

1 **Gene loss and relaxed selection of *plaat1* in vertebrates adapted to low-light environments**

2 Danielle H. Drabeck¹, Jonathan Wiese¹, Erin Gilbertson², Jairo Arroyave³, Dahiana Arcila⁴, S. Elizabeth
3 Alter⁵, Richard Borowsky⁶, Dean Hendrickson⁷, Melanie Stiasny⁸, and Suzanne E. McGaugh¹

4 ¹Department of Ecology, Evolution and Behavior, University of Minnesota Twin Cities, 1475 Gortner Ave, St. Paul, MN 55108 ²University of San
5 Francisco, Department of Epidemiology and Biostatistics, University of California, San Francisco, CA ³Instituto de Biología, Universidad Nacional
6 Autónoma de México (UNAM), Ciudad de México, México ⁴Marine Vertebrate Collection, Scripps Institution of Oceanography, University of
7 California San Diego, La Jolla, California, 92093, USA ⁵California State University Monterey Bay, Biology and Chemistry Department, Chapman
8 Academic Science Center, Seaside, CA ⁶Department of Biology, New York University, Washington Square, New York, NY, 10003, USA
9 ⁷Biodiversity Center, Texas Natural History Collections, University of Texas at Austin, Austin, TX 78758, United States ⁸Department of
10 Ichthyology, American Museum of Natural History, New York, NY 10024, USA

11 Gene loss is an important mechanism for evolution in low-light or cave environments where visual
12 adaptations often involve a reduction or loss of eyesight. The *plaat* gene family are phospholipases
13 essential for the degradation of organelles in the lens of the eye. They translocate to damaged organelle
14 membranes, inducing them to rupture. This rupture is required for lens transparency and is essential for
15 developing a functioning eye. *Plaat3* is thought to be responsible for this role in mammals, while *plaat1*
16 is thought to be responsible in other vertebrates. We used a macroevolutionary approach and
17 comparative genomics to examine the origin, loss, synteny, and selection of *plaat1* across bony fishes
18 and tetrapods. We show that *plaat1* (likely ancestral to all bony fish + tetrapods) has been lost in
19 squamates and is significantly degraded in lineages of low-visual acuity and blind mammals and fish. Our
20 findings suggest that *plaat1* is important for visual acuity across bony vertebrates, and that its loss
21 through relaxed selection and pseudogenization may have played a role in the repeated evolution of
22 visual systems in low-light-environments. Our study sheds light on the importance of gene-loss in trait
23 evolution and provides insights into the mechanisms underlying visual acuity in low-light environments.

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25 **Keywords:** Convergent Evolution, *plaat1*, HRASLS, gene loss, vision, eye loss, cavefishes

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37 **1. Introduction**

38 Though evolution via sequence divergence has been a major focus of studies of adaptation, gene loss
39 has been demonstrated to be an important mechanism of adaptive evolution, shaping both constructive
40 and regressive adaptations [1–3]. Gene loss can occur through various mechanisms such as genetic drift
41 and/or positive selection and is a common feature of evolution in low-light or cave environments where
42 visual adaptations often involve a reduction or loss of eyesight [4–6]. Generally, the degradation and
43 loss of eye-associated genes are thought to be adaptive via the loss of energy-intensive tissue or
44 through closure of a disease-vulnerable mucus membrane in fossorial species [7–10].

45 Though the eye is one of the most well-studied organs, the physiological and genetic basis of many
46 vision-related diseases remain unknown [4,11–13]. Previous comparative genomic work on cave-
47 dwelling and fossorial lineages with eye degradation has provided vital insights into the genetic basis of
48 eye-related gene function and disease [13–15]. In addition, insights from genomic studies of gene loss
49 have been critical to informing convergent adaptive function [16–19].

50 *Plaat* family genes, which are phospholipases involved in N-acylethanolamine (NAE) biosynthesis, play a
51 crucial role in lens clarification, as well as tumor suppression, satiation signaling, and viral translocation
52 [20,21]. Recently, *Plaat1* was found to play a critical role in the metabolism of lens organelles in
53 zebrafish (affecting eye clarity), as well as cardiolipin synthesis and remodeling, which may be a key
54 novel pathway for diabetes [22]. Specifically, the protein PLAAT1 is recruited to damaged organelle
55 membranes in the lens during development, causing the rupture and clearance of lens organelles which
56 is required for the development of a clear lens [21]. *Plaat1* is one of 5 paralogs (*plaat1-5*) present in
57 humans, and *plaat3* is thought to be the dominant phospholipase for lens organelle degradation in mice
58 [21]. While publicly available databases (e.g. Ensembl, NCBI orthology and gene tree tools) suggest that
59 duplication events are also apparent in primates, rodents, and shrews, the evolutionary history and
60 function of these orthologs across mammals has not been explored. Expression and knockdown data
61 suggest that *plaat1* is critical for eye clarity in zebrafish. Both zebrafish *plaat1* knockouts and mice
62 *plaat3* knockouts develop cataracts, and previous work suggests that *plaat1* is conserved across
63 vertebrates while *plaat3* is unique to mammals [20–21]. It remains unclear what role *plaat1* may play in
64 mammalian eye development or if this gene is central to eye development in fish exclusively.

65 While previous evidence suggested a role for the *plaat* gene family in the evolution of visual acuity, their
66 potential loss or pseudogenization and correlation with vision loss has not been investigated. Here we
67 utilize a comparative phylogenetic approach to examine *plaat1* across more than 300 vertebrates and
68 *plaat3* in mammals, including low visual acuity and blind (LVAB) mammals as well as several convergent
69 lineages of LVAB fishes (Figure 1). Our data support the newly discovered function of *plaat1* as a vital
70 eye-function gene showing strong evidence for relaxed selection and gene degradation in several
71 independent lineages of LVAB fishes and mammals. These data suggest *plaat1* is important for the
72 evolution of visual acuity in both fish and mammals and demonstrates the value of a comparative
73 evolutionary framework to inform gene function [21,23]. While the precise impact of the *plaat* gene
74 family on vertebrate macroevolution remains to be fully elucidated, our data show that gene loss, gene
75 degradation, and relaxed selection of these genes is strongly associated with reduced vision in specific
76 vertebrate clades.

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78 2. Methods

79 (a) Sequence Retrieval and Alignment

80 In total, 311 sequences from 310 species (two *Astyanax mexicanus*, one surface and one cave)
81 were aligned and used for downstream analysis. First, we used the NCBI ortholog database (accessed
82 Aug 2022, https://www.ncbi.nlm.nih.gov/genome/annotation_euk/process/#references), to download
83 one orthologous sequence of *plaat1* from each species from groups of interest—mammals, bony fishes,
84 and sauropsids (filtering processes for orthology databases can be found here:
85 https://www.ncbi.nlm.nih.gov/genome/annotation_euk/process/) (Supplementary file 3). Nucleotide
86 sequences were translated into protein sequence and aligned with Clustal Omega v1.2.2 [24]. Protein
87 alignments and DNA sequences were then used as input for PAL2NAL v14 [25] to generate protein-
88 informed codon alignments. Second, sequences annotated as *plaat1* were pulled from 13 newly
89 annotated cave and cryptophthalmic interstitial lithophilic river fish genomes (Supplementary Table 1),
90 part of a concurrent project (Drabek et al. *in prep*). To be sure that the full and correct sequence for
91 *plaat1* was recovered, genomes were searched using BLASTN as well as an hmm-based exon capture
92 pipeline (<https://github.com/lilychughes/FishLifeExonHarvesting>). Resulting hits were mapped to their
93 respective scaffolds in GeneiousPrime 2022.2.1 (<https://www.geneious.com>). Scaffolds were also
94 searched for syntenic genes using the map to reference function. Exons for *plaat1* were extracted and
95 aligned to the RefSeq transcript from *A. mexicanus*, and accessioned into Genbank (submission ID:
96 2721929:OR260033-45) (AstMex3_surface GeneID: 103036287).

97 Sequences were filtered for quality and paralogy through a multi-step process as follows. Using
98 unrestricted tests of selection (aBSREL, described below), we examined sequences which were found to
99 be under positive selection via a likelihood ratio test (n=8). Since these sequences were highly divergent
100 from the bulk of the alignment, we further scrutinized them. First, alignments were scrutinized by eye
101 and misplaced gaps were corrected (n=3: *Xiphophorus maculatus*, *X. couchianus*, and *Poecilia reticulata*).
102 We also used Ensembl's ortholog database (release 109 - Feb 2023,
103 https://useast.ensembl.org/info/genome/compara/homology_method.html) to check that NCBI
104 sequences matched and were the longest available transcript, and if they were not, sequences were
105 replaced with the longest available transcript (Ensembl n=2 *Struthio camelus*, *Crocodylus porosus*). If a
106 species was not available on Ensembl, we used *plaat1* sequence from a closely related species to
107 BLASTN against all available NCBI genomes for the species with a questionable sequence, and mapped
108 hits as well as syntenic genes to their respective scaffolds in Geneious v8.1. Sequences for which a
109 better sequence match was identified on the same scaffold as genes syntenic with *plaat1*, were deemed
110 mis-annotated and removed (n=3 *Trachemys scripta elegans*, *Haliaeetus albicilla*, *Pterocles gutteralis*).

111 Programs used to test for selection cannot handle stop codons, thus sequences were edited to remove
112 stop codons and contain only in-frame sequence in GeneiousPrime 2022.2.1. Three species
113 (*Stomatorhinus microps*, *Nemacheilus troglolactactus*, *Prietella phreatophila*) had premature
114 termination codons (PTCs) in the second or third exon after which the remnant of the gene was still
115 discernible. To test whether signal for relaxed selection was amplified when including both the coding
116 region and the gene remnants, we used both (selection analyses for alignments with and without gene
117 remnants) for downstream analyses. Alignments with and without gene remnants used for analyses are

118 available on Dryad (DOI: 10.5061/dryad.qfttdz0p0). To verify that premature stop codons were not a
119 result of assembly errors, we performed a BLASTn on the raw reads using the entire sequence with gene
120 remnants as a query, and included an alignment of all 500 top hits with the query in our Dryad database.
121 TimeTree (<http://www.timetree.org>) was used to generate a topology for all available sequences and
122 additional species were added in a text editor using topologies from the FishLife project
123 (www.FishTree.org) [26]. The same process was repeated for *plaat3* (for mammals only) (Figure 3;
124 Supplementary Data).

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126 **(b) Evolutionary hypothesis testing**

127 Several nested hypotheses about the evolution of *plaat1* were tested. To account for the diversity of
128 sequences included in this dataset, tests of selection were run using both the entire dataset as well as
129 two subsets: 1) Actinopterygii and 2) Mammalia. Foreground lineages included LVAB mammals,
130 cavefishes, cryptophthalmic interstitial lithophilic river fish (*Stomatorhinus microps*, *Mastacembelus*
131 *brichardi*) and the Asian swamp eel (*Monopterus albus*, which has reduced eyes but is also not a
132 cavefish) (Figure 1, Supplementary File 2). Hereafter we use simply 'LVAB' to reference the fish and
133 mammal foreground species. Because *plaat1* is missing entirely in squamates, and several sequences
134 within sauropsida appear to be pseudogenized, we test whether *plaat1* was evolving under relaxed or
135 positive selection in all sauropsids. To do this we designated sauropsids as the foreground taxa using
136 the entire 311 sequence dataset. To test for positive selection on foreground branches, we employed an
137 unrestricted branch site test, aBSREL as well as a gene-wide test of selection, BUSTED, in HyPhy v2.5.33
138 [28,29]. To test for relaxed selection, we employ the RELAX model test, specifying foreground lineages
139 as LVAB mammals, LVAB fishes, or sauropsids (Supplementary File 2) [30]. To identify sites under
140 positive selection in foreground lineages, we used the mixed effects model of evolution (MEME) [28,31].
141 All statistical tests for selection were employed within the HyPhy software suite v 2.5.33 [31].

142 The PLAAT proteins contain a transmembrane domain (TMD) which is vital for membrane identification
143 and attachment, as well as a lecithin retinol acyltransferase domain (LRAT) which is responsible primarily
144 for catalysis [20-21]. To test for enrichment of mutations that are likely to cause a loss of function, we
145 counted for presence of a deletion or premature stop codons located in the TMD, LRAT domain,
146 and the N-terminal (NTERM) region for both fish and mammals of *plaat1*. We used Fisher's exact tests in
147 R (version 4.2.3) to compare the occurrence of deletions and premature stop codons in either LVAB
148 mammals or LVAB fishes with sighted mammals and fish, respectively.

149 **(c) Confirming *plaat1* gene loss in squamates**

150 In both NCBI and Ensembl orthology databases, *plaat1* is present in mammals, archosaurs and turtles,
151 tuataras, amphibians, lungfish, coelacanth, and cartilaginous fishes, but is missing in squamates, and
152 therefore likely present in the most recent common ancestor (MRCA) of Gnathostomata.

153 Loss of *plaat1* in squamates was confirmed utilizing both automated and manual checks. First, to create
154 a BLAST query for *plaat1*, we used BioMART's orthology tool to compile nucleotide and amino acid
155 sequences from five mammals spanning broad phylogenetic range (Tasmanian devil, platypus, opossum,
156 cow, human), as well as gar and frog. This query was used to perform both BLASTN and tBLASTN
157 searches against 90 sauropsid reference genomes (all amniotes including mammals and avian/nonavian
158 reptiles), transcriptomes, and raw reads (Supplementary File 1). Losses were confirmed manually
159 utilizing both NCBI and Ensembl ortholog databases (accessed Aug 2022). Finally, manual examination of
160 syntenic regions was used to confirm gene loss and, if possible, identify gene remnants. To evaluate
161 syntenic regions, we identified conserved genes that were directly upstream and downstream to *plaat1*
162 using the NCBI orthology database in sauropsids (Figure 2). We then used these flanking genes to search
163 available genomes within squamates to identify the syntenic region and recover potential gene
164 remnants [27]. If flanking genes were clearly present but *plaat1* was missing, intergenic regions were
165 extracted and aligned using ClustalOmega in Geneious v8 to the best quality/ closest related genome
166 available that contained *plaat1* to recover any possible gene remnants [24].

167 **3. Results**

168 In brief, our comparative genomic approach recovered important losses and relaxed selection in
169 synteny-confirmed orthologs of *plaat1*. Losses were found predominantly in blind fishes, or fishes with
170 low visual acuity. Enrichment analysis revealed that deletions and premature stop codons were
171 significantly enriched in functional domains for both LVAB fishes and LVAB mammals. This suggests that
172 the *plaat1* gene has played an important role in the evolution of vision (and its subsequent loss) in many
173 vertebrate species.

174 **(a) Little evidence of positive selection in lineages of LVAB fishes for *plaat1***

175 Neither gene-wide (BUSTED) nor branch-site (aBSREL) revealed signatures of significant positive
176 selection across 85 teleost fish in a hypothesis-free test (Table 1). When LVAB were designated as
177 foreground species, positive selection was detected on the branch leading to the Asian swamp eel (Table
178 1; Supplementary Table 4), and tests for relaxed selection with all LVAB fishes designated as foreground
179 are marginally non-significant ($p = 0.06$). Mixed effects model of evolution (MEME) revealed no sites ($p <$
180 0.05) evolving under positive selection when LVAB fish lineages are placed in the foreground.

181 **(b) *plaat1* exon loss and relaxed selection in LVAB fishes**

182 *Plaat1* is a phospholipase which functions via enzymatic activity through binding to a substrate with a
183 hyper-conserved C-terminal transmembrane region, and it has been previously established that
184 removing this region renders PLAAT1's phospholipase activity nonfunctional by making it unable to
185 translocate to organelle membranes [21]. Because tests of selection are blind to insertions, deletions,
186 and other structural changes that significantly impact gene function, we examine the instance of
187 deletions and premature stop codons in LVAB species compared to sighted species. Among 16 fish
188 lineages designated as foreground species based on eye reduction, one cryptophthalmic interstitial
189 lithophilic river fish (*Stomatorhinus microps*) and two cave-dwelling species (*Prietella phreatophila*,
190 *Nemacheilus troglodactaractus*) are missing the C-terminal end of *plaat1* due to premature stop codons
191 in exon 3 (*S. microps*, *P. phreatophila*) or 2 (*N. troglodactaractus*) (Figure 2). Across 85 teleost fish, these
192 are the only sequences missing any portion of the C-terminal sequence (Figure 2). Two additional cave-

193 dwelling species (*Rhamdia laluchensis* and *R. macuspanensis*) as well as the Asian swamp eel
194 (*Monopterus albus*, which has reduced eyes but is not a cavefish) have large deletions in the middle
195 exon (53-85aa, 67-72aa, 59-68aa, respectively) which are also unique relative to all other teleost fishes
196 surveyed. Fisher's exact tests confirm that deletions and premature stop codons are significantly
197 enriched for LVAB and blind species in both the LRAT ($p = 0.0001$) and TMD ($p = 0.0068$) domains
198 (Supplementary Figure 1).

199 For three fish species with significant exon loss (*S. microps*, *P. phreophila*, *N. trogloteractus*), gene
200 remnants 3' to a premature stop codon were identifiable by eye. The majority of teleost fishes have a
201 PLAAT1 protein which is 166-170 amino acids in length, whereas *S. microps*, *P. phreophila*, and *N.*
202 *trogloteractus* have lengths 136, 124, and 133 respectively, all of which are restored to a full length
203 (166-170aa) when gene remnants are included. To test whether these regions were under relaxed
204 selection, we employed the same test of selection with these regions included. When the sequence 3' to
205 premature stop codons (gene remnants) were included in the same analyses, the branch leading to the
206 elephant fish (*S. microps*) was also identified as evolving under positive selection (aBSREL) (Table 1;
207 Supplementary Table 5). Similarly, when gene remnants were included in gene-wide tests of positive
208 selection (BUSTED) and relaxed selection (RELAX) signal for both were significant ($p =$
209 0.0011 , $p < 0.0001$ respectively), suggesting that regions 3' to the premature termination codon may
210 still be under negative selection.

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213 (c) *Plaat1* in low-visual acuity and blind mammals

214 Using the low-visual acuity and blind lineages of mammals highlighted in Figure 1, we used an array of
215 selection tests to identify whether evolutionary trajectories of eye loss were associated with sequence-
216 level signatures of relaxed or positive selection on *plaat1* in mammals. Though *plaat3* is thought to be
217 most strongly involved in lens clarification in mammals, it remains unknown how important *plaat1* is to
218 this function. Tests of branch-site selection (aBSREL) of *plaat1* did not recover evidence of positive
219 selection for any branches when specifying low visual acuity or blind mammals as foreground species
220 (Figure 1; Table 1). Tests for gene-wide episodic positive selection (BUSTED) were significant when
221 identifying LVAB mammals as a foreground lineage ($p = 0.0208$), but not significant when a foreground is
222 not specified ($p = 0.5$). Tests of relaxed selection using the same LVAB foreground species were also
223 significant (Table 1; Supplementary Table 6). Similar to fishes, Mixed Effects Model of Evolution (MEME)
224 reveals codon diversification as clustered largely at the C-terminal end (Table 1; Supplementary Table 7).
225 Particularly notable are deletions in the TMD for the Cape golden mole and the blind mole-rat.

226 (d) *Plaat3*

227 Because lens clarification via organelle degradation is thought to be more strongly influenced by *plaat3*
228 in mammals, we would expect *plaat3* to exhibit signatures of selection (either relaxed or intensified)
229 among LVAB mammals. To test this expectation, we aligned *plaat3* from 66 available mammals and
230 employed tests for both positive and relaxed selection. We found largely conserved synteny across
231 placental mammals (Figure 3) and recovered no evidence for positive selection or relaxed selection
232 across this gene. However, both *Myotis spp.* (mouse-eared bats) and *Chrysochloris asiatica* (Cape golden

233 mole) have very long branch lengths, and notable sequence divergence in the C-terminal region
234 (Supplementary Figure 2).

235 **(e) Clade-wide *plaat1* gene loss in squamates**

236 Searches of genome assemblies and raw reads, including exhaustive searches of the intergenic regions
237 of syntenic genes suggest that *plaat1* is lost in squamates (Figure 2). Because *plaat1* is present in the
238 gar, coelacanth, cartilaginous fish, toad, and mammals, the most parsimonious scenario for the
239 evolution of *plaat1* is that it was present in the MRCA of gnathostomes and subsequently lost in the
240 lineage leading to squamates.

241

242 **(f) Relaxed Selection of *plaat1* supported in Sauropsids**

243 Branch-site tests of positive selection (aBSREL) with sauropsids specified as the foreground, which
244 includes the stem as well as all branches within Sauropsida (Figure 1, Supplementary Figure 3),
245 recovered several branches within sauropsids under positive selection (Table 1, Supplementary Table 2).
246 To test for evidence of relaxed selection, we employed the HyPhy RELAX test. Specifically, RELAX
247 compares a model where both negatively selected sites *and* positively selected sites are shifting *towards*
248 neutrality in foreground lineages with respect to background lineages, versus a simpler model for
249 positive selection where positively selected sites are shifting *away from neutrality*. Tests of relaxed
250 selection using all sauropsids as foreground recovered evidence of gene-wide relaxation of selection
251 (Supplementary Table 3), while a gene-wide test of positive selection with sauropsids placed as
252 foreground (BUSTED) was not significant ($p=0.44$).

253

254

255 **4. Discussion**

256 Understanding the evolutionary history of gene families can shed light on the processes that drive
257 evolutionary change such as gene duplication, gene loss, and functional divergence. By tracing the
258 evolutionary trajectories of gene families across different lineages, we can gain insights into the
259 evolution and loss of important biological traits over time. Though functional work is needed to better
260 understand the importance of *plaat1* in eye development and function, our work found enrichment for
261 deletions, premature stop codons, and relaxed selection in lineages with species with reduced eyes, as
262 well as an ancient loss along the basal lineage of squamates. Overall, our study suggests that *plaat1*
263 may play a vital role in evolutionary shifts to sightlessness.

264

265 **(a) *Plaat1* gene degradation in multiple species of LVAB fishes**

266 Colonization of cave environments and the evolution of troglomorphy has occurred in over 200
267 species of teleost fish [46–49]. Our dataset included sixteen species with reduced eyes that represent
268 eight independent lineages of cave colonization, two cryptophthalmic interstitial lithophilic river fish
269 (*S. microps*, *M. brichardi*), and one non-cave dwelling species with greatly reduced eyes (*M. albus*)

270 (Figure 1) [50-51]. *Plaat1* zebrafish knockouts of the entire *plaat1* gene as well as knockouts of only the
271 C-terminal transmembrane domain (TMD), reliably produce fish with cataracts [21]. Two cavefish
272 lineages (the blind cave loach, *N. troglodactylus*; and the Mexican blindcat, *P. phreatophila*) and the
273 cryptophthalmic interstitial lithophilic river fish African elephant fish (*S. microps*) have
274 premature stop codons 5' to the TMD, and therefore likely lack a functional *plaat1* which is key for lens
275 clarification. However, because the remainder of this gene (including the catalytic triad) remains intact,
276 it is possible that phospholipid metabolism is conserved in some capacity (potentially avoiding negative
277 pleiotropy). Three additional large deletions occur in three additional blind/eye-reduced fishes (the La
278 Lucha blind catfish, *R. laluchensis*; the Olmec blind catfish, *R. macuspanensis*; and the Asian swamp eel,
279 *M. albus*). These deletions occur in the lecithin retinol acyltransferase (LRAT) domain which plays a vital
280 role in the availability and storage of retinol and are therefore very likely to cause large functional
281 disruptions [52].

282 Gene-wide and branch-site tests of selection which did not specify a foreground across teleosts are not
283 significant (Table 1). When LVAB fishes are specified as foreground species in branch-site tests (aBSREL),
284 the lineage leading to *M. albus* is identified as evolving under positive selection, and when gene
285 remnants are included in the same analysis, both *M. albus* and *S. microps* are identified as evolving
286 under positive selection (Supplementary Table 4-5). As both species have large deletions of major
287 functional regions (See Supplementary data annotation alignment), are identified on branches
288 evolving under positive selection, with marginally non-significant signal for relaxed selection, we can
289 surmise that positive selection may be contributing to loss of function. This contrasts with the case in
290 mammals where there is a clear negative result for all branch tests of positive selection, and clearer
291 difference in level support for relaxed ($p=0.0003$) vs positive selection (0.0208). These results suggest
292 that selection might contribute to loss of function mutations in these species, a phenomenon that has
293 been demonstrated for other cavefish [40].

294 While tests of relaxed selection specifying the same foreground are marginally non-significant ($p = 0.06$),
295 they become highly significant when gene remnants are included ($p < 0.0001$). Together this suggests
296 that both relaxed selection and positive selection may be important in the process of *plaat1* gene
297 degradation and are likely acting on different sites of the same gene [40-41].

298

299 **(b) Selection for *plaat1* but not *plaat3* is relaxed in low visual acuity and blind mammals**

300 Recovery of significant evidence for relaxed or positive selection in *plaat1* in mammals is found
301 exclusively in tests that designate low visual acuity and blind mammals as foreground species,
302 suggesting that *plaat1*, likely retains an important role in mammalian eye function and development.
303 Notably, the blind Cape golden mole, *Chrysochloris asiatica*, has two coding mutations which disrupt the
304 ultra-conserved H-box domain, thought to be vital for hydrolysis [37].

305 Though *plaat3* has been shown to have a vital role in lens clarification in mice, we did not see a signal of
306 positive or relaxed selection in LVAB mammals in our dataset for *plaat3*. However, branch lengths for
307 *plaat3* for the Cape golden mole and the little brown bat are noticeably long (though not significantly
308 so), with sequence divergence concentrated in the C-terminal end of the protein across the TMD, which
309 is vital for membrane translocation and lens clarification (Supplementary Figure 1 & 2).

310 Mammalian *plaat1* results are significant for both gene-wide episodic diversifying selection (BUSTED) as
311 well as relaxed selection (RELAX). Though fit across models cannot be directly compared, p-values are
312 much lower for relaxed selection, suggesting this model may be a better fit for these data. Additionally,
313 BUSTED results identified one site with an evidence ratio >10, suggesting that a small number of
314 positively selected sites (one) are driving the signal for positive selection [30]. Both selection and
315 Fisher's exact tests support the hypothesis that mammalian *plaat1* is degrading via relaxed selection in
316 species with reduced visual acuity and blindness. These data support recent work by Partha et al. [15]
317 which identifies HRASLS (PLA/AT) as a gene exhibiting convergent rate evolution in fossorial mammals
318 [45].

319 While signal for relaxed selection and gene degradation across convergent lineages of LVAB species
320 suggests that *plaat1* retains some functional importance in eye development in mammals, it is also
321 possible that *plaat1* is associated with pleiotropic physiologies that are also altered in dark
322 environments. For example, *plaat1* is known to be involved in insulin signaling pathways, cardiolipin
323 metabolism, as well as tumor suppression, which are also known to be important in shifts to darkness
324 [22,38,39]. Additionally, *plaat1* plays a role in p53-mediated apoptosis involved in starvation and
325 disease, both of which are greatly modified in low-light environments [23].

326

327 **(c) Loss of *plaat1* in squamates**

328 *Plaat1* is present in the tuatara (sister group of squamates) and absent in both lizards and snakes,
329 suggesting that it was lost along the branch leading to squamates. Shifts to fossorial niches and
330 subsequent reduction of eye-related genes occurred at several points across both modern and ancient
331 snakes and lizards [35]. Recent molecular work has shown that loss of eye related genes is apparent
332 across the squamate lineage, including in the squamate ancestor [35]. The apparent loss of *plaat1*
333 uncovered here agrees with recent work and identifies a novel pathway of eye reduction in ancient
334 squamates [35].

335

336 **(d) Relaxed selection of *plaat1* in sauropsids**

337 Because *plaat1* is lost in squamates, and several sauropsids appear to have highly degraded *plaat1*
338 sequences, we investigated whether relaxed selection was pervasive in all squamates, indicating a more
339 widespread loss of function, redundancy, or alternate function in this larger group. Results of selection
340 tests indicate significant relaxed selection across sauropsids in *plaat1* (Table 1, Supplementary Table 3).
341 Sequence divergence across sauropsids is quite variable, with likely non-functional variants and
342 potentially pseudogenized *plaat1* convergently arising in the tuatara, common box turtle, saltwater
343 crocodile, kea, and the red-throated loon (Supplementary Figure 3) [36]. Further investigation should
344 focus on the role of *plaat1* in sauropsids, and whether this apparent divergence is driven by convergent
345 physiological functional adaptation, ancient loss of function, or by neofunctionalization of sauropsid-
346 specific paralogs which may have rendered *plaat1* redundant.

347

348 **(e) Other important physiologies**

349 While we focus here on the newly discovered lens clarification function that is apparent for *plaat1*, it is
350 important to note that *plaat1* has several pleiotropic physiological interactions, which we do not fully
351 understand [20–22,36,39,52]. *Plaat* family proteins are important for tumor suppression, NAPE
352 biosynthesis (hormones released by the small intestine into the bloodstream to process fat), obesity,
353 and peroxisome formation [20–22,36,39,52,53]. Particularly noteworthy is *plaat1*'s role in facilitating
354 viral translocation, making viral coevolution another strong potential driver of convergent gene loss
355 and/or degradation in vertebrates [53]. When *plaat1* is suppressed in mouse and human cell lines, NAPE
356 levels decrease, suggesting that *plaat1* is an important N-acetyltransferase [20]. *Plaat1* deletions in
357 humans are associated with Poland Syndrome, a disorder which causes missing or underdeveloped
358 muscles on one side of the body [52,54].

359 *Plaat3*'s absence or divergence outside of mammals is also noteworthy, as it plays vital roles in obesity,
360 cancer invasion, and vitamin A storage [55–57]. Remarkably, in the course of this work, we observed
361 the venomous mammal *Sorex araneus* had more than 30 duplications of *plaat3*, which is also present in
362 the salivary transcriptome of another venomous shrew, *Blarina brevicaudata*, which suggests that
363 *plaat3* might have been recruited into the venom proteome in shrews.

364 **5. Conclusions**

365 There is a lack of research on the impact of the *plaat* gene family on vertebrate macroevolution.
366 Specifically, our work suggests that this gene family may have played a crucial role in the evolution of
367 visual acuity. We found a significant association between *plaat1* increased sequence divergence (due to
368 relaxed or positive selection), premature stop codons, and deletions in key functional domains across 12
369 lineages of low visual acuity and blind mammals, 16 lineages of low-light adapted fishes, and a complete
370 loss of *plaat1* in squamates. We identified several cases of convergent relaxed selection within
371 sauropsids that have notably reduced visual acuity or are night vision specialists (e.g., kiwi and
372 saltwater crocodiles, respectively). However, convergent relaxed selection in other groups (e.g. ostrich),
373 with high visual acuity, suggest that the functional significance of *plaat1* within sauropsids remains
374 unknown, and requires further exploration. Though there are at least 5 paralogs known in humans, and
375 public databases suggest duplication events elsewhere in the mammalian tree, further investigation of
376 ortholog and paralog function are needed to shed light on how labile lens clarification function is among
377 copies. A better understanding of how these enzymes have diversified, and how different species have
378 coped with losses and gains of function may be crucial to understanding disease pathways (e.g., eye
379 disease, insulin signaling, tumor suppression, and viral infection) and developing strategies for human
380 disease intervention[14].

381

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 394 Zoo for aiding in acquiring the tissues necessary for this and accompanying genomic work.

395 Data Accessibility

396 The data generated in this work including sequence alignments, species topologies, and scaffolds are
 397 stored in the Dryad repository which can be accessed with this link DOI: 10.5061/dryad.qfttdz0p0

398 Tables

399

400 **Table 1.** Summary of results for various tests for diversifying and relaxed selection on *plaat1* using low acuity and
 401 blind species (LVAB) mammals, LVAB fishes, and/or sauropsids as foreground taxa. LVAB fish sequences were run
 402 with and without gene remnants (GR) which included the sequence downstream of a premature stop codon which
 403 otherwise would have been within a coding exon. The designation ‘none’ means that no branch was specified as
 404 the foreground, and all branches were run and tested individually. P- values reported are corrected for multiple
 405 tests in HyPhy.

	Foreground Specified	aBSREL (episodic positive, branch-site)	BUSTED (episodic positive, gene-wide)	RELAX (relaxed, gene-wide)	MEME (episodic positive, site)
Mammals	LVAB	p > 0.05	p = 0.0208	p = 0.0003	p < 0.05 (4 sites)
	none	p > 0.05	p = 0.5000		
Fishes	LVAB	p < 0.04 ⁵	p = 0.4777	p = 0.0608*	p < 0.05 (0 sites)
	LVAB (GR) none	p < 0.0004 ⁴ p > 0.05	p = 0.0011 p = 0.4798	p < 0.0001	
Vertebrates	LVAB	p < 0.002 ²	p < 0.0001		p < 0.05 (6 sites)
	Sauropsids none	p < 0.04 ³ p < 0.03 ¹	p = 0.4441 p < 0.0001	p < 0.0001	

406 ¹*Crocodylus porosus*, p-value < 0.000011, *Terrapene carolina*, p-value < 0.000011, *Struthio camelus*, p-value < 0.000011 *Elephantulus edwardii*,
 407 p-value = < 0.000011, *Myotis davidii*, p-value = 0.02573

408 ²*Myotis davidii*, p-value = 0.00145

409 ³*Terrapene carolina*, p-value = < 0.000010, *Crocodylus porosus*, p-value = < 0.000010, *Struthio camelus*, p-value = < 0.000010, *Nestor notabilis*,
 410 p-value = 0.03369

411 LVAB: foreground set to include all blind/low visual acuity species in that group

412 ⁴*Stomatorhinus microps*, p-value < 0.000011, *Monopterus albus*, p-value = 0.0268

413 ⁵*Monopterus albus*, p-value = 0.03031

414 *marginal non-significance

415

416 Figure Legends

417 **Figure 1.** Topology of all species included in *plaat1* analysis. Blue lineages indicate cave, blind, and low-visual acuity
418 species designated as foreground for selection analyses. This dataset represents 311 species in total.

419 **Figure 2.** Generalized synteny of the *plaat1* gene across bony fish and tetrapod species. Synteny was identified
420 using the NCBI genome browser. Groups of species sharing the same syntenic pattern were collapsed on the
421 phylogeny and are denoted using the largest taxonomic group of which all species are included. The number in
422 parentheses indicates the number of species included in our analysis that fell in each group and that had a genome
423 assembly on NCBI. Each species in each group shared the same synteny pattern, with the following exceptions.
424 **Fish:** Osteoglossiformes: Old calabar mormyrid (*Paramormyrops kingsleyae*) missing *mfn1b*; Acanthomorpha:
425 Ocellaris clownfish (*Amphiprion ocellaris*) missing *clcc1* and *fts3*, Zig-zag eel (*Mastacembelus armatus*) missing
426 *mfn1b*, Crimson soldierfish (*Myripristis murdjan*) missing *clcc1* and *fts3*, Black rockcod (*Notothenia coriiceps*)
427 missing *clcc1*; Otophysi: Common carp (*Cyprinus carpio*) missing *rfc4*. **Mammals:** African elephant (*Loxodonta*
428 *africana*) missing *atp13a4*, Greater mouse-eared bat (*Myotis myotis*) missing *atp13a5*, Jamaican fruit bat (*Artibeus*
429 *jamaicensis*) missing *fgf12* and *mb21d2*, Baiji (*Lipotes vexillifer*) missing *atp13a5*, Blue whale (*Balaenoptera*
430 *musculus*) missing *atp13a5*, Northern elephant seal (*Mirounga angustirostris*) missing *fgf12*. **Aves:** Helmeted
431 guineafowl (*Numida meleagris*) missing *fgf12* and *mb21d2*. PLAAT1 protein illustrations are generic and not to
432 scale. LRAT = lecithin retinol acyltransferase domain, TMD = transmembrane domain.
433
434

435 **Figure 3.** Generalized synteny of the *plaat3* gene across selected vertebrate lineages. *Plaat3* orthologs were
436 identified using the Ensembl gene tree (ENSG00000176485). Synteny was identified using the NCBI genome
437 browser. Groups of species sharing the same syntenic pattern were collapsed on the phylogeny and denoted using
438 the largest taxonomic group of which all species are included. The number in parentheses indicates the number of
439 species included in our analysis. Each species in each group shared the same synteny pattern, with the following
440 exceptions: **Testudines:** Painted turtle (*Chrysemys picta*) missing *lgals12*, Three-toed box turtle (*Terrapene carolina*
441 *trunguis*) missing *plaat2*; Squamata: Eastern brown snake (*Pseudonaja textilis*) missing *or6n1*; Euarchontoglires:
442 mouse (*Mus musculus*) missing *plaat4* and *plaat2*, Coquerel's sifaka (*Propithecus coquereli*) missing *plaat5*;
443 Carnivora: harbor seal (*Phoca vitulina*) missing *plaat4*; Artiodactyla: camel (*Camelus dromedarius*) missing *rtn3*.

444

445 **Supplementary Figure Legends**

446 **Supplementary Figure 1.** Bar plot depicting the number of deletions or premature stop codons (PSCs)
447 in each region of *plaat1* for (A) sighted versus low visual acuity and blind (LVAB) mammals, and (B)
448 sighted versus LVAB fishes. Genes are counted only once if they have one or more PSC or deletion to
449 reduce bias caused by degraded genes having multiple deletions/disruptions. Numbers under bars
450 indicate total number of sequences (species) that fell into that category. P-values derived from Fisher's
451 exact tests.

452 **Supplementary Figure 2.** Gene tree of *plaat3* for all mammals included in selection analyses, generated
453 using aBSREL Full adaptive model (Smith et al. 2015).

454 **Supplementary Figure 3.** Topology of all sauropsids used in analyses next to the protein alignment of
455 *plaat1* for each species. Image generated in iTOL.

456

457

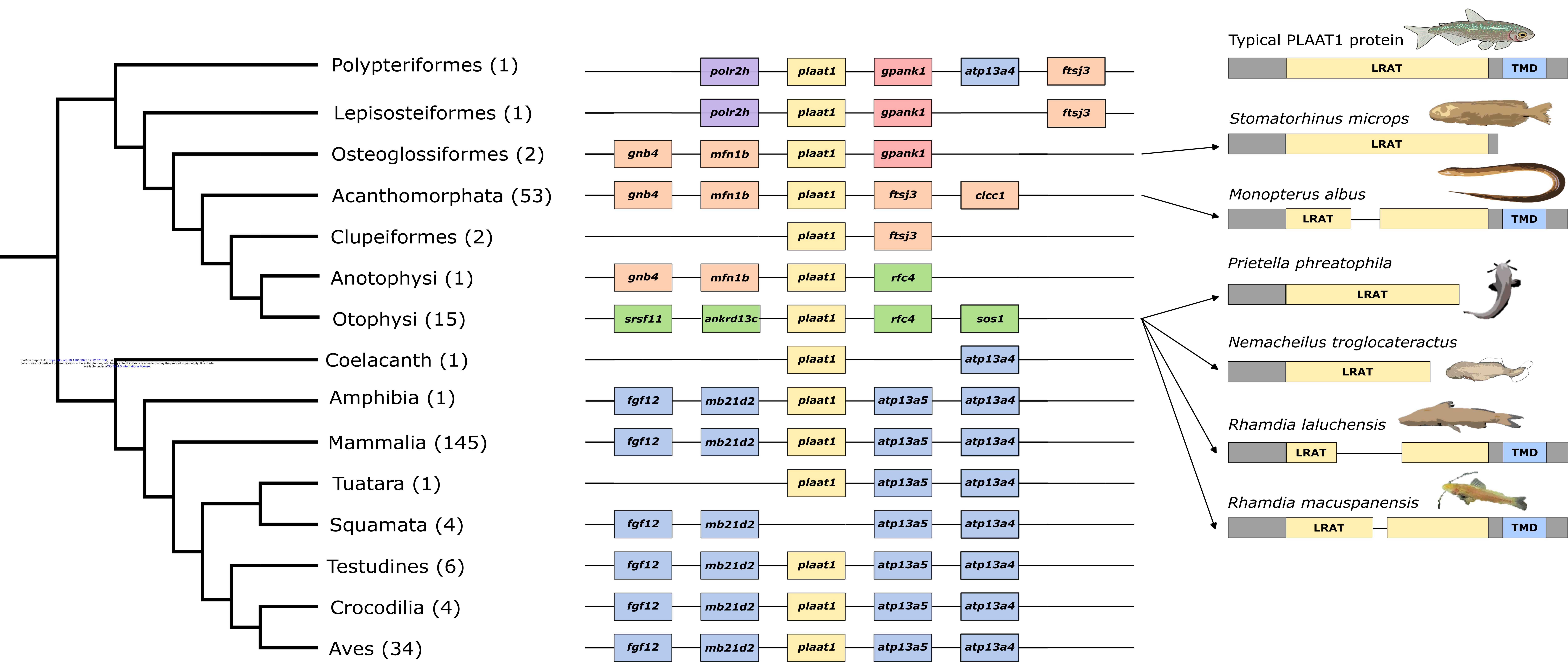
458 **References**

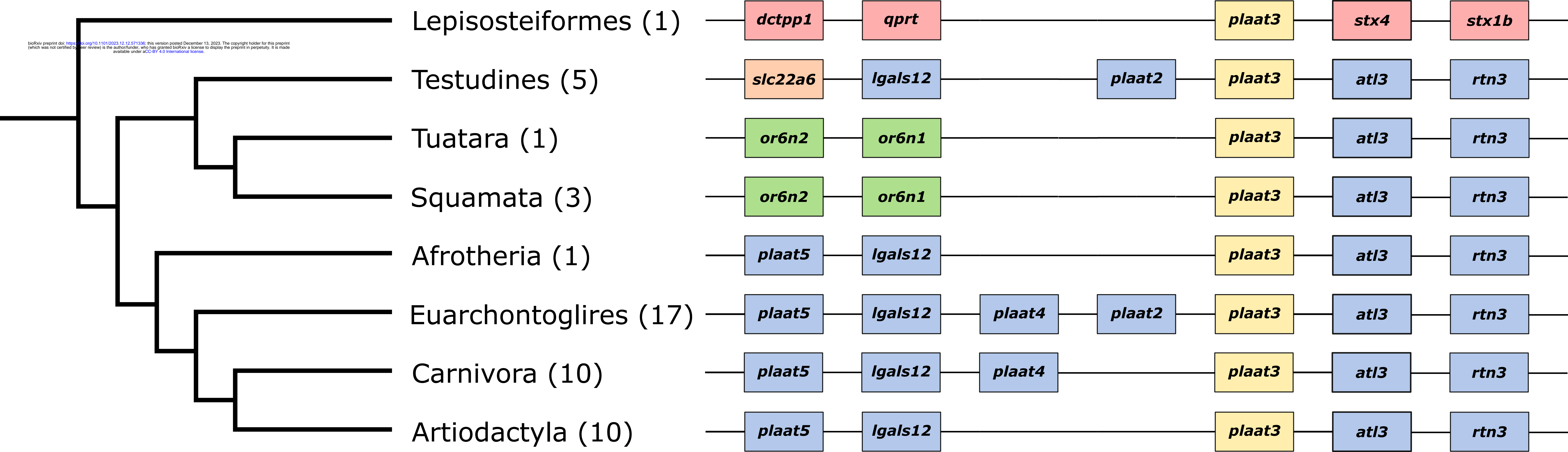
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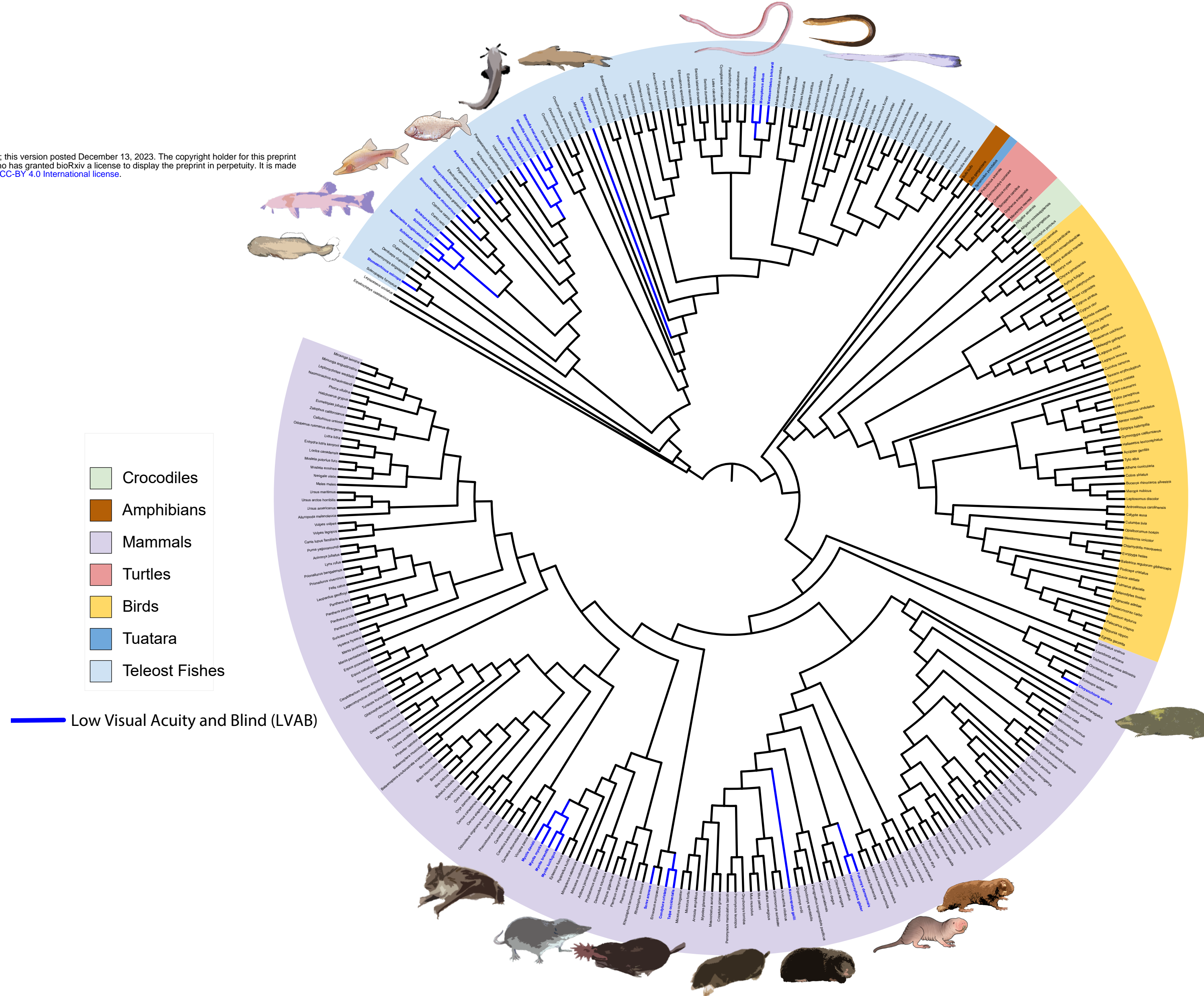
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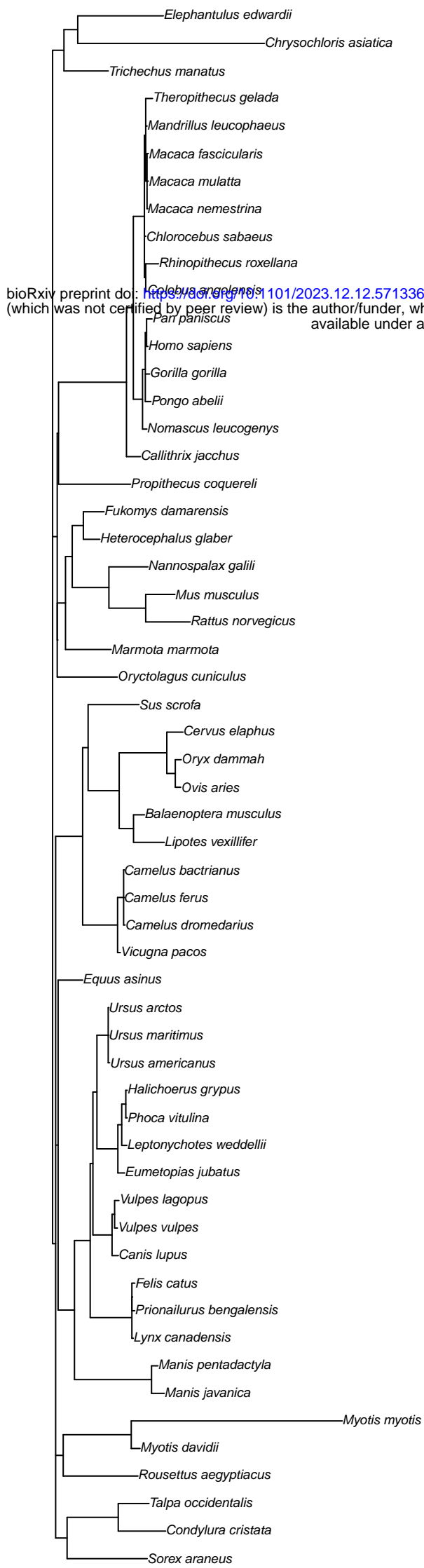
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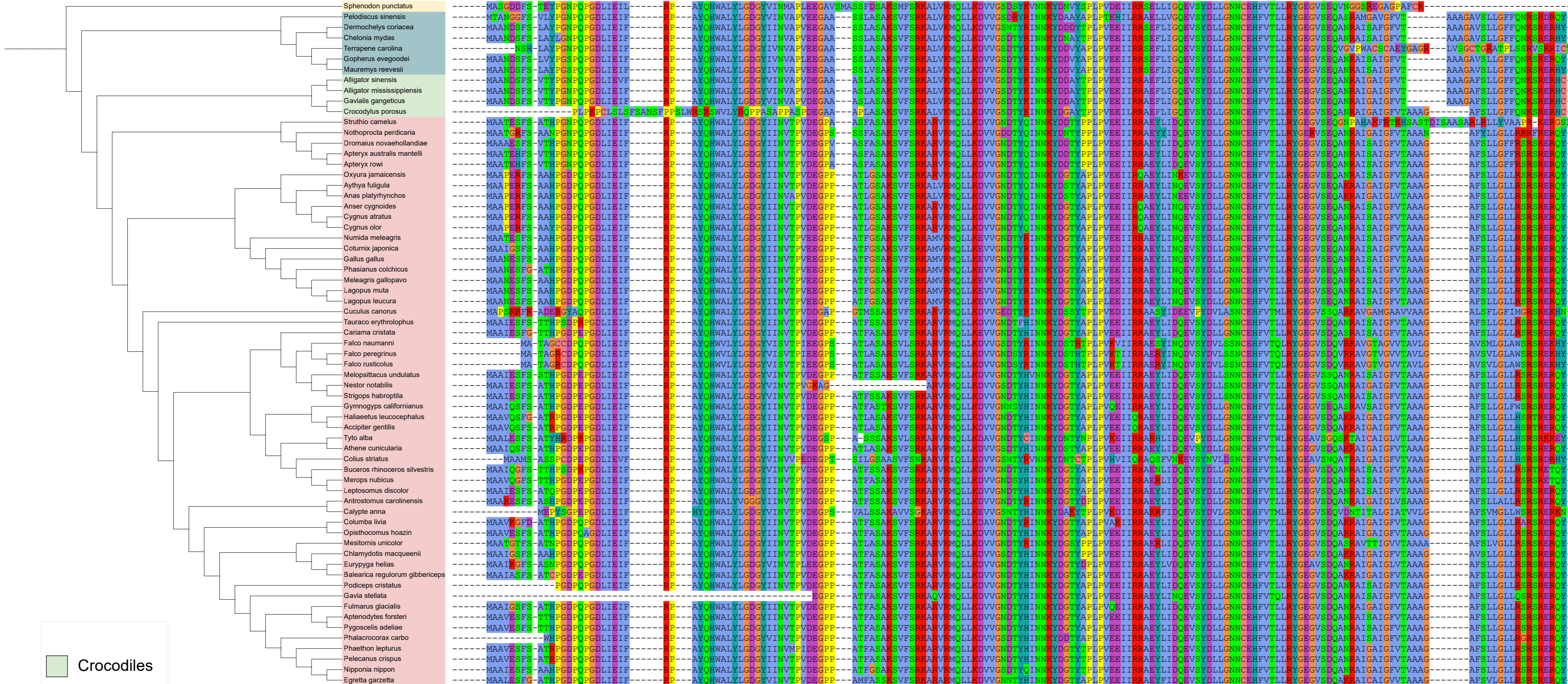






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0.4 0.3 0.2 0.1 0



Crocodiles
Turtles
Birds
Tuatara

