructions of palaeocolour in non-avialan dinosaur and early bird integument (Clarke *et al.*, 2010; Li *et al.*, 2010, 2012; Field *et al.*, 2013; Vinther *et al.*, 2016; Peteya *et al.*, 2017; Hu *et al.*, 2018). Despite mounting evidence from their structure, chemistry, and localisation patterns in favour of a melanosome identity for these fossil microbodies, many studies continued to argue in favour of a microbial identification (McNamara *et al.*, 2009; Iniesto *et al.*, 2013; Moyer *et al.*, 2014; Schweitzer, Lindgren & Moyer, 2015), while others suggested the identities of these structures should be determined on a case-by-case basis (Lindgren *et al.*, 2012, 2015).

Fossil colour reconstruction has progressed significantly since the early work on isolated feathers (Vinther *et al.*, 2008, 2010; Zhang *et al.*, 2010; Vitek *et al.*, 2013) with palaeocolour reconstructions produced for iconic fossils. A comparative dataset of melanosome morphology (i.e. aspect ratio and size) in 167 modern bird species and use of their shape–colour relationships to predict colour in fossil samples through quadratic discriminant analysis (QDA) has been used on the paravians *Anchiornis huxleyi* and *Microraptor gui* (Li *et al.*, 2010, 2012).

Melanosomes sampled from various regions of *Anchiornis* led to its reconstruction with reddish-brown head feathers, a grey feathered body, and black-tipped white feathers along the wings and tail (Li *et al.*, 2010). Saitta *et al.* (2018*c*) provided additional details on its feathering and a refined reconstruction sporting fluffy, likely open-vaned, bifurcated contour feathers. *Microraptor*, on the other hand was reconstructed to possess iridescent black feathers all over its body (Li *et al.*, 2012). The initial dataset (Li *et al.*, 2010, 2012) has been augmented by the addition of a further 129 modern taxa bearing iridescence-producing

melanosomes (Nordén *et al.*, 2018). However, QDA has its own caveats. Since the distributions of the melanosome morphology variables (aspect ratio, long axis, short axis, etc.) are not Gaussian, parametric predictive modelling like QDA yields limited statistical accuracy (63–73%) and cannot support nominal multistate variables in combination (e.g. melanosome shape and hollow/flat/solid). Multinomial logistic regression (MLR) proposed by Nordén *et al.* (2018) is much more accurate (yielding a statistical modelling accuracy of 83%) and does not possess the shortcomings of QDA. However, they also acknowledge that the expanded dataset may not be entirely representative of the morphological diversities of melanosomes in 10000+ modern avian species. Improvements to statistical methodology have the potential to revolutionise palaeocolour reconstruction.

Despite robust statistical analysis, a whole-body reconstruction might still require extensive destructive sampling of the fossil because colour can vary across the body of an organism and specimens are often too large to be viewed whole in an electron microscope. To reduce permanent damage to fossil specimens, palaeontologists are often limited in the extent of their sampling, which may be insufficient to ascribe patterns of melanin-based hues across the entire body.

Ideally, methods to determine fossil pigmentation should be non-destructive and quantitative. Through combined use of synchrotron rapid-scanning X-ray fluorescence (SRS-XRF), X-ray absorption near-edge structure (XANES) and X-ray absorption spectroscopy, the preservation of trace metal cations chelated by melanin within fossils has been studied (Wogelius *et al.*, 2011; Manning *et al.*, 2013, 2019). Cu^{2+}/Zn^{2+} ions can be chelated to melanin during its synthesis and were suggested to be biomarkers mapping the colouration of fossils. However, there is no strong support for (*i*) trace metal chelation happening uniquely during the synthesis of eumelanin/phaeomelanin *versus* the synthesis of other biomolecules, and (*ii*) the ability of these techniques to distinguish ions incorporated during eumelanin synthesis from those taken up secondarily during *post-mortem* taphonomic processes. Additionally, in cases where oxidative weathering has removed the carbonaceous fossil melanin from certain regions in the fossil, a lack of Cu^{2+}/Zn^{2+} associated with the mouldic impressions of melanosomes in sediment could lead to the erroneous conclusion that these regions were unmelanised *in vivo*. Therefore, Cu^{2+}/Zn^{2+} does not meet the definition of a biomarker for melanin in that it is not specific to melanin and is not expected to persist when the melanin it is chelated to is lost (Vinther, 2015). Indeed, many organic compounds other than melanin chelate copper ions, including other pigments (e.g. porphyrins) and humic acids from decomposed organic substances (Premović *et al.*, 2000). A widespread presence of Cu^{2+} has also been found in a 48-million-year-old Dawn Redwood (*Metasequoia*) leaf (Edwards *et al.*, 2014), which would not have possessed melanin. In a more recent work on a 3-million-year-old fossil mammal (Manning *et al.*, 2019), if the Cu^{2+}/Zn^{2+} ions were bound to phaeomelanin benzothiazole moieties and organosulphur residues from the diagenesis of keratin, the signal is likely to be of limited use on account of variability and significant peak overlap of the phaeomelanin signal with background remnants of keratin breakdown products, beyond the issue of potential taphonomic incorporation of Cu^{2+}/Zn^{2+} ions.

Incomplete preservation remains a major hurdle for palaeocolour reconstruction. For example, the single supposedly matte black feather (Carney *et al.*, 2012), whose ascription to *Archaeopteryx* has been questioned (Kaye *et al.*, 2019*b*), is clearly not enough confidently to reconstruct the colour pattern of the entire animal. Among the various theropod specimens for which palaeocolour has been reconstructed, many have large patches of missing soft tissue preservation such as the tail feathers of the *Anchiornis* specimen studied by Li *et al.* (2010), the leg region of *Caudipteryx* (Zhang *et al.*, 2010; Li *et al.*, 2014), the neck region of *Beipiaosaurus* (Zhang *et al.*, 2010), tail portions of *Caihong* (Hu *et al.*, 2018), and parts of the abdominal region of *Sinosauropteryx* (Zhang *et al.*, 2010; Smithwick *et al.*, 2017). In some instances, such gaps in carbonaceous staining might not simply have represented white colouration due to absence of melanin considering that other pigments and/or structural mechanisms could have been present but either not preserved (e.g. protein-based structural colour) or yet to be detected reliably in fossil feathers and scales (e.g. carotenoids or porphyrins) (Table 1). However, certain patterns of carbonaceous soft-tissue preservation might be very consistent with a truly non-pigmented, white reconstruction (e.g. countershading or complex repeating patterns such as spots or stripes). Furthermore, much as the osteological completeness of fossil specimens is often less than 100%, incomplete soft tissue preservation could be due to scavenging, physical perturbation, or microbial/autolytic decay of even relatively thermally stable biomolecules. Another consideration is that in non-avialan dinosaurs with simple feathers (e.g. non-pennaceous or barbule-lacking) and presumably higher predation risk due to terrestrial lifestyles, colouration might have been more limited to drab or cryptic melanin-based colours compared to the diversity of plumage colours seen in modern birds.

Fossil feather morphologies are critical factors in relation to colour and must be considered in an evolutionary perspective. The organisation of barbs and barbules into the planar vanes of feathers might have permitted the evolution of structural iridescence through nanoscale alignment of reflective keratin–melanosome arrays (Koschowitz, Fischer & Sander, 2014). Coinciding with the evolution of pennaceous feathers in maniraptorans, a pleiotropic increase in melanosome morphological diversity linked to physiological factors such as elevated metabolic rates has been suggested to have greatly expanded the integumentary colour gamut (Li *et al.*, 2014). Prior to this, melanosome morphological diversity was reported to be limited in filamentous integumentary structures of pterosaurs and non-maniraptoran dinosaurs, which were then hypothesised to have plesiomorphically used spherical to sub-spherical

Table 1. Current lines of evidence for palaeocolour reconstruction

Observational evidence	Examples	Considerations
Macroscopic carbonaceous stains (Vinther *et al.*, 2008; Zhang *et al.*, 2010; Smithwick *et al.*, 2017)	Colour patterns (e.g., stripes, mottling, bars, 'bandit masks', countershading).	Absence of stains may be due to: (*i*) early taphonomic processes (e.g. scavenging, decay, physical perturbation); (*ii*) non-pigmented integument; (*iii*) structurally coloured integument that lacks fossilisation potential through diagenesis; (*iv*) non-melanin-based pigmentation; (*v*) late taphonomic processes (e.g. oxidative weathering of organics).
Melanosome morphology and organisation (Vinther *et al.*, 2008, Li *et al.*, 2010, 2012, Zhang *et al.*, 2010)	Aspect ratio, shape, and size from organic preservation or mouldic impression in sediment (e.g. oblong, oblate, platelet). Internal structure (e.g. solid/hollow).	Melanosome organic structure can be lost through oxidative weathering or through aqueous conditions during thermal maturation/diagenesis.Thermal maturation/diagenesis results in some minor (<10%) shrinkage of melanosomes.
	Arrangement of melanosomes relative to each other can reveal structural colouration (e.g. melanosome lattices).	Many structural colour arrays involve proteinaceous components that likely do not fossilise.
Organic chemistry (Colleary *et al.*, 2015)	Chemical signatures consistent with fossil pigment – precise signature dependent on the pigment and analytical technique used (e.g. secondary ions, pyrolysates, infrared absorption spectra, etc.).	Some sulphur moieties can be derived from phaeomelanin or from taphonomic incorporation of sulphur into eumelanin. Chemical makeup of biomolecules can alter during diagenesis.

melanosomes to confer black, brown, or grey colours (Li *et al.*, 2014). Li *et al.* (2014) also suggest that the lack of diversity in melanosome shapes of non-maniraptoran archosaurs actually calls into question the attribution of brown colour to filamentous integuments by QDA (Li *et al.*, 2010, 2012) simply on the basis of spherical/sub-spherical melanosomes. This limited melanosome diversity might be viewed as analogous to the limited diversity seen in modern day ratites (Eliason *et al.*, 2016), although characterising ratites as having low metabolic rates as per the metabolic hypothesis is likely inappropriate when viewed within the broader archosaur phylogeny, and because their flightlessness may have limited colour/melanosome diversity due to predation pressures. However, this metabolic hypothesis of low melanosome morphology (Eliason *et al.*, 2016; Eliason & Clarke, 2018) is difficult to test in extinct animals, and it would be surprising if pterosaurs capable of powered flight had low metabolic rates. Additionally, non-maniraptoran archosaurs, particularly those with a filamentous integument, are under-represented compared to maniraptorans in palaeocolour studies, and so their limited melanosome diversity may be influenced by small sample size or terrestrial lifestyles with high predation risk. If melanosome diversity is instead increased to compensate for a lack of alternative colour-producing mechanisms in dead filamentous tissue as compared to metabolically active skin, then the fact that melanosome diversity is high in more endothermic animals might represent correlation rather than causation since animals with filamentous integument tend towards elevated metabolisms, at least in extant species. Therefore, the shift of colour production from skin to filaments remains the main alternative hypothesis (Vinther, 2015) to the pleiotropic metabolic hypothesis (Li *et al.*, 2014) for the evolutionary drivers of increased melanosome diversity.

Methods predicting palaeocolour using melanosome characteristics might need to account for diagenetic effects on melanosome morphology and chemistry. McNamara *et al.* (2013*a*) conducted thermal maturation experiments on feathers, however their study erroneously reported their methodology. Colleary *et al.* (2015) subjected feathers of different colours to a pressure of 250 bars and temperature regimes of 200°C and 250°C and yielded comparable and quantifiable results. With increasing maturation conditions, melanosomes shrank by no more than 10% in the experiments of Colleary *et al.* (2015) due to volatile loss and dehydration with potentially minimal effects on aspect ratio. McNamara *et al.* (2013*a*) reported as much as 20% shrinkage, but their experimental duration was only 1 h and not 24 h as originally reported (McNamara *et al.*, 2017). Additionally, the chemical signature of the samples of Colleary *et al.* (2015) as determined by time of flight–secondary ion mass spectrometry (TOF-SIMS) approached that of fossil melanin samples with increasing maturation conditions and remained distinct from non-melanin controls. Aqueous maturation conditions, however, can completely obliterate melanosome morphology (possibly those with high phaeomelanin concentration in particular), even though the amorphous pigments might still remain, similar to that seen in some fossils (Colleary *et al.*, 2015). Although fossil melanin is chemically altered from the original molecule (Glass *et al.*, 2013; Colleary *et al.*, 2015) and can also diagenetically incorporate sulphur (McNamara *et al.*, 2016*a*), melanin is still considered to be relatively stable through diagenesis (Briggs & Summons,

2014), and is therefore capable of being fossilised due to its innate thermal stability. A novel method of sediment-encased maturation of feathers and lizards (Saitta *et al.*, 2018*a*) has supported the hypothesis that melanosomes remain exposed on the sediment while surrounding keratin protein is lost during diagenesis (Saitta *et al.*, 2017, 2018*d*). The method promises a means to test the efficacy of palaeocolour reconstruction. Predictions about the original colour of the feather based on methods used to study fossil feathers could be validated against the known colour of the experimental feather prior to its maturation. It might also be used to determine what chemical signatures would be expected from non-melanin pigments in fossils and if these pigments can leave behind any macro- or microscopic staining or textures. One weakness of the current method, however, is that due to a lack of concurrent compaction during thermal maturation, melanosome organisation is lost, hindering comparisons to fossil feathers with reported structural colouration based on preserved melanosome arrangement (Saitta *et al.*, 2018*d*).

Palaeocolour reconstructions (see Table 2 for a list of fossil specimens for which colours have been reconstructed, and Fig. 5 for illustrations of key taxa) have been used to infer aspects of extinct organisms' ecology or behaviour, such as sexual displays and camouflage. Iridescent plumage in *Microraptor* (Li *et al.*, 2012) and *Caihong* (Hu *et al.*, 2018), rufous head feathers and spangled remiges and coverts *of Anchiornis* (Li *et al.*, 2010), striped retrices in *Caudipteryx* (Qiang *et al.*, 1998), melanised rectrices in *Jeholornis* (O'Connor *et al.*, 2013), elongate ribbon-like rectrices in *Epidexipteryx* (Zhang *et al.*, 2008), and possibly sexually dimorphic streamer-like retrices, spotted wings, and head feathers in *Confuciusornis* (Zhang *et al.*, 2010; Li *et al.*, 2018), among others, might be consistent with the presence of sexual selection and visual displays, at least among non-avialan maniraptoran dinosaurs and early birds (Clarke, 2013).

Countershading is a form of camouflage in which an animal's body is lighter in the ventral regions and darker in the dorsal regions (Stevens & Merilaita, 2009; Penacchio *et al.*, 2015). This pigmentation pattern counteracts the shadow the body casts when it is illuminated from above to reduce three-dimensional appearance to a relatively two-dimensional flattened appearance. This makes the animal more difficult to recognise against its background. Countershading is widespread among animals and is consistent with the ventrally non-pigmented abdomen in *Psittacosaurus* (Vinther *et al.*, 2016), *Borealopelta* (Brown *et al.*, 2017), and *Sinosauropteryx* (Smithwick *et al.*, 2017). Additionally, a bandit mask, common in modern diurnal birds and mammals possibly to reduce glare in open environments and mask the presence of eyes, has been reported in *Sinosauropteryx* (Smithwick *et al.*, 2017). One type of analysis of countershading in fossils involves the creation of a uniformly grey three-dimensional volume-rendering model, considering soft tissues such as musculature, that is photographed in different natural and laboratory light conditions. The negative images of the illuminated model are then matched against the carbonaceous stains of macroscale pigmentation patterns in the fossil to determine which lighting conditions most closely estimate the countershading pattern of the animal. Animals in open, well-illuminated habitats normally show sharp and more dorsally situated boundaries between dark and light colours, whereas smoother and ventrally situated boundaries are common among animals in diffusely lit habitats. Such analysis of countershading patterns enables ecological hypotheses for extinct taxa to be proposed: closed, shaded environments for *Psittacosaurus* (Vinther *et al.*, 2016) *versus* open, illuminated environments for *Sinosauropteryx* (Smithwick *et al.*, 2017). Predator–prey interactions have also been predicted by comparing the relationships between body size and extent of countershading in modern animals with those of fossils in order to predict predation pressure (Brown *et al.*, 2017). Once predation pressure is reduced due to the evolution and development of large body size, countershading can be lost, allowing for comparisons of predation pressure on large-bodied animals across time. However, it must be kept in mind that fully articulated fossils with well-preserved pigment preservation capable of being reasonably modelled in three dimensions are the exception rather than the norm.

VI. DISCUSSION

Under the framework presented herein (Fig. 6), the fossil specimen is first identified and placed within a phylogenetic framework, followed by macroscopic examination of carbonaceous stains and visible colour patterns in the integument (e.g. stripes, spots, splotches, spangles, etc.) in both the part and counterpart, if present. Since, the macrostructure of feathers (pennaceous *versus* plumulaceous vane, branching patterns, shapes of barbs and barbules) can be critical to colour production, particularly structural colour, a range of imaging is recommended to visualise their preserved morphology as far as possible. For example, UV imaging can clarify integumentary details and sometimes reveal hidden information (Frey *et al.*, 2003; Tischlinger, 2005; Chiappe & Göhlich, 2010; Hone *et al.*, 2010; Kellner *et al.*, 2010; Rauhut *et al.*, 2012; Tischlinger & Arratia, 2013; Foth, Tischlinger & Rauhut, 2014). Laser stimulated fluorescence (LSF) is a next-generation fluorescent imaging method that offers substantial benefits in these areas (Mayr *et al.*, 2002; Kaye *et al.*, 2015, 2019*a*,*b*; Falk *et al.*, 2016; Vinther *et al.*, 2016; Wang *et al.*, 2017; Yang *et al.*, 2019). The utility of these fluorescent imaging methods appears to stem from the preservation of soft tissues like patagial membranes, skin, scales, or keratinous sheaths *via* endogenous phosphates (Murphy *et al.*, 2006; Christiansen & Tschopp, 2010). Although fossil melanin does not fluoresce, fluorescing compounds in the sediment matrix can often backlight the fossil to reveal the morphology of organically preserved integument (Kaye *et al.*, 2015; Wang *et al.*, 2017). For feathers, unless the rachis/calamus is calcified, then it is likely that it will not fluoresce and will have to be visualised through backlighting. LSF can also help identify

Table 2. Predictions of palaeocolour in fossil amniotes with gaps in current knowledge

Specimens	Colour patterns predicted	Gaps in knowledge
THEROPOD DINOSAURS		
Inkayacu (Clarke *et al.*, 2010)	Secondary remiges and an isolated body contour feather were brown, whereas preserved covert and tertials were grey.	Remaining body colour unknown. No chemical data available.
Eocypselus (Ksepka *et al.*, 2013)	Feathers on the top of the head were glossy black.	Melanosomes were also found in the wing feathers but have not been analysed or interpreted as any specific colour. No chemical data available.
Messelornis (Colleary *et al.*, 2015)	A single feather was found to be iridescent.	Unclear exactly where the sample was taken but appears to have been a wing feather. No chemical data available.
Primotrogon (Nordén *et al.*, 2018)	Black, iridescent, and grey colours detected.	Incomplete preservation. Differentiating between covert and body feathers is difficult on both specimens. No chemical data available.
Eocoracias (Babarović *et al.*, 2019)	First instance of a fossil bird showing non-iridescent structural blue colour.	Colour reconstructed using ancestral state reconstruction of non-iridescent structural blue in Coraciidae. However, the morphology of melanosomes producing this colour overlaps significantly with grey colour producing ones.
Scaniacypselsus (Nordén *et al.*, 2018)	Black and grey wings with grey body.	Reconstruction based on combined data from three different individuals. Incomplete preservation in all three fossils leaves gaps in reconstruction. In two sampling points, statistical predictions offer equal probability for black/iridescent and grey/brown colours. No chemical data available.
Changzuiornis (Huang *et al.*, 2016)	Preserved wing and tail feathers were black.	Remaining body colour unknown. No chemical data available.
Unnamed enantiornithine CUGB P1202 (Peteya *et al.*, 2017)	Feathers of nape, head, and body were iridescent.	Melanosomes were also found in a wing feather but had degraded too much to determine colour based on morphology. No chemical data available.
Confuciusornis (Li *et al.*, 2018; Zheng, 2009)	Contour feathers were black all over the body with lighter wings. One specimen (CUGB P1401) had small spots on the wings, coverts, crest, and throat. Cryptic colouration in wing coverts, crest, and throat.	Sexual dimorphism in pair of long tail feathers unclear. No chemical data available.
Eoconfuciusornis (Zheng *et al.*, 2017)	Feathers making up the wing coverts, nape, and tail were black. A dark spotted pattern is visible on the secondary remiges. Feathers on the hindlimb and top of the head were grey. Feathers on the throat were brown.	Colour not predicted beyond identifying melanosome morphology. No chemical data available.
Archaeopteryx (Carney *et al.*, 2012)	The isolated holotype feather (possibly a covert wing feather) was black.	Remaining body colour unknown. Uncertain if feather belongs to *Archaeopteryx* (Kaye, Pittman, Mayr *et al.*, 2019*b*).
Anchiornis (Li *et al.*, 2010)	Body contour feathers were dark grey. Forelimb and hindlimb coverts and remiges were white with black tips. Feathers on the top of the head were reddish brown. Flecks of reddish brown were also present on the face.	Colouration of tail feathers unknown, as the specimen examined did not preserve a tail, but other specimens' tails show similar spotting as in the wings. No chemical data available.
Caihong (Hu *et al.*, 2018)	Iridescent feathers as in hummingbirds.	Precise hue created by light scattering from the platelet-like melanosomes cannot be reconstructed because it is determined by the spacing of the photonic nanostructures *in vivo* as well as the distribution of keratin.
Microraptor (Li *et al.*, 2012)	Feathers sampled across the body were iridescent. Most conservative possibility is that they were glossy black.	
Sinornithosaurus (Zhang et al., 2010)	Rod and spherical melanosomes detected in different samples, interpreted as black and rufous feather colouration.	Feathers are from pieces of the counterpart with uncertain location. No chemical data available.

Table 2. *(Cont.)*

Specimens	Colour patterns predicted	Gaps in knowledge
Caudipteryx (Zhang *et al*., 2010; Li *et al*., 2014)	Feathers sampled across the body were black. Tail feathers preserve a visible banding pattern.	Colour not predicted beyond identifying melanosome morphology. No chemical data available.
Yi (Xu *et al*., 2015)	Feathers near the skull contain both oblong (as do feathers near neck, tibia and ulna) and oblate (as do membranous tissue) melanosomes.	Colour not predicted beyond identifying melanosome morphology. No chemical data available.
Beipiaosaurus (Li *et al*., 2014)	Feathers sampled from the neck were brown.	Remaining body colour unknown. No chemical data available.
Sinosauropteryx (Smithwick *et al*., 2010)	Feathers sampled from the tail were reddish brown with intervening non-melanised bands. More detailed examination of preserved feather distribution across several specimens suggests a counter shaded pattern on the body and a "bandit mask" on the face.	Reconstruction based on combined data from different individuals of different sizes. No chemical data analysed for a taxon that is suggested by some (Eliason, Shawkey & Clarke, 2016; Li *et al*., 2014) to lack the correlation between melanosome morphology and colour.
Ornithischian Dinosaurs		
Psittacosaurus (Vinther *et al*., 2016)	Scales sampled across the body were melanised. The face, ankle, ischial region, cloacal region, and some large scales on the shoulder were particularly heavily pigmented. Countershading present.	Colour reconstruction purely based on melanosome morphology. No chemical data available.
Borealopelta (Brown *et al*., 2017)	No melanosomes with preserved structure were found, but chemical signatures suggest high concentrations of phaeomelanin based on benzothiazole detected from TOF-SIMS interpreted to give a reddish-brown colouration. Countershading present.	No melanosomes preserved, so the colour predictions are based purely on chemistry.
Marine Reptiles		
Stenopterygius (Lindgren *et al*., 2018)	Abundant branched melanophores and melanosomes across the flank but conspicuously absent in belly - evidence of dark dorsum and a light ventrum (i.e., countershading).	Skeleton and soft tissue preservation not complete. Parts of snout and regions beyond the pelvis missing. Incomplete model for melanophore preservation mechanism.
Platecarpus (Lindgren *et al*., 2010)	Melanosomes preserved in the eye. Macroscopic colour patterns in preserved scales.	Colour not reconstructed.
Pterosaurs		
Pterorhynchus (Czerkas & Ji, 2002)	Macroscopic colour patterns noted in the head crest.	Colour not reconstructed.
Tupandactylus (Pinheiro *et al*., 2012)	Swaths of melanosomes preserved in large head crest misinterpreted as fossilised microbial consortia.	Colour not reconstructed.
Anurognathid pterosaurs NJU-57003, CAGS-Z070 (Yang *et al*., 2019)	Most likely covered in pycnofibres with various degrees of branching. Melanosomes of diverse morphologies reported.	Brown and black colour in filaments. Colour patterns not reconstructed.
Mammals		
Palaeochiropteryx (Colleary *et al*., 2015)	Phaeomelanin signature identified using TOF-SIMS. Spherical to oblate melanosomes observed in hair filaments. Brown pelage.	Entire body colour not reconstructed.
Hassianycteris (Colleary *et al*., 2015)	Phaeomelanin signature identified using TOF-SIMS. Spherical to oblate melanosomes observed in hair filaments. Brown pelage.	Entire body colour not reconstructed.
Apodemus (Manning *et al*., 2019)	Phaeomelanin pigment remnants reportedly mapped using Cu^{2+}/Zn^{2+} ions bound to organosulphur residues through a combination of SRS-XRF and XAS. Brown pelage.	The use of chelating metal ions is not a reliable method of colour reconstruction.

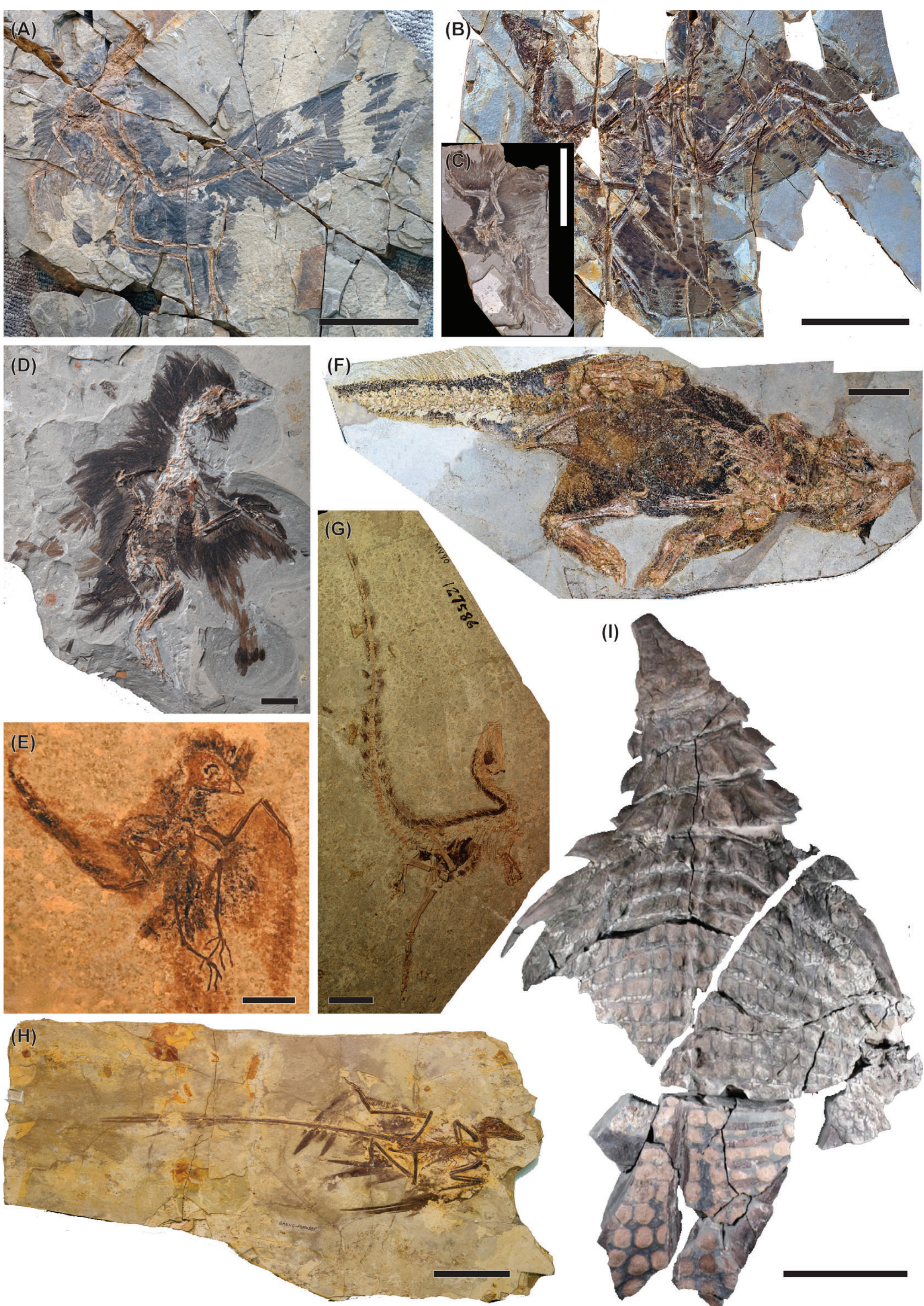

Fig. 5. Key fossil taxa used for palaeocolour reconstruction: (A) *Caihong juji* (Hu *et al.*, 2018), (B) a pristine specimen of *Anchiornis huxleyi* showing macroscale colour patterns (image credit: Xiaoli Wang), (C) specimen of *Anchiornis* used for prediction of plumage colour (Li *et al.*, 2010), (D) *Eoconfuciusornis zhengi* (O'Connor & Claessens, 2005; Pan *et al.*, 2016), (E) *Psittacosaurus* (Vinther *et al.*, 2016), (F) *Eocypselus rowei* (Ksepka *et al.*, 2013), (G) *Sinosauropteryx prima* (Zhang *et al.*, 2010; Smithwick *et al.*, 2017), (H) *Microraptor gui* (Li *et al.*, 2012), and (I) *Borealopelta markmitchelli* (Brown *et al.*, 2017). Scale bars: A, 10 cm; B, 10 cm; C, 10 cm; D, 20 mm; E, 20 mm; F, 10 cm; G, 50 cm; H, 10 cm; I, 0.5 m.

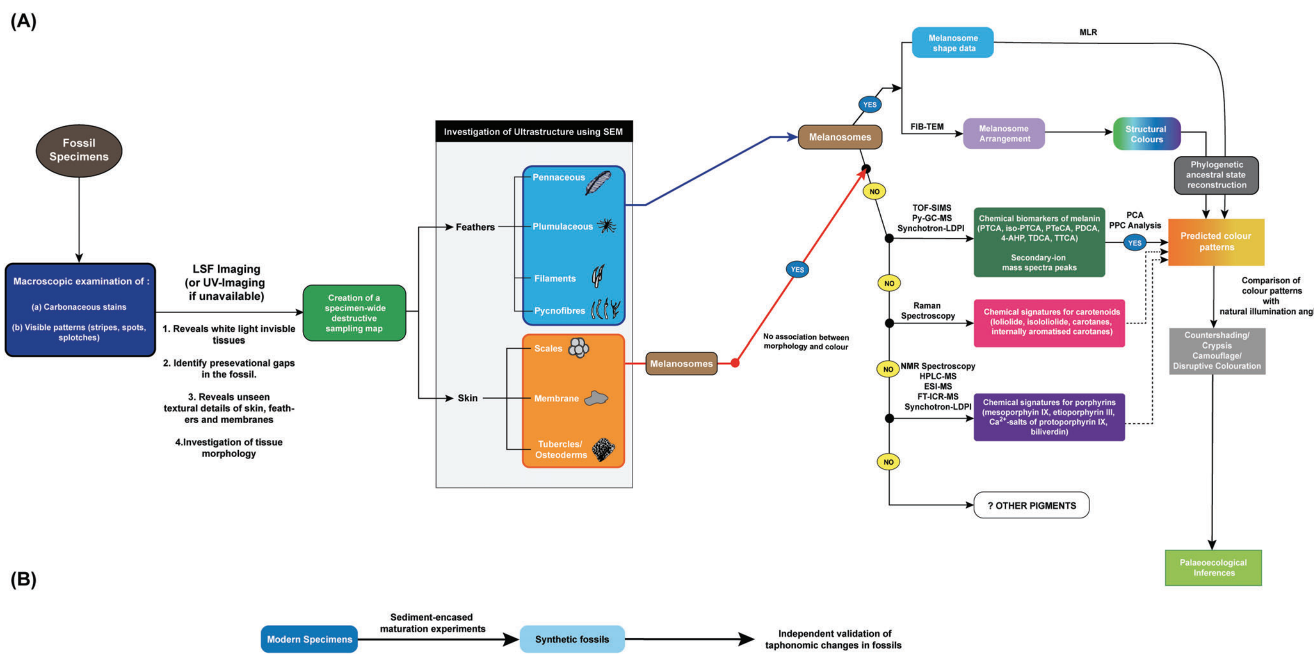

Fig. 6. (A) Proposed holistic schematic framework for the reconstruction of fossil colour. Solid lines indicate confirmed steps; dashed lines indicate potentially useful, but yet untested, steps in palaeocolour reconstruction. (B) Sediment-encased maturation can be used to experimentally validate taphonomic changes in fossils. ESI-MS, electrospray ionisation mass spectrometry; FIB-TEM, focused ion beam-transmission electron microscopy; FT-ICR-MS, Fourier-transform ion cyclotron resonance mass spectrometry; HPLC-MS, high performance liquid chromatography; LSF, laser stimulated fluorescence; MLR, multinomial logistic regression; NMR, nuclear magnetic resonance; PCA, principal components analysis; PPC, peak probability contrast; Py-GC-MS, pyrolysis-gas chromatography-mass spectroscopy; synchotron-LDPI, laser desorption-ionisation; TDCA, thiazole-4,5-dicarboxylic acid; TOF-SIMS, time of flight secondary-ion mass spectroscopy; UV, ultra-violet.

preservational gaps within the fossil, to provide an estimate for the loss of information due to partial decay and disarticulation (e.g. *Anchiornis*: BMNHC PH828 and *Yi*: STM 31-2), as well as any artificial reconstructions added to the fossil (Mateus, Overbeeke & Rita, 2008; Kaye *et al.*, 2015). While several types of pigment biomolecules (e.g. porphyrins) can fluoresce, their preservation potential is either uncertain or their biomarkers cannot be reliably linked to their original sources and distinguished from environmental sources.

Once both white-light visible and white-light invisible tissues have been identified, the next step would ideally be to create a detailed map that encompasses all identified integumentary tissue types and macroscopic colour patterns for destructive sampling. While destructive sampling is carried out by flaking off small regions of organic stains, care must be taken to acquire the least material that is necessary. Following this, samples can be coated with gold/silver for ultrastructural analysis using scanning electron microscopy (SEM) to determine melanosome morphology and organisation. Additionally, if the fossil specimen is small enough to be placed into the SEM chamber, it can imaged uncoated under variable pressure conditions (VP-SEM) (Vinther *et al.*, 2008; Zhang *et al.*, 2010). If melanosome morphology is preserved (i.e. not taphonomically altered into amorphous fossil melanin) and appears minimally undistorted by diagenesis as evidenced, for example, by organic melanosomes residing inside mouldic impressions with minimal shrinkage, then the melanosome shape data can be compared to that of modern species using QDA or MLR (Nordén *et al.*, 2018). If melanosomes seem to be arranged in ordered nanostructural arrays, then sections perpendicular to barb/barbule long axes made using focused ion beam-transmission electron microscopy (FIB-TEM) can at least partially reveal the nature of the repeating units in three-dimensional space (Hu *et al.*, 2018).

Even if the melanosome morphology is obliterated or rendered unusable by diagenesis, melanin chemistry can still be exploited to identify the diagenetically altered melanin and ascertain the type of melanin pigment. Different variants of mass spectroscopy techniques such as synchotron-LDPI (Liu *et al.*, 2014), TOF-SIMS (Colleary *et al.*, 2015), or peroxidation followed by pyrolysis-gas chromatography-mass spectroscopy (Py-GC-MS) (Glass *et al.*, 2012, 2013) can be used to detect chemical biomarkers of melanin. Melanin-based colours can be estimated through comparison with modern bird feathers through statistical methods such as peak probability contrast (PPC) (Liu *et al.*, 2014), which identifies the most important melanin peaks for identifying colour in a training database of modern birds and predicts the colour in fossil samples based on the presence/absence of those peaks. One point to note is that the morphology of melanosomes which produce non-iridescent structural colours is not readily distinguishable from those of grey-colour-imparting ones and this has important bearings

on palaeocolour reconstruction (Babarović *et al.*, 2019). In these cases, simple statistical classifications or chemical analysis may not be sufficient to pin-point exact colours in fossils. Phylogenetic ancestral state reconstruction has shown much promise in adequately distinguishing between grey and non-iridescent-colour-producing melanosomes in fossil birds (Babarović *et al.*, 2019).

Although pigments other than melanin have yet to be fully utilised in vertebrate palaeocolour reconstructions, we present some possible analytical techniques here. Carotenoid biomarkers (e.g. loliolide, isololiolide and carotanes) can be probed for by Raman spectroscopy. Porphyrin biomarkers (e.g. metallo-DPEP and metallo-BiCAP) can be detected with nuclear magnetic resonance (NMR) spectroscopy, HPLC-MS, electrospray ionisation mass spectrometry (ESI-MS), Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), or synchotron-LDPI (Mironov *et al.*, 2017; Zheng *et al.*, 2018). However, caution is urged in linking these biomarkers with carotenoids or porphyrins in carbonaceous fossils since they might be derived from exogenous, environmental sources.

A systematic understanding of the molecular changes that occur through pigment diagenesis will help to guide a search for chemical signatures in fossils, and so researchers attempting to reconstruct palaeocolour may wish to validate their conclusions under experimental frameworks. Experimental taphonomy, particularly thermal maturation, should help to identify chemical and morphological signatures of fossil melanin and other pigments and might lead to the possibility of using pigments other than melanin for palaeocolour reconstruction (Colleary *et al.*, 2015; Saitta *et al.*, 2017, 2018*d*).

VII. CONCLUSIONS

(1) There is currently no single formalised methodological framework that can be referenced for all cases of fossil colour reconstruction. Additionally, limitations on the preservation of biological tissues, sampling, and diagenesis should be accounted for. For example, information loss in fossilised feathers due to incomplete preservation of structural colour-imparting keratin protein arrays or uncertainties in the preservation/identification of other fossil pigments should be borne in mind as combined pigmentary and structural mechanisms may have produced colour blends in extinct taxa that are undetectable in fossils.

(2) The correlation between melanosome morphology and the chemistry of the melanin pigment within, and therefore the hue produced, does not persist in tissues other than feathers and hair (i.e. non-filamentous integument) (Vinther, 2015). Other workers have gone further by suggesting that the relationship does not persist in some non-maniraptoran dinosaurs based on the hypothesis that melanosome morphology is dependent on pleiotropic gene relationships with other metabolic processes (Kellner *et al.*, 2010; Li *et al.*, 2014), but this hypothesis is currently untestable in extinct organisms.

(3) Given that colour patterns, such as camouflage and countershading, are now being used to inform ecological aspects such as habitat preference and predator–prey interactions (Vinther *et al.*, 2016; Brown *et al.*, 2017; Smithwick *et al.*, 2017), it has become especially important to discuss thoroughly the nuances of palaeocolour reconstruction. Herein, we synthesised different methodologies to provide a thorough framework to recognise melanin and fossilisable structural colour-based patterns in fossils, especially among amniotes.

(4) Only once colour patterns have been reconstructed can predictions be made regarding the visual ecology and behaviour of ancient animals, such as signalling, camouflage, habitat preference, or predator–prey interactions. Strong caution has been advised by Negro, Finlayson & Galván (2018) in inferring colour patterns and predicting life-history traits in fossils. While some of their criticisms are quite valid, it should be kept in mind that there are numerous limitations commonly faced by palaeontologists, such as an incomplete fossil record, poor preservation, destructive sampling techniques, and the inability to conduct observational or experimental behavioural or ecological studies on extinct organisms, all of which will hinder palaeocolour reconstruction efforts. Although biases should be recognised and accounted for, these difficulties should not preclude attempts by researchers to conduct objective palaeobiological investigations to the best of their abilities, thus making the field of palaeocolour reconstruction an exciting and stimulating endeavour.

(5) This review provides a framework incorporating a comprehensive battery of tests informed by taphonomy that can serve to guide palaeocolour reconstruction, especially among amniotes.

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