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Transcriptomic data support a nocturnal bottleneck in the ancestor to gecko lizards

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Abstract

Geckos are a species-rich clade of primarily nocturnal lizards and adaptations to nocturnality has dramatically reshaped the gecko eye. Perhaps the most notable change is the loss of rod cells in the retina and subsequent “transmutation” of cones into a rod-like morphology. While many studies have noted the absence of the rod opsin RH1, in geckos, these studies have focused on a handful of species that are nested deep in the gecko phylogeny. Thus, it is not clear whether these changes are ubiquitous across geckos or restricted to a subset of species. Here, we used eye transcriptomes from five gecko species, representing the breadth of extant gecko diversity, to show that geckos lost expression of multiple rod cell phototransduction genes in the eye, distinct from other reptiles. Furthermore, all sampled species lost expression of the SWS2 visual opsin. Together, these results suggest a “nocturnal bottleneck” in the MRCA of geckos.

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Introduction

Behavioral shifts between different light environments, such as changes from diurnal to nocturnal activity patterns, have led to major modifications to the eye over evolutionary time (Walls 1934; Walls 1942; Davies et al. 2012). These modifications involve changes to photoreceptor cell morphology and alterations to the phototransduction gene complement utilized by these cells (Lamb and Hunt 2017; Simões et al. 2015; Walls 1942). Two types of photoreceptors are present in most vertebrate retinas, rods, used for low light vision, and cones, used in daylight vision (Kojima et al. 1992; Lamb 2013). Rods and cones possess significant differences in their sensitivities to light (rods being more sensitive than cones) and phototransduction speed (cones transmit signals faster than rods) (Li et al. 2010), providing tradeoffs in the selective forces driving adaptation to differing light environments (Schott et al. 2016; Simões et al. 2015). Indeed, these tradeoffs have led to changes in photoreceptor cell morphology in some lineages when they adapt to dramatic shifts in light environment, with some rods becoming cone-like and some cones becoming rod-like, a process known as 'transmutation' (Pedler and Tilley 1964; Schott et al. 2016; Tansley 1964; Underwood 1970; Walls 1934; Walls 1942; Zhang et al. 2006). Groups that have experienced stark evolutionary changes in diel activity, such as mammals, snakes, and geckos, have seen concomitant changes to phototransduction gene complement and/or transmutations occur. Thus, characterizing diverse visual systems in a phylogenetic framework can lead to insights into how cellular morphology and signaling pathways respond to changing environments (Serb and Oakley 2005).

The ancestral tetrapod eye utilized 5 visual opsins, 4 in cone cells: LWS, RH2, SWS1, and SWS2; and 1 in rod cells: RH1 (Okano et al. 1992; Davies et al. 2012; Lamb and Hunt 2017). However, lineage-specific losses of photoreceptor gene-complement have

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been repeated in adaptations to nocturnal, fossorial, and low-light aquatic environments; such as: crocodilians (Emerling 2017a), burrowing rodents (Emerling and Springer 2014), snakes (Davies et al. 2009; Simões et al. 2015), whales (Levenson and Dizon 2003), and geckos (Crescitelli et al. 1977; Kojima et al. 1992; Yokoyama and Blow 2001;). The loss and transmutation of photoreceptors can be characterized by analyzing the presence/absence of the components of signaling pathway – particularly opsins and key members of the phototransduction cascade. These components may be lost simultaneously in the most recent common ancestor (MRCA) to extant taxa, which would be evidence of a "nocturnal bottleneck", as occurred in ancestral mammals (Walls 1942; Menaker et al. 1997; Gerkema et al. 2013) or in a step-wise manner, such as in cetaceans (Springer et al. 2016).

Geckos have been an important model for investigating how changes in light environment impact vision (Walls 1934 and 1942; Kojima et al. 1992; Röll 2000; Roth & Kelber 2004). This is because geckos are thought to be ancestrally nocturnal with multiple, independent reversions to diurnality throughout their evolutionary history (Walls 1942; Gamble et al. 2015). The examination of opsins and other phototransduction genes have shown that the tokay gecko (*Gekko gecko*) has lost its rods and has a pure cone retina, even though the photoreceptors have a rod-like morphology, supporting the transmutation hypothesis (Walls 1942; Crescitelli et al. 1977; Kojima et al. 1992; Röll 2000; Yokoyama and Blow 2001; Zhang et al. 2006). Despite their historic importance for studying visual system evolution, nearly all of the studies concerning molecular components of the gecko visual system were performed within the genus *Gekko* (mostly *Gekko gecko*) and various species of Malagasy day geckos (*Phelsuma* ssp.) (Crescitelli et al. 1977; Kojima et al. 1992; Liu et al. 2015; Loew et al. 1994; Taniguchi et al. 1999; Taniguchi et al. 2001; Yokoyama and Blow 2001; Roth et al. 2009). Both genera are in the family Gekkonidae, which is

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nested within the infraorder Gekkota (composed of 7 families) and, thus, fail to show whether these changes are gecko-wide or specific to Gekkonidae (Fig. 1). Furthermore, examination of pseudogenes in the *Gekko japonicus* genome suggests a step-wise loss of phototransduction genes with loss of SWS2 approximately 202 million years ago (mya) preceding the loss of the rod opsin, RH1, about 80.9 mya (Emerling 2017b), well after the divergence of extant gekkotan families (Gamble et al. 2011; Gamble et al. 2015). Thus, it is not clear whether rod loss and transmutation occurred simultaneously in the MRCA of extant geckos, in which we expect no expression of rod-based phototransduction genes or SWS2 in the eyes of extant geckos (Fig. 1b). Alternately, these losses could have occurred in a step-wise manner, after the divergence of extant gekkotan lineages, where we would expect to find rod-based phototransduction genes or SWS2 expressed in the eyes of some extant gekkotan lineages, but not others (Fig. 1c). Here, we combine data from two published gecko genomes (Liu et al. 2015; Xiong et al. 2016) with six *de novo* assembled eye transcriptomes (5 geckos, that span extant gekkotan diversity; and an outgroup that possesses all vertebrate ancestral opsins, *Chamaeleo calyptratus*, Pinto et al. 2019) to test the nocturnal bottleneck hypothesis in geckos.

Materials and Methods

RNAseq and Transcriptome Assembly

We euthanized and removed whole eyes from 5 geckos, representing the breadth of extant gecko diversity (Fig. 1; *Correlophus ciliatus, Gehyra mutilata, Hemidactylus turcicus, Lialis burtonis*, and *Phelsuma laticauda*). All species are nocturnal except *P. laticauda*, which is diurnal. Tissues were flash frozen at -80˚C in TRIzol™ reagent. RNA extraction, library prep, and transcriptome assembly processes are identical to those described by Pinto et al. (2019). Briefly, we extracted RNA using the Qiagen RNeasy™ Mini Kit and

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prepared RNAseq libraries with KAPA® Stranded mRNA-Seq Kit (KR0960 [v5.17]). Libraries were sequenced on an Illumina® HiSeq 2500 (paired-end 125 bp reads). We assembled *de novo* transcriptomes for each species using the *De novo* RNA-Seq Assembly Pipeline (DRAP) [v1.91] (Cabau et al. 2017), which is a compilation of automated assembly (Trinity [v2.4.0]; Grabherr et al. 2011) and quality-control tools to reduce redundancy.

Ortholog Identification and Phylogenetic Analyses

To identify genes present in the visual phototransduction of geckos, we downloaded a set of 35 key phototransduction genes (Schott et al. 2018), assumed to be present in the ancestor to all tetrapods, for nine species (Supplemental Table 3) from Ensembl [v91.0]. We used BLAST, implemented in Geneious® [v11.1.2] (Altschul et al. 1990; Kearse et al. 2012) to identify orthologs to these genes from annotated CDS's from published genomes of nine additional species and transcriptomes from a chameleon (Pinto et al. 2019) and five geckos described above (Supplemental Table 3).

Four transcripts, GNAT2 in *Correlophus*, GNGT2 and GUCY2D in *Lialis*, and SAG in *Hemidactylus*, were not found in the assembled transcriptomes. However, since the numbers of assembled *de novo* transcripts can vary greatly when assembling from short-reads (Zhao et al. 2011), we suspected that these 'missing' transcripts were sequenced, but not assembled. We verified their presence by mapping quality-filtered RNAseq reads to the respective transcript of their closest sampled relative using Geneious® [v11.1.2]. Similarly, two genes (GNAT1 and GUCY2F) had functional copies present in gecko genomes but were not assembled in any gecko transcriptomes. We mapped RNAseq reads to GNAT1 and GUCY2F CDSs from the *G. japonicus* genome but recovered no transcript for either gene in any sampled geckos. To visualize these data, we produced a character matrix indicating presence/absence of each phototransduction gene from the genome or

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transcriptome for every sampled species (Fig. 3) using *phytools* [v0.6-60] (Revell 2012) in R (R Core Team 2008).

The difficulty assigning orthology from BLAST alone prompted us to use phylogenies to ascertain orthology for several gene families. Sequences were translation aligned using MAFFT [v7.388] implemented in Geneious® [v11.1.2] (Katoh et al. 2002; Kearse et al. 2012). We generated gene trees for a subset of gene alignments (Fig. 2; Supplemental Fig. 1–3) on the CIPRES portal (Miller et al. 2010) using the RAxML Blackbox [v8.2.10] (Stamatakis, 2014). CNGA3 sequences were constrained as monophyletic – following Lamb & Hunt (2017).

Results and Discussion

We assembled *de novo* eye transcriptomes for five gecko species and one chameleon, *Chamaeleo calyptratus* (assembly statistics and benchmarking information are in Supplemental Table 1). We recovered the same 25 (out of 35) phototransduction genes in the five gecko eye transcriptomes and 31 (out of 35) in the chameleon transcriptome (Fig. 3). Eight rod-specific genes, including RH1, were missing in all the gecko transcriptomes, which supports the hypothesis that rod cells were lost in the MRCA of extant geckos (Fig. 1b). Similarly, the cone-specific opsin, SWS2, was missing from all sampled geckos and present in chameleon. Maximum-likelihood phylogenies from visual opsins (Fig. 2) and several other phototransduction genes were largely concordant with previously published gene trees (Lamb and Hunt 2017) and allowed us to confirm orthology of sequences initially identified via BLAST (Fig. 2).

While there was broad concordance between our transcriptomic data and the genome data from the geckos *Eublepharis* and *Gekko*, there were two genes that were not

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expressed in the eye but still had functional copies in the genomes. Our transcriptomic data indicates loss of expression of the rod-specific GNAT1, which retained functional copies in the *Gekko* and *Eublepharis* genomes, suggesting an additional function for this gene outside of the eye. Similarly, the rod and cone gene GUCY2F was not expressed in gecko or chameleon eyes although functional copies were found in all sampled squamate genomes. Given that expression is also missing in snakes (Schott et al. 2018), the loss of GUCY2F expression in the eye is likely squamate-wide.

We observed no differences in the occurrence of core phototransduction transcripts in the sampled gecko eyes (Fig. 3), which rejects the step-wise loss of phototransduction genes in extant geckos (Fig. 1c). Indeed, our data are consistent with the MRCA of geckos going through a nocturnal bottleneck, similar to that of placental mammals and crocodilians, that resulted in the ancient loss of rod cells and the transmutation of cones into a rod-like morphology (Fig. 1b; Gerkema et al. 2013; Emerling et al. 2017a). Thus, within squamate reptiles, two visual bottlenecks (fossoriality in snakes and nocturnality in geckos) accompanied a different set of phototransduction gene losses and subsequent transmutations. In some snakes, all-cone retinas have been observed via a rod-to-cone transmutation (Schott et al. 2016). However, in geckos, the loss of RH1 and accompanying rod phototransduction genes provides conclusive evidence of a single loss of ancestral rod cells and a transmutation of ancestral cones to a rod-like morphology (Walls 1942).

Additional lines of evidence also support a nocturnal ancestor in geckos. These include numerous adaptations to a low-light lifestyle, including: widespread acoustic communication (Gans and Maderson 1973; Marcellini 1977); sustained locomotion at low temperatures (Autumn et al. 1999); olfactory specialization (Schwenk 1993); and eye modifications such as increased size, pupils capable of extreme constriction and dilation,

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and retinas lacking foveae (Röll, 2001). Finally, comparative phylogenetic analyses of diel activity patterns of extant geckos also indicate the MRCA of extant geckos was nocturnal (Gamble et al. 2015). Taken together, these lines of evidence overwhelmingly support a "nocturnal bottleneck" of the MRCA of extant gecko lizards leading to a dramatic restructuring of the eye to adapt to dim light vision. While most gecko species are nocturnal, diurnality has evolved repeatedly in this clade (Gamble et al. 2015). Future work should attempt to elucidate the molecular evolution of visual opsins and other phototransduction genes as they adapt from ancestral nocturnality to diurnality across the breadth of independently-evolved shifts in diel activity pattern.

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Data Availability

SRA project for data generated in this study is available in Supplemental Table 1. Assembled transcriptomes and alignments are available via Figshare (TBD).

Author Contributions

BJP and TG developed project aims and assessed gene orthology. BJP, SVN, and TG conducted fieldwork and wrote the manuscript. BJP assembled and annotated transcriptomes. SVN extracted RNA and made sequencing libraries.

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Figures

Figure 1: (A) Phylogeny, pruned from Gamble et al. (2015), depicting relationships among sampled geckos (*Correlophus ciliatus* :: Diplodactylidae; *Lialis burtonis* :: Pygopodidae; *Eublepharis macularius* :: Eublepharidae; and *Phelsuma laticauda, Gekko japonicus, Hemidactylus turcicus,* and *Gehyra mutilata* :: Gekkonidae). Data from transcriptomes or genomes are indicated. (B) Hypothesis #1, a "nocturnal bottleneck" in the most recent common ancestor of geckos shows a simultaneous loss of phototransduction gene expression that is conserved across extant geckos. (C) Hypothesis #2, a step-wise loss of gene expression in the phototransduction pathway leading to a disparity in gene expression profiles across extant geckos. For (B) and (C), filled boxes represent gene expression of key photoreception genes, while hollow boxes indicate lack of expression.

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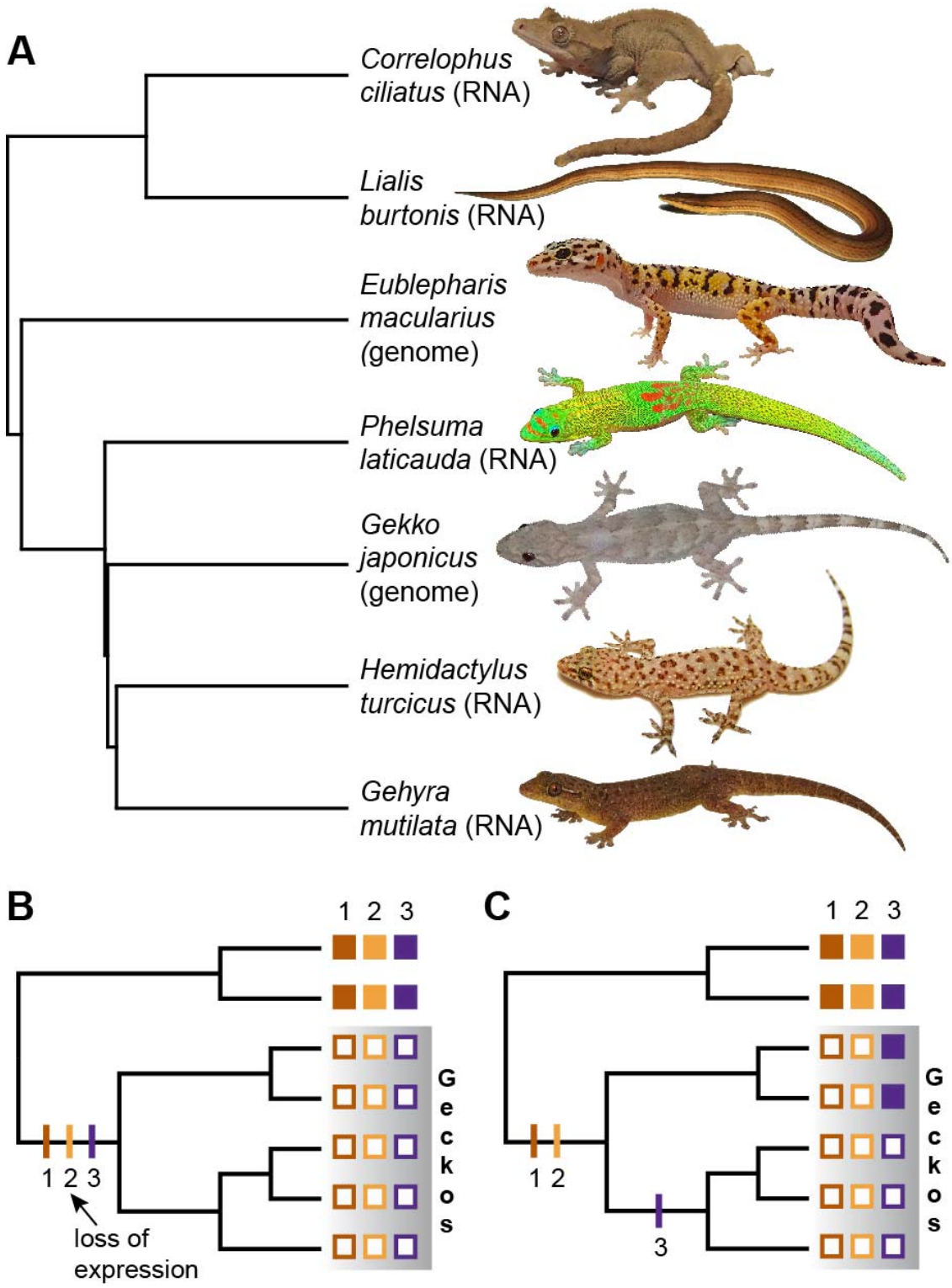

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Figure 2: Maximum likelihood phylogeny of five visual opsins in tetrapods illustrating the absence of SWS2 and RH1 in geckos. Cone opsins are shaded lighter, (*orange, online only version*) and rod opsin is shaded darker (*violet, online only version*). Black filled circles at nodes indicate bootstrap values > 70; open circles at nodes indicate bootstrap values < 70. Tree is rooted using pineal opsin sequences (OPNP).

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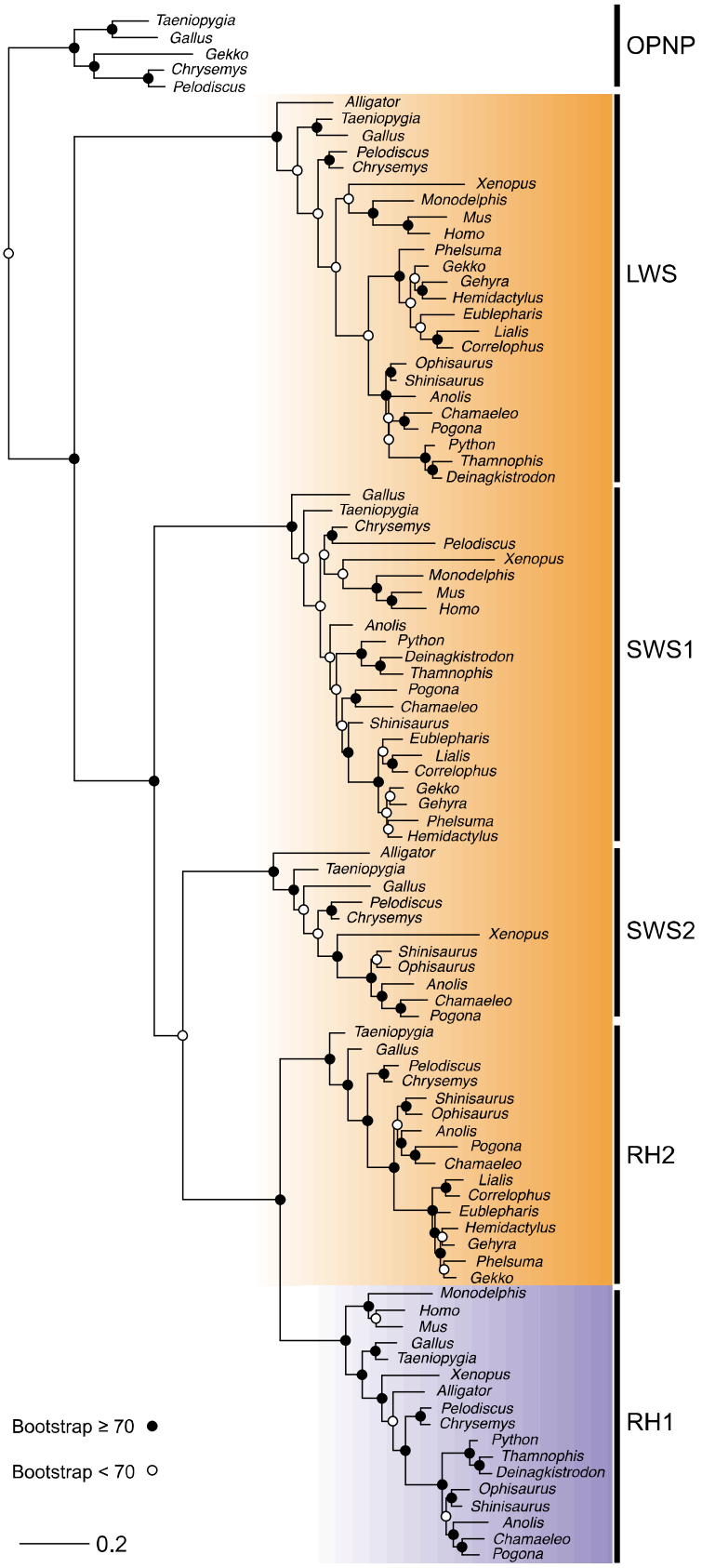

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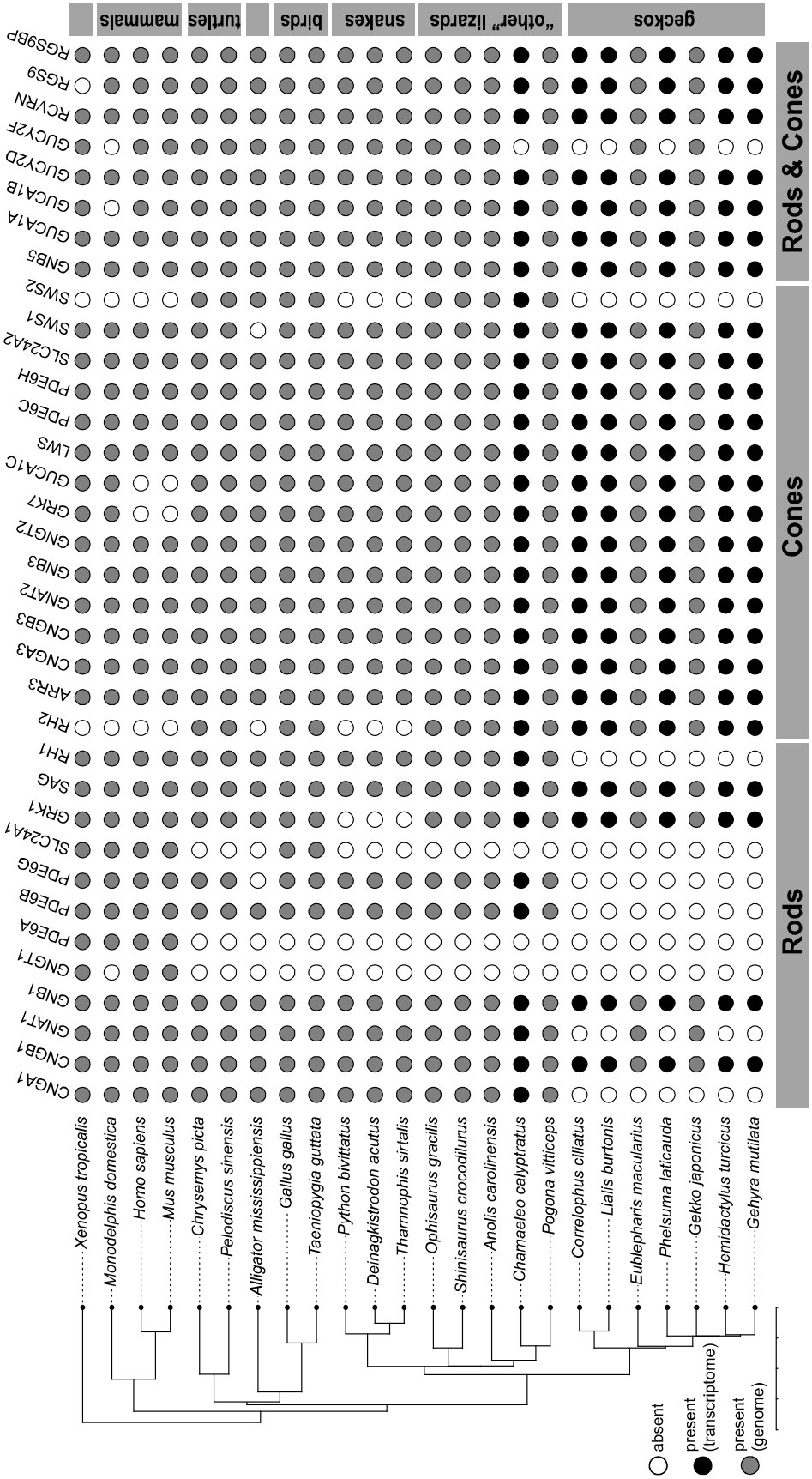
Figure 3: Presence/absence of 35 ancestral phototransduction genes for sampled tetrapod species. Filled gray circles indicate presence in the sampled genome, filled black circles indicate presence in the sampled transcriptome, white circles indicate absence. Expression in rods, cones, or both cell types is indicated along the bottom of the matrix. Phylogeny is a composite from Gamble et al. (2015) – geckos – and Irisarri et.al. (2017) – other vertebrates.

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