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2 **Ecology drives spectral transmission in the ocular lenses of frogs and salamanders**

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42

43 **Conflicts of Interest**

44 None of the authors have any conflicts of interest.

45

46 **Author Contributions**

47 RHD, DJG, JWS and KNT designed the study with input from RCB and MKF. JWS, DJG, RCB,
48 MKF, CFBH, CGB, RAM, CLC, KNT, and HCL collected samples. KNT, JWS, DJG, RCB, MKF, RKS,

49 and CFBH assigned ecological traits to species. RHD and KNT collected spectral transmission
50 measurements from lenses, analysed the data, and drafted the manuscript. HCL ran
51 evolutionary model comparisons. All authors participated in revising the draft manuscript
52 and gave final approval for publication.

53

54 **Data availability statement**

55 All data presented here and details for individual specimens (including museum catalogue
56 numbers) are available from the Natural History Museum Data Portal:

57 <https://doi.org/10.5519/a7ficgr8> (Thomas et al., 2021). Genetic data used to help identify
58 19 species are accessioned on GenBank (MW830045-MW830063). R code to reproduce
59 analyses is available at <https://github.com/knthomas/amphibian-lens-transmission>.

60

61 **Abstract**

- 62 1. The spectral characteristics of vertebrate ocular lenses affect the image of the world
63 that is projected onto the retina, and thus help shape diverse visual capabilities.
64 Here, we tested whether amphibian lens transmission is driven by adaptation to
65 diurnal activity (bright light) and/or scansorial habits (complex visual environments).
- 66 2. Spectral transmission through the lenses of 79 species of frogs and 6 species of
67 salamanders was measured, and data for 29 additional frog species compiled from
68 published literature. Phylogenetic comparative methods were used to test ecological
69 explanations of variation in lens transmission and to test for selection across traits.
- 70 3. Lenses of diurnal (day-active) and scansorial (climbing) frogs transmitted significantly
71 less shortwave light than those of non-diurnal or non-scansorial amphibians, and
72 evolutionary modelling suggested that these differences have resulted from
73 differential selection.
- 74 4. The presence of shortwave-transparent lenses was common among the sampled
75 amphibians, which implies that many are sensitive to shortwave light to some
76 degree even in the absence of visual pigments maximally sensitive in the UV. This
77 suggests that shortwave light, including the UV, could play an important role in
78 amphibian behaviour and ecology.
- 79 5. Shortwave-absorbing lens pigments likely provide higher visual acuity to diurnally
80 active frogs of multiple ecologies and to nocturnally active scansorial frogs. This new
81 mechanistic understanding of amphibian visual systems suggests that shortwave-
82 filtering lenses are adaptive not only in daylight conditions but also in scotopic
83 conditions where high acuity is advantageous.

84 **Keywords:** Activity period, Anura, Caudata, diurnal, scansorial, sensitivity, UV

85 **Introduction**

86 Animal vision is extraordinarily diverse, and animals obtain different information from their
87 external world depending on the optics, structure, and physiology of their eyes. Eyes may
88 differ in, for example, the colours they can detect (spectral sensitivity), the level of detail
89 they can resolve (spatial acuity), and the amount of light they can capture (sensitivity), and
90 these differences affect how animals interact with each other and their environment (Land
91 & Nilsson, 2012). When light passes through an eye, it may encounter ocular filters that
92 change the spectrum of light that ultimately reaches the photoreceptors, and these are
93 diverse in form and function across animals (reviewed in Douglas & Marshall, 1999). In
94 vertebrates, as well as a few cephalopods (e.g. Thomas et al., 2017) and spiders (Hu et al.,
95 2012, 2014), the lens or cornea can act as long-pass filters with important implications for
96 sensitivity, acuity, and colour vision. Here, we examine the spectral characteristics of
97 amphibian lenses and test whether aspects of ecology that affect visual environment and
98 behaviour drive differences across species.

99

100 In a camera-type eye, the main function of the lens is to focus light from the external world
101 onto the retina, yet spectral properties of lenses are also important in shaping vision. Light
102 from the environment must pass through the ocular media (cornea, lens, humours) to
103 activate the visual pigments within the retina for visual perception to occur. The longest
104 wavelengths of light a vertebrate can see are determined by the spectral sensitivity of its
105 visual pigments, because transmission by the ocular media is uniformly high at longer
106 wavelengths (Douglas & Marshall, 1999). At short wavelengths (ca. 300–450nm), however,
107 the limits of vision in vertebrates are determined primarily by the lens (Douglas & Marshall,
108 1999).

109

110 Light below approximately 300 nm will not reach the retina in any vertebrate because it is
111 absorbed by the nucleic acids and structural protein components of the cornea, lens, and
112 aqueous and vitreous humours (Douglas & Marshall, 1999). Between about 300–450 nm,
113 however, ocular media transmission varies among species. In some vertebrates, all the
114 ocular media are as transparent as biologically possible and transmit short wavelengths
115 well. In others, the lens (and in some fishes, the cornea) contains pigments that absorb
116 short wavelengths, preventing them from reaching the retina (Douglas & Marshall, 1999 for
117 review). Species with shortwave-transparent ocular media presumably benefit from
118 increased absolute sensitivity and potential perception of short wavelengths, including
119 ultraviolet (UVA = 315–400nm). Species with pigmented ocular media, on the other hand,
120 sacrifice sensitivity to protect the retina from damaging short wavelengths that can induce
121 retinal toxicity (Glickman, 2011; Van Norren & Vos, 2016) and potentially improve spatial
122 acuity by removing that part of the spectrum most prone to chromatic aberration and
123 Rayleigh scattering (Douglas & Jeffery, 2014). Thus, the balance of costs and benefits of
124 shortwave-filtering lenses should vary across species that are active in distinct light
125 environments or have different visual needs, potentially leading to different evolutionary
126 trajectories for spectral lens transmission.

127

128 Shortwave-absorbing ocular filters are found in the lenses of diverse species across all major
129 vertebrate groups examined, including fishes (Douglas & McGuigan, 1989; Thorpe et al.,
130 1993; Siebeck & Marshall, 2000, 2001, 2007), humans (Kessel et al., 2010) and other
131 mammals (Douglas & Jeffery, 2014), reptiles (Simões et al., 2016; Gower et al., 2019), birds
132 (Lind et al., 2014; Olsson et al., 2021) and frogs (Yovanovich et al., 2019, 2020). However, we

133 currently have little understanding of the forces driving the evolution of lens pigmentation
134 in any group, and only recently have studies started to use phylogenetic comparative
135 methods to investigate the evolution of this trait (e.g. Yovanovich et al. 2020, Olsson et al.
136 2021). To our knowledge, no study has found evidence for correlated evolution of lens
137 transmission and any aspect of ecology. Considering the important implications of spectral
138 lens transmission for acuity, sensitivity, and colour vision, studying the distribution and
139 evolution of this trait not only provides insights into how ecology drives sensory evolution,
140 but also into the potential visual capabilities of diverse animals.

141

142 Previous surveys have highlighted two factors likely to be important in the evolution of
143 shortwave lens transmission in vertebrates. Many vertebrates with shortwave-transparent
144 lenses are nocturnal, presumably to maximize absolute sensitivity in scotopic environments,
145 while most vertebrates with pigmented lenses that absorb short wavelength light are
146 diurnal. For instance, low shortwave transmission has been observed in the lenses of
147 diurnally active species of birds (Lind et al., 2014), mammals (Douglas & Jeffrey, 2014),
148 snakes (Simões et al., 2016), and fishes (Douglas & McGuigan, 1989; Thorpe et al., 1993;
149 Seibeck & Marshall, 2001; Seibeck & Marshall, 2007). Exceptions are mesopelagic fishes
150 (and squids), which live in the equivalent of permanent nocturnality, yet have lenses
151 containing shortwave-absorbing filters that are presumed to increase the visibility of
152 bioluminescent signals and camouflage (Douglas & Thorpe, 1992; Muntz, 1976; Thomas et
153 al., 2017). Lens pigmentation has also been noted in vertebrates with morphologies,
154 physiologies, and behaviours associated with high acuity, such as mammals with high cone
155 densities in the retina (Douglas & Jeffrey, 2014), raptors with large tubular eyes and high
156 electrophysiological spatial resolution (Lind et al., 2014; Gaffney & Hodos, 2003), and snakes

157 that are highly visual hunters (Simões et al., 2016). Thus, explanations for the scattered
158 presence of vertebrate lenses containing shortwave-absorbing pigments rely mostly on
159 loose or anecdotal associations with ecological, anatomical, and physiological variables.
160 Here, using extensive taxonomic sampling paired with phylogenetic comparative methods,
161 we aimed to test whether activity period and the need for high acuity are forces driving the
162 adaptive evolution of lens pigmentation in a vertebrate group.

163

164 Frogs (Amphibia: Anura; includes toads) and salamanders (Amphibia: Caudata; includes
165 newts) exhibit a range of ecologies and natural histories that make them good candidates
166 for testing hypotheses about the adaptive evolution of lens transmission. Although
167 predominantly nocturnal, amphibians have repeatedly evolved diurnal activity patterns
168 across diverse families (Anderson & Wiens, 2017) and therefore may benefit from the
169 protection of shortwave-absorbing pigments in their lenses. Further, many frogs inhabit
170 ground- and water-associated habitats, but scansorial species specialized for climbing and
171 jumping in foliage may require high spatial acuity for navigating visually complex
172 environments. Therefore, scansorial species may benefit from the increased acuity that lens
173 pigmentation can provide. Little previous work on amphibian lens transmission has been
174 carried out. Recent surveys of spectral lens transmission among 36 of the approximately
175 7,370 living species of frogs reported similar variation to that observed among other
176 vertebrates, but perhaps surprisingly found no correlations with potential ecological drivers
177 of lens pigmentation, including activity pattern (Yovanovich et al., 2019, 2020).

178

179 The aims of the current study were to measure spectral lens transmission in an expanded
180 sample of ecologically and phylogenetically diverse amphibians in order to test several

181 explanatory hypotheses, including 1) that in amphibians, lens transmission is not driven by
182 ecology and species variation is primarily explained by phylogeny (Yovanovich et al., 2020),
183 2) that diurnal activity drives low shortwave lens transmission as an adaptation to bright
184 environments, and 3) that scansorial behaviour drives low shortwave lens transmission as
185 an adaptation to complex visual environments where high acuity is advantageous. We
186 measured ocular lens transmission in 79 species of frogs and six species of salamanders
187 distributed within 29 families, and, together with published data for 29 anuran species, used
188 phylogenetic comparative methods to test for ecological and evolutionary drivers of spectral
189 transmission in amphibian lenses.

190

191 **Materials and Methods**

192

193 ***Animals***

194

195 The spectral transmission (300–700 nm) of 171 lenses from 123 post-metamorphic
196 individuals belonging to 85 species of frogs ($n = 79$) and salamanders ($n = 6$) was examined
197 (Table 1). Both lenses from two tadpoles (*Dendropsophus minutus* and *Alytes muletensis*)
198 were also measured. Most animals were collected in the field, although 40 specimens
199 representing 27 species were obtained from commercial dealers or captive colonies as
200 noted in Table 1 (see also archived data), with ethical approval and collection permits from
201 relevant organizations for each site (see Acknowledgments for granting bodies and project
202 numbers). Animals were sacrificed by immersion in 5g/L Tricaine methanesulfonate (MS-
203 222) buffered with sodium bicarbonate to a pH of 7.0–7.5 for approximately 5–10 minutes.
204 Lenses were removed from enucleated eyes and frozen dry, then transported to City,

205 University of London (UK) on dry ice for scanning. In most cases, species were identified
206 based on morphology, but mitochondrial 16S rRNA molecular barcoding was used to assist
207 identifications for 19 specimens. Briefly, DNA was extracted from adult muscle or liver tissue
208 and tadpole tail snips, then universal 16S AR/BR primers were used to amplify and sequence
209 a ~580 bp fragment following Jameson et al. (2021). Sequences were aligned and cleaned
210 using Geneious Prime v.2019.2.3 and species identities confirmed using an initial NCBI
211 BLAST search of GenBank followed by reference sequence verification using primary
212 taxonomic literature. Spectral lens transmission data from fresh lenses of 29 additional
213 species of frogs were obtained from published literature (Yovanovich et al. 2019, 2020).

214

215 ***Transmission measurement***

216

217 Lenses were thawed, briefly rinsed in phosphate buffered saline (PBS) and placed in an
218 aluminium holder that fit into a cuvette mounted in front of an integrating sphere within a
219 Shimadzu 2101 UVPC spectrophotometer. The lenses were positioned within holes in the
220 aluminium blocks, ranging in diameter from 1.0–9.0 mm in 0.5 mm increments, so that they
221 were in line with the measurement beam of the spectrophotometer (Supplementary
222 Material S1). Several lenses were small (equatorial diameter <2mm; Table 1), so the scans
223 were sometimes noisy, in which case data from multiple scans per lens were averaged.

224

225 To further reduce noise, the average scan for each lens was smoothed by an 11-point
226 running average and transmission at 700 nm taken as 100%. The only exceptions were
227 lenses from four species (*Ambystoma mexicanum*, *Cynops pyrrhogaster*, *Lithobates pipiens*,
228 *Salamandra salamandra*), which were scanned from 300–500 nm as part of an earlier study

229 (Thorpe, 1991) and were normalised at 500 nm. Some lenses were slightly smaller than the
230 hole in the holder in which they were placed, especially those that were < 1 mm in
231 diameter. This resulted in some light bypassing the lens, so transmission did not reach zero
232 at 300 nm as it should, since wavelengths below this do not penetrate the structural
233 components of the lens (Douglas & Marshall, 1999). Thus, all scans were further adjusted so
234 that transmission at 305 nm was 0%. Lens transmission is expected to be high and
235 approximately spectrally flat at longer wavelengths (Douglas & Marshall, 1999), so scans
236 from lenses whose transmission at 450 nm was below 70% were assumed to be
237 compromised and excluded (see Supplementary Material S2).

238

239 Supplementary experiments indicated freezing and thawing had no effect on the spectral
240 transmission of *Rana temporaria* lenses (Supplementary Material S3). This view is supported
241 by the observation that there was little difference between the transmission spectra of
242 fresh lenses measured in a previous study (Yovanovich et al., 2020) and those measured
243 here in previously frozen lenses of the same species ($n = 7$, Supplementary Material S4).

244

245 Right and/or left lenses were scanned. When both lenses from the same animal were
246 scanned, there was no systematic difference between them and the adjusted curves from
247 each lens were averaged to find mean lens transmission for the specimen. Post-
248 metamorphic individuals sampled within a species were then averaged to represent the
249 spectral transmission of the lenses for that species. The only exception to this was *Hyllodes*
250 *phyllodes*, where a larger and a smaller individual were kept separate because they showed
251 substantially different transmission spectra (Fig. 1b).

252

253 The degree of shortwave transmission by a lens is expressed in this study by both the
254 wavelength of 50% normalized transmission (t50) and the total amount of UVA light
255 transmitted by the lens (%UVA). Although t50 is the traditional measure of lens
256 transmission, we believe the %UVA gives a more accurate representation of a lens's overall
257 transmission at short wavelengths (Supplementary Material S5).

258

259 ***Measurement of lens size***

260

261 The amount of shortwave radiation transmitted by a lens depends not only on the
262 concentration and identity of any shortwave-absorbing pigment within it but also on its axial
263 length (thickness/pathlength), with larger lenses transmitting less light (Douglas & Marshall,
264 1999). Thus, it is important to determine the dimensions of each lens as closely as possible.
265 Although aquatic tadpoles have lenses that are close to spherical, the lenses of terrestrial
266 adult amphibians are to some degree flattened, with the equatorial diameter exceeding the
267 axial length (Sivak et al., 1985). Because thawed amphibian lenses are somewhat fragile and
268 prone to lose their shape when manipulated, pathlength could not be measured directly,
269 but we were able to approximate equatorial lens diameter by the size of the hole in the
270 mounting holder in which the lens fit most closely without deformation (see above; Fig. S1).
271 Although the degree of asphericity of the adult amphibian lenses varies with species, the
272 equatorial diameter is never more than 22% greater than the axial dimension and is usually
273 significantly less than this (e.g. Sivak et al., 1985; Volonteri et al., 2017). Thus, any error
274 involved in estimating equatorial rather than axial diameter will be relatively modest.

275

276 ***Species traits***

277

278 Adult ecology was categorized into binary states for activity pattern and habitat using peer-
279 reviewed literature, online natural-history resources, field guides, and field observations: 1)
280 diurnal or non-diurnal, and 2) scansorial or non-scansorial (Table S1). Categorizations were
281 simplified versions of those used by Thomas et al. (2020). Species were classed as diurnal if
282 adults are primarily active in daylight above ground; crepuscular, cathemeral, and nocturnal
283 species were classified as non-diurnal. Species were categorized as scansorial if adults are
284 primarily found climbing among plants. Ideally, we would have scored species for visual
285 acuity directly, but such data are unavailable for the vast majority of amphibians (see
286 Discussion). Scansoriality was a readily scored trait that is likely reliant on visual acuity for
287 navigating complex environments and for precision jumping.

288

289 ***Phylogeny***

290

291 Comparative analyses used the phylogenetic hypothesis of Jetz and Pyron (2018), which
292 combined a molecular phylogeny of 56% of extant amphibians with taxonomic inference of
293 remaining species to produce a full amphibian species tree. Tip labels of the phylogeny were
294 matched to species in the dataset with AmphiNom v.1.0.1 in R (Liedtke, 2019), which uses
295 the Amphibian Species of the World online database (Frost, 2021) to match taxonomic
296 synonyms across datasets. The phylogeny was pruned to the species in the dataset and
297 polytomies were resolved randomly with the multi2di function in ape v.5.4.1 (Paradis et al.,
298 2004; Paradis & Schliep, 2019). Data were plotted alongside the phylogeny using ggtree
299 v.2.4.1 in R (Yu et al., 2016; R Core Team, 2019).

300

301 ***Testing ecological explanations of variation in lens transmission***

302 To test whether activity pattern or habitat are correlated with spectral lens transmission
303 while accounting for evolutionary relationships, the comparative approach of Freckleton et
304 al. (2002) was used. Phylogenetic linear models with maximum-likelihood (ML) estimation of
305 phylogenetic signal (λ) were implemented with caper v.1.0.1 in R to test for differences in
306 lens transmission (t50 and %UVA, separately) between binary ecological states (Orme et al.,
307 2018). These models did not include lens size, because previously published transmission
308 data did not include diameters of the lenses scanned (Yovanovich et al., 2019; 2020).
309 Species means for adult lens transmission measured in this study ($n = 85$) and from the
310 literature ($n = 29$) were both included in these models; however, because %UVA was
311 defined as total transmission from 315–400 nm, %UVA for 10 species from the literature
312 where transmission was not measured to 315 nm could not be calculated. This led to
313 differences in species sampling for models using t50 ($n = 114$) and those using %UVA ($n =$
314 104). Analyses exploring the relationship between lens size, spectral transmission, and
315 ecology can be found in the Supplementary Materials (S6).

316

317 ***Testing models of evolution across ecological groups***

318 To test whether species with different ecological phenotypes are under different selective
319 regimes for lens transmission, different models of evolution were fit to the lens transmission
320 data and compared. These models included only the post-metamorphic species data
321 collected in the present study ($n = 85$) so that lens size (approximating pathlength) could be
322 accounted for as a covariate, and included identical species sampling for t50 and %UVA.
323 First, ancestral states for each node of the phylogeny were estimated separately for activity
324 pattern (diurnal or non-diurnal) and habitat (scansorial or non-scansorial). Node states were

325 determined based on marginal likelihoods calculated from 99 stochastic character maps
326 constructed using make.simmap in phytools v.0.7.70 (Revell, 2012), with Q set to
327 “empirical” and an equal rates transition model. These reconstructions (Supplementary
328 Materials S7) were then used to fit four models of the evolution of lens transmission
329 (separately across activity patterns and habitats) with log lens size as a covariate in
330 mvMORPH v.1.1.4 (Clavel et al., 2015): 1) a single-parameter Brownian motion (BM) model
331 (BM1), 2) a single-parameter Ornstein-Uhlenbeck (OU) model (OU1), 3) a multiple-
332 parameter BM model (BMM), and 4) a multiple-parameter OU model (OUM). The multiple-
333 parameter models fit different parameter regimes to branches or clades reconstructed to
334 have different activity patterns or habitat preferences. The best-fit model was determined
335 by ranking Akaike information criterion (AIC) scores for each model, with a $\Delta AIC \geq 2$
336 between models considered a significant difference in model fits.

337

338 **Results**

339

340 ***Variation in spectral lens transmission across species***

341

342 Post-metamorphic spectral lens transmission among the 85 amphibian species measured
343 here varied substantially. The spectral transmission curves for the lenses of representative
344 species highlighting the diversity of shortwave transmission are shown in Fig. 1, and those of
345 all species measured are given in Supplementary Materials S8 (Figs. S8a–i). Table 1 gives
346 further details of all species studied.

347

348 The lenses of 52% of the species examined showed no evidence of substantial pigmentation,
349 displaying a smooth decline in transmission below ca. 350 nm and transmitting on average
350 56% of incident UVA (range 92%–37%). A few species' lenses (13%) contained high pigment
351 concentrations resulting in a sharp decline in transmission around 390–420 nm, with little
352 transmission of UVA (mean 12%, range 21%–2%). Between these two extremes, the
353 remaining lenses (35%) contained lesser amounts of shortwave-absorbing pigments,
354 resulting in an irregular decline in transmission in the UVA (mean 30%, range 55%–17%).

355

356 The most highly pigmented lenses were measured from a diurnal frog, *Hylodes phyllodes*,
357 with 2 mm diameter lenses. A smaller individual of the same species with 1 mm diameter
358 lenses still showed evidence of pigmentation but transmitted substantially more UVA light
359 (Fig. 1b, Table 1). We sampled more tadpole diversity than presented here, but due to the
360 small size of tadpole lenses (often < 0.5 mm) the scans were noisy, and most did not meet
361 our data quality standards (above). The two species of tadpoles successfully measured,
362 *Alytes muletensis* and *Dendropsophus minutus*, both had shortwave-transparent lenses with
363 no evidence of pigmentation. We also measured the transmission of adult *A. muletensis*
364 lenses, which were also unpigmented but removed slightly more short-wavelength radiation
365 than the tadpole lens, consistent with the increased pathlength of the larger lens (Fig. 1b).
366 Although a range of adult lens sizes were scanned in several other species, we observed no
367 changes in lens transmission relating to size within species or across species (Fig. S6).

368

369 ***Ecological explanations of shortwave lens transmission***

370

371 Both activity pattern and habitat had significant effects on shortwave transmission by
372 amphibian lenses (Figs. 2 & 3). Activity patterns differed significantly in t50 (PGLS: $F_{(1, 112)} =$
373 6.72 , $R^2_{\text{adj.}} = 0.05$, $\lambda = 0.78$, $p = 0.01$) but not %UVA (PGLS: $F_{(1, 102)} = 2.00$, $R^2_{\text{adj.}} = 0.01$, $\lambda =$
374 0.79 , $p = 0.16$), with the lenses of diurnal species ($n = 14$, $\mu = 366$, $SE = 15.1$) exhibiting
375 higher t50s than non-diurnal species ($n = 100$, $\mu = 347$, $SE = 7.5$; $t = 2.59$, $p = 0.01$). Diurnal
376 species ($n = 10$, $\mu = 46$, $SE = 12.6$) also had a lower mean %UVA than nondiurnal species ($n =$
377 94 , $\mu = 55$, $SE = 6.4$), though this difference was not statistically significant.

378

379 Habitats differed significantly in t50 (PGLS: $F_{(1, 112)} = 9.04$, $R^2_{\text{adj.}} = 0.07$, $\lambda = 0.82$, $p = 0.003$)
380 and %UVA (PGLS: $F_{(1, 102)} = 10.9$, $R^2_{\text{adj.}} = 0.09$, $\lambda = 0.73$, $p = 0.001$), with scansorial species
381 exhibiting higher t50s ($n = 42$, $\mu = 367$, $SE = 6.8$; $t = 3.01$, $p = 0.003$) and lower %UVAs ($n =$
382 37 , $\mu = 39$, $SE = 5.23$; $t = -3.30$, $p = 0.001$) than non-scansorial species (t50: $n = 72$, $\mu = 346$,
383 $SE = 13.8$; %UVA: $n = 67$, $\mu = 56$, $SE = 9.7$). Thus, the lenses from both scansorial and diurnal
384 species prevented more UV light from reaching the retina compared to non-diurnal and
385 non-scansorial animals.

386

387 ***Patterns of evolution across ecological groups***

388

389 Comparisons of neutral and non-neutral models of evolution suggested that, when
390 modelled together with lens size, species active at different times of day are under different
391 evolutionary regimes for the spectral transmission of their lenses. For t50, models that
392 allowed different evolutionary parameters for diurnal vs. non-diurnal species (BMM and
393 OUM) performed significantly better than those estimating a single parameter across all
394 species (BM1 and OU1; Table 2). While the OUM model had the lowest AIC, it was not

395 significantly better than the BMM ($\Delta AIC = 1.4$). The BMM model suggested that the rate of
396 neutral change (drift) is higher among diurnal species ($\sigma = 29.3$) than for nondiurnal species
397 ($\sigma = 3.7$), while the OUM model estimated a higher evolutionary optimum (θ) for t50 in
398 diurnal species ($\theta = 401$ nm) than for nondiurnal species ($\theta = 351$ nm). For %UVA among the
399 same species, the OU model allowing for different parameter estimates for diurnal and non-
400 diurnal species (OUM) performed significantly better than the three other models ($\Delta AIC >$
401 9). This model estimated a lower optimum for UVA transmission in diurnal species ($\theta =$
402 22.1%) than in non-diurnal species ($\theta = 50.5\%$).

403

404 Similar modelling of evolution across habitats consistently suggested that scansorial and
405 non-scansorial species are under different selective regimes for spectral lens transmission.
406 For t50, the OUM model was the best fit ($\Delta AIC > 12$), with a higher estimated optimum t50
407 for scansorial species ($\theta = 396$ nm) than for non-scansorial species ($\theta = 352$ nm). Likewise,
408 for %UVA the OUM model was best supported, with a lower optimum for UVA transmission
409 in scansorial species ($\theta = 22.3\%$) than in non-scansorial species ($\theta = 51.2\%$).

410

411 **Discussion**

412

413 Among vertebrates, the spectral properties of ocular lenses determine how much
414 shortwave light is transmitted to the retina, with implications for visual sensitivity and
415 spatial acuity (Douglas and Marshall, 1999). Yet, no study to date has found evidence for
416 correlated evolution of lens transmission and any aspect of ecology. This study generated
417 new data for spectral lens transmission across ecologically and phylogenetically diverse
418 amphibians and found evidence for ecologically driven patterns of lens transmission. Both

419 diurnal and scansorial ecologies were associated with decreased shortwave lens
420 transmission across sampled species. Further, evidence suggests that species active at
421 different times of day or utilizing different habitats are under different selective regimes for
422 the spectral transmission of their lenses. The evolutionary context and functional
423 implications of these findings for vertebrate vision are discussed below.

424

425 ***Shortwave filtering in the lenses of diurnal species***

426

427 Amphibians active primarily during the day transmitted less shortwave light through their
428 lenses than non-diurnal (catheameral, crepuscular, or nocturnal) species. This difference was
429 significant when shortwave transmission was summarized by the wavelength at which
430 transmission falls to 50% (t50), but not when quantified by the proportion of UVA light that
431 passed through the lens (%UVA). This was likely due to reduced species sampling, because
432 4/14 diurnal species in the comparison of t50 had to be dropped from the comparison of
433 %UVA due to lack of available data (see Methods); indeed, with the same four species
434 dropped from analysis of t50 we had less evidence for the difference ($p = 0.06$). A previous
435 study on frogs did not find any correlation between lens transmission (quantified by t50)
436 and activity period with eight diurnal species in a sample of 37 species (Yovanovich et al.,
437 2020). High variation in lens transmission among species and small sample sizes driven by
438 the relative rarity of diurnality among amphibians (Anderson & Wiens, 2017), as well as the
439 difficulty of scoring complex behavioural traits into discrete bins, may make correlations
440 with activity pattern difficult to detect in small samples.

441

442 Diurnal amphibians will benefit from lenses that reduce shortwave transmission because
443 this will both reduce light-induced retinal toxicity (Glickman, 2011; Van Norren & Vos, 2016)
444 and improve image quality by removing those wavelengths most prone to chromatic
445 aberration and scattering (Douglas & Marshall, 1999), while involving minimal costs from
446 the reduced photon capture in their well-lit environments. In contrast, non-diurnal species
447 will benefit from unpigmented ocular media, as the fundamental requirement of the visual
448 system is to capture enough photons to activate the photoreceptors. Thus, amphibians
449 living in photon-limited environments, such as experienced by nocturnal species, will benefit
450 from ocular media that are as transparent as possible.

451

452 As in other vertebrates, low shortwave transmission was not observed in all diurnal
453 amphibians sampled. For example, among diurnal dart frogs (family Dendrobatidae),
454 *Dendrobates leucomelas* had shortwave-transparent lenses, while congener *D. auratus* had
455 highly pigmented, shortwave-filtering lenses. Similarly, *Atelopus varius* was the only diurnal
456 toad (family Bufonidae) in our dataset but it had more shortwave-transmissive lenses than
457 the seven other, non-diurnal toads. If lens pigmentation is serving a protective function in
458 species occupying bright habitats, then one might predict that the amount of UV radiation
459 present in the environment could exert selection on the spectral characteristics of lenses. A
460 previous study on 37 frogs found no associations between elevation or latitude (two proxies
461 for UV light) and lens transmission (Yovanovich et al., 2020). Further study is needed to
462 disentangle the relative importance of shortwave-induced retinal toxicity and sensitivity in
463 the evolution of shortwave-filtering lenses among frogs.

464

465 ***Shortwave filtering in the lenses of scansorial species***

466

467 The lenses of scansorial amphibians, which are primarily found climbing in trees and other
468 plants, transmit significantly less shortwave light than those of species associated with
469 ground or water habitats. Scansorial behaviour has evolved multiple times in frogs and is
470 repeatedly associated with low shortwave lens transmission. Evidence for shortwave-
471 absorbing lens pigmentation was seen across major radiations of tree frogs, such as
472 hyperoliids (e.g. *Hyperolius riggenbachi*), rhacophorids (e.g. *Polypedates leucomystax*),
473 centrolenids (e.g. *Cochranella granulosa*), and hylids (e.g. *Boana faber*, Figs. 2 & S8). Among
474 41 scansorial frogs measured to date, only four have lenses that transmit more than half of
475 incident UVA. While indirect, this evidence is consistent with the hypothesis that increased
476 acuity is adaptive for climbing species.

477

478 Because chromatic aberration and Rayleigh scattering are both greatest at short
479 wavelengths (Douglas & Marshall, 1999; Cronin & Bok, 2016), the removal of this part of the
480 spectrum by the lens may serve to increase spatial acuity in scansorial amphibian species.
481 Climbing species tend to inhabit more complex 3-dimensional worlds compared to, for
482 example, ground-dwelling animals, so high spatial acuity likely benefits several aspects of
483 their visually guided behaviours. A tree frog, for example, might need to negotiate narrow
484 twigs or make potentially precarious leaps to avoid a predator, capture prey, or simply move
485 from one location to another to encounter a mate. Such movements would be aided by the
486 ability to see fine detail.

487

488 High degrees of lens pigmentation have previously been observed alongside increased
489 spatial acuity in non-amphibian vertebrates. For example, lens pigmentation is associated

490 with high cone densities (a prerequisite for high spatial acuity) in mammals (Douglas &
491 Jeffrey, 2014), with high-acuity aerial hunting in birds (Lind et al., 2014), and with visual
492 predation in snakes (Simões et al., 2016). If lenses that filter out short wavelengths do
493 indeed serve to improve the spatial resolution of the retinal image for scansorial
494 amphibians, one might expect such species to have high acuities.

495

496 Spatial acuity is most reliably determined by behavioural means, which has been
497 investigated in few amphibians (Birukow, 1937; Manteuffel & Himstedt, 1978; Aho, 1996).
498 However, because the ability of vertebrate eyes to resolve spatial detail depends largely on
499 the focal length of the eye and the density of retinal neurons (Land & Nilsson, 2012), these
500 can be used to calculate a theoretical spatial resolving power for a species, which often
501 closely matches that determined by behavioural means (Pushchin, 2019). Large eyes and an
502 increased density of cones and retinal ganglion cells potentially result in higher spatial
503 resolution. Unfortunately, such anatomically based estimates of spatial resolution have only
504 been calculated for four anuran species (Pushchin, 2021 for review) and ganglion cell
505 density alone has been determined in an additional eight (Pushchin, 2019 for review). From
506 these limited behavioural and morphological data, maximum resolving power was highest in
507 a scansorial hylid, *Boana (Hyla) raniceps* (Bousfield & Pessoa, 1980).

508

509 Thus, it is congruent that the lenses of scansorial species tend to absorb more shortwave
510 radiation than those of non-scansorial amphibians (Fig. 3) and the highest theoretical spatial
511 acuity of any amphibian was also calculated for a tree frog. Furthermore, scansorial frogs
512 tend to have large relative eye size compared to other frogs (Thomas et al., 2020),
513 potentially elevating spatial resolving power. All this is consistent with shortwave-filtering

514 lenses being an adaptation for increased spatial resolution in scansorial amphibians.
515 However, not all scansorial species have lenses that filter out shortwave light (e.g.
516 *Phyllomedusa vaillantii*, Figs. 2 & S8g, Table 1), nor do they always have higher maximum
517 ganglion cell densities than non-scansorial amphibians (Pushchin, 2019, 2021). Such
518 variability in the relationship between lens transmission and acuity among scansorial species
519 suggests no single explanation will account for the distribution of shortwave-filtering lenses
520 in frogs.

521

522 One explanation for this might be that most amphibians, including the scansorial species in
523 our dataset, are nocturnal, and lenses that block short wavelengths come with the cost of
524 reduced absolute sensitivity for those species that are active in low light. Thus, some
525 scansorial species may benefit more from the increased acuity afforded by a shortwave-
526 absorbing lens, while for others the enhanced sensitivity resulting from a clearer lens will be
527 at a premium. Morphological and behavioural study of acuity in tree frogs would be an
528 interesting realm for future study and would allow for a direct examination of whether
529 scansorial frogs with pigmented lenses have higher spatial acuity.

530

531 ***Evidence for selection***

532

533 Comparisons of different evolutionary models suggested that the evolution of shortwave
534 lens transmission differs across habitats and activity periods in amphibians. Scansorial frogs
535 appeared to be under selection for increased lens pigmentation, whereas non-scansorial
536 species were under selection for more transparent lenses. Diurnal and non-diurnal species
537 also showed consistent differences in evolutionary dynamics for lens transmission, but it

538 was less clear whether this resulted from an increased rate of neutral change (drift) or
539 selection for reduced shortwave transmission in diurnal species. To our knowledge this is a
540 first attempt to examine the evolutionary processes driving lens pigmentation, but the use
541 of complex Ornstein-Uhlenbeck models compared via likelihood can be prone to error in
542 small datasets and should be interpreted with caution (Cooper et al., 2016). As spectral
543 transmission is measured in more species, it will be appropriate to undertake larger
544 macroecological studies disentangling the ecological and functional drivers of patterns
545 within and across diverse groups. Future work might examine whether selection for acuity,
546 sensitivity, and retinal protection shape patterns of shortwave-absorbing lens pigmentation
547 across amphibians and other vertebrate groups.

548

549 ***Lenticular pigments***

550

551 The variation in shortwave lens transmission we observed across amphibians is almost
552 certainly due to the presence of shortwave-absorbing pigments in the lenses of many
553 species, and their absence in others. The identities of the lenticular pigments responsible for
554 shortwave-filtering in vertebrates are known in detail only for mammals and fishes. In most
555 mammals (Douglas & Marshall, 1999 for review; Haines et al., 2006; Douglas & Jeffery,
556 2014) and some fishes (Truscott et al., 1992; Thorpe et al., 1992) they have been identified
557 as kynurenine-related tryptophan derivatives. By contrast, most shortwave-filtering in fish
558 lenses is performed by a series of mycosporine-like amino acids (Dunlap et al., 1989; Thorpe
559 et al., 1993). For other groups of vertebrates, the identity of the lens pigments is largely
560 unknown, although an unidentified pigment with an absorption maximum around 345–347
561 nm has been extracted from the lenses of the frog *Lithobates pipiens* (Kennedy & Milkman,

1956; Thorpe, 1991). Although pigments were not extracted from the lenses in this study, their absorption maxima can be approximately determined when present in low concentrations because they result in an irregular decline in lens transmission at short wavelengths. Although most such pigments, in agreement with the pigment found in *L. pipiens*, absorb maximally around 350 nm (e.g. *Aplastodiscus leucopygius* & *Scinax rizibilis*, Figs. 1a & S8), it seems likely that, as in fishes, pigments absorbing most strongly at longer wavelengths may also be involved in some species (e.g. *Hylodes phyllodes*, Figs. 1b & S8). Future study of the identity, spectral characteristics, and distribution of lens pigments would shed light on how this trait has evolved across amphibians and among diverse vertebrates.

Implications for sensitivity to short wavelengths

Visual sensitivity at short wavelengths, including in the UV, is widespread among both vertebrates and invertebrates and can serve to increase overall sensitivity and extend the spectral range of vision or contribute to more specific visual tasks such as navigation, mate choice, foraging, predator and prey detection, or communication (Cronin & Bok, 2016). There is limited behavioural (Dietz, 1972; Przyrembel et al., 1995; Han et al., 2007; Secondi et al., 2012; Kats et al., 2012) and electrophysiological (Govardovskii & Zueva, 1974; La Touche & Kimeldorf, 1979; Perry & McNaughton, 1991; Deutschlander & Phillips, 1995; Martin et al., 2016) evidence thus far that some amphibians are also sensitive in this part of the spectrum. Representatives of all classes of vertebrate (Bowmaker, 2008), including amphibians (Ma et al., 2001; Korenyak & Govardovskii, 2013; Mège et al., 2016; Martin et al., 2016; Secondi et al., 2017), have visual pigments with maximum sensitivity (λ_{\max}) in the UV, although among amphibians, only salamanders have been demonstrated to have cones

586 with a UV λ_{\max} , whereas the shortest wavelength cones identified in frogs all have maximum
587 sensitivity in the violet (Donner & Yovanovich, 2020).

588

589 Not having a visual pigment with a λ_{\max} in the UV, however, does not preclude UV-
590 sensitivity. The range of wavelengths absorbed by visual pigments is broad, and all visual
591 pigments have a secondary absorption maximum in the UV (the cis-peak or β -band).

592 Therefore, as long as a species has ocular media that are transparent to short wavelengths,
593 they will be sensitive to light in this part of the spectrum even in the absence of a visual
594 pigment with a λ_{\max} in the UV (Douglas & Jeffery, 2014). With the exception of a few fish
595 corneas, within vertebrates the lens always absorbs more short wavelengths than the
596 cornea or aqueous and vitreous humours (Douglas & Marshall, 1999). Thus, because most
597 amphibian lenses transmit substantial amounts of UVA radiation, these wavelengths will
598 impinge on the retinae of many species, resulting in sensitivity to the UVA portion of the
599 spectrum. This suggests that UVA light may have important roles and consequences for
600 visual ecology and evolution across amphibians. We hope the transmission spectra provided
601 in this manuscript will be of value to providing a more thorough understanding of colour
602 vision in amphibians once more visual pigment data become available.

603

604 ***Diversity and ontogeny***

605

606 This study produced some of the first measurements of lens transmission in salamanders,
607 but only six species were sampled and all showed substantial shortwave lens transmission.

608 Salamanders are rarely diurnal as adults, but we did sample a diurnal terrestrial eft of

609 *Notophthalmus viridescens* and saw no evidence of lens pigmentation or difference from

610 adult transmission spectra. Further study of salamanders, including scansorial species (e.g.
611 several plethodontids: Waldron & Humphries, 2005; Capshaw et al. 2019), is needed to
612 make any generalizations about lens transmission in this group. Lens transmission data are
613 unavailable for the third major lineage of extant amphibians, the mostly fossorial caecilians
614 (Order Gymnophiona), though they have reduced visual systems and we expect their lenses
615 to lack filtering pigments. Many amphibians have distinct life stages, and anurans are known
616 to exhibit changes in visual systems at metamorphosis (e.g. Sivak et al., 1985; Shrimpton et
617 al., 2021; Schott et al., 2021). Although we found that the tadpoles of two frog species had
618 shortwave-transparent lenses, the adult stage of *Alytes muletensis* also had shortwave-
619 transparent lenses (Fig. 1b). It is unknown how lens pigmentation may change across life
620 stages in most vertebrates (except fishes: Thorpe & Douglas, 1993), and this could have
621 important implications for colour vision, acuity, and sensitivity through ontogeny. Continued
622 study of the visual systems (lens transmission, acuity, sensitivity, spectral sensitivity),
623 behaviours, and natural histories of diverse vertebrates will make larger-scale and more
624 powerful macroevolutionary studies of their visual evolution possible.
625

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856 **Tables and Figures**

857 **Table 1. Summary transmission data for species in the current study.** Shown are: taxonomic
 858 information according to Frost (2021; Or. = Order, A = Anura, C = Caudata), number of animals and
 859 lenses scanned for each species, the approximate equatorial diameters (diam) of the lenses, and the
 860 species means for transmission at 450 nm (t450), the wavelength of 50% transmission (t50), and the
 861 amount of UVA transmitted (%UVA). All data are derived from scans normalised at 700 nm except
 862 for *Ambystoma mexicanum*, *Cynops pyrrhogaster*, *Lithobates pipiens*, and *Salamandra salamandra*,
 863 where scans began and were normalised at 500 nm. *Hylodes phyllodes* is listed twice, because lenses
 864 from differently sized individuals produced different results at short wavelengths. Scans are from
 865 post-metamorphic individuals unless specified (tad = tadpole). Species are ordered by %UVA
 866 transmitted by the lens. An asterisk (*) indicates data collected from specimens originating from
 867 commercial dealers or captive colonies; all other data are from wild-caught animals.

Or.	Family	Species	lens <i>n</i>	animal <i>n</i>	diam (mm)	450t	t50	%UVA
A	Hylodidae	<i>Hylodes phyllodes</i>	1	1	2.0	84.6	423	2.0
A	Hyperoliidae	<i>Hyperolius riggenbachi</i> *	2	2	2.0	84.9	407	5.7
A	Hylidae	<i>Boana faber</i>	1	1	5.5	82.3	399	6.1
A	Hylidae	<i>Osteopilus septentrionalis</i> *	2	1	3.0	85.0	397	10.3
A	Mantellidae	<i>Boophis erythrodactylus</i>	2	1	3.0	80.6	396	12.9
A	Hylidae	<i>Acris blanchardi</i>	5	4	1.5	89.6	394	13.3
A	Hylidae	<i>Osteocephalus taurinus</i>	6	4	3.5-5.0	78.2	395	14.4
A	Ranidae	<i>Rana temporaria</i>	5	4	2.0-4.0	97.5	392	15.2
A	Ranidae	<i>Lithobates pipiens</i> *	16	8	2.7-3.9	99.9	392	15.4
A	Rhacophoridae	<i>Polypedates leucomystax</i> *	1	1	4.0	91.3	391	16.6
A	Hylidae	<i>Dendropsophus minusculus</i>	2	1	1.5-2.0	69.9	402	17.7
A	Hylidae	<i>Boana calcarata</i>	1	1	3.0	77.0	398	18.5
A	Hylidae	<i>Scinax cruentomma</i>	1	1	2.5	72.2	397	20.6

A	Hylidae	<i>Boana diabolica</i>	2	1	2.5-3.0	70.0	396	20.9
A	Hylidae	<i>Bokermannohyla circumdata</i>	1	1	4.0	89.9	389	20.9
A	Mantellidae	<i>Mantella betsileo*</i>	2	1	1.5	78.7	389	21.2
A	Hylidae	<i>Boana cinerascens</i>	2	1	2.0	80.1	389	21.8
A	Hylidae	<i>Scinax rizibilis</i>	1	1	2.5	93.3	388	22.8
A	Dendrobatidae	<i>Epipedobates tricolor*</i>	1	1	1.0	79.8	413	23.1
A	Hyperoliidae	<i>Heterixalus alboguttatus*</i>	1	1	1.5	88.0	397	23.5
A	Leptodactylidae	<i>Leptodactylus rhodomystax</i>	2	1	2.5	71.4	397	24.1
A	Hylidae	<i>Boana pardalis</i>	1	1	4.5	89.4	387	25.2
A	Leptodactylidae	<i>Leptodactylus knudseni</i>	2	1	6.0-7.0	78.6	380	25.7
A	Craugastoridae	<i>Pristimantis zeuctotylus</i>	2	1	2.5	74.5	388	25.9
A	Hylidae	<i>Scinax hayii</i>	1	1	3.5	88.2	387	26.6
A	Hyperoliidae	<i>Hyperolius fusciventris*</i>	2	1	1.5-2.0	86.8	394	29.2
A	Hylidae	<i>Aplastodiscus leucopygius</i>	1	1	2.5	89.5	385	30.5
A	Megophriidae	<i>Megophrys nasuta*</i>	1	1	1.5	92.3	374	30.5
A	Ranidae	<i>Lithobates sphenoccephalus</i>	2	1	3.5	87.7	381	33.1
A	Hylidae	<i>Dendropsophus leucophyllatus</i>	5	4	1.5-3.0	75.4	384	33.7
C	Plethodontidae	<i>Batrachoseps attenuatus</i>	3	2	1.0	73.3	360	34.4
A	Alytidae	<i>Alytes cisternasii*</i>	2	1	1.5	76.0	380	37.1
A	Leptodactylidae	<i>Leptodactylus fuscus</i>	1	1	3.0	88.4	370	37.2
A	Hylidae	<i>Boana punctata</i>	1	1	1.5	80.7	379	37.8
A	Mantellidae	<i>Mantella viridis*</i>	1	1	1.5	77.0	368	37.9
A	Hylidae	<i>Scinax boesemani</i>	1	1	1.5	85.2	376	41.5
A	Leptodactylidae	<i>Leptodactylus podicipinus</i>	1	1	2.5	79.1	365	42.2
A	Hylidae	<i>Dryophytes cinereus</i>	1	1	2.5	76.0	370	42.5
A	Bufonidae	<i>Rhaebo guttatus</i>	2	1	8.0	78.3	365	43.4
A	Bufonidae	<i>Rhinella marina</i>	2	1	3.5	79.3	362	43.9
A	Leptodactylidae	<i>Lithodytes lineatus</i>	4	2	2.5-3.0	81.4	366	44

A	Bufonidae	<i>Sclerophrys maculata*</i>	2	1	3.0	86.7	359	44.1
A	Leptodactylidae	<i>Leptodactylus pentadactylus</i>	3	2	2.5-6.0	81.1	364	45.6
A	Craugastoridae	<i>Pristimantis chiastonotus</i>	1	1	1.0	74.6	364	46.3
A	Centrolenidae	<i>Vitreorana uranoscopa</i>	1	1	2.0	89.1	361	46.6
A	Ceratophryidae	<i>Lepidobatrachus laevis*</i>	1	1	2.0	94.2	359	47.6
A	Centrolenidae	<i>Hyalinobatrachium cappellei</i>	5	4	1.0-1.5	80.7	359	47.9
A	Brachycephalidae	<i>Brachycephalus rotenbergae</i>	1	1	1.0	91.7	356	48.6
A	Eleutherodactylidae	<i>Eleutherodactylus marnockii</i>	1	1	2.0	76.0	355	49.3
A	Scaphiopodidae	<i>Spea bombifrons</i>	2	1	3.5	82.0	349	49.3
A	Microhylidae	<i>Gastrophryne olivacea</i>	1	1	1.0	76.2	349	49.4
A	Hylidae	<i>Itapotihyla langsdorffii</i>	1	1	2.0	98.6	368	49.9
A	Leptodactylidae	<i>Physalaemus cuvieri</i>	1	1	1.5	87.6	360	50.0
A	Scaphiopodidae	<i>Spea multiplicata</i>	1	1	4.0	89.9	354	50.6
A	Alytidae	<i>Alytes muletensis*</i>	2	1	1.0	77.8	351	51.5
A	Hylidae	<i>Boana polytaenia</i>	1	1	2.5	91.6	365	51.5
A	Ceratobatrachidae	<i>Cornufer guentheri*</i>	1	1	4.0	90.5	358	52.8
A	Pipidae	<i>Xenopus laevis*</i>	5	3	2.0	89.4	352	52.9
A	Rhinophrynidae	<i>Rhinophrynus dorsalis</i>	4	3	1.0-1.5	81.9	349	53.5
A	Phyllomedusidae	<i>Phyllomedusa vaillantii</i>	2	1	4.0	85.1	348	54.6
A	Dendrobatidae	<i>Dendrobates leucomelas*</i>	2	1	1.0	89.1	326	54.8
A	Phyllomedusidae	<i>Phyllomedusa distincta</i>	1	1	4.5	93.8	362	55.2
A	Microhylidae	<i>Phrynomantis microps*</i>	2	1	2.0	85.2	343	56.7
C	Salamandridae	<i>Notophthalmus viridescens</i>	3	3	1.0	85.1	347	57.1
A	Hemisotidae	<i>Hemisus marmoratus*</i>	1	1	1.5	84.9	345	57.3
A	Ascaphidae	<i>Ascaphus truei</i>	3	2	2.5-3.0	91.0	349	57.5
A	Microhylidae	<i>Chiasmocleis shudikarensis</i>	2	2	1.0	83.3	341	58.2
A	Microhylidae	<i>Kaloula pulchra*</i>	4	3	2.0-3.0	84.0	339	58.5
A	Scaphiopodidae	<i>Scaphiopus couchii</i>	2	2	4.5-5.0	86.7	342	59.4

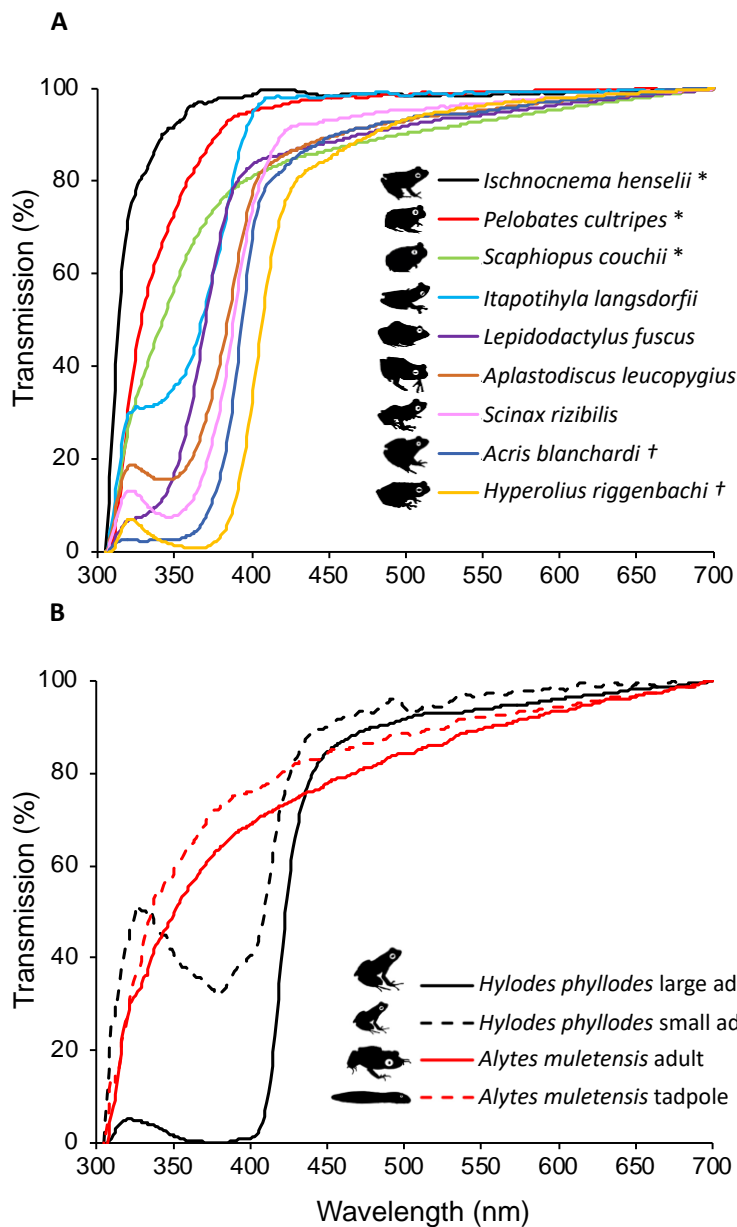
A	Alytidae	<i>Discoglossus galganoi</i>	2	1	2.0	92.0	346	59.6
C	Salamandridae	<i>Lissotriton vulgaris</i>	2	1	1.0	87.5	342	59.9
A	Hyperoliidae	<i>Kassina senegalensis*</i>	1	1	3.0	97.0	348	60.1
A	Leptodactylidae	<i>Adenomera andreae</i>	1	1	1.0	86.8	337	63.4
A	Bufo	<i>Rhinella icterica</i>	1	1	6.5	92.0	338	63.5
A	Microhylidae	<i>Glyphoglossus guttulatus*</i>	1	1	2.5	91.1	337	64.3
A	Bufo	<i>Bufo bufo</i>	1	1	3.5	98.0	341	66.8
A	Brachycephalidae	<i>Ischnocnema parva</i>	1	1	1.0	83.4	322	67.3
A	Odontophrynidae	<i>Proceratophrys boiei</i>	1	1	1.0	91.5	332	69.2
A	Microhylidae	<i>Dyscophus guineti*</i>	1	1	2.0	93.4	331	69.9
C	Ambystomatidae	<i>Ambystoma mexicanum*</i>	1	1	1.6	99.4	318	73.4
A	Pelobatidae	<i>Pelobates cultripes</i>	2	1	4.0	97.9	328	73.6
A	Craugastoridae	<i>Haddadus binotatus</i>	1	1	1.5	98.3	327	73.7
C	Salamandridae	<i>Salamandra salamandra*</i>	2	1	1.9-2.0	97.1	317	73.9
C	Salamandridae	<i>Cynops pyrrhogaster*</i>	2	1	0.8-0.9	97.2	318	74.0
A	Brachycephalidae	<i>Ischnocnema henselii</i>	1	1	2.0	98.2	314	91.5
A	Hylodidae	<i>Hylodes phyllodes</i> (small)	1	1	1.0	90.0	409	40.8
A	Hylidae	<i>Dendropsophus minutus</i> (tad)	2	1	1.0	74.0	359	48.2
A	Alytidae	<i>Alytes muletensis</i> (tad)	2	1	1.0	84.4	336	59.3

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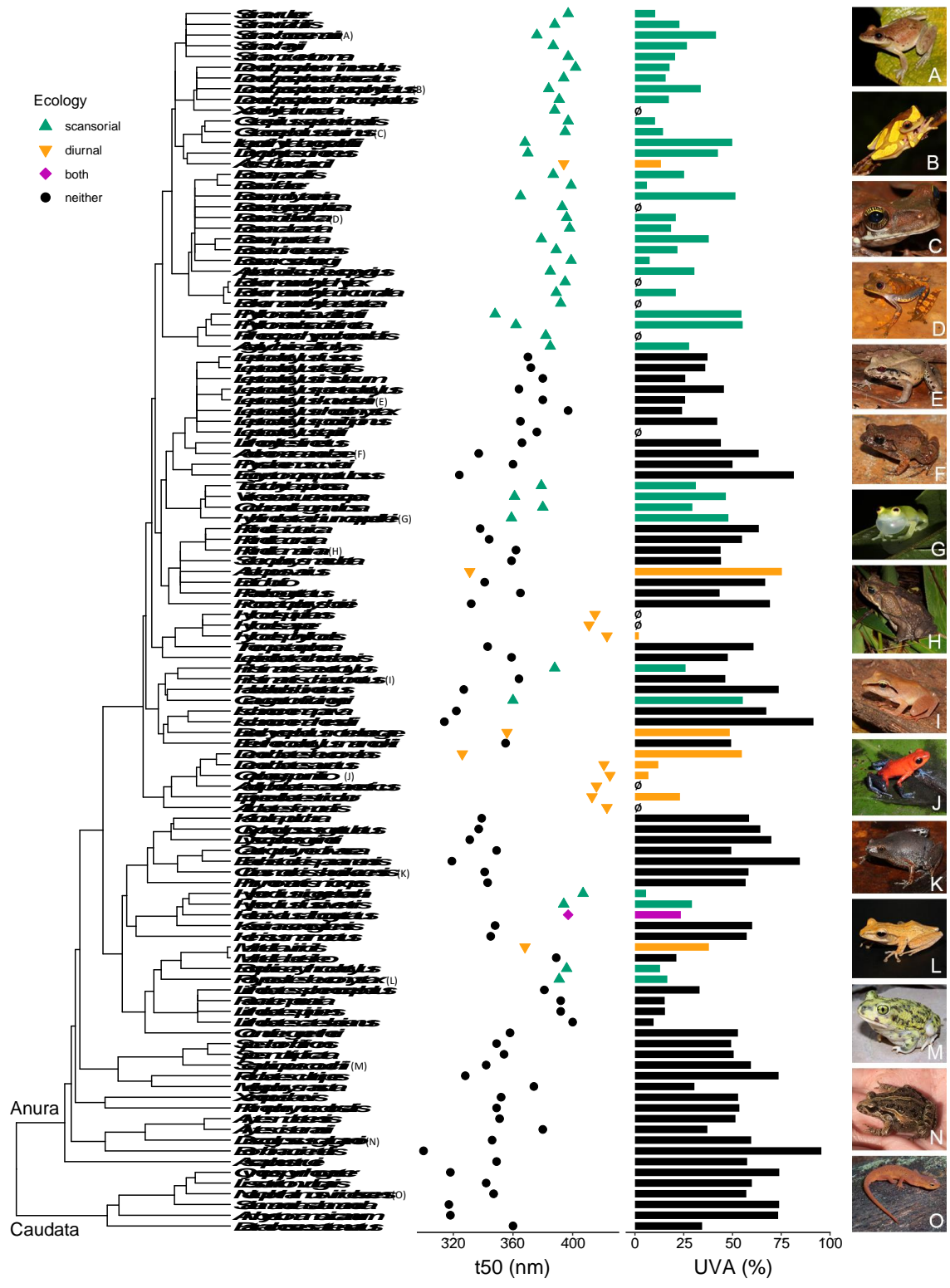
870 **Table 2. Model comparisons for the evolution of lens transmission.** AIC scores comparing models of
 871 the evolution of spectral lens transmission across binary ecological regimes, with lens diameter as a
 872 covariate. Models included neutral evolution with a single parameter across binary regimes (BM1),
 873 neutral evolution with multiple parameters (BMM), adaptive evolution with a single parameter
 874 across regimes (OU1) and adaptive evolution with different parameters across regimes (OUM). Best-
 875 fit model(s) are indicated in bold.

		t50				%UVA			
		BM1	BMM	OU1	OUM	BM1	BMM	OU1	OUM
Habitat	AIC	744.8	744.5	744.3	731.8	713.3	714.4	705.3	695.8
	Δ AIC	13	12.7	12.5	0	17.5	18.6	9.5	0
Activity period	AIC	744.8	732.8	744.3	731.4	713.3	711.1	705.2	695.9
	Δ AIC	13.4	1.4	12.9	0	17.4	15.2	9.3	0



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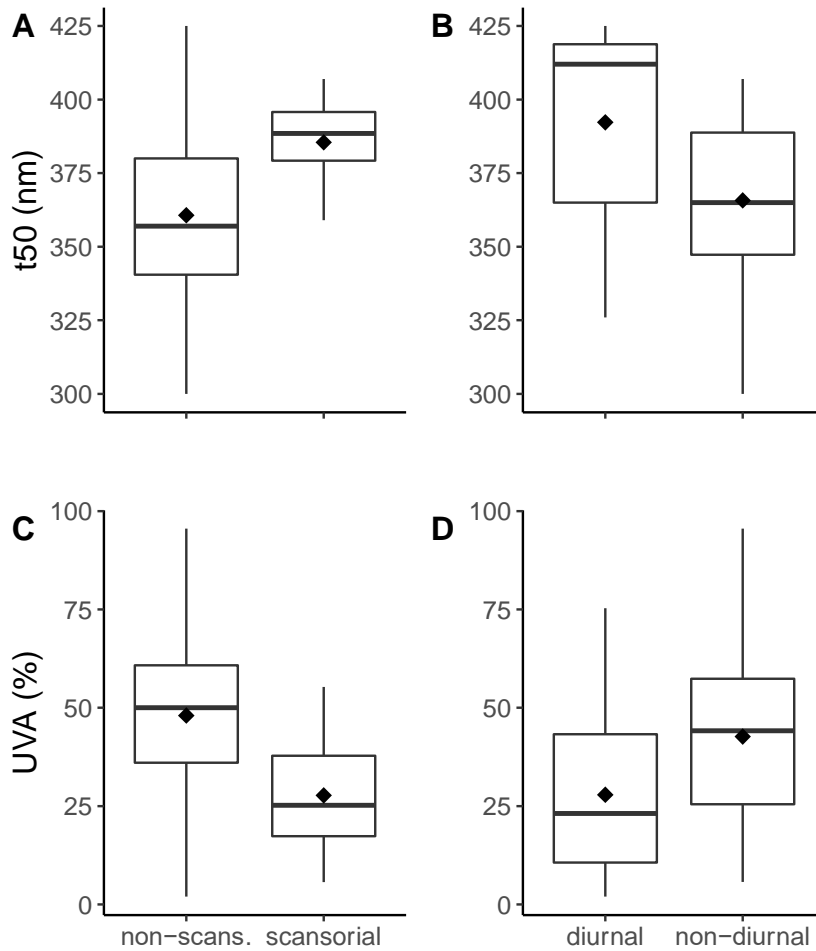
880 **Figure 1.** Representative examples of the spectral transmission of amphibian lenses. (A) Shortwave
 881 lens transmission varies across different species of anurans; * denotes an unpigmented (shortwave-
 882 transparent) lens, and † the presence of high concentrations of shortwave-absorbing pigment in the
 883 lens. The other species had lower levels of lens pigmentation. (B) Lens transmission in two *Hylodes*
 884 *phyllodes* males, one large adult (SVL = 34.4 mm) with a ca. 2 mm equatorial lens diameter, and one
 885 small adult (SVL = 29.1 mm) with a ca. 1 mm lens diameter, as well as a comparison of lens
 886 transmission in an adult and a tadpole of *Alytes muletensis*.



887

888 **Figure 2.** A phylogeny of frogs and salamanders used in tests for associations between ecology and
 889 lens transmission, with the dot plot depicting the wavelength of 50% transmission (t50) and the bar
 890 plot showing the proportion of UVA light transmitted (%UVA). Species taken from the literature
 891 (Yovanovich et al., 2019, 2020) and lacking data for %UVA because transmission spectra were not

892 measured to 315nm are denoted by \emptyset . Alphabetic annotations following species names correspond
893 to photographs of specimens taken by J. Streicher (H, J, L-O) and C. Cox (A-G, I, K). Both scansorial
894 and diurnal species show significantly lower shortwave transmission than non-scansorial or non-
895 diurnal amphibians.



896

897 **Figure 3.** Boxplots of wavelength of 50% transmission (t50) and amount of UVA light transmitted
 898 (%UVA) for species across ecological categories for adult habitat (scansorial or non-scansorial; A,C)
 899 and activity pattern (diurnal or non-diurnal; B,D). Filled points indicate data collected for this study,
 900 and open points show data compiled from published literature. Black diamonds indicate the mean
 901 and black horizontal bars the median for each group.

902

903 **Supporting Information**

904 Additional supporting information may be found in the online version of this article.

905 S1. Spectrophotometry setup

906 S2. Artefactual decline in transmission from long to short wavelengths

907 S3. Effect of freezing on lens transmission

908 S4. Comparison of transmission data across studies

909 S5. Quantification of lens spectral transmission

910 S6. Lens size, ecology, and spectral transmission

911 S7. Ancestral state estimations of activity period and habitat

912 S8. Spectral transmission of lenses from all species examined

913 Table S1. Categorical assignment of adult species ecology

914 Supplementary References