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Habitat light sets the boundaries for the rapid evolution of cichlid fish vision, while sexual selection can tune it within those limits

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Abstract

Cichlid fishes' famous diversity in body coloration is accompanied by a highly diverse and complex visual system. Although cichlids possess an unusually high number of seven cone opsin genes, they express only a subset of these during their ontogeny, accounting for their astonishing interspecific variation in visual sensitivities. Much of this diversity is thought to have been shaped by natural selection as cichlids inhabit a variety of habitats with distinct light environments. Also, sexual selection might have contributed to the observed visual diversity, and sexual dimorphism in coloration potentially co-evolved with sexual dimorphism in opsin expression. We investigated sex-specific opsin expression of several cichlids from Africa and the Neotropics and collected and integrated data sets on sex-specific body coloration, species-specific visual sensitivities, lens transmission and habitat light properties for some of them. We comparatively analysed this wide range of molecular and ecological data, illustrating how integrative approaches can address specific questions on the factors and mechanisms driving diversification, and the evolution of cichlid vision in particular. We found that both sexes expressed opsins at the same levels—even in sexually dimorphic cichlid species—which argues against coevolution of sexual dichromatism and differences in sex-specific visual sensitivity. Rather, a combination of environmental light properties and body coloration shaped the diversity in spectral sensitivities among cichlids. We conclude that although cichlids are particularly colourful and diverse and often sexually dimorphic, it would appear that natural rather than sexual selection is a more powerful force driving visual diversity in this hyperdiverse lineage.

KEYWORDS

cichlid, co-evolution, cone, opsin expression, rod, run-away selection, sensory drive

Ralph F. Schneider and Sina J. Rometsch contributed equally.

1 | INTRODUCTION

Cichlid fishes are evolutionary model organisms for studying the process of speciation, mostly due to their exceptionally high diversity and ultra-fast rates of phenotypic divergence (Genner & Turner, 2005; Henning & Meyer, 2014; Kocher, 2004; Meyer, Kocher, Basasibwaki, & Wilson, 1990). Cichlids famously vary in morphological traits, such as body coloration and their trophic morphology, but also in behavioural traits, such as social systems and parental care (Meyer, 1993; Seehausen, 1997; Stiassny & Meyer, 1999; Wisenden et al., 2015). This remarkable diversity is thought to be the result of both natural and sexual selection (Fryer & Iles, 1972; Kocher, 2004; Seehausen, 2006; Selz, Thommen, Pierotti, Anaya-Rojas, & Seehausen, 2016; Wagner, Harmon, & Seehausen, 2012). While natural selection is widely believed to be the driving force behind the evolution of a wide range of foraging modes and associated morphologies (Keenleyside, 1991), sexual selection pushes the evolution of a plethora of body colorations (Allender, Seehausen, Knight, Turner, & Maclean, 2003; Deutsch, 1997). This colour diversity is found across species, but also at the intraspecific level, for example as sexual dimorphism: males show different and usually much brighter colours and higher colour intensities than females (Fryer & Iles, 1972; Seehausen, Mayhew, & Van Alphen, 1999). As males typically exhibit more conspicuous body coloration patterns, female preference was suggested to be the main driver of sexual dimorphism in cichlid fishes' body colorations (Seehausen, Van Alphen, & Witte, 1997).

A trait that is also particularly variable among cichlids is their visual system (Carleton, 2009; Carleton, Dalton, Escobar-Camacho, & Nandamuri, 2016), and this lineage has become a model system for the study of visual ecology and evolution. Cichlids have an unusually high number of opsin genes (seven cone opsins and one rod opsin) and also opsin expression patterns were found to be especially variable (different combination of typically three out of the seven cone opsins are expressed at a given time; Carleton, 2009; Carleton et al., 2016). Previous research suggested that the diversity in visual sensitivities, in large part due to variation in opsin gene expression patterns, reflects the diversity of habitats and thus light regimes cichlids inhabit (Maan, Hofker, van Alphen, & Seehausen, 2006; Seehausen et al., 2008; Smith, van Staaden, & Carleton, 2012). Light generally attenuates when transmitted through water and thus habitats are getting darker with increasing depth. In addition, clear water absorbs light of long wavelengths ("red" light) and very short wavelengths ("UV" light) particularly fast (Anthes, Theobald, Gerlach, Meadows, & Michiels, 2016; Lythgoe, 1988). Thus, with increasing depth the ambient light becomes spectrally narrower and short to middle-long light wavelengths dominate (blue-green light). Furthermore, algae and particles suspended in the water change these transmission properties (Cronin, Johnsen, Marshall, & Warrant, 2014). Finally, natural selection has been implied to affect the evolution of cichlid visual sensitivities by other factors besides the depths at which they live, such as the trophic niche a fish occupies (Carleton et al., 2016; Hofmann et al., 2009).

Abiotic factors, such as water depth and clarity, as well as other environmental properties that shape visual sensitivities can, however, also indirectly affect the evolution of body coloration through sensory biases (i.e., sensory drive; Figure 1a; Boughman, 2002; Ryan & Cummings, 2013). The sensory bias model assumes that signals of the sender evolve to be transmitted efficiently in the species' respective habitat and for being detected easily by the intended receiver due to sensory biases, such as environmentally-tuned sensitivities of the receiver (i.e., the mating partner) (Boughman, 2002; Endler, 1992; Endler & Basolo, 1998). This mechanism is assumed to have contributed to species divergence in Lake Victoria's *Pundamilia* cichlids (Seehausen et al., 2008). Populations of the common ancestor of two *Pundamilia* species are thought to have experienced disruptive selection on visual sensitivity caused by different light environments at different water depths, and in turn visual adaptation to these divergent light environments might have affected the evolution of nuptial coloration through sensory drive (Carleton, Parry, Bowmaker, Hunt, & Seehausen, 2005; Seehausen et al., 2008). Sensory biases may also result from innate preferences for environmental stimuli associated with fitness benefits. Selection is then predicted to further strengthen such preferences by improving visual sensitivity for the beneficial signal (Figure 1b). For example, the evolution of conspicuous colour patterns in Trinidadian guppies has been demonstrated to be driven by a sensory bias towards orange colours that initially facilitated the finding of prey (Rodd, Hughes, Grether, & Baril, 2002).

In addition to natural selection, sexual selection can potentially be a force affecting the evolution of vision (Arikawa, Wakakuwa, Qiu, Kurasawa, & Stavenga, 2005; Bloch, 2015; Briscoe et al., 2010), although less evidence supports a role of sexual compared to natural selection in driving the evolution of visual sensitivities (Kelber & Osorio, 2010). This can happen if sensory biases are projected on conspecifics, such as mates. Then, sexual selection could become the primary driver, predicted to exaggerate the preference for the signal and, potentially, simultaneously imposing selection on the linked visual sensitivity to improving mate evaluation through runaway processes. A co-evolutionary feedback-loop through mate choice can drive the evolution of increasingly exaggerated signals, which in turn might result in further evolution of preference and sensitivity for them (Bloch, 2015; Fisher, 1930). This mechanism might underlie some of the extreme diversity in body coloration in cichlid fishes (Figure 1c). Finally, dimorphism in any of the involved traits (e.g., signal, signal preference, and sensitivity for the signal) could evolve if such traits become coupled with sex, particularly when there is a division into a choosy (typically females) and a chosen (typically males) sex (Figure 1d; e.g., Kelber & Osorio, 2010; Bloch, 2015). Considering the diversity of body coloration and visual systems in cichlids, it is plausible that run-away selection has contributed to divergent visual sensitivities between sexes (Sabbah, Laria, Gray, & Hawryshyn, 2010).

Here, we investigate the relative strength of natural and sexual selection on cichlid opsin expression by integrating species-specific photic environmental data, spectral body coloration measurements

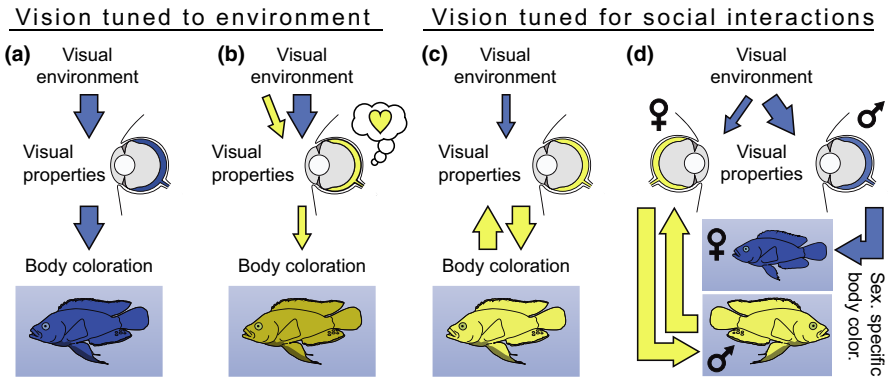

FIGURE 1 Evolutionary mechanisms potentially affecting the evolution of body coloration in cichlids. (a) The light environment of a cichlid is thought to be a main determinant of the fish's visual properties. According to the "sensory drive"-hypothesis, nuptial colour patterns evolved to match the environmental light as this optimizes visual perception by conspecifics. (b) Arbitrary preferences (e.g., "sensory biases") for beneficial environmental signals can be linked to improved visual sensitivities for such signals. If preferences are projected on conspecifics, sexual selection for body colours that deviate from the dominant environmental light regime can be the result. (c) For social interactions, mate-choice then replaces ecological selection as driver of preference and visual sensitivity. A co-evolutionary feedback-loop can evolve between continuously increasing preference and linked visual sensitivity, and increasingly exaggerated body coloration, as predicted by Fisher's runaway sexual selection-model. (d) An extreme outcome of this model may be found in species with a choosing sex and a chosen sex, as it is the case for many cichlids. Here, sex and the preference for specific nuptial body colorations may become evolutionarily uncoupled (e.g., by evolving sex chromosomes). Thus, while matching visual sensitivity for the mates' body coloration is particularly adaptive for choosing females, in males it is more adaptive to evolve their visual properties according to their nonsocial visual environment. In turn, males have to display (potentially costly) nuptial body colorations, while females can remain inconspicuous. Therefore, sexual dimorphism is expected to be found in body coloration as well as in visual properties

and opsin expression data from both sexes in sexually dimorphic and monomorphic species in terms of body coloration. We discuss whether sexual dimorphism in coloration is associated with differences in opsin expression among sexes, we evaluate the relative importance of the environmental light versus mate body coloration on cichlid visual properties, and we investigate whether sexually dimorphic species in terms of coloration use different opsin arrangements than sexually monomorphic species.

2 | MATERIALS AND METHODS

2.1 | Species selection and maintenance

The studied cichlid species included representatives of African (i.e., *Pundamilia nyererei*, *Labidochromis caeruleus*, *Pseudotropheus lombardoi*, *Melanochromis auratus* and *M. johannii*) and Neotropical cichlid radiations (*Hypsophrys nematopus*, *H. nicaraguensis*, *Apistogramma agassizii*, *A. cacatuoides*, *A. ortegai*, details on species habitat in Table 1; Figure S1). The selected species exhibit varying levels of sexual dimorphism in body coloration and/or brightness (Table 1). Furthermore, chosen cichlids occur in different habitats and different water depths (Table 1). Fish were either captivity-bred and raised under artificial light until sexual maturity, or wild caught (only *Apistogramma* species, but seven of 12 *A. ortegai* individuals were laboratory-bred). All individuals were kept for at least 2 weeks under artificial light in the laboratory (Figure S2). Two weeks were deemed a sufficiently long time period for plastic changes in adult visual system to be concluded (Härer, Karagic, Meyer, & Torres-Dowdall, 2019; Nandamuri, Yourick, & Carleton, 2017), as well as for male fish to establish territories in the acclimatization tanks and thus to develop characteristic nuptial colorations. Fish were euthanized by an overdose of tricaine methanesulfonate (MS222) in the early afternoon (from 14:00 to 16:00 hr). For each fish, both eyes were dissected and the lenses extracted. The lens-less eyes were transferred to RNAlater (Sigma) and stored at −20°C or directly processed (see below), whereas the lens was transferred to PBS for short-term storage (<1 hr). Fish were euthanized under University of Konstanz permit (T16/13TFA).

2.2 | Determination of opsin expression using quantitative PCR

Cichlids have a total of seven unique cone opsin genes, each with a characteristic sensitivity peak (Carleton, 2009). Nonetheless, only three of those opsins are usually expressed in cichlids at any time, resulting in a functionally trichromatic visual system (Carleton & Kocher, 2001; Fernald, 1981, 1984; Hofmann et al., 2009; Levine & MacNichol, 1979). To determine which subset of opsins were expressed differentially by sex, RNA was extracted from between 12 to 17 individuals per species (sample size per sex per species in Figure 2) whole lens-less eyes using an RNeasyMini Kit (Qiagen), including the optional on column gDNA digestion step, and concentration and purity were determined using a Colibri microvolume spectrometer (Titertek Berthold). Equal amounts of extracted RNA (750 ng) were used for first-strand cDNA synthesis (GoScript Reverse Transcription System; Promega). Primer pairs for all opsin genes were designed

TABLE 1 Details on the examined species' habitats and ecology

Species	Conti-nent	Specific origin	Water clarity[a]	Habitat depth[a]	Adult food	Depth in analysis	Body colour sexual dimorphism?
H. nematopus	Neotropics	Nicara-gua and Costa Rica	Depending on specific habitat, relatively clear to very murky (Great Nicaraguan Lakes)	1–5 m	Aufwuchs	NA	Only brightness
H. nicaraguensis				1–5 m	Insects, detritus, leaves		Colour
A. agassizii		Ama-zonas drainage rivers	Black/clear/white water	<1 m	Small arthropods, leaf debris, algae		Colour
A. ortegai			Very clear	<0.5 m			Colour
A. cacatuoides			Clear/white water				Colour
P. nyererei	Afrotropics	Lake Victoria	Murky, brownish	3–8 m	zooplancton	5 m	Colour and brightness
L. caeruleus		Lake Malawi	Clear	10–30 m, mostly 25 m	Algae, Aufwuchs	25 m	Only brightness
P. lombardoi				6–30 m, mostly 10 m		10 m	Colour and brightness
M. auratus				3–8 m		6 m	(Colour and) brightness
M. johannii				3–8 m		6 m	Colour and brightness

[a]References: Britzke et al. (2014), Froese and Pauly (2014), Konings (1990), Kullander (1986), Maan et al. (2006), Rodrigues, Zuanon, Del-Claro, and Carvalho (2012), Römer (1998), Römer and Hahn (2008), Witte and Van Oijen (1990).

using NCBI PRIMER-BLAST (Ye et al., 2012) and reference sequences retrieved from GenBank (*rh2a* primers were designed to amplify both *rh2aα* and *rh2aβ*; details, primer sequences and reference taxa for primer design, see Table S1). Targeted regions always included intronic regions (except for *rh1*, as this gene has no introns) to avoid unintended amplification of the gDNA sequence. Primer pairs were tested for each species separately and an efficiency value was calculated for each opsin and species combination (following Carleton & Kocher, 2001). Quantitative PCR (qPCR) conditions were adjusted for each primer pair, resulting in efficiency values between 0.79 and 1.1, with those genes that showed considerable expression typically with primer efficiencies closest to 1 (Table S2). qPCR was performed on a CFX96 Real-Time System (Bio-Rad; using a GoTaq qPCR mix, Promega). Melt curves were performed after each qPCR run, which confirmed that all amplified product was of a similar size, suggesting that there was no unspecific amplification. To test for gDNA contamination in our RNA due to a potentially insufficient DNA digestion during RNA extraction, noRT-qPCRs were run for 20 samples using *rh1* primers, as these do not cover an intronic region and are expected to amplify gDNA. The mean Cq differences between cDNA and RNA samples was 15, while the lowest, i.e., the "worst", was 6.6 (i.e., ~1% of signal was due to gDNA). For the opsin genes that were not expressed at a significant level, no reliable efficiency could be determined (we assume an efficiency of 1.0 for downstream analyses; Table S2), which is in accordance with previously published data (e.g., Escobar-Camacho, Ramos, Martins, & Carleton, 2016).

Opsin gene expression was then calculated following Fuller, Carleton, Fadool, Spady, and Travis (2004), Carleton and Kocher (2001) and Yourick et al. (2019):

$$T_{i,j} = 1/\left(\left(1+E_j\right)^{Ct_{i,j}}\right),$$

where $T_{i,j}$ is the absolute expression value of the opsin gene *j* from individual *i*. E_j is the species-specific efficiency of *j's* primer and $Ct_{i,j}$ is the critical cycle number obtained from individual *i* using opsin primer *j*. For all six cone opsin primer pairs, individual relative expression values were then calculated by dividing each absolute cone opsin expression value by the sum of all cone opsin expression values. Complementary, opsin gene expression standardization for single-cone opsins and double-cone opsins is provided but not considered in subsequent analyses (Figure S3) as a single visual sensitivity curve per species was computed across cone types (see e.g., Rennison, Owens, Heckman, Schluter, & Veen, 2016). For the rod opsin rhodopsin (*rh1*), its absolute expression was divided by the sum of all opsin absolute expression values. Thus, *rh1* expression was normalized to the level of overall opsin expression. We used this approach rather than normalizing by housekeeping genes, as we were not interested in daily variation in *rh1* expression (we sampled all fish between 14:00 and 16:00 hr, see above), but rather in the differences between sexes in expression patterns for which relative-to-opsin-expression appears to be a better approach (although not tested for *rh1;* Yourick et al., 2019). For each opsin separately, *t* tests (for unequal variances)

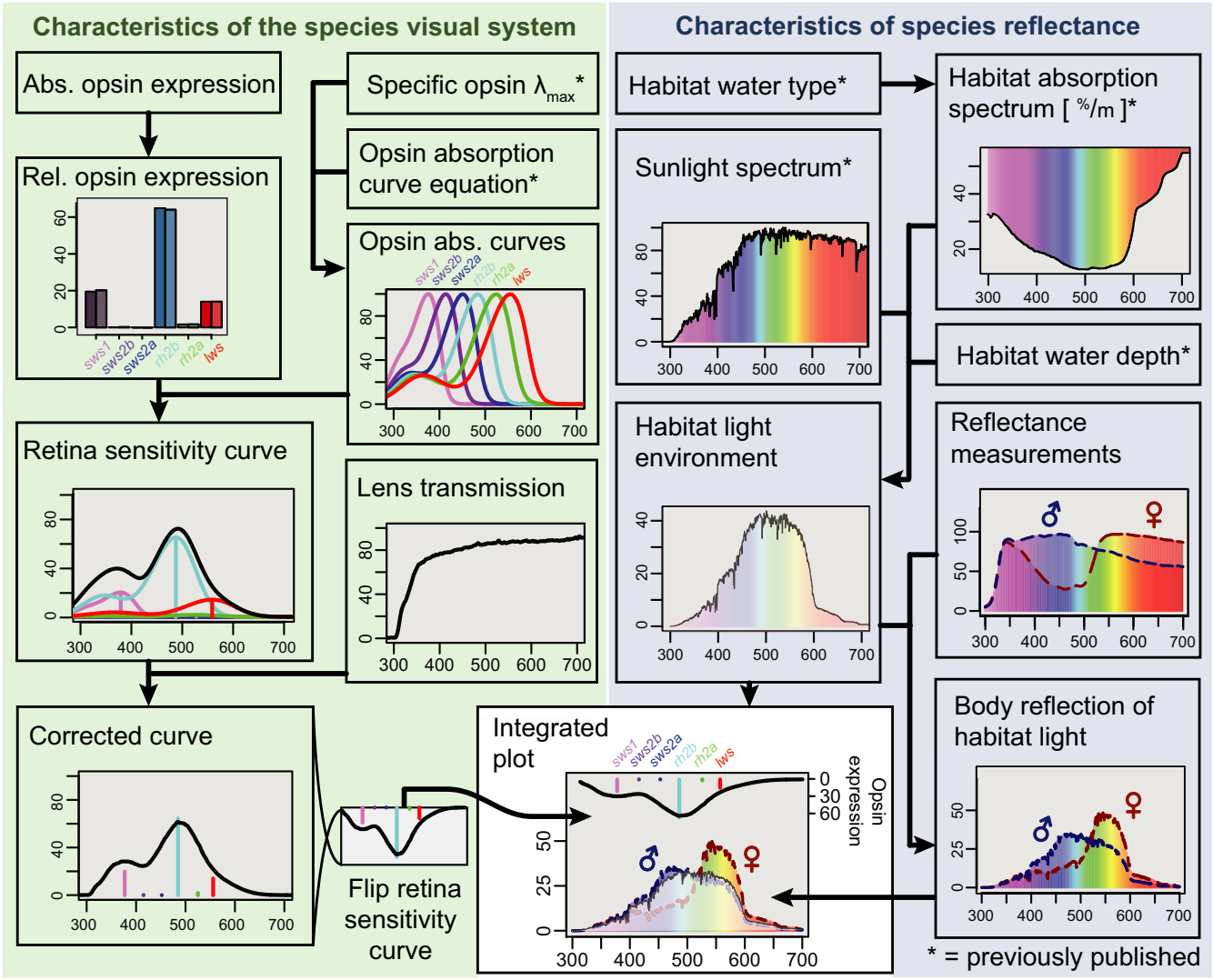

FIGURE 2 Relative opsin expression of male (darker columns) and female (lighter columns) individuals of the 10 investigated cichlid species. Mean relative expression levels of the six cone opsin genes and the rod opsin gene *rh1* were at the same level between sexes. In Neotropical cichlids (a–e) the same set of expressed opsins with some variation in relative expression levels were found while pronounced differences in relative *rh1* expression could be detected. In African Cichlid (f–j) opsin expression patterns were more variable, including *rh1*. Error bars reflect the standard error

were used to test for differences in opsin expression between sexes. The *p*-values were species-wise adjusted for multiple testing using the false-discovery-rate correction (Table S3). For *A. ortegai*, potential differences between wild-caught and laboratory-raised individuals were investigated using a Scheirer-Ray-Hare test (using opsin gene, rearing background and their interaction as independent variables) on relative opsin expression values (Table S4), followed by opsin-wise two-factorial Scheirer-Ray-Hare test (using rearing background, sex and their interaction as independent variables) to test if rearing background affected opsin expression in sexes differently (Table S5).

2.3 | Modelling African cichlids' visual sensitivity

To explore factors influencing cichlid vision, the properties of each species' visual apparatus (e.g., variations in lens transmittance and visual pigment sensitivity curves) were measured in more detail, as outlined below. In addition, the reflectance spectrum of each sex of each species in their respective habitat was modelled (see Figure 3 for an overview of the analysis pipeline). While we report the lens transmission for all investigated species, the remaining part of the analysis was restricted to the African cichlids because we found no reliable information on the visual environment of the studied South American species. Only few studies took those measurements (e.g., Escobar-Camacho, Pierotti, et al., 2019; Escobar-Camacho et al., 2016; Torres-Dowdall et al., 2017), but those are restricted to specific habitats. Additionally, due to dramatic changes in melanophores during anesthesia, South American cichlids' reflectance measurements were unreliable (see below).

Optical filters, such as the lens, can modify the incoming light, e.g., by filtering out potentially harmful light of very short wavelengths (UV-light; Douglas & Marshall, 1999) and therefore affect overall visual sensitivity. Light transmittance of cichlid lenses was determined by

measuring the lenses' relative light transmittance of a given broad-range light. First, the spectrum of the used Ocean Optics PX-2 pulsed xenon light source was determined by pointing the light source on a Spectralon diffuse white standard (Figure S4) and measuring the reflected light spectrum, which spanned a wavelength range of ~220–800 nm, with an Ocean Optics USB2000-UV-VIS-ES photospectrometer (operated with OCEAN OPTICS OCEANVIEW v.1.6.7 software). Relative light transmittance of each lens was determined within 1 hr after dissection. For this purpose, the lens was mounted between the light source and the measurement probe. Black tracing paper showing a circular whole with a diameter smaller than the lens' diameter was positioned directly behind the lens to guarantee that all measured light had passed through the lens. All measurements were conducted in a dark room with no other light source than the PX-2. Photon count was measured in 0.4 nm wavelength bins (initially from 320 to 800 nm). Per lens spectrum, the absolute transmittance was converted to relative transmittance by dividing its photon count per wavelength bin by the photon count of the respective bin of the light source (Figure S5):

$$t_{\mathrm{rel},\lambda} = \frac{t_{\mathrm{abs},\lambda}}{I_{\mathrm{whiteLight},\lambda}},$$

where $t_{\mathrm{rel},\lambda}$ is the relative transmittance for a given wavelength λ, $t_{\mathrm{abs},\lambda}$ is the absolute transmittance intensity measurement at λ, and $I_{\mathrm{whiteLight}\,\lambda}$ is the absolute intensity measurement of the light source at λ.

Also, we calculated the commonly used T50 values (the wavelength below which light of short wavelength is absorbed by the lens) using sigmoid fit curves fitted to each species' average lens transmission curve (Table S6, Figure S5; Hofmann, O'Quin, Marshall, & Carleton, 2010). Firstly, an average relative lens transmittance spectrum was computed for each species. To do so, all lens transmittance spectra had to be standardized for each species towards each other. This was achieved by approximating the integral of each transmittance spectrum from 300 to 700 nm by computing the sum of all transmittance values per wavelength from 300 to 700 nm, and the spectrum was then divided by this sum:

$$t_{\mathrm{st.rel},\lambda} = \frac{t_{\mathrm{rel},\lambda}}{\sum t_{\mathrm{rel}}},$$

where $t_{\mathrm{st.rel},\lambda}$ is the standardized relative transmittance for a given wavelength λ, $t_{\mathrm{rel},\lambda}$ is the relative reflectance intensity at the wavelength λ, and t_{rel} is the sum of the relative transmittance spectrum across all wavelengths.

After this procedure, per wavelength step, the mean of all transmittance spectra was calculated, which produced one average transmittance spectrum per species. Additionally, the standard error per wavelength was calculated for plotting (see Figure S2). For each of these mean spectra, we used R's nonlinear least squares function (*nls()*) to approximate the parameters of a sigmoid fit

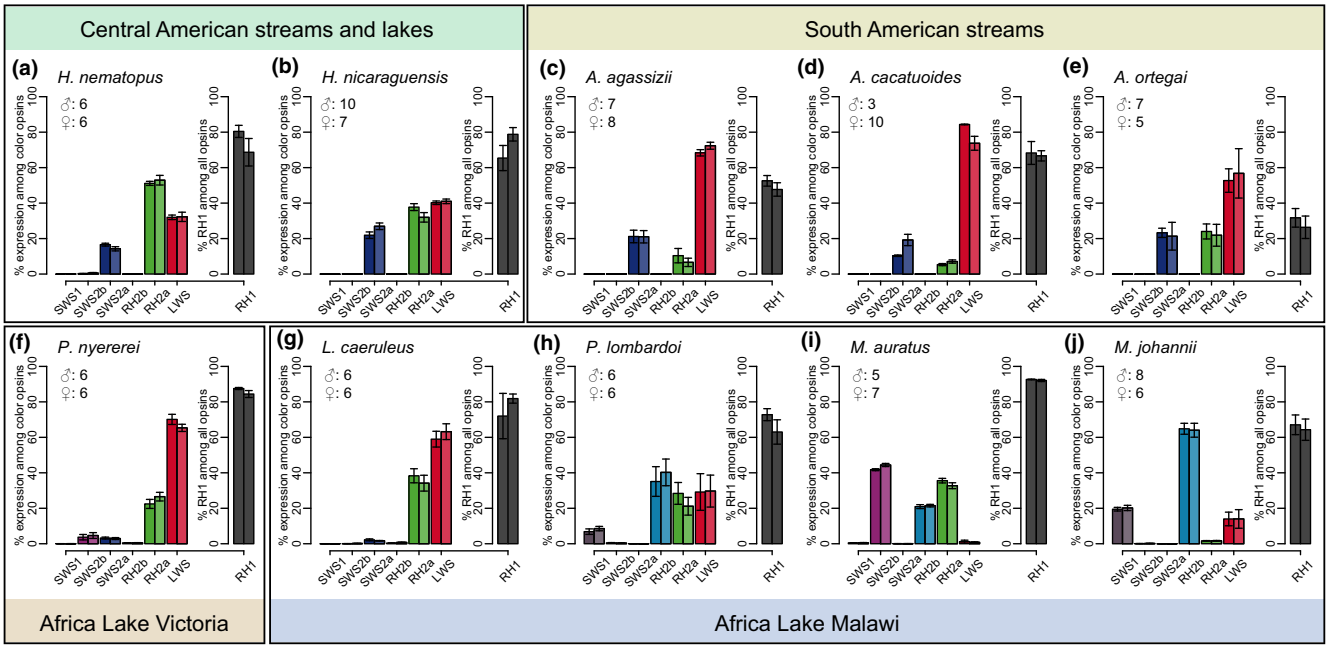

FIGURE 3 Pipeline to integrate opsin expression, body reflectance and natural habitat conditions. To characterize the visual system of investigated species, absolute opsin expression values were used to compute relative values. Opsin absorption peak wavelength and a general opsin absorption curve equation were used to calculate opsin absorption curves, which were weighted by the species-specific opsin expression profile and the transmission properties of the lens. For each species, the natural light environment was estimated by integrating a spectrum of the sunlight, the habitat water's specific absorption properties (taken from previously published literature) and the depth of the fish's natural habitat. The reflectance of each species and sex was characterized by measuring its relative reflectance, multiplying it with its habitat light spectrum and standardization. Visual capacities and reflective properties were then integrated for each species and measured spot in a common plot, showing the average modelled reflectance spectra for both sexes (left axis) and the weighted retina sensitivity curve and relative opsin expression levels upside down (right axis)

TABLE 2 Wavelengths at which the integrals of the (a) lens-corrected weighted retina sensitivity curves, (b) habitat light spectra, and (c) male and female reflectance spectra in habitat illumination per species (for most chromatically conspicuous spot, see Table 3) were divided into integral quarters and the wavelength difference between the first and third quarter borders. This difference was used to reflect the overall width of the sensitivity curves

		Integral cutoff (nm)			
		λP_{25}	λP_{50}	λP_{75}	Width (nm) = $\Delta\lambda$
(a) Visual sensitivity					
P. nyererei		502	544	580	78
L. caeruleus		500	541	577	77
P. lombardoi		455	507	554	99
M. auratus		424	469	517	93
M. johannii		419	476	515	96
(b) Habitat light					
P. nyererei		516	548	578	62
L. caeruleus		458	488	520	62
P. lombardoi		446	487	527	81
M. auratus		442	488	534	92
M. johannii		442	488	534	92
(c) Body reflectance					
P. nyererei	♂	558	584	618	60
	♀	537	568	599	62
L. caeruleus	♂	498	532	554	56
	♀	509	536	556	47
P. lombardoi	♂	470	518	555	85
	♀	455	489	528	73
M. auratus	♂	463	517	561	98
	♀	535	559	581	46
M. johannii	♂	443	478	525	82
	♀	496	542	571	75

curve: *nls(y_transmittance ~ a/(1 + exp(−b * (x_wavelengths − c))), start = list(a = 0.8, b = 0.5, c = 250))*. The T-50 value corresponds to the resulting "*c*" parameter in aforementioned equation.

To model visual sensitivity in more detail, the visual pigment sensitivity peaks (λ_{max} values) for each cone opsin were obtained from the literature (the "default" set; Table S6). In some cases, these default λ_{max} were adjusted when species-specific λ_{max} or those of closely related species were available (see Table S6 for details and references, and lens T50 values). Standardized cone opsin absorption curves were calculated according to Govardovskii, Fyhrquist, Reuter, Kuzmin, and Donner (2000) considering the species-specific λ_{max} of each opsin. Subsequently, these were weighted according to the respective opsins' relative expression levels, as done in Hofmann et al. (2009). Retina sensitivity curves were then calculated by adding up the integrals of all cone opsin absorption curves (as was done in Rennison et al., 2016). Finally, the sensitivity curves of each species were corrected for the

TABLE 3 Divergence between habitat and body colour spectra after standardization in % ("chromatic conspicuousness"; i.e., small values indicate that reflectance spectra approximate the ambient light spectrum while large values indicate a divergent spectral composition) for males (MC) and females (FC), and the percentage to which this divergence differs between sexes after additional standardization (SCD; i.e., small values suggest that sexes differ from the habitat at similar wavelength ranges, while large values suggest that these wavelength ranges differ and fish are sexually dichromatic). Discussions in the manuscript were focused on measurement spots with the highest chromatic conspicuousness averaged between sexes per species ([MC + FC]/2; shaded here in grey; see arrowheads Figure 4)

	Cheek			Back			Belly			Anal fin			Egg spots	Other		
	MC	FC	SCD	MC	FC	SCD	MC	FC	SCD	MC	FC	SCD	MC	MC	FC	SCD
P.ny	3	5	32	31	4	43	5	3	75	11	9	40	10	-	-	-
L.ca	12	27	16	35	43	4	35	35	21	3	27	95	12	37	38	7
P.lo	19	21	97	17	20	100	22	16	93	14	16	92	35	-	-	-
M.au	9	27	44	6	7	68	14	38	15	15	58	20	49	7	7	59
M.jo	25	31	94	8	40	32	11	36	97	23	35	92	21	29	39	97

Abbreviations: *L.ca*, *L. caeruleus*; *M.au*, *M. auratus*; *Mjo*, *M. johannii*; *P.lo*, *P. lombardoi*; *P.ny*, *P. nyererei*.

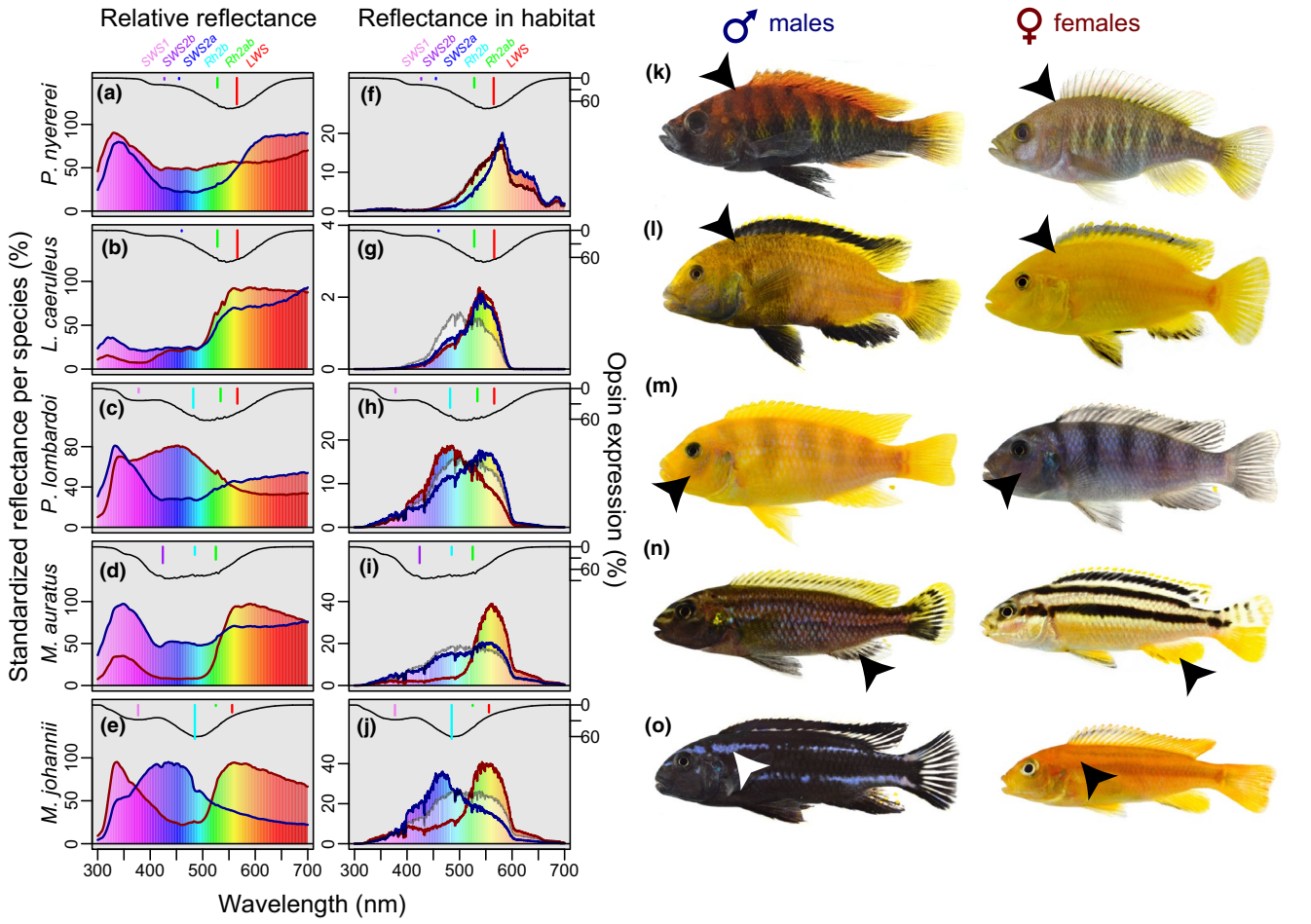

FIGURE 4 Modelled African cichlid relative (a–e) and absolute body reflectance patterns at habitat depths (f–j; reflectance spectra represent the most chromatically conspicuous measurement spots on the fish as indicated by the arrowheads in k–o). The grey line and white shade represents the standardized habitat light spectrum. Additionally, the retina sensitivity curves and mean opsin expression levels are indicated bottom up on the right axis of each plot. Assumed habitat depths are 5, 25, 10, 6 and 6 m for f–j, respectively. Species with broader reflectance spectra in their habitat appear to express a broader array of opsins (expression levels <3% not shown)

species-specific long-pass filter effects of the lens by multiplying the sensitivity curves with the average transmission curve per species (Figures S5 and S6). Thus, a lens-corrected weighted retina sensitivity curve was estimated for each species. To facilitate the comparison of the spectral widths of these lens-corrected weighted retina sensitivity curves, wavelengths that divide the integral of these visual sensitivity curves in 25% sections were calculated (λP_{25}, λP_{50} and λP_{75}) and the wavelength difference between the 25% and 75% wavelengths were calculated ($\Delta\lambda = \lambda P_{75} - \lambda P_{25}$) to reflect the widths of the visual sensitivity curves (Table 2; as in McFarland & Munz, 1975; Sabbah et al., 2011).

2.4 | Modelling African cichlids' body reflectance in their natural habitat

For the five African cichlid species, the reflective properties of the fish's bodies were measured at five to six spots on the fish, corresponding to cheek, back, belly, anal fin (anterior part), egg spots (if applicable) and an optional species-specific additional spot, representing a particular conspicuous or colourful patch (Figure S7 for more details). Fish euthanized with MS222 tended to expand their melanophores, which led to generally darker fish compared to their coloration when alive. Melanophores typically mostly alter the brightness of fish and have a more limited effect on the relative spectral composition of reflected light as melanin itself does not have an absorption peak in the visible light wavelength range (Zonios et al., 2008). Nonetheless, melanophores can cover other pigment cells with melanin and thus indirectly affect body colour in addition to brightness—effectively homogenizing the spectral composition of the body colour (Nilsson Sköld, Aspengren, & Wallin, 2013; Wucherer & Michiels, 2014). In studied African cichlids, we did not observe pronounced colour changes by the naked eye due to anesthesia; however, we did observe such changes in the South American *Apistogramma* species, rendering the measurements on this species too unreliable. For African cichlids, the PX-2 pulsed xenon lamp was used to illuminate each measured spot via a bifurcated fibre with a single probe that also allowed us to also measure the spot at the

same time and angle using the aforementioned spectrometer. Before each measurement, the respective spot was superficially dried with a paper tissue and then the probe was held at a 40°–60° angle directly onto the skin to measure the integrated reflective properties of an approximately 0.5 cm^2 spot. Nonmeasured areas were covered with black tracing paper to minimize noise due to scattered light. Nonetheless, particularly small structures (e.g., egg spots) may also contain some noise from unintentionally illuminated and measured surrounding skin. Comparisons to measurements conducted within water submerged fish and probe showed no relevant differences from the above-surface water measurements. Reflectance measurements were recorded from 300 to 700 nm. The relative reflectance spectrum per fish and spot (Figure S8) was calculated by dividing the absolute spectrum by the illumination spectrum, which was determined using a Spectralon diffuse white standard, and subsequent standardization:

$$I_{\text{rel.ref},\lambda} = \frac{I_{\text{abs.ref},\lambda}}{I_{\text{whiteLight},\lambda}},$$

where $I_{\text{rel.ref},\lambda}$ is the relative reflectance intensity for a given wavelength λ, $I_{\text{abs.ref},\lambda}$ is the absolute reflectance intensity measurement at λ, and $I_{\text{whiteLight}\,\lambda}$ is the absolute intensity measurement of the light source at λ.

To obtain estimates of species- and sex-specific body reflectance spectra of cichlids in their natural habitat, body light reflectance spectra had to be integrated with natural illuminance at their habitat. Due to random noise (e.g., variable melanophore spread levels of sampled fish and somewhat variable tissue-probe-distances during measurements) raw relative reflectance measurements are not necessarily comparable across individuals and spots. To allow comparisons of spectral peaks, relative reflectance spectra heights were standardized to have equal integrals between sexes' average spectra, i.e., the sum off all reflectance values per wavelength step were summed up per spectrum and the whole spectrum was divided by this value:

$$I_{\text{st.rel.ref},\lambda} = \frac{I_{\text{rel.ref},\lambda}}{\sum I_{\text{rel.ref}}},$$

where $I_{\text{st.rel.ref},\lambda}$ is the standadized relative reflectance intensity for a given wavelength λ, $I_{\text{rel.ref},\lambda}$ is the relative reflectance intensity at the wavelength λ, and $\sum I_{\text{rel.ref}}$ is the sum of intensities across the whole relative reflectance spectrum. When multiple reflectance measurements per spot were taken, an average spectrum was produced per sex and species.

Thus, spectral peaks and valleys of reflectance are readily recognizable in both sexes. However, all information on luminance (i.e., spectra heights) was therefore lost. Object luminance can affect an individual's colour discrimination abilities, particularly when object luminance is relatively low and background luminance is high, and we note that our approach unfortunately cannot consider effect (Lythgoe, 1988).

The habitat type for each species was determined based on previously published literature (Table 1 for details and references). To model the particular habitat ambient light environment, firstly the light attenuation properties of the water of the respective habitat were assigned to one of two water-types: "Lake Malawi rock habitat" (very clear water; for all Malawi cichlids) and "Lake Victoria Makobe" (murky water, for *P. nyererei*). We obtained the relative light attenuation spectrum per meter depth for the Lake Malawi habitats from Sabbah et al. (2011), which was only possible for downwelling light. Habitat light was then calculated by multiplying a sun light spectrum (sunlight spectrum obtained from http://rredc.nrel.gov/solar/spectra/am1.5/) with the habitat's transmission (which is one minus attenuation) spectrum to the power of the respective habitat's depth:

$$\text{HL}_{\text{dep},\lambda} = \text{SL}_{\lambda} \times \left(1 - A_{\lambda}\right)^{\text{dep}},$$

where $\text{HL}_{\text{dep},\lambda}$ is the habitat light intensity at a given depth dep (in meter) and wavelength λ, SL_{λ} is the sun light intensity at wavelength λ, and A_{λ} is the attenuation coefficient at wavelength λ. Obtained spectra were then standardized as outlined above for reflectance spectra. The Lake Victoria habitat relative light absorption spectra per meter depth were calculated using data from Maan, Seehausen, and Van Alphen (2010) (Figure S9) and processed the same. Again, λP_{25}, λP_{50}, λP_{75} and $\Delta\lambda$ were calculated (Table 2).

Species reflectance patterns in bright sunlight above water (Figure S10) and in their natural habitats (Figure S11) were then modelled. In aquatic habitats, fish in the water column are, however, illuminated not only by downwelling light, but also by side-welling and upwelling light. Downwelling light has the highest intensity among these and illuminates particularly surfaces oriented upwards (typically the back of the individual). Surfaces averted to the water surface are illuminated by side- or upwelling light. The wavelength spectrum of these is slightly more restricted compared to downwelling light, i.e., they are more similar to the spectra at a slightly greater water depth (Dalton, Cronin, Marshall, & Carleton, 2010; Sabbah et al., 2011). As fish body shapes are complex and individuals move within the water column, estimating which proportion of which light illuminates which part of a fish's body is challenging and was outside of the scope of this study. For example, while haplochromine anal fins and egg spots are oriented perpendicularly to the water surface most of the time, during mating males typically present them oriented towards the water surface and downwelling light illuminates them (authors' own observations, and also see Theis, Salzburger, & Egger, 2012). Finally, side-welling light spectra were not available for *P. nyererei*'s habitat, which is why only downwelling light is considered as ambient light of the fishes' habitats. Body coloration spectra were then modelled by multiplying the corrected relative reflectance spectra with the respective light spectrum:

$$I_{\text{hab.ref},\lambda} = I_{\text{st.rel.ref},\lambda} \times \text{HL}_{\text{dep},\lambda},$$

where $I_{\text{hab.ref},\lambda}$ is the intensity of reflectance under habitat light at wavelength λ, $I_{\text{st.rel.ref},\lambda}$ is the standardized relative reflectance intensity for a given wavelength λ, and $\text{HL}_{\text{dep},\lambda}$ is the intensity of the habitat light at wavelength λ.

Another standardization of obtained spectra followed (i.e., male and female reflectance curves as well as the habitat sunlight curve were adjusted to the same integral). It should thus be noted that estimated colour spectra of body surfaces averted to the water surface are expected to be estimated slightly broader than reality this way.

We combined the visual sensitivity of African cichlid species and the modelled colour patterns of males and females in their respective habitats by combining the weighted retina sensitivity curves, peak wavelength positions of each expressed opsin and the reflectance patterns in single plots (Figure 3, Figure S12). To quantify how chromatically conspicuous each reflectance spectrum is in its natural habitat light, the percentage of its integral not overlapping with the habitats' ambient light spectrum was calculated by first subtracting for each wavelength the habitat light from the body reflectance and then dividing the sum of absolute distances across all wavelengths by the integral of the habitat light spectrum (=integral of reflectance pattern), and then multiplying it by 100 to obtain percentages:

1. Calculation of divergence spectrum

$$\Delta I_{\lambda} = \mathrm{abs}(I_{\mathrm{st.hab.ref},\lambda} - \mathrm{HL}_{\mathrm{st.dep},\lambda}),$$

where $\Delta I\lambda$ is the absolute value of the difference between the standardized body reflectance intensity under habitat light at the wavelength λ ($I_{\mathrm{st.hab.ref},\lambda}$) and the standardized habitat light intensity at the wavelength λ ($\mathrm{HL}_{\mathrm{st.dep},\lambda}$) (Figure S13).

2. Calculation of spectral divergence

$$C = \frac{\sum \Delta I}{\sum \mathrm{HL}_{\mathrm{st.dep}}} \times 100,$$

where C is the chromatic conspicuousness (either MC in males or FC in females; Table 3).

Small chromatic conspicuousness values thus suggest body colour evolution to be driven either by sensory drive (or by nonsocial drivers, e.g., for camouflage), while larger values may argue for reciprocal evolution of body colour and the visual system by sexual selection. Again, the downwelling light spectrum was used although it is likely that side-welling light may more commonly be in the background of a cichlid fish observed by a conspecific. However, as mentioned before, side-welling light spectra were unfortunately not available for Lake Victoria habitats and while there are measurements for Lake Malawi habitats (e.g., Dalton et al., 2010; Sabbah et al., 2011) published data did not allow us to calculate a reliable attenuation curve.

Subsequently, sexual chromatic divergence per species and measurement spot was estimated by first standardizing aforementioned curves reflecting differences between each sexes' reflectance patterns and the habitat light (i.e., ΔI spectra; Figure S13) to equalize absolute integrals between them, and then calculating their percentage of overlap (as was done to obtain FC and MC values). For example, *L. caeruleus*' "back" reflectance spectra have male and female chromatic conspicuousness values of MC = 35% and FC = 43%, while those of *P. lombardoi* are "only" 17% and 20%, respectively. However, sexual chromatic divergence values for *L. caeruleus* and *P. lombardoi* are 4% and 100%, respectively. This illustrates that, while both sexes of *L. caeruleus* are much more chromatically conspicuous than any *P. lombardoi* sex, the sexes of the former species differ from the habitat light at the same wavelength ranges (despite one being darker than the other), while these ranges differ to 100% in *P. lombardoi*. Results of these analyses are presented in the main manuscript only for one measurement spot per species (the one with the highest average chromatic conspicuousness; Table 3), but data for all spots are presented in Figure S13.

Finally, as for the weighted and corrected retina sensitivity curves and the habitat light, λP_{25}, λP_{50}, λP_{75} and $\Delta\lambda$ were calculated (Table 2). Final sample sizes for all measurements are reported (Table S7). Data processing, visualization and statistical analyses were conducted in R (v. 3.5.1; R Core Team, 2013).

2.5 | Simplifications and assumptions

The used analytical pipeline integrated a wide range of data, however, several aspects of cichlid ecology and physiology had to be simplified as sufficient data was either not available or too complex. Simplifications of the ecology include the usage of only two water types, the negligence of seasonality effects on those (Smith et al., 2012) and the fact that fish occur at a range of water depths and only the mean depth is used here. Also, our analysis does not account for changes in water absorption properties with increasing depth and only considers downwelling ambient light (also for chromatic divergence calculations, albeit side-welling ambient light or rock reflectance may be more appropriate; Rennison et al., 2016). Further simplifications include the negligence of chromophore usage (A1/A2 ratio, though its effect on the visual systems of Lake Malawi cichlids is probably small, Carleton & Kocher, 2001), the structuring of cones in single- and double cones and potential shifts in diurnal cycle between opsin classes, although recent studies reveal that standardizing using overall opsin expression is a valid approach, especially as samples were processed in the early afternoon (Yourick et al., 2019). Furthermore, we do not account for opsin expression variation during ontogeny (only sexually mature fish were used and, among those, no relationship of opsin expression and age could be found) or due to plasticity (e.g., Härer et al., 2019; Hofmann, O'Quin, O'Quin, Smith, & Carleton, 2010; Nandamuri et al., 2017), although a minimum of 2 weeks acclimatization to artificial lighting was used to avoid bias in plasticity. Additionally, measuring physiological variables (opsin expression, lens transmission) can only approximate visual capabilities while actual visual capabilities can only be determined using behavioural approaches (e.g., Escobar-Camacho,

Marshall, & Carleton, 2017; Escobar-Camacho, Taylor, et al., 2019; Kalb, Schneider, Sprenger, & Michiels, 2015; Kelber, Vorobyev, & Osorio, 2003; Kröger, Knoblauch, & Wagner, 2003; Schluessel, Fricke, & Bleckmann, 2012). Finally, we only consider the measured spots and neglected any body patterns that differed due to variation in brightness.

3 | RESULTS

3.1 | Opsin expression and lens transmittance vary among species but not between sexes

Our analyses showed that sexes did not differ in their patterns of opsin expression after species-wise correction for multiple testing (Figure 2, Table S3, Table S5). Generally, all neotropical cichlids examined expressed the same three cone opsin genes (*sws2a*, *rh2a* and *lws*). However, the expression ratio of double-cone opsins *rh2a* to *lws* varied considerably among species, with *A. cacatuoides* expressing much more *lws* and *H. nematopus* expressing more *rh2a*. African species expressed more diverse combinations of opsin genes among species (Figure 2f–j) and no single opsin was expressed in all African species (*lws* and *rh2a* were each expressed in all but one species; *lws* only ~1% in *M. auratus* and *rh2a* only ~2% in *M. johannii*). Rhodopsin 1 (*rh1*) was expressed in all species with high variation among species: whereas in *M. auratus rh1* expression exceeded 92% of all expressed opsins, it was slightly less than 30% in *A. ortegai*. Between species, lenses were found to have very variable transmittance for light of very short wavelengths: whereas lenses of *P. nyererei* do not allow UV light to pass (T50 of 396 nm), much lower T50 values were found in species with UV-sensitive retinas (Figure 4, Table S6), most notably, *P. lombardoi* and *M. johannii* had average T50 values of 337 and 349 nm, respectively. Within species lenses varied only little, particularly in T50 values (Figure S5). Finally, we also note that in *A. ortegai*, despite no overall effect of rearing background was detectable (Table S4), in opsin wise analyses this factor seemed to have affected *sws1*, *rh2a* and *rh1* expression. *Sws1* and *rh2a* genes were virtually not expressed in *A. ortegai* (both <0.1% of expressed cone opsins) but notably *rh1*, whose expression is approximately 30% of all expressed opsins, was more highly expressed in laboratory-raised individuals than in wild-caught ones. Even when rearing background was considered, sexes did not differ in opsin expression in this species (Table S5).

3.2 | Visual sensitivity of African cichlids is associated with their light environment

Variation in their visual sensitivity of African cichlids was partially correlated to their habitat light spectrum. The centre wavelength (λP_{50}; Table 2) of the investigated cichlids' lens-corrected visual sensitivity curves showed considerable variation, with *Melanochromis* species being most visually sensitive in the short light wavelength range (469 and 476 nm for *M. auratus* and *M. johannii*, respectively), while *P. lombardoi*'s sensitivity is at intermediate wavelengths (507 nm), and *L. caeruleus* and *P. nyererei* were visually sensitive in the long wavelength range (541 and 544 nm, respectively; Table 2). This variation was reflected in the centre wavelength of the respective habitat light spectra: especially the habitat light spectrum experienced by *P. nyererei* was shifted particularly to the long wavelength range (centre wavelength was 548 nm) compared to the Lake Malawi light spectra (all at 487–488 nm). We also found that the widths of the visual sensitivity spectra were narrower in species with a narrower habitat light (*P. nyererei* and *L. caeruleus* habitat light widths: $\Delta\lambda$ = 62 nm in both cases), except for *P. lombardoi*, which showed broad visual sensitivity despite living in a slightly more restricted light environment than the *Melanochromis* species (*P. lombardoi* and *Melanochromis* habitat light widths: $\Delta\lambda$ = 81 and 92 nm, respectively; Table 2).

3.3 | Body reflectance of African cichlids is highly dependent on their natural light environment

Relative reflectance patterns of all African cichlid species showed a pronounced reflection peak at around 340 nm light wavelength (i.e., UV-light; Figure 4a–e; Figure S8). Reflectance at surface-level sunlight (Figure S10) showed that almost all species reflected sunlight at a wide range of wavelengths, although darker individuals reflected overall less light prior standardization. Estimated reflectance patterns (and thus body colour) changed dramatically when the natural light environment was considered (Figure 4, Figure S11). As expected, due to the restricted wavelength compositions in the natural habitats the estimated reflectance spectra became narrower and the reflectance peak in the UV light as well as the reflectance shoulder in the long wavelength light range of some species was reduced or even disappeared. Particularly striking was this effect in *P. nyererei* and *L. caeruleus*, for which the water column was predicted to absorb considerable portions of the downwelling light due to dissolved particles in the water column and habitat depth, respectively (habitat light width: $\Delta\lambda$ = 62 nm in both cases; Table 2). For these two species, chromatic conspicuousness was determined to be very high and the most conspicuous measured spot in both species was the "back" (Figure 4a,b,f,g; Figure S13; Table 3). This spot in males and females of *P. nyererei* and *L. caeruleus* showed very distinct and narrow reflectance spectra (body reflectance: $\Delta\lambda$ = 60 nm & 62 nm and 56 nm & 47 nm, respectively; Table 2). While in *P. nyererei* the male showed much higher chromatic conspicuousness than the female at this spot, in *L. caeruleus* both sexes showed very high conspicuousness values (Table 3).

Cichlids inhabiting the relatively shallow waters of Lake Malawi, where the light spectra were predicted to be relatively broad include *P. lombardoi* and both *Melanochromis* species (habitat light width: $\Delta\lambda$ = 81 and 92 nm, respectively; Table 2). These species reflected considerable amounts of UV-light, while this was

virtually absent in *P. nyererei* and *L. caeruleus* under habitat light. Their male and female chromatic conspicuousness values were intermediate to very high (Table 3) on their most chromatically conspicuous spot (Figure 4c–e,h–j; Table 3). While *P. lombardoi* and *M. johannii* reflectance spectra showed intermediate male and female body reflectance spectra widths (body reflectance: $\Delta\lambda$ = 85 nm & 73 nm and 82 nm & 75 nm, respectively; Table 2), in *M. auratus* the males were found to have a less distinct reflection peak, whereas females had a very distinct one (body reflectance: $\Delta\lambda$ = 98 and 46 nm). Finally, in *P. lombardoi* and *M. johannii*, divergence in reflectance spectra between sexes extended clearly into the short wavelength light range around 400 nm and their sexes' chromatic conspicuousness did almost not overlap (sexual chromatic divergence both 97%; Table 3; Figure S13), while overlap was much stronger for all other species in which conspicuousness was high in both sexes.

3.4 | Light environment, reflectance and spectral sensitivity in African cichlids

Lastly, we combined our approximations of fish reflectance with the modelled weighted and corrected retina sensitivity curves (Figure 4, Figure S6; Table 2). Species with a single clearly defined reflectance peak appeared to show most pronounced opsin expression in close proximity to this peak and the visual sensitivity curve is rather narrow (*P. nyererei* and *L. caeruleus*; Table 2: λP_{50} values). For *P. nyererei* this reflectance peak also coincides well with the environmental light spectrum, but this is not the case for *L. caeruleus* (Figure 4g). In species with divergent reflectance peaks between sexes and thus large sexual chromatic divergence values, expressed opsins appeared to cover a wider range of wavelengths (*P. lombardoi*, *M. johannii*, Tables 2 and 3), which is reflected in wider visual sensitivity curves (Figure 4; Table 2). Furthermore, species with sexual dichromatism and a reflectance peak at a shorter wavelength (*M. johannii* and *P. lombardoi*) also expressed the short-wavelength sensitive opsin *sws1*. *M. auratus* individuals did not express this opsin, but instead *sws2a*, despite inhabiting a very similar habitat as *M. johannii* that provides light of short wavelengths and its lens allowing UV light to reach the retina (albeit in *M. auratus* to a lesser extent than in *M. johannii* and *P. lombardoi*; T50 at 366, 337 and 349 nm, respectively). Still, in our study, the *M. auratus*' retina sensitivity curve appears to overlap more with the broad habitat light spectrum than its rather specific body reflectance curves.

4 | DISCUSSION

Cichlid fishes are famous for their astonishing diversity in body colorations, both across and within species (e.g., Mcelroy, Kornfield, & Everett, 1990; Seehausen & van Alphen, 1998; Seehausen et al., 2008; Stiassny & Meyer, 1999). However, whether and how this diversity is linked to the species' visual systems, environment, and body coloration remains unresolved in many cases (Cronin et al., 2014; Dalton et al., 2010; Price, 2017). In this study we integrated ecological, physiological, and molecular data to link ecology and visual abilities, and thus provide new insights in the evolutionary forces that shaped cichlid colour and their vision systems.

4.1 | No evidence of sexually dimorphic visual sensitivities in cichlid fishes

Our study found no evidence for sexual dimorphism in visual sensitivities of any of the investigated cichlid species, and thus found no support for the hypothesis that run-away sexual selection might be shaping sex specifically opsin expression in dimorphic cichlid species (Figure 1a–d). Few studies have pointed to the likelihood of sexual dimorphisms in visual sensitivity co-evolving with dimorphic nuptial coloration (Bloch, 2015; Kelber & Osorio, 2010; Sabbah et al., 2010), but this does not seem to be generally the case for cichlids. Across the African and Neotropical cichlid radiations, and across a wide range of body coloration including monomorphic and dimorphic species no sexual dimorphism in visual sensitivity was found. For instance, while in *H. nematopus* both sexes' exhibit grey and rather dull body coloration, albeit brightness and pattern can change during mating season, in the closely related species *H. nicaraguensis* sexes differ strikingly in red and green body coloration. However, neither of them shows evidence for differences in opsin expression patterns between sexes.

A previous study (Sabbah et al., 2010) suggested that there could be differences in opsin expression between sexes of *M. auratus* and one of the species we also included in our study. Sabbah et al. (2010) calculated opsin expression indirectly, based on the frequency of cone pigments from spectral sensitivity data obtained from electroretinograms. Our data does not support that conclusion, and is thus in agreement with findings of no differences in colour discrimination capability between sexes in this species (Coniam, 2014). However, it cannot be fully excluded that other species may show expression differences or that differences in opsin expression might be too small to capture in this study (see also below). The evolution of dimorphic visual systems may have been hampered in the investigated species due to highly complicated mechanisms of sex determination that can vary across species: while in some cichlid species sex clearly is determined entirely genetically (Gammerdinger, Conte, Sandkam, Penman, & Kocher, 2019; Gammerdinger et al., 2018; Parnell & Streelman, 2013; Ser, Roberts, & Kocher, 2010), hormone titres can also affect sex determination in others. Hormone titres were also shown to affect sex-specific body coloration, which might have promoted the evolution of sexual dimorphism in colour, but although hormones can affect visual sensitivity in cichlids, there is no evidence that this effect can be different between sexes (Baroiller, Chourrout, Fostier, & Jalabert, 1995; Baroiller & D'cotta, 2001; Dijkstra et al., 2017; Francis & Barlow, 1993; Härer, Torres-Dowdall, & Meyer, 2017). Thus, linking opsin expression to a specific sex may, evolutionarily

speaking, not be as straightforward as in species where sex is solely genetically determined.

4.2 | Is diversity in Neotropical cichlid vision constraint by phylogenetic history?

Expression levels of cichlid opsin genes varied considerably among the investigated cichlid species (Figures 1d and 2), however, compared to studied African cichlids, Neotropical cichlids showed no interspecific diversity in the set of expressed opsins, despite diverse body colorations and pronounced sexual dimorphisms in most of them. Nonetheless, expression levels within expressed genes varied. For instance, both sampled Central American cichlids expressed the *rh2a* and *lws* at the same relative level, while sampled South American *Apistogramma* species always expressed at least twice as much *lws* than *rh2a*—in agreement with data from other South American cichlids (e.g., Escobar-Camacho, Pierotti, et al., 2019). Sampled Central American species inhabit a variety of different water bodies, ranging from very clear to rather murky water (based on our own observations in the field). It is thus not surprising to find a lack of *sws1* expression as UV light is probably virtually absent in many more murky habitats, dominated by green-brown light wavelengths. However, sampled *Apistogramma* species inhabit the Amazonian drainage system and while *A. agassizii* and *A. cacatuoides* are relatively widely distributed species and occur in a variety of waters, *A. ortegai* has been found exclusively in extremely clear forest streams, and all sampled *Apistogramma* species inhabit shallow waters (Britzke, Oliveira, & Kullander, 2014; Römer, 1998). Thus, the exceptionally high *rh2a* and (very) low *rh1* expression levels in these species may reflect an adaptation to these bright light environments. Absence of *sws1* expression in all of them despite inhabiting water depths and clarities which allow UV light to reach them argues for a strong phylogenetic constraint. However, closed canopy cover may overshadow particularly smaller streams and thus remove UV light effectively from their habitat, rendering *sws1* expression unnecessary. Escobar-Camacho et al. (2016) found that the same three opsins were expressed in all of three different studied Amazonian cichlids, suggesting that Amazonian cichlids generally have a limited opsin repertoire. Although sampled representatives of Central American lineages expressed the same three cone opsins in our study, they appear to be generally less constrained in their visual repertoire as recent studies found more visual diversity among them, with at least some of them also expressing *sws1*, *sws2b* and *rh2b* in certain habitat types or developmental stages (Härer, Meyer, & Torres-Dowdall, 2018; Karagic, Härer, Meyer, & Torres-Dowdall, 2018).

4.3 | Natural selection can partially explain variation in the relative rod opsin expression

Rh1 sequence evolution has previously been identified to be under divergent selection in cichlids (e.g., Hauser et al., 2017; Schott, Refvik, Hauser, López-Fernández, & Chang, 2014; Torres-Dowdall, Henning, Elmer, & Meyer, 2015), and is commonly found to be dominant in the total opsin expression (e.g., Yourick et al., 2019), which is expected given the relative abundance of rod versus cones on retinas of fish. However, we found that the proportion of *rh1* expressed relative to all expressed opsins is highly variable among the studied species, making up as little as only ~30% of total opsin expression in the Neotropical cichlid species *A. ortegai* (Figure 3; unpublished transcriptome on another *Apistogramma* species show similarly low levels). Although at this point the causes of this variation are unknown, we speculate that this could be due to variation in the light environment of the habitats these species occupy or the result of different patterns of daily activity. For example, the studied African species that live in rather dim-light habitats, such as *P. nyererei* and *L. caeruleus*, expressed rather high amounts of *rh1* (86% and 77%, respectively) compared to two species living in brighter environments, *P. lombardoi* and the *M. johannii* (68% and 66%, respectively). An exception to this pattern is *M. auratus*, that lives in approximately the same habitat as *M. johannii* but shows the highest *rh1* expression among the investigated fish (92%). Reasons for this may include behavioural deviations from the other species (e.g., activity of this species could be during the dimmer dusk and dawn, instead of during bright daylight). We therefore encourage in the future to investigate the potential causal relationship between *rh1* expression and ecological parameters. Determining whether expression differences in *rh1* are caused by varying numbers of rod cells in the retina, varying expression levels of *rh1* per rod or a combination of both was beyond the scope of this study but may also foster future research.

4.4 | Habitat light explains some, but not all variation in cichlid's visual sensitivities

A central aim in visual evolutionary ecology is to determine the major mechanisms that drive and tune visual perception (Levine & MacNichol, 1979; Lythgoe, 1984). Ambient light was already proposed to be a major determinant of opsin gene expression and visual pigment sensitivity (Cronin et al., 2014; Cummings & Partridge, 2001; Seehausen et al., 2008); yet, we found that this effect may vary considerably among species. For example, species whose habitat light is deprived of short-wavelengths (UV) always expressed less than 1% *sws1*, supporting the hypothesis that ambient light affects opsin expression. Furthermore, for the most part lens T50 values negatively correlated with *sws1* expression. This co-evolutionary pattern argues for the ecological importance of short-wavelength light perception, e.g., to recognize conspecifics or food items, which both can reflect short-wavelength light (Hofmann et al., 2009; Hofmann, O'Quin, Marshall, et al., 2010). This is most strikingly demonstrated in *M. johannii*, whose T50 is lower than any of those previously measured in other cichlids (Hofmann, O'Quin, Marshall, et al., 2010) and comparable with some coral reef fish (Siebeck & Marshall, 2001). While negligible levels of short-wavelength light thus probably predicts a lack of short-wavelength vision in fish, UV light availability does not

guarantee UV visual sensitivity: *M. auratus* lives in water depths in which considerable amounts of short-wavelength light are still available, and which are also reflected by the fish's body. But this species does not seem to exploit the short-wavelength portion of the available light to gather visual information, even though its lens does allow some UV light to reach the retina.

In addition to ecological selection via the habitat light conditions and other factors, body coloration may be another factor affecting vision in cichlids (e.g., via sexual selection). Our results suggest that cichlid vision in the rather murky Lake Victoria habitat is driven primarily by the constraint light availability compared to Lake Malawi and less so by sexual selective forces (Figure 4). In Lake Victoria, the light environment is narrow and enriched in long wavelength light (Figure 4; Seehausen et al., 2008). Body reflectance patterns of females of the species inhabiting this lake, *P. nyererei*, match almost perfectly the ambient light spectrum. Males' reflectance peak also coincides with the ambient light peak, but overall reflectance is considerably shifted towards the long-wavelength range resulting in a moderate chromatic dimorphism. Furthermore, as previously described (Carleton et al., 2005), *P. nyererei* showed a long wavelength shifted visual sensitivity matching the light environment. Our study thus confirms the ascribed role of sensory drive in these fish, in which the habitat light putatively shaped the visual sensitivity of the fish and nuptial coloration evolved correspondingly to their visual sensitivity (Figure 1a; Carleton et al., 2005; Seehausen et al., 2008).

The light environment of Lake Malawi is spectrally broader than that of Lake Victoria (Dalton et al., 2010; Sabbah et al., 2011), which is reflected in high diversity in the visual system of the native cichlid species (Carleton et al., 2016; Hofmann, O'Quin, Smith, et al., 2010; this study). Additionally, we also found variation in alignment patterns among the spectral curves of the light environment, body coloration and visual sensitivity (Figure 4). *L. caeruleus* was the only species from Lake Malawi in which no sexual dichromatism was detected. Its body reflectance patterns do not exploit the whole range of the available light spectrum (as reflected in very large male and female chromatic conspicuousness values), but it is shifted towards longer wavelengths, reflecting almost exclusively the little light in the yellow part of the spectrum that reaches the depth they inhabit. Their visual system also appears to be particularly tuned towards the yellow part of the spectrum of the light present in their environment. Thus, in this species visual sensitivities do fall within, but do not completely span the prevalent light environment. Importantly, visual sensitivities overlap with body coloration, suggesting that the visual system may have co-evolved with body coloration. The other three species (*P. lombardoi* and *M. johannii*, and to a lesser degree *M. auratus*) are strongly chromatically dimorphic (i.e., sexual dimorphism is not solely due to variations in melanin and thus brightness, Figure 4). The opsin expression patterns of *P. lombardoi*, *M. auratus* and *M. johannii* suggest that their visual systems evolved to exploit the whole range of light available in their environment. These species have relatively broad visual sensitivities and the dominant sensitivity peaks roughly coincide with the intensity peak of the spectrally broad habitat light (Figure 4h,j, Figure S12, Table 2). Two of these species are also pronounced chromatically sexually dimorphic, with the sexes falling at opposite ends of their habitat light spectrum (Figure 4h,j, Figure S12, Table 3). Assuming that the ancestral pattern of opsin expression of cichlids colonizing Lake Malawi include *sws2a*, *rh2a* and *lws* (O'Quin, Hofmann, Hofmann, & Carleton, 2010), two potential evolutionary scenarios linking environmental light, visual abilities and body patterns emerge: (a) Initially, given the broad light spectra of the environment, ecologically relevant visual cues across a broad spectral range selected for fish with a broad visual sensitivity. Secondarily, broad sensitivity allowed dichromatism to evolve within the sensitivity range, for example driven by sensory biases for such ecological cues. (b) Alternatively, sexual dichromatism in body coloration may have co-evolved with the visual system. However, at this point we cannot determine if the evolution of the sensory systems of the studied chromatically dimorphic species was initially driven by ecologically relevant cues for which a broad visual sensitivity spectrum was selected, and/or by a reciprocal co-evolution of vision and the body coloration of their sexually dichromatic conspecifics. Nonetheless, both of these evolutionary scenarios require the bright and spectrally broad ambient light present in these species' habitats compared to species living in deeper or spectrally more restricted habitats (Price, 2017).

Contrasting these species, the visual sensitivity peak of *M. auratus* best exploits the short wavelength part of the spectrum available in its habitat (although not in the UV light range), and it is particularly short wavelength shifted compared to its body reflectance pattern (λP_{50} are 469 nm, 488 nm and 561/581 nm, respectively, Table 2, Figure S12). The dominant reflectance peak of *M. auratus* (particularly of females) is at the long-wavelength tail of the ambient light intensity peak (Figure 4i, Figure S12), and, thus, there is no evidence for body coloration in *M. auratus* being the result of sensory drive, or that its visual sensitivity is shaped by conspecific body coloration. Thus, it is possible that other factors have shaped *M. auratus*' visual system. For example, it is plausible that contrast detection and resolution plays a more important role in this species as both sexes show particularly striking stripe patterns on their body flanks and fins, which are significantly altered in dominant males compared to females and submissive males (Figure 4n). It should be noted that many *M. auratus* males used or described in other publications did show some blue reflectance, which can also be seen in the photos illustrating the species in Figure 4 as well as the spectra illustrating relative reflectance (compare Figure 4b and d, where *M. auratus* shows a subtle reflectance peak in the short blue wavelength range; Dalton et al., 2010). However, this blue reflectance was not picked up very strongly by our spectrometric measurements, potentially due to a specific population that does not exhibit strong blue reflectance or the blue reflectance not being at a measured spot. Thus, we cannot discard that the relatively broad visual sensitivity observed in *M. auratus* (Table 2) may be linked to this blue body reflectance.

In sum, the broader light spectrum of Lake Malawi seems to allow for high variation in the pattern of opsin gene expression of cichlid fish, with some species having the ability to perceive the complete spectrum of available light and others tuning to particular parts of

the spectrum, either matching the spectral reflectance of nuptial coloration or not. Indeed, expressing opsins with absorption spectra not well coinciding with the ambient light spectrum argues that the habitat's light environment is visually not too restrictive (Loew & Lythgoe, 1978). Expanding this approach to other lineages might help to determine the generality of these result and highlight important aspect of cichlid vision evolution. For instance, the inclusion of riverine haplochromine species that ecologically resemble those lineages that have colonized Lake Malawi and Lake Victoria and initiated their adaptive radiations would be valuable additions. Integrating the phylogenetic history by using comparative methods would thus further our understanding of the evolutionary drivers of cichlid visual ecology.

4.5 | Methodological approach and future directions

Besides providing insights in cichlid visual evolution, we applied an integrated set of measurements that allowed the quantification of parameters that are often hard to quantify, such as "chromatic conspicuousness", "sexual chromatic divergence", and "spectra widths" (Table 2, Table S6, Figure S13). This approach is meant to complement, rather than replace, methods that aim to estimate colour discrimination abilities (e.g., colour distance and just noticeable differences; e.g., Carleton et al., 2016; Dalton, De Busserolles, Marshall, & Carleton, 2017; Escobar-Camacho, Taylor, et al., 2019). Our approach allowed us to work with all expressed opsin genes and we investigated their relative expression levels. However, we could not integrate luminance into our models, although it probably affects colour discrimination abilities, particularly in dim habitats (Brown, 1951). Furthermore, in this study we interpreted opsin expression without considering the cone type (Carleton et al., 2005; but see Figure S3) or how signals from different cones types are integrated. While there have been some recent advances in this field (e.g., Dalton et al., 2017), it still not fully understood how visual signal strength is composed in cichlids, i.e. to which extent cell type number and/or opsin expression within these cell types contribute to visual perception. Finally, phenotypic plasticity is known to strongly affect opsin expression (e.g., Härer et al., 2019; Hofmann, O'Quin, Smith, et al., 2010; Nandamuri et al., 2017). While we aimed here to avoid any bias due to plasticity by first acclimatizing studied cichlid species to laboratory light conditions in order to compare the genetically determined portion of these diverse species' visual systems (Carleton et al., 2016; Nandamuri et al., 2017), studies comparing cichlid species' performances within a specific field setup can also sample environmental parameters and fish retinas simultaneously. The difference in *rh1* expression between *A. ortegai* rearing backgrounds (Table S5) furthermore illustrates that developmental plasticity may contribute to adult opsin expression levels, which we also could not account for with our acclimatization period. Despite our best efforts to incorporate as much ecological and molecular information as possible, the natural habitat of cichlids will always be so much more complex then we accounted for in our study (see Section 2.5). Nonetheless, by integrating more information on the molecular underpinnings of cichlid vision as well as their ecology future studies will be provided with more statistical power for correlative analyses.

In conclusion, freshwater visual environments are often much more complex and diverse than those of marine or terrestrial environments, partially due to a higher variability in light-transmission properties (Cronin et al., 2014). Therefore, it is challenging to determine the importance of factors that may have affected fish vision. Our integrative approach sheds new light on the degree to which fish vision evolved due to selection for their respective habitats, e.g., by adjusting opsin expression levels according to the habitat's prevalent light regime. Opsin expression varied within the range of available light wavelengths and habitat light appears as the primary selective force on visual sensitivities. However, our evidence suggested that cone opsin expression patterns also evolved to optimize perception of conspecific coloration, directly or indirectly. We provide additional knowledge to previous findings by excluding sexual visual dimorphism as being common and thus a considerable factor in cichlid visual ecology. More data on ecological parameters but also visual properties of individual cichlid species are necessary to discern the relative contributions of alternative evolutionary forces driving the diversity of visual ecologies in these colourful fish.

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DATA ACCESSIBILITY

All raw data for opsin expression, lens transmission and body reflectance are deposited in Dryad (Schneider, Rometsch, Torres-Dowdall, & Meyer, 2020; https://doi.org/10.5061/dryad.9w0vt4bbp). Primer reference sequence sources are found in the supplements.

AUTHOR CONTRIBUTIONS

J.T.D., R.F.S., S.J.R., and A.M. conceptualized the project. S.J.R., and R.F.S. conducted the experimental work and analysed the data. R.F.S., and S.J.R. wrote the first draft of the paper and all authors revised the manuscript in later stages.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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