Visual Evolution of Scincoidea

A dissertation by

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“What is this life if, full of care,

We have no time to stand and stare?”

-W.H. Davies

Summary

Several clades are now known to have undergone nocturnal bottlenecks, changing their eye morphology, photoreceptor morphology and opsin complement. Within Squamata, snakes and geckos are the most widely studied clades that are known to have undergone nocturnal bottlenecks. The visual evolution of representatives of most major clades within Squamata have been examined, yet Scincoidea are a particularly understudied clade, considering their species richness, cosmopolitan nature and diversity in body form and habitat. With this diverse clade practically unexplored in the field of visual evolution, I investigated the visual and non-visual opsins of Scincoidea.

Within Scincimorpha there are several clades that have transitioned to a fossorial lifestyle, and exhibit morphological adaptations consequently. Of particular interest are *Lerista,* a large and diverse clade with a spectrum of adaptation across its species. There have been several independent digit and limb loss events in *Lerista*, and the full range of limb configurations is seen within, from pentadactyl to limbless. This contrasts with *Ctenotus*, its sister clade, whose species are strictly pentadactyl. The transitions to low light in this clade are an excellent system in which to examine the effect of recent scotopic transitions on the visual opsins. I sequenced the visual opsins of 86 *Lerista* and *Ctenotus* using gene capture, finding all five ancestral visual opsins in every species. Selection tests were then conducted on each opsin to see if selection correlated with any morphological indicator of low light adaptation. We found several selected sites, though the relevance of these in terms of spectral tuning is as yet unknown. We also found that LWS is under relaxed selection in *Lerista* that have experienced limb and digit loss, and that there are site changes in RH1 that may have implications for the structural stability of the opsin. Thus we found that colour vision retains importance for fossorial skinks, and that eye size reduction in fossorial species may only reduce acuity, and reduce the maximum distance at which they can focus, which we hypothesise is of lower importance to skinks that spend their time manoeuvering through sand, leaf litter and interstitial spaces.

I then expanded our focus to all of Scincoidea, with a view to comparing their non-visual opsin complement to other clades of squamate. Snakes, geckos and mammals have all undergone nocturnal bottlenecks, and in addition to visual opsin loss, they are now known to have lost several non-visual opsins. The implications of these losses are not yet fully understood, but many of the lost genes have roles in circadian rhythm regulation, photoentrainment, sensitivity to polarised light and skin pigmentation changes. There have recently been studies that suggest skinks have had a non-diurnal ancestor, but the retinal morphology of the few skinks studied does not provide clear evidence of a dim-light ancestry in snakes and geckos. I took non-visual opsins from publicly available genomes and transcriptomes, and adding to those my gene capture data and skink transcriptomes we synthesised, we compiled a database of presence and absence of 15 non-visual opsins across 70 species of squamate. Supplementing our skink dataset with sequences from the *Tiliqua rugosa* genome, we were able to infer that Scincoidea as a clade has lost at least three, and possibly as many as six non-visual opsins, many of which are convergently lost in snakes and

geckos. These results provide the first evidence of non-visual opsin loss in Scincoidea, support previous hypothesis of a mesopic bottleneck in Scincoidea, and have also allowed us to infer that the pineal gland of skinks, as in snakes and mammals, may not be photosensitive. I also uncovered previously unknown opsin losses in diverse squamate clades Gymnopthalmoidea and Acrodonta, related to parietal eye and pineal gland function, respectively.

Our final investigation built on our previous one, as we then tested selection on non-visual opsins across Squamata. Taking the same dataset as in Chapter 3, sampling across Squamata, various selection tests uncovered that snakes and geckos showed signals of relaxed selection in most non-visual opsins, compared to other squamate clades such as lacertids and anguimorphs. Skinks however showed enhanced constraint in five opsins, OPN5, OPN3, OPN4m, OPN4x and RRH. This is in stark contrast to snakes and geckos, and differs even from findings in lacertids and anguimorphs. From this I inferred that skinks, having experienced a mesopic bottleneck and lost several non-visual opsins, as I found previously, had then emerged and readopted diurnality, increasing selective pressures on the remaining non-visual opsins. Parapinopsin and parietopsin, parietal eye genes, have been maintained in skinks, while pinopsin, the ancestral pineal photopigment, has been lost. Based on this I suggest that the parietal eye opsins have assumed the role of melatonin regulation from this inactivated opsin.

This thesis provides deep insight into the adaptation of visual systems to ecological transitions, from the lateral visual pigments within a clade of Scincoidea, to the extra retinal photopigments across Squamata. It also represents the first molecular evidence of non-visual opsin loss due to a mesopic bottleneck in Scincoidea, and purifying selection pressures resulting from their hypothesised re-adoption of diurnality. It provides evidence of a loss of function in an important circadian rhythm mediator, and co-option of existing opsins to compensate for the loss of others. Finally it demonstrates the diversity of photic adaptation in all Squamata, and particularly in the previously overlooked Scincoidea, which I show to be a rich system for understanding the evolution of visual and non-visual photoreception.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Contributions

Chapter 2: Retention of the full visual opsin repertoire in Australian scincid lizards

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Status: Unsubmitted, prepared in publication format

Chapter 3: More than meets the eye: Molecular analyses reveal repeated losses of non-visual opsins in Squamata

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Chapter 4: Shifting evolutionary constraints on non-visual opsins in squamates suggest divergent selective pressures on skinks

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Chapter 1: Introduction

Vision, Eye Morphology and Photosensitivity

Vertebrate eye morphology is largely conserved throughout modern vertebrates that have retained full vision, with only minor adaptive and idiosyncratic changes among lineages. Generally speaking, the vertebrate eye is a hollow sphere made of the white sclera with a small frontal aperture, the pupil, through which light enters (Walls, 1942). The cornea is a transparent surface that covers the pupil and iris and is continuous with the outer layer ('white' or 'sclera') of the eye (Walls, 1942). The size of the pupillary aperture can be adjusted with the iris, a circular structure which alters the amount of light that can enter the eye using sphincter muscles and dilatory muscles (Walls, 1942). Behind the iris lies the lens, a crystalline structure that is responsible for focusing light on the retina (Walls, 1942). The retina is where the light is detected by photoreceptor cells that initiate a chemical cascade, resulting in a signal to an adjoined ganglion cell that sends an impulse along the optic nerve to the brain (Walls, 1942; Kolb, 2012).

In most vertebrate eyes, there are two main types of photoreceptor cell, rods and cones (Walls, 1942; Kolb, 2012). Rods and cones share the same basic cell structure. They can be divided up into 4 sections: the outer segment, which is where the visual pigments are housed; the inner segment, which holds most of the organelles of a cell, including the golgi apparatus, mitochondria and the endoplasmic reticulum; the cell body; and the synaptic terminal, which connects to neurons (Walls, 1942; Kolb, 2012).

Most of the cone protrudes upwards through the lacuna of the external limiting membrane which holds it in place (Walls, 1942). Directly above this is the inner segment, which houses the nucleus of the cell and most other organelles (Walls, 1942). The nucleus lies at the proximal end of the inner segment, and at the distal lies the ellipsoid (Walls, 1942; Lamb, 2013). In some animals, an oil droplet is embedded in the ellipsoid, though in other animals such as placental mammals including humans, this is lost (Walls, 1942; Kolb, 2012; Lamb, 2013). The rod shows less variation among species than the cone, and largely shares the same basic structure between species (Walls, 1942; Lamb, 2013).

Rods are responsible for scotopic (low light) vision, an idea first proposed by Schultze (1866; 1867) when he examined the eyes of many vertebrates and found that the eyes of nocturnal animals were dominated by rods (Schultze, 1866; 1867; Crescitelli, 1972). Rods are exceptionally sensitive to light and can detect single photons incident upon them (Walls, 1942; van der Velden, 1946; Rieke and Baylor, 1998), but will saturate in very low light (Lamb, 2009). Rods also have a much slower reset rate after saturation compared to cones: approximately 20 minutes in a mammalian rod (Thomas and Lamb, 1999), but only 20ms in a cone (Kenkre et al, 2005; Lamb, 2009). The morphological differences between the two types of cell are not easily recognised in all animals (Walls, 1942). This has led some scientists to define rods and cones independently of outer segment shape and size, for example defining rods as any photoreceptor cell that can detect single photons (Lamb, 2009; 2013). Cones have also been defined as a light-adapted photoreceptor that maintains functionality (is not

saturated) at high light intensities (Lamb, 2009). Rods tend to express one opsin gene, Rhodopsin 1 (RH1) (Simões et al, 2016b). Multiple rods are often connected to single bipolar cells, and multiple bipolar cells may be connected to a single ganglion cell (Walls, 1942; Kolb, 2012). This convergence of pathways increases sensitivity in rods, because multiple rods sending a signal to a bipolar are more likely to excite that cell than a single rod (Walls, 1942). However, this also reduces acuity, as it cannot be determined which rod was the one that received the light, and light upon different rods connected to the same bipolar will create an indistinguishable image (Walls, 1942). Therefore, the image generated by such cells is diffuse and has low resolution (Walls, 1942).

Cone cells are responsible for photopic vision, i.e. vision in high light levels (Walls, 1942; Kolb, 2012). They are generally conical in shape, which is what gives them their name, however in mammals they tend to be more cylindrical, like rods (Lamb, 2009; 2013). In contrast to rods, the outer segment lamellae are continuous with the plasma membrane. They are not highly sensitive (or activated by a single photon) like rod cells, but they do have high resolution, due to their shorter response times and the low convergence of cones compared to rods (Fu, 2010). Often, several rods will synapse to one bipolar cell, several of which in turn synapse on one ganglion cell (Kolb, 2007). This means any one of these rods may be triggered and provide an impulse to these cells, whereas cones, which have lower convergence and in some cases a 1:1:1 ratio of photoreceptor to bipolar cells, and thus have high spatial resolution (Kolb, 2007). All these factors mean that cone cells produce very high-resolution images. Cones are usually associated with cone photopigments, which include Short Wavelength Sensitive 1 (SWS1) , Short Wavelength Sensitive 2 (SWS2), Long Wavelength Sensitive (LWS), and Rhodopsin 2 (RH2). RH2 actually occurs in the cone and is a cone photopigment; however, RH1 was found to have been derived from RH2, showing that rod and scotopic vision evolved much later than cones (Okano, 1992; Collin and Trezise, 2004; Davies et al, 2007). The photopigments have a wide spectral range and cover most of the visible colour spectrum and can even be ultraviolet sensitive. The λ_{max} values for the photopigments are as follows: RH1=~500nm; RH2=480-530nm; LWS=500-570nm, SWS2=400-470nm and SWS1=355-445nm (Yokoyama 2000; Bowmaker 2008; Davies 2011).

Non-Visual Opsins

There are also a number of opsins that have functions other than image forming. There are known as non-visual opsins and are around 19 families in vertebrates, 16 of these genes are known to be expressed in lepidosaurs (OPN4m, OPN4x and pinopsin (Frigato et al, 2006), OPN5-8 (Davies et al, 2015), OPN3 and TMT1-3 (Beaudry et al, 2017), Parapinopsin and Parietopsin (Wada et al, 2012), RGR and RRH (Emerling, 2017; Perry et al, 2018), and VAOP (Beaudry et al, 2017)).. Some such as the melanopsin OPN4 are expressed in the retina, but many are also expressed in several other extra-ocular tissues, such as the pineal gland (Su et al, 2006), parietal eye in lizards (Wada et al, 2012), adrenal glands (Ohuchi et al, 2012), and the skin (Crowe-Riddell et al, 2019). Of particular importance are photosensitive structures such as the pineal gland, which contains pinopsin, parapinopsin and parietopsin in lepidosaurs (Okano et al, 1994; Frigato et al, 2006; Wada et al, 2012). This is a major centre

of hormonal control, mediating hormone suppression and release (Sapède and Cau, 2013). Some clades of lizards also possess a parietal eye, which makes a complex with the parietal gland, and contains parapinopsin and parietopsin (Su et al, 2006; Wada et al, 2012). The parietal eye is known to be a detector of polarised light (Beltrami et al, 2010), and may be able to discriminate between wavelengths (Wada et al, 2012). In chickens, pinopsin has been shown to give the pineal gland photosensitivity, and thus enable the secretion and suppression of melatonin released from the pineal gland to be entrained to light cycles (Okano et al, 1994;). In the skink *Tiliqua rugosa*, the photoreceptive parietal eye has been proven to be able to detect the polarisation of light, and use it to navigate to home ranges (Freake, 1999; 2001).

Spectral Tuning

Spectral tuning is a phenomenon whereby the photopigments can be altered to be sensitive to slightly different wavelengths of light though a change in an amino acid in the opsin, or a change in the chromophore from 11 cis retinal to 3,4 dihydroretinal, which can raise the maximum wavelength the pigment can absorb ($\boldsymbol{\lambda}_{max}$) by 20 nm. An example of this is the F86Y in SWS1, where changing the amino acid at the 86 position along the photopigment from phenylalanine to tyrosine shifts $\boldsymbol{\lambda}_{max}$ from 359nm to 438nm, which means it is now similar in $\boldsymbol{\lambda}_{max}$ to SWS2 (Cowing et al, 2002; Simões et al, 2020).

The spectral sensitivities of all major groups of NVOs have been measured using spectrophotometry and mutagenesis and found to range from 360nm in the OPN5 gene (Yamashita et al, 2014) to 522nm in the parietopsin gene (Wada et al, 2012). Among-lineage variation in maximal absorption has been reported for some NVOs, notably in OPN5 where the visible light maxima for non mammalian vertebrates was 474nm, but in humans and mice it was 469nm (Yamashita et al, 2014). OPN3 is blue sensitive, with absorption maxima of 465nm in zebrafish and ~470nm in chickens (Sugihara et al, 2016). Many different optimal maxima have also been reported in pinopsin, from 460nm to 482nm (Okano et al, 1994; Max et al, 1995; Kawamura and Yokoyama, 1998; Nakamura et al, 1999). Many non-visual opsins are bistable, which means they can have two absorption peaks. Unlike visual opsins, bistable opsins do not become thermally unstable and disassociate the retinal chromophore after excitation by light ('bleaching'), and instead are reverted to their original state by subsequent light absorption (Peirson et al, 2009). This gives these opsins two thermally stable stages corresponding to two absorption maxima (Hubbard and St George, 1958). For example, parapinopsin is UV sensitive with an absorption maximum of 370nm, but after isomerisation, it becomes maximally sensitive at 515nm, meaning it is UV sensitive and green sensitive in different states (Koyanagi et al, 2004). OPN5 is also bistable, with spectral peaks of 360nm and 500nm (Yamashita et al, 2014).

Adaptive Significance of Visual Systems

Sight is arguably the most important sense in the animal kingdom. Nearly all vertebrates possess the sense of sight and even animals that live in low light (nocturnal, fossorial or deep-sea conditions) often retain some element of visual system. Sight is

important for a wide variety of functions in the animal kingdom, from sighting predators and sighting prey, to finding a suitable mate. These are two of the most important parts of an animal's life, staying alive and reproducing. Therefore, it is easy to see why vision is a highly evolvable trait. For example, many different animals use striking colouration and visual cues to attract mates. Visual signals such as the elongated tail feathers of the male long tailed widowbird, *Euplectes progne*, signal to the female the fitness and sexual appeal of the male. This cue is so important that even when artificially elongated in an experiment, the females will preferentially choose a male with a longer tail, although the length of the tail decreases the male's ability to fly (Andersson, 1982). Sight also has been found to play a part in lizard mate selection, with males of *Platysaurus broadleyi* using sight to choose larger females for the competitive advantage a larger body size may bestow to offspring (Whiting and Bateman, 1999).

Squamates and Nocturnal Bottlenecks

The general structure of a vertebrate eye can be described as previously stated but there are many adaptations to different lifestyles in different clades of the animal kingdom, some of which have evolved convergently because of similar ecological niches. Some of the most drastic adaptations come from adaptations to light intensity during the time in the day they are active. For example, the retinal pigment epithelium, the layer of the retina behind the photoreceptor cells, possesses long processes which interdigitate with the rod cells in bright light and block any light entering at too great an angle (Kolb, 2012). The visual cells themselves may also move. When the pigment migrates as just described, the cones move away from the pigment, thus they are never shielded by the pigment (Walls, 1942). Rods, however, are not so migratory and if they move at all, it is toward the pigment (Walls, 1942).

The rods of nocturnal animals have also been found to have adaptations to that lifestyle. The nucleus of the rod has many differences in nocturnal animals from diurnal animals and other types of cell that are clear adaptations to nocturnal low light vision. Solovei et al (2009) investigated this and found that, in mice, the architecture of the rod nucleus is inverted. This is not due to the small size of a typical rod nucleus, because the nucleus of a spleen cell, perhaps the smallest nucleus in the mouse, has normal nuclear configuration, as do many other cells in the mouse's body. Solovei et al (2009) investigated 22 other species, both nocturnal and diurnal animals, and found that the inverted pattern predominates in nocturnal animals. This showed an even stronger correlation with other scotopic adaptations in the eye. They found a much higher density of refractive heterochromatin in the nucleus, which mirrors the cone mitochondrial morphology of the tree shrew and may have a similar effect in acting as a lens and directing eyes to the outer segments.

A study in two species of sympatric anolid lizards has revealed that they partition their habitat according to their photic environment (Leal and Fleishman, 2002). *Anolis cooki* and *A. cristatellus* are sympatric but occupy different microhabitats with distinct light intensities and spectra (Leal and Fleishman, 2002). *A. cooki* lives in microhabitats with greater intensity across a broad spectral range than *A. cristatellus*, whereas *A. cristatellus* habitats peak at 550nm, but are significantly less intense than *A. cooki* habitats at longer and shorter wavelengths (Leal and Fleishman, 2002). This is due to a lack of green vegetation

over *A. cooki* microhabitats (Leal and Fleishman, 2002). This seems to be mirrored in spectral sensitivity, where *A. cristatellus* has a peak sensitivity around 560nm, whereas with *A. cooki*, sensitivity rises from 450nm, plateaus at 550-600nm, and decreases around 610nm, but no sharp peaks in absorbance are seen anywhere along the spectrum (Leal and Fleishman, 2002). Both species are UV sensitive, but sensitivity is somewhat higher in *A. cristatellus* (Leal and Fleishman, 2002). Finally, reflectance was measured for the dewlaps of males of both species, and it was found that the dewlap of *A. cooki* reflects significantly less UV than that of *A. cristatellus* (Leal and Fleishman, 2002). While the dewlaps of the two species are so similar in the visible spectrum as to be indistinguishable to humans, the difference is obvious when taking UV reflectance into account (Leal and Fleishman, 2002).

The evolutionary history of snakes has long been discussed and debated in scientific literature and is often closely linked to the study of their vision. Walls (1942) saw that there were losses of certain visual features in snakes such as lost cones and hypothesised that this was due to a fossorial period in snake history. However, Underwood (1967) noted that most snake eyes are noticeably different to those of fossorial lizards, and Simões et al (2015) found that the ancestral snake retained genes and eye structures that are lost in the most dedicated modern burrowing snakes, the scolecophidians. Therefore he proposed a modified version of Walls' hypothesis, where snakes went though a nocturnal phase rather than being dedicated burrowers (Underwood, 1977; Simões et al, 2015).

Transmutation

Because of a period in the evolutionary history of nocturnality, crepuscularity or fossoriality in some snakes and geckos, they might have adapted their cone cells to be more similar in morphology to rods (Walls, 1942). Noting changes in anatomy, Walls (1942) suggested that perhaps these squamates had managed to develop rod-like functionality in some of their cones, effectively re-evolving rod cells. The transmutation hypothesis was first investigated in geckos and found to have some substance. Geckos first went through a stage of being diurnal and evolved all cone retinas, before becoming primarily nocturnal as a group (Walls, 1942; Röll, 2000). Now around 343 of the 1552 species of gecko are again diurnal and possess all phenotypically rod-like photoreceptors in their retinas (Röll, 2000; Gamble et al, 2015). Examination of the eye structure of the diurnal geckos *Phelsuma guentheri* and *Rhoptropus barnardi* showed that many rod cells in these gecko retina display features which are predominantly associated with cone cells (Röll, 2000). Zhang et al (2006) found that in the Tokay gecko (*Gekko gecko*), which has an all rod retina, the main photopigment is P521, a cone type pigment most similar to those found in chicken cones (Crescitelli et al, 1977). The gecko cone also possesses another photopigment which is similar to chicken RH2, found in rods and cones. More recently, Pinto et al (2019) found that geckos express SWS1, RH2 and LWS, and do not express SWS2 and RH1 (Liu et al, 2015; Pinto et al, 2019). So, although the morphology of photovisual cells in the gecko eye is rod-like, they express cone pigments (Pinto et al, 2019). This suggests that the morphological characteristics are more plastic than the molecular characteristics, which are still like those further back in gecko evolutionary history (Kojima et al, 1992). Further evidence for a nocturnal bottleneck in geckos comes from the non-visual opsins. Geckos have lost parietopsin, parapinopsin,

OPN4m and OPN8 (Emerling, 2017), and have also lost their parietal eye (Gundy et al, 1975) in which parietopsin and parapinopsin would normally be expressed in other lizards (Wada et al, 2012).

Schott et al (2016) followed by Simões et al., (2016b) became the first to provide molecular evidence for transmutation in snakes. Microscopy revealed that all *Thamnophis proximus* photoreceptor cells are cone-like in morphology. It was also found that they possess three visual pigments, which correspond to LWS, RH1 and SWS1 (Schott et al, 2016). However they found that RH1 RNA is expressed in one type of single cone (Schott et al, 2016). Further investigation of these cones revealed that they possess some rod characteristics like an outer segment encased on a plasma membrane. This suggests transmuted rods. Inspection of the RH1 expressed in these rods showed the RH1 to be highly blue shifted with an absorbance peak of 481nm (Schott et al, 2016). So this shows that RH1 is conserved and expressed in transmuted rod cells (Schott et al, 2016). Shortly after, Simões et al (2016b), provided more evidence across a diverse range of snakes that only three visual opsins, LWS, SWS1 and RH1 were retained and expressed in the rod-like and cone-like photoreceptors. Like geckos, snakes have also lost two visual opsins, RH2 and SWS2 (Simões et al, 2015). And also like geckos, they have lost numerous non-visual opsins (pinopsin, parapinopsin, parietopsin, OPN4m)(Emerling, 2017; Hauzmann et al, 2019) even showing parallels with geckos, having lost many of the same genes. Also like geckos, snakes have lost the parietal eye (Kunkel, 1915), one of the main extra retinal photosensitive organs in reptiles.

Scincoidea (scincid lizards) - an overlooked system for studies of visual evolution

Scincoidea is a large taxon of lizards, encompassing the families of Scincidae, Cordylidae, Xantusiidae and Gerrhosauridae (Pyron et al, 2013). The family of Scincidae are particularly diverse, with approximately a quarter of all lizard species, some 1743 lizards, being in Scincidae (Uetz et al, 2022). In Australia specifically, there are ~450 species of Scincidae, of which 205 species belong to the genera of *Lerista* and *Ctenotus* (Uetz et al, 2022).

It is puzzling why two genera of skink comprise nearly two thirds of the species diversity in the Australian sphenomorphine clade, an Australian clade of skinks comprising at least 232, including the genera of *Lerista* and *Ctenotus*. It has been noted that the majority of species in *Lerista* and *Ctenotus* inhabit the arid and semi arid habitats, which during the past 25 mya have seen considerable expansion in Australia, now occupying more than three quarters of the surface area of the country (James and Shine, 2000; Rabosky et al, 2007). This same expansion appears to have been accompanied also by sclerophyllous plants (Crisp et al, 2004) and agamid lizard diversification (Hugall and Lee, 2004; Rabosky, 2006). Rabosky et al (2007) showed that the diversification times for *Lerista* and *Ctenotus* broadly match the times for these other lineages too, and with the known time for aridification in Australia, suggesting perhaps that the expansion of the Australian arid zones facilitated species diversification in *Lerista* and *Ctenotus* (Rabosky et al, 2007). Considering these facts, it is

surprising that so few other Australian sphenomorphine species have succeeded in colonising the vast arid and semi arid habitats. This suggests some form of niche conservatism, such as that which limits the dispersal of lineages between tropical and temperate climates (Wiens et al, 2006), and between elevational clines on mountains (Wiens et al, 2007). This may not be the only explanation, however, as there are four other species of sphenomorphines in Australia that inhabit arid and semi arid zones, which means that two of three or four (depending on whether the common ancestor of *Ctenotus* and *Lerista* were arid adapted, or whether this happened independently in the ancestry of both genera) Australian sphenomorphine species failed to diversify (Rabosky et al, 2007). The reason for this may be that *Ctenotus* has a much higher critical thermal maximum than many other genera, including *Eremiascincus*, one of the genera containing arid and semi arid adapted species (Garland et al, 1991). Further research would be necessary to determine if *Lerista* has a similar level of critical thermal maximum and the remaining arid adapted species in *Notoscincus* do not, but this may go some way to explaining the much vaster radiation of these two genera of sphenomorphine skinks relative to the rest (Rabosky et al, 2007). Sensory adaptations, which are the interface between the organism and the environment, may also have contributed to the success of *Lerista* and *Ctenotus.*

Lerista are a genus of squamates in the family Scincidae, originating between 12.9 to 22.1mya (Rabosky et al, 2014). They are endemic to Australia and found in a wide range of habitats all over the country. They are notable for highly diverse limb configurations and digit numbers, from the limbless *Lerista apoda* to the fully limbed pentadactyl *Lerista bougainvillii*. They are most widely used as a model system to study evolutionary changes in morphology over a short space of time due to their repeated and exceptionally rapid digit and limb loss (Skinner et al, 2008). It was found that limb reductions have occurred at least ten times from a pentadactyl state, with at least a further seven from a tetradactyl state (Skinner et al, 2008; Wiens, 2009). There have been at least four independent total reductions to no limb state in the genus (Skinner et al, 2008; Wiens, 2009). What is more, the age of the genus was determined to be around 13.4 million years, and the quickest limb reduction from pentadactyly to no limb happened in as little as 3.6 million years (Skinner et al, 2008; Wiens, 2009). This shows that the genus has exceptionally high phenotypic plasticity. There is also some evidence that digits may be regained in certain species of *Lerista* (Skinner et al, 2008; Wiens, 2009). It was found that in the hind limb, the estimated rate of digit gain was higher than the rate of loss, suggesting possibilities of digit loss reversal (Wiens, 2009). Further research in this area is necessary to more conclusively state that digit loss reversal occurred, but if it did this may be one of the few cases that goes against Dollo's Law, which states that once lost, structures will not be regained in exactly the same way (Skinner et al, 2008).

In contrast to *Lerista*, the closely related genus of *Ctenotus* is extremely phenotypically stable, with no digit or limb loss described in the genus, despite similar levels of ecological and habitat diversity with *Lerista*, and sharing a similar probable origin date (Rabosky et al, 2014). In *Ctenotus*, rates of morphological change were found to have decreased by 10x compared to the common ancestor of the genus (Rabosky et al, 2014). This is surprising because it is well known that digit and limb reduction and loss is intrinsically linked to habitat in skink species, and there is even an index (Scincidae Ecological Niche Index, SENI) that allows on to predict the ecological niche of a skink species based on the

length of its digits (Schnirel, 2004). *Ctenotus* species all maintain pentadactyly on both fore and hind limbs, and also exist in the majority of terrestrial Australian habitat types.

Given that these two lineages are the result of a rapid species radiation as result of occupying multiple climatic and ecological niches, we might expect these species' vision genes to show signals of ecological adaptation that are not obscured by phylogenetic history, allowing to link genetics and eye anatomy changes.

Because of the similar evolutionary history between some burrowing skinks and snakes, both of which have at some point adapted to a fossorial lifestyle according to the leading hypothesis in snakes, we can draw hypotheses about what we may expect to see in *Lerista* and *Ctenotus* visual evolution. There are already a number of similarities in morphology between snakes and the more fossorial skinks, including the obvious reduction in limbs, but also fused eyelids and some even have increased presacral vertebrae numbers, which seem to increase with limb loss and increasingly fossorial lifestyle (Greer, 1987; 1990). If the fossorial lizards follow a similar evolutionary path to the snakes, we would expect to see several differences in photochemistry and morphology to the more terrestrial species of skinks.

However there are a number of key differences to take into account between non-snake squamates (lizards) and snakes. Firstly, most non-gecko lizards as a group have retained all 5 visual pigments, whereas snakes have lost SWS2 and RH2 (Davies et al, 2012; Simões et al, 2015). Snakes do not possess oil droplets, whereas most diurnal lizards do (Robinson, 1994). As stated previously, double cones of Colubridae snakes are vastly different to any seen in the rest of the animal kingdom, lizards included (Walls, 1942). While snakes and geckos do not have a parietal eye, most lizard species in other clades do (Gundy et al, 1975). And while geckos and snakes have convergently lost certain NVOs, these opsins have been found in other lizard clades (Su et al, 2006; Wada et al, 2012; Beaudry et al, 2017).

The morphology of the eye in skinks has been examined in a few species, and skinks appear highly varied. The previously mentioned shingleback, *Tiliqua rugosa*, is a diurnal skink which possesses oil droplets in its photoreceptors, only single and double cones, no rods, and no fovea (New et al, 2012), unlike other lizards which normally have two foveae (Röll, 2001). However the rod photopigment RH1 has been found to be expressed in these cone-like cells in the retina of *Tiliqua rugosa* (New et al, 2012). This is reminiscent of geckos and their re-evolved cones, albeit in reverse. This is not the case with all skinks however, as the also diurnal *Cryptoblepharus boutonii* does possess a clear fovea and oil droplets as well as an immovable spectacle and fused eyelids like a gecko, while still only having single and double cone morphologies (Röll, 2001). The sand swimming skinks *Eumeces schneideri* and *Scincus scincus* however, both possess rod-like photoreceptors that express RH1 (Canei et al, 2020) and there is a case of transmutation. A fossorial skink, *Acontias orientalis*, possesses a highly degenerated eye, while the eye of *Typhlosaurus vermis* has degenerated to the point of vestigiality (Zhao et al, 2020). Overall, skinks do show some similarities to geckos, but the morphological evidence for a nocturnal bottleneck is inconclusive (for more detail see Fleming, 2022).

Despite Squamata as a whole being a heavily studied clade in terms of visual evolution, skinks are largely absent from these studies. So, despite these lineages offering an almost unique set of ecological and evolutionary circumstances in which to study and

understand regression and re-emergence of visual traits and the genetics behind them, there have been relatively few studies in the evolution of their visual systems. This thesis seeks to address this underrepresentation of skinks in the study of visual evolution, and uncover any adaptive variation in their opsins in comparison to other clades of Squamata.

Broad Aims

I aimed to elucidate on the visual evolution of Scincoidea, first within the clade using an endemic genus of skink. Then by comparing the clade as a whole to other equivalent clades in Vertebrata. I use molecular methods, and computational analyses to do this, and draw on a wide range of morphological, behavioural and ecological studies to examine how visual evolution has impacted skinks.

Aim 1

Understand how genetic variation underpins lizard visual system anatomical and physiological diversity: I will identify and analyse the 5 opsin genes in 91 species of *Lerista* and *Ctenotus* and across the Scincidae family. I will align them and contrast with outgroups to identify any gene losses or mutations which may make them non-functional or may alter the spectral tuning of the opsin, and conduct selection tests to detect variations in selective pressure according to gene and ecological divisions.

Aim 2

To investigate the loss and retention of non-visual opsins in Scincoidea, and compare these to losses in other vertebrate clades, especially snakes, geckos and mammals, which have undergone gene losses during nocturnal bottlenecks. I will use publicly available genomes to search for non-visual opsins, as well as using sequences from published transcriptomes, and gene captures and transcriptomes from skinks generated for this study. I will then compare opsin complements among clades, and use these results to infer ecological transitions and effects on physiological mechanisms due to losses of opsins.

Aim 3

Using the non-visual opsin sequences generated in Aim 2, I will test for positive/purifying versus relaxed selection pressures on each opsin, at the level of individual amino acid sites, gene-wide, and according to phylogenetic (major squamate clades) and ecological (e.g. nocturnal versus diurnal) divisions. I identify shifts in signals of selection on the non-visual opsin genes in skinks and other lepidosaurs, and interpret my findings in the context of the role of low light transitions in shaping the evolution of non-visual photoreception in squamates.

Thesis Outline

Visual evolution is a long studied and incredibly important field of research, and one of the most studied clades across vertebrates is the Squamata. Ever since Gordon Walls' (1942) seminal book on the subject, first detailing the curious phenomenon of transmuted cones and rods in snakes, squamates have been one of the foremost clades in visual evolution research. Despite plentiful research into snake, gecko, lacertid and other squamate clades, skinks have received very little attention in this area. This is surprising since skinks are one of the biggest families of squamate, they are found on many continents and occupy several different photic, temporal and spatial niches. In particular, the genus *Lerista* comprises a recent radiation across Australia, occupies several different habitats from tropical forest to arid desert and, most interestingly, exhibits varying degrees of fossorial behaviour and limb loss.

Chapter 2

The first chapter of this thesis undertakes a deep sampling of *Lerista* and examines the visual opsins of these skinks. Despite the wide range of fossorial behaviours, and previous findings that low light transitions in snakes, geckos, and other clades have led to loss of visual opsins, no losses were found across all skinks. Selection tests revealed that though some sites were selected for, including one that destabilised rhodopsin, selection pressures did not differ significantly among the several ecological groupings that were tested.

Chapter 3

The second chapter of this thesis focuses on non-visual opsins across Squamata. Compared to the visual opsins, non-visual opsins are much less researched and are poorly understood. No previous research has defined the non-visual opsin complement across every group in squamates. Our research set out to do just that. We found that key non-visual opsins lost in mammals were also lost in snakes and geckos, indicating their loss as a result of nocturnal bottleneck during periods that these groups have experienced. This study showed that skinks have also lost several of these same genes, perhaps providing evidence for a possible low light period in the evolutionary history of Scincoidea.

Chapter 4

The third chapter of this thesis conducted selection tests carried out on the non-visual opsins generated for Squamata in Chapter 3. Because certain groups have lost genes during putative nocturnal bottlenecks, we hypothesised that retained genes may show divergent signals of selection among clades, especially the snakes, geckos and skinks. Results showed that few specific sites were reliably selected. However there was evidence of a relaxation of selection in many non-visual opsins of snakes and geckos when compared to those of other lepidosaurs. Moveover, skinks showed evidence of intensification of selection when compared to other lepidosaurs in many of these same opsins. These results were used to generate testable hypotheses for the roles of extraocular photoreceptors in skinks and other squamates.

This thesis sheds light on a previously unexplored clade of Squamata, and has uncovered hitherto unknown phenomena that may suggest a low light period in the evolutionary history of skinks, similar to those theorised in snakes and geckos. Evidence also suggests however that skinks have retained all visual opsins, unlike snakes and geckos, and that selection on retained non-visual opsins and the visual opsin LWS is very dissimilar to that found in those two previously studied clades. This research significantly contributes to knowledge in visual evolution, and the evolutionary history and ecology of Scincidae.

Chapter 5
Discussion

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By signing the Statement of Authorship, each author certifies that:

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Chapter 2: Retention of the full visual opsin repertoire in Australian scincid lizards

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Abstract

Australian scincid lizards in the sister-genera *Lerista* and *Ctenotus* are a prominent system for understanding adaptation in the transition from surface to fossorial life. The approximately 205 species in this group exhibit extreme diversity in morphology and ecology. *Lerista* and *Ctenotus* both include diurnal and surface-active species that are fully pentadactyl, whereas *Lerista* also contains many specialised limbless and limb-reduced sand-swimmers. To understand how the visual systems of these lizards have responded to their highly varied photic environments, we examined the five opsins genes encoding the pigments that mediate colour and dim-light vision in tetrapods. These genes were sequenced for 59 species of *Lerista* and *Ctenotus* and analysed using codon models to detect variation in selection pressures among amino acid sites and across branches in the species tree. We found that all five opsins are present and intact in all species of *Lerista* and *Ctenotus* examined, and further identified signals of positively selected substitutions in four of the five opsin genes –RH1, which mediates dim-light vision, and three cone opsins associated with bright-light vision (SWS1, SWS2, LWS). Most comparisons of site-, branch- and clade-specific selection pressures did not show significant differences according to broad ecological divisions. Only the LWS opsin gene showed a signal of relaxed selection in sand-swimming (limb reduced) versus less fossorial (fully limbed) *Lerista* under models of gene-wide selection pressures. These results suggest that photopic visual abilities are retained across both clades, even in sand-swimming species with externally reduced eye size and fused eyelids. Our study highlights a need for studies of the visual ecology of Australian skinks, and prompts caution with regards to generalisations about degenerate vision in fossorial squamates.

Introduction

Evolutionary transitions from bright-light (photopic) to low-light (scoptopic) environments are important drivers of change in the evolution of visual systems (Walls, 1942; Zhao et al, 2009; Pinto et al, 2019). In many taxa that rely on vision for vital tasks such as feeding, predator avoidance and mating, reduced light availability is compensated by increased corneal diameter and visual sensitivity (Hall and Ross, 2006; Hall, 2006). However, while indispensable for many taxa, eyes and optic neural tissue are also energetically expensive to develop and maintain , and these costs are often linked to evolutionary degeneration of eyes and vision in scotopic habitats (Moran et al, 2015; Protas et al, 2007). Indeed, the repeated loss of visual function in scotopic-adapted mammals, reptiles, amphibians, fish and insects is

a prominent example of convergent evolution (Sadier et al, 2018; Mohun et al, 2010; Simões and Gower, 2017; Musilova et al, 2021; Sharkey et al, 2017). In these taxa, the degree of visual conservation or degeneration is expected to vary with ecological context. The subcutaneous eye of the obligate fossorial *Spalax* mole-rats is retained only for a non-visual, circadian function (Avivi et al 2002; Haim et al, 1983), whereas the primarily subterranean blind snakes (Simões et al, 2015; Gower et al., 2021) and star-nosed moles (Emerling & Springer, 2014) have degenerated eyes and reduced colour discrimination. There is often strong selective pressure on burrowing animals to converge on a variety of morphological adaptations, one of which is reduced eye size (Gans, 1975; Pough et al, 1998).

Changes to the visual pigments in the retina are fundamental steps during adaptation to new photic environments (Emerling and Springer; 2014; Simões et al, 2015; 2020, Hauser et al, 2021). Visual pigments activate the phototransduction cascade that transforms light into an electrochemical stimulus (Cronin et al, 2014) and are encoded by opsin genes. In the ancestral tetrapod (and squamate) visual system, five spectrally distinct opsin pigments are expressed in two main photoreceptor types (Yokoyama, 2000; Solomon and Lennie, 2007). Cone photoreceptors are primarily responsible for photopic vision and contain short (~360nm) to long (~600nm) wavelength sensitive opsins that enable colour discrimination; rod photoreceptors contain rhodopsin 1 (RH1) and are used for vision in scotopic conditions (Walls, 1942; Crescitelli, 1977; Yokoyama, 2000; Bowmaker, 2008; Hunt et al, 2009; Davies, 2011).

Transitions to scotopic environments are thought to be linked to selective losses of cone opsins, and preferential retention of rod-expressed opsins, in several lineages of snakes, crocodilians, mammals and amphibians (Simões et al, 2015; Gower et al, 2021; Emerling, 2017; Emerling & Springer, 2014; Mohun & Davies, 2019). Several times in vertebrate evolution, some of these opsins and associated visual transduction pathway genes have been lost due low light transitions such as nocturnal bottlenecks. In squamates, this is known to have been the case in geckos (Pinto et al, 2019) and snakes (Simões et al, 2015; Gower et al. 2021). Gene losses occur when purifying selection is reduced due to a relaxation of selective constraints, resulting in the accumulation of inactivating mutations. In some echolocating bats, evidence of relaxed selection on the SWS1 opsin is associated with the loss, or 'pseudogenisation', of this gene (Wertheim et al, 2015; Simões et al, 2019). Alternatively, cone opsins might be intact but evolve at accelerated rates indicative of relaxed selective constraints during ancestral periods in low-light environments (Emerling and Springer, 2014; 2015). Where opsins are retained and function conserved, purifying selection is expected to remove harmful variants (Veilleux et al, 2013). Alternatively, positive selection might favour an adaptive variant that changes some aspect of opsin function, such wavelength sensitivity. These signals of positive selection are often detectable by the amino-acid make-up at so-called spectral tuning sites that influence the spectral sensitivity of the visual pigments (Fasick & Robinson, 1998; Cowing et al, 2002; Yokoyama, 2008 and citations therein). One of the most important sites is site 86 on SWS1 (Fasick and Robinson, 1998; Yokoyama & Takenaka, 2005; Carvalho et al, 2007; Carvalho et al, 2012). In mammals, snakes and birds, the ancestral state at this site, Phenylalanine (F86), has been changed in some species to

confer violet sensitivity rather than UV sensitivity (Fasick and Robinson, 1998; Hunt et al, 2009; Carvalho et al, 2007; Cowing et al, 2002; Yokoyama & Takenaka, 2005). Evidence from mammals suggests that UVS/VS transitions have been guided by photic niche, with transitions from UVS to VS corresponding with transitions to more day time activity (Emerling et al, 2015).

Clades of closely related taxa that have undergone replicate shifts between photic environments provide powerful systems for understanding visual evolution during ecological transitions. Australian scincid lizards in the genera *Lerista* and *Ctenotus* are an attractive system in this respect. The ~205 species (Uetz et al, 2022) in this clade share a common ancestor ~31 Mya (million years ago) (Rabosky et al, 2014; Skinner et al, 2013; Hedges et al, 2015; Zheng et al, 2016; Tonini et al, 2016; Pyron et al, 2014; Wiens et al, 2006; Wright et al, 2015). All *Ctenotus* are surface active, but while all species are primarily day-active in bright light desert environments, some, most notably *Ctenotus pantherinus*, show increased crepuscular and (less commonly) nocturnal activity or occupy light-limited habitats such as leaf litter in mesic woodlands (Gordon et al, 2010). In contrast to *Ctenotus*, *Lerista* species are highly diverse in habitat and morphology (Cogger, 2014). All *Lerista* are day and surface active, though none exclusively, as all species are active in surface vegetation, loose leaf litter, sand, or interstitial spaces. Across *Lerista*, there have been many independent adaptations of morphology to better suit this more cryptozooic lifestyle, such as limb loss (Greer, 1987; Greer, 1990; Skinner et al, 2008), elongation of the body (Mann, 2020) and alteration of the skull (Pough et al, 1997)(see also Fig. 2). Some lineages are more diurnal and surface-active, and have well-developed eyes and a pentadactyl, tetrapodal limb morphology, whereas several independent lineages are more fossorial and show anatomical regression of the eyes and burrowing adaptations such as limb loss and eyelid fusion (Storr, 1976; Storr, 1990; Greer, 1987; Greer, 1990; Skinner et al, 2008; Couper et al, 2016). There are also several 'transitional' forms, with a continuum of intermediate morphologies characterised by loss of digits (often with fewer digits on the forelimbs than hindlimbs), some even having lost the forelimb whilst retaining the hindlimb with 1 or 2 digits (Greer, 1983; Greer, 1987; Greer, 1990; Skinner et al, 2008). While the ancestral state of *Lerista* is not definitively known, it is thought that the ancestor of the genus would be tetrapodal and probably pentadactyl (Morinaga and Bergmann, 2017), as limb loss is unlikely to be reversed (Skinner et al, 2008; Skinner, 2010; but see also Wagner et al, 2018; Bergmann et al, 2020).

The repeated shifts in photic environments during the evolution of *Lerista* provide a valuable opportunity to identify adaptive responses of visual systems to environmental factors. We might hypothesise that the opsin genes of highly fossorial skinks will show signals of relaxed selection and/or regressive evolution. However, we emphasise that these skinks must experience complex and variable selection pressures on their visual systems, given that no two species are expected to have identical combinations of limb morphology, eye-lid condition, activity patterns and habitat use (Fig. 1). Moreover, we lack specific information on each species' visual ecology with which to make robust links between visual and

molecular adaptation.

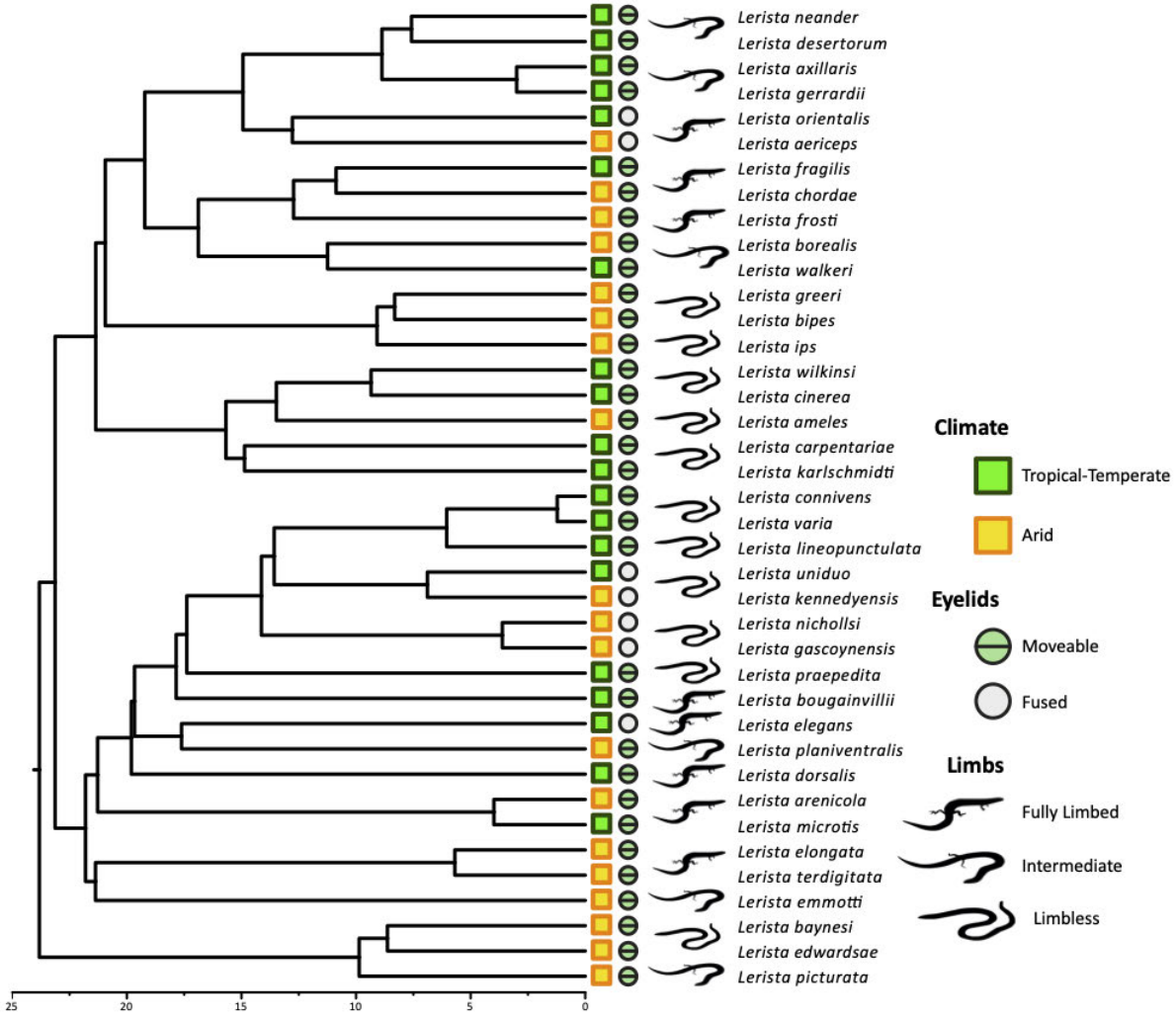

Figure 1: Phylogenetic tree of *Lerista* showing the time divergence (in MYA) of the species included in this study and their habitat, eyelid morphology and limb state. Time tree obtained from VertLife phylogeny subsets.

This study used molecular analyses to explore the diversity of visual traits in *Lerista* and *Ctenotus* and, more specifically, determine the consequences of transitions to fossorial habits in *Lerista.* To do this, we sequenced all visual opsins across 59 species, including diverse surface-active species and spanning multiple lineages that have independently acquired transitional or highly fossorial habits (Skinner et al, 2008, Fig. 1). This sampling allowed us to perform comparative tests of opsin selection across repeated transitions in habitat and diel activity. The recent timing of these transitions, from 3.6 to 11.8 million years (Skinner et al, 2008), reduces the possibility of diversity in opsins being due to factors associated unrelated or subsequent to the transition in photic environment (Rabosky et al, 2007; Skinner et al, 2013; Rabosky et al, 2014). In these respects our paper provides a substantial contribution to the literature on the nature of visual opsin adaptations to low light conditions in Squamata. We will be among the first to analyse the visual opsin complement of Scincidae, a previously understudied part of Squamata with regards to visual pigment evolution. This will also make

this the first study to examine vision in a comparative framework in squamates outside of geckos and snakes. Finally we will examine fossorial adaptations of *Lerista*, and their relation to vision. This paper samples a diverse range of skinks, with the full spectrum of digit configurations and many ecological transitions represented in our deeper sampling of *Lerista*. These factors combined with the comprehensive nature of the sampling should give a clear picture of the phenomena at work here and shed light on potential spectral tuning of skink opsins and how they relate to ecology and phenotype in Scincidae.

Figure 2: Four *Lerista* showing different limb and eyelid states. Clockwise from top left: *Lerista microtis*, tetrapodal and pentadactyl; *Lerista edwardsae*, hind limbs only, bidactyl; *Lerista punctatovittata*, showing movable eyelids; *Lerista aericeps*, showing immovable eyelids and a spectacle covering the eye. Photos courtesy of Mark Hutchinson

Methods

Taxon sampling and DNA Extraction

We extracted DNA for a total of 127 species in 23 genera of skinks (Squamata: Scincidae) obtained via fieldwork (35 samples) and from The Australian Biological Tissue Collection (ABTC) of the South Australia Museum (92 samples) (SI Table 2.1). Additionally, we extracted total RNA from the eyes of 13 species for transcriptome sequencing (SI Table 2.1). Fieldwork and tissue collection was approved by permits from the Animal Ethics Committee of the University of Adelaide (21877) and from the Government of South Australia (Q26642-5) and Queensland (WA0009193). In addition, we downloaded the opsin sequences of *Bos taurus*, *Anolis carolinensis*, *Sphenodon punctatus*, and *Python bivittatus* from GenBank (SI

Table 2.1). DNA was extracted from liver and muscle using DNeasy Blood and Tissue kits (Qiagen, Chadstone, VIC, Australia) and following the manufacturer protocols. Samples were quantified using a QuBit™ 2.0 (ThermoFisher Scientific, Waltham, MA, USA).

Library preparation and sequencing

Genomic DNA was sheared to ~400-500bp using a Bioruptor Pico Sonicator (Diagenode, Denville, NJ, USA). Fragments over 200bp were selected for using Ampure XP (Beckman Coulter Inc, Brea, CA, USA) with 8.5% PEG for bead clean-up (Li et al, 2013). DNA was then prepared for Illumina sequencing based on the protocol of Meyer and Kircher (2010), using the on-bead method of Fisher et al (2011) and Li et al (2013). The DNA library underwent blunt end repair followed by adaptor ligation and ligation fill-in and the samples were double indexed by PCR amplification (Meyer and Kircher, 2010). The libraries were combined in equimolar amounts in pools of five samples each and then centrivapped down to 7µl and hybridised to custom RNA baits (Daicel Arbor Biosciences, Ann Arbor, Michigan, USA). We used MyBaits targeted capture protocols version v4 (http://www.arborbiosci.com/mybaits-manual/) with baits designed on the *Anolis*, *Pogona*, *Python* and *Gecko* genomes, where targeted vision genes were PCR-amplified in 16 cycles after capture with streptavidin beads (Dynabeads, ThermoFisher). Once amplified, these pools were all checked for concentration with the Qubit and fragment size distribution was measured using a HS D1000 ScreenTape run on a Tapestation 2200 (Agilent Technologies, Santa Clara, CA, USA). The capture libraries were pooled in equimolar quantities to give a single sample at 10 nM DNA with an average fragment size of 400 bp. Illumina NovaSeq SP sequencing using 50bp paired end 100 cycle runs (single lane) were performed by Australian Genomics Research Facility (AGRF).

RNA extraction, library preparation and assembly

Eyes of 11 specimens of skinks stored in RNAlater and frozen at -80ºC (see SI table X) were macerated in TRIzol and the RNA was purified using the PureLink™ RNA Mini Kit (ThermoFisher Scientific, Waltham, MA, USA) using the manufacturer's protocol. The mRNA-Seq library was double-indexed and prepared with the mRNA-Seq Library Prep Kit v2 (Lexogen, Vienna, Austria) following the manufacturer's protocol. It was sequenced with 56 other libraries in equimolar concentrations in one lane of an Illumina NovaSeq S4. Low quality reads were identified and removed using Trimmomatic (Bolger et al, 2014). The remaining reads were assembled and scaffolded using Trinity (Grabherr et al, 2011).

Sequence assembly and annotation

Eleven RNA-seq samples were selected to provide reference sequences that are representative of the phylogenetic breath of the gene capture samples (SI Table 1). The transcriptome assembly software Trinity v2.10.0 (Grabherr et al, 2011) was used to assemble the RNA-seq data into transcripts, with the Trimmomatic (Bolger et al, 2014) argument specified to trim sequences prior to assembly. TransDecoder v5.5.0 (github.com/transdecoder) was then used to predict putative coding regions within the transcripts for each assembly. Candidate coding sequences were first predicted using

TransDecoder.LongOrfs. These sequences were screened against the Uniprot/SwissProt curated protein dataset using BLAST, along with the PFAM-A protein domain database using HAMMER, to obtain homology information to improve overall coding sequence prediction. Finally, TransDecoder. Predict was used to generate the final high-quality coding sequence predictions. The coding sequences for each assembly were then searched for 156 genes of interest using BLAST. A multi-fasta file containing the genes of interest was manually compiled by searching the *Alligator mississippiensis*, *Alligator sinensis*, *Protobothrops mucrosquamatus*, *Gallus gallus*, *Chrysemys picta*, *Anolis carolinensis*, *Python bivittatus*, *Pantherophis guttatus*, *Gekko japonicus*, *Columba livia*, *Serinus canaria*, *Aquila chrysaetos*, *Pseudopodoces humilis*, and *Cuculus canorus* (SI Table 2.6) gene annotations for the genes of interest. BLAST hits were filtered for the best hit using bitscore, while also ensuring that the subject and target sequence lengths were comparable. For each transcriptomic reference, the best hit genes were extracted into a multi-fasta file, which formed the reference files for the gene capture data.

Gene capture data was processed through a custom consensus pipeline based on the methods from Schott et al (2017). FastQC v0.11.9 (FastQC, 2015) was first used to assess the quality of each gene-capture sample. The data was then quality trimmed using trimmomatic to remove adapter sequences and any low quality bases from the ends of reads. Gene capture samples were then aligned to one of the eleven reference files based on their evolutionary distance. The software BWA (Li and Durbin, 2009) was used to align the gene-capture samples to their respective reference files with parameters -B 2 to reduce the mismatch penalty (default value 4) and -M to mark split alignments as secondary. Unmapped reads were then removed from the alignment files using SAMtools (Li et al, 2009; Danacek et al, 2021). Genotyping was performed using the SAMtools/BCFtools mpileup and call pipeline (Li, 2011). SAMtools mpileup was run with parameters -d 5000 to increase the maximum depth, -Q 20 to control the mapping quality and -q 20 to control the base quality. Genotype calls were then converted to variant calls using BCFtools call. Variants were normalised using BCFtools norm, with multiallelic SNPs being joined into single records using -m +any. Finally, consensus sequences for each gene-capture sample relative to the reference genes of interest were generated using BCFtools consensus with argument -H 1 to use the first allele from the GT field.

We complemented this dataset with complete CDS regions of opsins for other squamates available on GenBank (SI Table S1). Additionally, full annotated genomes sourced from Genbank were BLASTed for individual exons of opsin genes. BLAST hits were then curated and concatenated to construct full coding sequences. These were then aligned and any intron sections or UTRs still present were identified and deleted.

The opsin genes were aligned using Clustal Omega in Geneious Prime (v2020.2.4). We identified spectral sites based on Yokoyama, 2008a (and references therein), as well as other influential sites as listed in the entries on NCBI and Uniprot to determine the sites known to alter spectral sensitivity in the opsins. Leading into the analyses, 39 *Lerista* and 20 *Ctenotus* species from gene capture remained in the alignments. Opsin identity was confirmed by

assembling a larger alignment combining (using MAFFT; Katoh et al, 2002) all the opsin sequences and generating a tree in order to verify the identifications of the opsins. Firstly, a nucleotide evolution model was selected using ModelTest-NG (Darriba et al, 2020) in raxmlGUI 2.0 (Edler et al, 2020). The chosen evolutionary model (JTT+G4+F) was then used to construct a tree with RAxML-NG (Koslov et al, 2019), also using raxmlGUI 2.0 (Edler et al, 2020). The outgroup was set to be the LWS opsin cluster (Terakita, 2005). This tree was then checked visually for opsin clustering to support their likely identity.

Analyses of molecular evolution

Firstly, we downloaded the opsin sequences of *Bos taurus*, *Anolis carolinensis*, *Sphenodon punctatus*, and *Python bivittatus* from GenBank (SI Table 2.1), and aligned them with the skinks gene captures in order to quickly identify any changes to important regions in skinks.

To detect changes in selection on skink opsin genes, CodeML analyses were conducted using ete3 (Huerta-Cepas et al, 2016), using PAML version 4.8a (July 2014). CodeML allows comparison of codon-based models of selection based on the ratio of non-synonymous (dN) to synonymous (dS) substitution rates (dN/dS, or ω). dN and dS are expected to occur at the same rate ($\omega \approx 1$) under a model of neutral evolution, whereas sites evolving under purifying (negative) selection are expected to have $\omega < 1$, and those under diversifying selection are expected to have $\omega > 1$. However in tests such as the branch test, $\omega > 1$ is not often seen as diversifying selection would not be expected across most sites. It is worth noting here that these CodeML models struggle to distinguish an increase in positive selection intensity, and a relaxation of either purifying or positive selection (Wertheim et al, 2015). Therefore CodeML tests will also be supplemented by RELAX tests, which use a more comparative method to demonstrate whether selection has been relaxed or intensified in a given subset of branches in a phylogeny (Wertheim et al, 2015).

To implement CodeML (Yang, 2007) a skink species tree based on a well-resolved molecular phylogeny (Pyron et al, 2013) was used as the input tree in all analyses. This tree was generated from Pyron et al (2013) phylogeny, reconstructed using maximum likelihood [RAxML] analysis of a concatenated alignment of up to 12896 base pairs composed of 7 nuclear and 5 mitochondrial genes. This was then reduced by pruning tips from the tree, retaining only the 95 skink species sampled for opsin genes in the present study. We also ran PAML analyses through the ete3 evol program (Hueta-Cepas et al, 2016). This program uses the same CodeML models as in PAML (Yang, 2007). Analyses and models are referred to by their PAML designations. Site models were performed on all skinks species, whereas branch, branch-site and clade models were performed only on *Lerista*.

We used site models - M0, M1a, M2a, M7 β, M8 β&ω to estimate the ratio of synonymous and non-synonymous substitution rates (ω) across amino-acid sites across the skink opsin genes. Likelihood ratio tests (LRTs) were used to compare the fit of null model M1a to the alternative model M2a, and null model M7 β to the alternative M8 β&ω. Bayes Empirical Bayes was used to infer the sites under positive selection under the M2a and M8 β&ω models.

Branch models (one ratio model, two ratio model, free ratio model) allow ω to vary according to branches on the phylogenetic tree (see Fig. 1). These analyses were performed only for *Lerista* species because these show the highest ecological and morphological diversity. The groupings of *Lerista* were: climate, in which the 19 species found in arid and semi-arid habitats (hereafter termed '*arid*' group) were the foreground and 20 species that occupy tropical and temperate habitats (hereafter '*temperate-tropical*' group) were the background; eyelid condition, where 7 species with fused eyelids ('*fused*' group) were the foreground and 32 with unfused eyelids ('*unfused*') the background; extreme limb condition, where 18 species with 0-0, 0-1, and 0-2 limbs configurations are the foreground and 21 species with other configurations were the background (Fig. 1: Limbless, '*reduced limbs*'); and mild limb condition, where 27 species with 0-0, 0-1, 0-2, 1-2 and 2-2 limb configurations were the foreground and 12 other configurations were in the background (Fig. 1: Limbless and Intermediates, '*reduced limbs and intermediates*'). Limb configuration information was obtained from Cogger (2014).

Branch-site models (model A, model A1, model B) allow ω to vary across both sites and branches and this was used to determine if positive selection was present at certain sites and differed according to the ecological or morphological groupings of *Lerista*. The null model to model A is model A1, where ω2=1 as according to Zhang et al (2005).

Clade analyses (CmC, CmD) have site classes such that $\omega<1$, $\omega=1$ and $0<\omega<\infty$, and assume that some sites evolve conservatively across the phylogeny whereas a class of sites can evolve freely. Analyses were performed between *Lerista* and *Ctenotus* groups. The null model for CmC is M2a_rel (Weadick and Chang, 2012), which constrains CmC such that ω2=ω3.

When comparing gene wide omega values, as with branch, it is difficult to distinguish positive and relaxed selection (Wertheim et al, 2015). Therefore we also conducted tests using the RELAX analysis on Datamonkey.com (Wertheim et al. 2015; Weaver et al, 2018). This tests the gene-wide strength (intensification versus relaxation) of selection on user selected branches of a given tree using the given alignments. Firstly, test and reference branches are selected by the user, then a null model is compared to an alternative model, where the null model has a selection intensity parameter (k) = 1, and the alternative has k as a free parameter. An LRT then determines if the alternative model has better fit to the data than the null model. If so, this indicates a significant difference in selection intensity on the test branch compared to the reference branch, with $k<1$ indicating relaxation of selection and $k>1$ indicating intensification of selection. We used the same trees, alignments and branch selections as in the branch and branch-site tests (see above).

In all M2a and M8 β&ω site analyses, and branch site analyses, sites potentially under positive selection were detected by the Bayes Empirical Bayes method with posterior probabilities of >0.95 (Yang et al, 2005). Any selected sites were reported using the residue number associated with Bos taurus RH1.

Results

Targeted capture of skink opsins

From a total of 92 samples, full length sequences were obtained from 87 species for LWS, 88 for SWS1, and partial sequences were obtained from 85 species for RH1 (630bp), 95 for RH2 (492bp) and 87 for SWS2 (438bp) (SI Table 2.1). All opsin genes were found in 59 *Lerista* and *Ctenotus* sequenced. Each opsin resolved as a monophyletic grouping (SI Figure 2.6). No gene duplications were found. We found some full sequences in closely related species, so we feel confident in reporting these sequences are retained in our study species, just not fully captured. *Lerista chalybura* (*zietzi*) and *L. timida* yielded low quality sequences that were discarded. From transcriptomes, full sequences of each 12 of 13 sequences were found in LWS, with *Melanoseps occidentalis* being near complete. In RH1, 11 partial sequences were found, again with *Melanoseps occidentalis* being near complete. RH1 was not found in *Eutropis macularia* and *Tiliqua rugosa*. In RH2, 11 sequences were found, with one full sequence in *Platysaurus broadleyi*, and near complete sequences in *Chalcides ocellatus*, *Eumeces schneideri*, *Eutropis macularia*, and *Glaphyromorphus punctulatus*, with six partial sequences for other species. RH2 was not found in *Melanoseps occidentalis* and *Tiliqua rugosa*. In SWS1, 12 sequences were recovered. 10 were complete, while *Eutropis macularia* and *Melanoseps occidentalis* were near complete. SWS1 was not found in *Tiliqua rugosa*. In SWS2, 12 sequences were recovered. Full sequences were found in *Chalcides ocellatus* and *Platysaurus broadleyi*, and partial sequences were found in the other species. SWS2 was not found in *Tiliqua rugosa* (SI Table 2.1).

One limiting factor in this study is the fact that RH1, RH2 and SWS2 were not recovered as full sequences for any of the gene captures conducted. SWS2 and RH2 recovered here in particular are quite short (438bp and 492bp respectively), at less than half of their complete size (1092bp and 1068bp respectively in *Anolis carolinensis*). This means many known tuning sites on these opsins are not represented in this data (7 for RH1, 2 for RH2, 4 for SWS2 were recovered), and thus the potential variance within cannot be examined. Therefore we must estimate that the sensitivity of the opsins of *Lerista* and *Ctenotus* are similar to previously described skink species (*Tiliqua rugosa*) and are as follows: LWS ≈ 560nm; RH1 ≈ 491nm; RH2 ≈ 495nm; SWS1 ≈ 360nm and SWS2 ≈ 440nm (Nagloo et al, 2016). Were the full sequences present in this study, we would have a much clearer picture of spectral tuning in all opsins and could potentially discover variances at known spectral tuning sites.

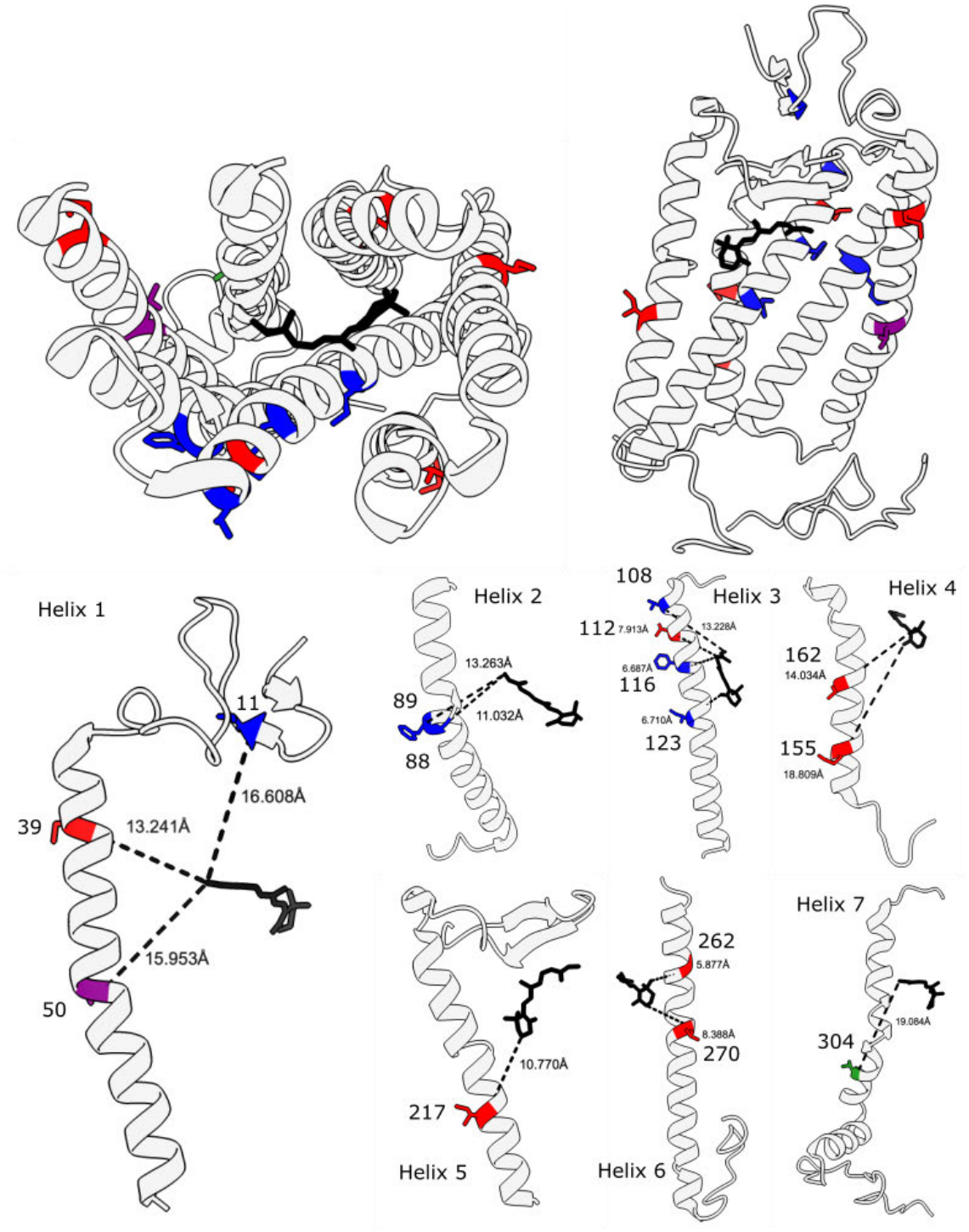

Figure 3: Depiction of bovine RHO (1u19) with positively selected sites found in this study shown as follows: LWS, red; SWS1, purple; SWS2, blue; RH1, green. The chromophore is shown in black. The left image has had the extracellular portions removed for better clarity. The right image has had helices 6 and 7 removed for better clarity

Analyses of molecular evolution

Site models show strong signals of positive selection at the codon level in four of the five opsins; only RH1 exhibits weak evidence of positive selection (Table1; SI Table 2.2). However, most branch model tests did not find statistically significant differences in selection pressures between partitions. These results are described for each gene below.

Long wavelength-sensitive (LWS) opsin: The LWS gene shows evidence of positive selection at seven amino acid sites. Models M2a and M8 β&ω are a significantly better fit than their respective null models, M1a and M7β. According to Bayes Empirical Bayes (BEB) for M2a and M8 β&ω, sites 39 (Helix 1), 112 (first extracellular loop), 155 (Helix 4), 217 (Helix 5), 270 (Helix 6) (according to Bovine LWS) are under positive selection (sites 162 (Helix 4) and 262 (Helix 6) are inferred to be under positive selection under M8 β&ω but not under M2a). None of these sites are known to have an impact on spectral tuning of LWS, but sites 262 and 270 are one amino acid directly after a spectral tuning site 285 (by bovine LWS), site 178 is 2 amino acids from a spectral tuning site (180 by bovine LWS) and site 128 is the amino acid directly before a retinal chromophore binding site (Table 1; SI Table 2.2).

Branch models found that ω did not differ significantly between lineages grouped according to eyelid condition, but ω values were lower in the *mobile* (ω=0.0723) versus *fused* (ω=0.140) groups. The ω values were lower in *temperate-tropical* (ω=0.110) versus *arid* climate groups (ω=0.132), however this difference was not significant (p=0.589). Similarly, in the comparison of *reduced limbs* versus non-reduced limbs and intermediates, the reduced limbs group had a higher ω (ω=0.146) than non-reduced limbs (ω=0.0883) but the differences were not significant (p=0.214). The *reduced limbs and intermediates* analysis that included intermediates produced a significant difference between groups (p=0.0393), with the *reduced limbs and intermediates* group having a higher ω (ω=0.151) than the non-limb reduced group (ω=0.0664). Giving each branch its own ω did not produce significantly different results to all branches having a single fixed ω (SI Table 2.3).

Branch-Site models reveal that for the arid climate group and both *reduced limbs and intermediates* and *reduced limbs*, site 112 (first extracellular loop) is under positive selection. For the limb groups site 270 is also under selection. No other branch-site analyses revealed significant results (SI Table 2.4). In clade analyses, CmC is a significantly better fit to data than M2a_rel for all opsins (SI Table 2.5). In the eyelid analysis, *mobile* (ω=0.00723) versus *fused* (ω=0) groups. The ω values were lower in *temperate-tropical* (ω=0) versus *arid* climate groups (ω=0.009). Similarly, in the comparison of *reduced limbs* versus non-reduced limbs and intermediates, the reduced limbs group had a higher ω (ω=0.009) than non-reduced limbs (ω=0). R*educed limbs and intermediates* group had a higher ω (ω=0.009) than the non-limb reduced group (ω=0) (SI Table 2.5).

LWS was also the only gene that passed the significance cut-off for model testing using RELAX. In this gene, the test branches with *reduced limbs with intermediates* showed a significant relaxation of the intensity of selection (K=0.73; p=0.014) relative to the reference branches (*fully limbed* group).

Models	Sites Under Positive Selection (BEB)
***sws1* opsin gene**	
M8 (β&ω)	50
***lws* opsin gene**	
M2a	39 - 112 - 155 - 217 - 270
M8 (β&ω)	39 - 112 - 155 - 162 - 217 - 262 - 270
***rh2* rhodopsin gene**	
M8 (β&ω)	304
sws2 rhodopsin gene	
M2a	11 - 88 - 108 - 116
M8 (β&ω)	11- 88 - 89 - 108 - 116 - 123

Table 1: Significantly selected sites according to BEB. Numbering based on the bovine rhodopsin.

Rhodopsin (RH1): The RH1 gene shows evidence of positive selection in two amino acid sites (SI Table 2). Models M2a and M8 β&ω are not a significantly better fit than their respective null models, M1a and M7 β, however M7 β/M8 β&ω is not far from significance (p=0.081). According to Bayes Empirical Bayes (BEB) for M8 β&ω, sites 201 and 219 are under positive selection. Neither of these sites are known to have an impact on spectral tuning of RH1, but site 201 is at a zinc metal binding site. In eyelid state comparisons,the ω values were similar in *mobile* (ω=0.124) and *fused* eyelids (ω=0.125). The ω values were higher in *temperate-tropical* (ω=0.147) versus *arid* climates (ω=0.104); *reduced limbs* had a lower ω (ω=0.127) than non-reduced limbs and intermediates(ω=0.123); *reduced limbs and intermediates* had a lower ω (ω=0.119) than the non-reduced limbs group (ω=0.139). Differences in ω values were insignificant in all of these among-group comparisons. Giving each branch its own ω did not produce significantly different results to all branches having a single fixed ω (SI Table 2). Branch-site models reveal that no sites were significantly selected for in any group. In clade analyses, CmC is a significantly better fit to data than M2a_rel, apart from in the *reduced limbs* analysis (p=0.62, SI Table 3). The *fused* vs *mobile* eyelids analysis showed significant site specific positive selection in both partitions (SI Table 4). RELAX analyses yielded no significant results.

Short wavelength-sensitive 1 (SWS1) opsin: The SWS1 gene shows evidence of positive selection at one amino acid site. Model M8 β&ω is a significantly better fit than its null

model M7 β, but M2a was not a significantly better fit than M1a. According to Bayes Empirical Bayes (BEB) for M8 β&ω, site 50 (Helix 1) is under positive selection. M2a did not show any selected sites. Site 50 is not known to have an impact on spectral tuning of SWS1, but it is the amino acid directly after a spectral tuning site (site 49) (Table 1; SI Table 2.2). In eyelid state comparisons, the ω values were lower in *mobile* (ω=0.0001) versus *fused* (ω=0.0565). The ω values were higher in *temperate-tropical* (ω=0.0582) versus *arid* (ω=0.0402). Similarly, the *reduced limbs* had a higher ω (ω=0.0561) than non-reduced limbs and intermediates (ω=0.0677). The *reduced limbs and intermediates* had a higher ω (ω=0.0561) than the non-limb reduced group (ω=0.0201). None of these branch analyses produced significant differences. Giving each branch its own ω did not produce significantly different results to all branches having a single fixed ω (SI Table 2.3). Branch-site models reveal that no sites were significantly selected for in any group (SI Table 2.4). In clade analyses, CmC is a significantly better fit to data than M2a_rel for all groups, but no evidence of site specific positive selection was found (SI Table 2.5). RELAX analyses yielded no significant results.

Short wavelength-sensitive 2 (SWS2) opsin: The SWS2 gene shows evidence of positive selection in six amino acid sites. Models M2a and M8a are a significantly better fit than their respective null models, M1a and M7 β. According to Bayes Empirical Bayes (BEB) for M2a and M8 β&ω, sites 11 (5' Extracellular region), 88 (Helix 2), 108 (first extracellular loop) and 116 (Helix 3, retinal chromophore binding pocket) are under positive selection (sites 89 (Helix 2) and 123 (Helix 3, retinal chromophore binding pocket) are inferred to be under positive selection under M8 β&ω but not M2a) (Table 1; SI Table 2.2). None of these sites are known to have an impact on spectral tuning of SWS2. Branch models found that ω values were lower *mobile* (ω=0.234) versus *fused* (ω=0.267). The ω values were lower in *temperate-tropical* (ω=0.258) versus *arid* (ω=0.262). Similarly, *reduced limbs* had a lower ω (ω=0.245) than non-reduced limbs and intermediates (ω=0.274. The *reduced limbs and intermediates* had a lower ω (ω=0.248) than the non-limb reduced group (ω=0.288). None of these branch analyses produced significant differences. Giving each branch its own ω did not produce significantly different results to all branches having a single fixed ω (SI, Table 2.3). Branch-site models reveal that for the *arid* group, sites 2 and 108 were under positive selection. For *reduced limbs* analysis, sites 11, 89 and 108 were under positive selection. For *reduced limbs and intermediates*, sites 2, 11, 50, 88, 89, and 108 were under positive selection. There was no evidence for positive selection at any site in the eyelid analysis (SI, Table 2.4). In clade analyses, CmC is a significantly better fit to data than M2a_rel for all groups, but RELAX analyses yielded no significant results (SI Table 2.5).

Rhodopsin 2 (RH2): The RH2 gene shows positive selection at one amino acid site. Models M2a and M8 β&ω are a significantly better fit than their respective null models, M1a and M7 β. According to Bayes Empirical Bayes (BEB), only site 304 (Helix 7) is under positive selection; however this is only under M8 β&ω but not M2a. This site is not known to have an impact on spectral tuning of RH2 (Table 1; SI Table 2.2). The ω values were higher in *temperate-tropical* (ω=0.668) versus *arid* (ω=0.344). The ω values were lower in *mobile* (ω=0.124) when compared to *fused* (ω=0.12). The *reduced limbs* had a lower ω (ω=0.452)

than non-reduced limbs and intermediates(ω=0.475). The *reduced limbs and intermediates* group had a lower ω (ω=0.437) than the non-limb reduced group (ω=0.524). None of these group comparisons produced a significant difference between groups. Giving each branch its own ω did not produce significantly different results to all branches having a single fixed ω (SI, Table 2.3). Branch-site models reveal that for the arid climate group, sites 72, 79, 96 and 113 were under positive selection. For *reduced limbs* analysis, sites 72 and 79 were under positive selection. For *reduced limbs and intermediates*, sites 72, 79 and 96 are under positive selection. There was no evidence for positive selection at any site in the eyelid analysis (SI, Table 2.4). In clade analyses, CmC is a significantly better fit to data than M2a_rel for all groups (SI, Table 2.5). RELAX analyses yielded no significant results.

Discussion

Our study provides a detailed view of the molecular evolution of visual opsins across a recent radiation of 126 ecologically diverse scincid lizards (86 *Lerista* and *Ctenotus*). Sequence analyses of full-length SWS1 and LWS and (mostly) partial SWS2, RH1 and RH2 sequences suggested that these visual opsins have been retained - no stop codons or frameshift mutations were observed in any of the sampled species. Consistent with this, we detected significant signals of positive selection in four of the five opsins (LWS, SWS1, SWS2, RH1). Most comparisons of site-, branch- and clade-specific selection pressures did not show significant differences according to broad ecological divisions between lineages inhabiting arid versus temperate-tropical climates, and lineages with diurnal surface-active habits versus highly fossorial forms with small, recessed eyes and fused eyelids. A significant signal of relaxed selection pressure was detected only in the LWS of limb reduced versus fully limbed *Lerista.* The retention and ongoing positive selection on visual opsins, particularly cone opsins associated with bright-light vision, in these ecologically diverse species, has important implications for understanding the impact of photic transitions on visual function and evolution in lizards. These are discussed below.

Conservation versus loss of visual genes in scotopic squamates

Our results contribute to the growing literature on visual evolution during ecological transitions in squamates. Previous studies of visual characteristics in fossorial and dim-light adapted alethinophidians, scolecophidians (Simões et al, 2015; Gower et al., 2021), and geckos (Pinto et al, 2019) have revealed multiple opsin losses. All snakes have lost RH2 and SWS2, with the alethinophidian *Anilius scytale* having lost the SWS1 and LWS (Simões et al, 2015) and the fossorial scolecophidians having further lost SWS1 (Gower et al., 2021). Geckos have lost RH1 and SWS2 but sand-swimmers *Lialis burtonis* retained SWS1 and LWS (Pinto et al, 2019). Estimates of the age of these lineages, and therefore the maximum time these losses have had to occur, are around 66-63.3Ma for scolecophidia (Standhardt, 1986), and 112Ma for the extant gekkotans (Daza et al, 2012; Daza et al, 2014), with the origin of nocturnality being close to the root of gekkota (Anderson et al, 2017, Gamble et al, 2015). However, other fossorial squamates, of similar evolutionary age, show retention of vision genes and visual capabilities. In particular, the amphisbaenian lizards are highly

fossorial and have reduced eyes yet have retained and express a full complement of visual opsins (Simões et al, 2015). In another example, the nocturnal, sand-burrowing lizard *Calyptommatus* appears to have retained photopic visual capabilities based on morphological and transmittance data (Yovanovich et al, 2019).

These variable patterns of opsin retention and loss in scotopic squamates prompt the question of whether the *Lerista* lineages analysed here are simply too recent to have accumulated inactivating mutations in their opsin sequences. Based on fossil-dated molecular relationships (Skinner et al, 2008), these lineages represent several independent transitions from surface active to fossorially adapted forms over the last 13.4 million years. There is limited comparative data on the rate of visual gene loss during ecological shifts in most taxa. However, evolutionary regression is expected to proceed most quickly if this is driven by selection favouring e.g. energy saving, while longer timeframes are expected if degeneration is a consequence of genetic drift due to lack of function. In cavefish *Lucifuga dentata*, relaxation in pseudogenised vision genes is estimated to have begun only ~1.3-1.5mya, or 370,000 generations ago (Policarpo et al, 2021). Eye degeneration is even more recent in the Mexican cavefish *Astyanax mexicanus*, with the energetic cost of developing eyes in low nutrient habitats driving regression within only 20,000 years since the origin of the cave populations (Moran et al, 2015; Fumey et al, 2018). *Lerista* species that move headfirst through solid substrates might also be expected to experience adaptive selection for eye loss; eyes are naturally delicate instruments and reducing their size obviously reduces the potential of damage. However, given the age of our sampled *Lerista* (Fig. 1), and evidence of relaxation of purifying selection for limb reduced species in LWS, we can conclude that these lineages are unlikely to have experienced direct selection for opsin regression. In neotropical cichlids, many species have lost blue sensitive opsin genes (Hauser et al, 2021), and where most clades that have undergone these losses are >90my old, *Uaru fernandezyepezi* may have diverged from its closest relative ~15mya (López-Fernández et al, 2012). This is around the age of *Lerista* (Skinner et al, 2008), with the loss of all limbs from species such as *Lerista ameles*, *apoda* and *stylis* having occurred in a period of 11.8my (Skinner et al, 2008). The fastest rate of limb loss from pentadactyl or tetradactyl to adactyl may have occurred in as little as 3.6MY (Skinner et al, 2008). Perhaps then many of these transitions in *Lerista* have occurred too recently for inactivation due to relaxed selection pressure.

Relaxation of purifying selection in the LWS of limbless and limb reduced *Lerista* suggests that detection of longer wavelengths is not as tightly constrained in these lizards compared to fully limbed *Lerista*, indicating somewhat smaller reliance (though far from relaxed as it still under purifying selection) on long wavelength detection. This is very unexpected given that very few organisms have been reported to have lost this opsin previously. Ordinarily, fossorial species tend to show spectral shifts to longer wavelengths, and if any opsins are lost or pseudogenised, LWS is often the least likely candidate (Peichl et al, 2001; David-Gray et al, 2002; Jacobs, 2013; Simões et al, 2019). In many species across the animal kingdom, fossorial or nocturnal species, or species with scotopic visual traits, lose function in opsins tuned to shorter wavelengths, maintaining LWS (Jacobs, 2013; Sadier et al, 2018; David-Gray et al, 2002; Davies, 2011). For instance, the obligate fossorial mole rats possess

subcutaneous eyes and are unable to respond to visual images, but still maintain LWS monochromacy for photoentrainment (David-Gray et al, 2002). Among vertebrates, the most notable losses of LWS have been in deep sea fish (Musilova et al, 2019) and deep diving whales (Meredith et al, 2013; Schweikert et al, 2016; Springer et al, 2016), which occupy photic habitats dominated by shorter wavelengths (Warrant and Johnsen, 2013), thus have little ecological parallel with our study system. A more detailed understanding of the visual ecology of *Lerista* may shed light on the cause and significance of relaxed selection on their LWS gene.

The link between visual function and eye-reduction in fossorial skinks

Absolute eye size is known to affect several aspects of visual function, not least of which is visual acuity (Walls, 1942). As eye diameter increases, and assuming all other eye parameters are constant, posterior nodal distance (PND) increases and creates a larger retinal image (Veilleux and Kirk, 2014; Hughes, 1977). Walls (1942) suggests that because of the higher sampling by photoreceptors, visual acuity should be increased, and various studies have since provided some support for this in mammals (Kirschfeld, 1976; Veilleux and Kirk, 2014). In smaller eyes, photoreceptors can narrow in order to make up for the loss of acuity due to shorter focal length, however this also means lower light collection per photoreceptor and thus there is a trade off between acuity and sensitivity (Caves et al, 2018).

We do not fully know the extent to which these lizards are diurnal and surface active. What can be certain is that all *Lerista* have periods of activity both out in the open, however brief, and periods of sand swimming, running through loose leaf litter or soil, i.e. in partial or low light environments. In addition to this, all *Lerista* will also have periods of diurnal activity as well as crepuscular and nocturnal (Meiri, 2019). Any difference in their overall exposure to light and light conditions will be a difference of degree rather than of kind. Therefore it is reasonable to assume that visual function is still highly important to all *Lerista*, regardless of the extent to which they appear to be adapted to cryptozoic or fossorial lifestyle. Even the most heavily adapted species will still need to be surface active to thermoregulate, disperse etc. It should also be noted that although *Lerista* do possess many adaptations associated with a heavily fossorial lifestyle, they also all possess an external ear (Greer, 1967), something often lost in heavily fossorial squamates. They also all possess a dark peritoneum, the abdominal cavity lining (Hutchinson, personal communication), which has been associated with diurnal activity (Greer, 2002). The branch codeml results seem to support this, with all but one of the tests we conducted on the various opsins and groups finding no significant selection. However these results here do show that there is selection at certain points along LWS, RH2, SWS1 and SWS2, and some of these sites are associated with the groups in this study according to branch site tests.

A plausible explanation for the retention of visual opsins in fossorial skinks is that in all lizards but the most fossorial and averse to photic conditions, high levels of acuity and colour vision maintain an evolutionary benefit and losing these attributes would lower fitness. We tentatively suggest that the reductions in eye size as seen in *Lerista ips* for example, might not reduce acuity significantly or effectiveness of colour vision, but only reduce maximum

effective focal distance. Hall (2008) found that scotopic lizards have increased corneal diameter in order to increase visual sensitivity when compared to photopic lizards, however this result does not specifically address fossorial lizards. Making the eye smaller whilst maintaining the same refractive index of the lens could potentially only naturally reduce the maximum distance from which such an eye could focus an image. In species that are surface-active in open habitats, this may be expected to be detrimental to fitness. In contrast, it is reasonable to expect that the distance at which fossorial species need to focus images is considerably shorter, given that they move through loose ground matter where the visible distance would be dramatically reduced. Therefore, reduction of eye size would not negatively impact the fitness of heavily fossorial species. Perhaps only in obligate fossorial species, where the vast majority of activity is performed in none or low photic environments, and when eye size is so drastically reduced that focussing a quality image becomes near impossible at any useful distance, is when we may start to see opsin function reduction or loss, and thence loss of eye structures and the eye itself in those species thoroughly adapted to life underground. Some support for a similar hypothesis in geckos exists (Schmitz and Higham, 2018) but more investigation and experimentation would be needed to verify or reject this hypothesis.

Adaptive opsin evolution during the *Ctenotus-Lerista* radiation

The results of the site model analysis seem to show that there is indeed selection at many sites, and some of the variation at these sites can be explained by ecological categories according to branch site models. Climate and both types of limb analyses produced significant results in LWS, RH2 and SWS2 (SI Table 2.4). Of the limb analyses, the *reduced limbs with intermediates* seems to explain variation better than just *reduced limbs*, as more selected sites were captured in the branch analyses in these tests (SI Table 2.3), and the only significant result in branch testing came from this group also. Therefore, it seems that selection pressures differ more significantly between more fully limbed species and any limb reduced ones, compared to between heavily limb reduced and limbless species, and all others. Without biochemical analyses, we cannot know the significance of these changes, whether they impact spectral sensitivity, or to what extent. Many of the sites listed as positively selected in this study seem to be close to previously known tuning sites. It is well known that changes at tuning sites can significantly alter the peak spectral sensitivity of the photopigment, but it is less clear how any changes in other positions along the opsin might affect tuning, if at all. There are a few changes that seem to happen mostly in conjunction with one another and occur in close proximity to one another (sites 97 and 98, SWS2 most notably). The phenomenon of previously undescribed positively selected sites being found in close proximity to previously known ones has also recently been described in anurans (Schott et al, 2022). Perhaps here too, these previously unidentified and unexplored sites may have minor effects on spectral tuning or other functions of the opsin, such as retinal release and binding, rates of retinal bleaching and recovery and so on.

One of the most interesting findings of the site analyses is the selected site at 201 on RH1. Although the site did not quite meet probability thresholds ($p<0.05$), there is variation at this site, and skinks as a group seem to have diverged from other groups. This site has been

previously identified as a zinc binding site (Toledo et al, 2009; Norris et al, 2021). Zinc is known to have a number of binding sites on rhodopsin, with site 201/279 possibly being a non-specific one (Toledo et al, 2009). The zinc binding sites increase the stability of the pigment, especially between the first extracellular loop and the sixth helix (Park et al, 2007). Its sister site, site 279, is consistent across all skinks, *Anolis* and Python and has the same amino acid as *Bos*, Glutamine. However site 201 seems to differ in skinks, and is not consistent throughout the clade, with only *Liopholis striata* possessing Glutamic acid at this site. Other skinks possess either Arginine and Lysine, with only *Plestiodon fasciatus* possessing a different amino acid, Glutamine. None of these amino acids are one of the four that have been found to commonly bind to zinc (Shu et al, 2008). Therefore it would appear that this zinc binding site may have been made non-functional across Scincidae. Interestingly, *Sphenodon* also seems to have changed this site too. The fact that this site seems to have changed so thoroughly, yet other clades in Squamata and even Mammalia have maintained this site, suggests a strong evolutionary advantage in decreasing the stability of this opsin. The other sites which were found to be under selection in this study also fall into the region that is particularly destabilised by the absence of zinc binding (Park et al, 2007). Though the exact effects of the loss of this one particular zinc binding site are unclear, one may speculate that it would have a destabilising effect, and perhaps accentuate any spectral tuning and tertiary structure changes that the selected sites could cause. Helices 5 and 6 are known to undergo conformational changes when rhodopsin is activated (Zhou et al, 2012) and destabilisation of this region by the removal of zinc binding may have implications for the activation and function of rhodopsin. Again, one can only highlight these interesting results, speculate on their significance and suggest further study in order to understand the findings presented here more clearly.

Conclusions

This study used thorough taxon sampling spanning replicate ecological transitions to investigate visual changes in skinks. Losses of cone opsins may have been expected in the most specialised fossorial lineages, as happened in geckos and snakes in historical low light transitions. Instead, all five opsins are retained in even the most dedicated fossorial lineages. We suggest that visual systems in these species have retained adaptive value in habitats with reduced visible distance. Future studies could elaborate on the exact impact of these selected sites on the spectral tuning of the opsins, and morphological studies could examine if there is a link between the extent of selection found here and the adaptations to a fossorial lifestyle in the skull, ie reduced eye size, elongated skull etc. The *Lerista* clade maintains its status as a key system for low light adaptive studies, but the results of this study suggest that the variations and extremity of adaptations are much more subtle than previously described, and research would be necessary to understand the species' activity patterns and extent of their fossorial activity. Further research into the particular effects of changes to the zinc binding sites, and the potential implications this may have for spectral tuning in the destabilised regions, would also help to shed light on the significance of the findings of this paper.

Supplementary Materials

Table 2.1. List of specimens with SA Museum ABTC numbers, NCBI numbers, type of sequence and presence of each visual opsin

Table 2.2. Site CodeML results

Table 2.3. Branch CodeML results

Table 2.4. Branch site CodeML results

Table 2.5. Clade CodeML results

Figure 2.1: Phylogenetic tree showing group partitions, and selected sites in RH2 with corresponding amino acids

Figure 2.2: Phylogenetic tree showing group partitions, and selected sites in LWS with corresponding amino acids

Figure 2.3. Phylogenetic tree showing group partitions, and selected sites in SWS1 with corresponding amino acids

Figure 2.4. Phylogenetic tree showing group partitions, and selected sites in SWS2 with corresponding amino acid

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https://github.com/TransDecoder/TransDecoder/releases

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Chapter 3: More than meets the eye: Molecular analyses reveal repeated losses of non-visual opsins in Squamata

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Abstract

Non-visual opsins (NVOs) mediate many vital responses to light, including the regulation of circadian and thermoregulatory rhythms. Accordingly, losses of the genes encoding NVOs have been linked to evolutionary transitions to low-light environments. Such 'nocturnal bottlenecks' in mammals and crocodiles are thought to explain substantial losses of NVOs in the early ancestors of these lineages. Parallel losses have been also reported in geckos and snakes, which similarly experienced low-light bottlenecks in their early ancestry. However, shallow genomic and taxon sampling in these (and other) squamate groups has left major gaps in knowledge of the patterns of NVO losses in squamates and the extent that these show convergence with other vertebrates. We addressed this by generating 104 sequences for 15 NVO genes from 100 squamates using gene capture and transcriptomics, and combining these new data with 37 sequences mined from publicly available genomes. This expanded dataset was used to infer broadscale patterns of retention of full genes, the presence of partial and pseudogenic fragments, and gene absences, across all major lineages of squamates. Our results showed losses of seven and five NVOs in snakes and geckos, respectively, of which five losses were shared by the two groups. Broader convergent patterns between mammals, crocodiles, snakes and geckos were seen for four genes lost in these groups. In contrast to the snakes and geckos, we found near-complete repertoires of NVOs in the lacertids and anguimorphs consistent with their diurnal ancestry. Within these groups, only teiids and chameleons were found to have lost NVOs, which might be expected in light of the secondary regression of these species' parietal organs. An intermediate pattern of NVO evolution was revealed for skinks (Scincomorpha). In this group, we found that while nine NVOs were retained, six NVOs appear to have been lost in common with snakes and geckos, which suggests the possibility of a mesopic bottleneck in ancestral skinks. Especially notable is the convergent loss of the pinopsin gene in snakes and skinks. In light of the regression of these species' pineal tissues, this finding indicates independent losses of the photosensitive function of snake and skink pineal organs in parallel with mammals.

Introduction

Opsins are a large group of photoreceptive proteins that mediate the conversion of photons into an electrochemical stimulus (Nathans, 1992; Yokoyama, 2000; Arshavsky et al, 2002; Terakita, 2005; Davies et al, 2012). The role of ecological factors in shaping the evolution of visual opsins has received intense research attention (Davies et al, 2012). Much less well-understood is the evolution of so-called non-visual opsins (NVOs). Unlike the visual opsins, these are not used to form images on the retina, and instead are involved in functions such as circadian rhythm mediation (Nakamura, 1999; Hankins et al, 2008; Peirson et al, 2009; Guido et al, 2020), pupillary light reflex (Lucas et al, 2001; Lucas et al, 2003), thermoregulation, and colour-changing camouflage (Bertolesi and McFarlane, 2018). The first evidence of extraocular photoreception was reported in 1911 using blinded and pinealectomised minnows to show light-induced skin colour changes (von Frisch, 1911). Since then there have been numerous confirmations of non-visual photoreceptor cells found both in and outside of the eye in all major groups of vertebrates (Beaudry et al, 2017).

RNA sequencing has revealed NVO gene expression in a wide range of sensory and visceral tissues. In vertebrates, many NVO genes been have identified in skin, including two forms of Melanopsin (Mammal-like OPN4m: Provencio et al, 2000; Terakita, 2005; Xenopus-like OPN4x: Provencio et al, 1998; Terakita, 2005), and Neuropsin (OPN5/NEUR1: Tarttelin et al, 2003), which have been associated with skin colour change (Shiraki et al, 2010; Gerkema et al, 2013), phototaxis (Crowe-Riddell et al , 2019), pupillary reflex (Lucas, 2003; Hattar et al, 2003; Spitschan, 2019), and photoentrainment of circadian rhythms (Panda et al, 2002; Ruby et al, 2002; Nelson and Zucker, 1981; Foster et al, 1991). Brain tissue has been found to express least 13 NVOs: OPN4m and OPN4x (Bellingham et al, 2006), Neuropsin (NEUR1)) and Neuropsin-like 2 (NEUR3) (Ohuchi et al, 2012), Encephalopsin (OPN3) (Blackshaw and Snyder, 1999), Retinal G protein-coupled Receptor (RGR) and Peropsin (RRH) (Bailey and Cassone, 2004), Pinopsin (PIN) (Okano et al, 1994; Nakamura, 1999), Parapinopsin (PP) and Parietopsin (PRT) (Wada et al, 2012), Teleost Multiple Tissue opsin and Teleost Multiple Tissue (TMT) opsin A (Sakai et al, 2015), and Vertebrate Ancient opsin (VAOP) (Kojima et al, 2000). The expression of these genes in the brain, and Melanopsin Mammal-like, and Melanopsin Xenopus-like in the pineal gland in particular, has been linked to the entrainment of circadian rhythms of rest-activity and sleep-wake patterns over the 24 hour light-dark cycle (Bertolesi et al, 2021).

Especially prominent in studies of NVO expression is the parietal eye, which is associated with the pineal gland in the brain of non-mammalian vertebrates, including *Sphenodon* and some lizards, amphibians and fish (Blackshaw and Snyder, 1997; Kawamura et al, 1997; Koyanagi et al, 2004; Su et al, 2006; Wada et al, 2012). In reptiles this structure is located on the top of the head and is covered by a variably transparent scale, hence the term ‘third-eye’. PRT and PP have been shown to be expressed in the parietal eye but not in the associated pineal tissue (e.g. in the lizards *Iguana iguana* and *Anolis carolinensis*: Wada et al, 2012), whereas PIN, PP, and VAOP have been reported to be expressed in pineal tissues

(Okano et al, 1994; Kojima et al, 2000; Philp et al, 2000) of studied species with the exception of PP in *Iguana iguana* (Wada et al, 2012). In species with ectothermic metabolisms, the pineal-parietal complex has been linked to fine-tuning of the timing of thermoregulatory and seasonal reproductive behaviours (Hutchinson and Kosh, 1974; Ralph et al, 1979; Firth et al, 1980; Sapède and Cau, 2013), as well as melatonin suppression (Nakamura et al, 1999; Kawano-Yamashita et al, 2014) and the detection of polarised light (Beltrami et al, 2010). The parietal eye is particularly well developed In diurnal lizards and the tuatara, and is thought to be an important structure for reproductive cycle synchronicity and thermoregulation in these taxa (Ralph et al, 1979; Firth et al, 1980; Sapède and Cau, 2013).

Convergent macroevolutionary patterns of retention and losses of NVOs suggest an important role for ecological transitions in shaping the repertoires of these genes in vertebrates. In mammals and crocodiles, losses of NVO genes have been interpreted as evidence that ancestral periods of nocturnality (known as 'nocturnal bottlenecks') may constrict the NVO repertoire in parallel with the visual gene repertoire (Gerkema et al, 2013; Borges et al, 2018). In mammals, some NVOs (OPN3, OPN4m, OPN5, RRH, RGR; TMT in marsupials) have been retained alongside losses of other OPN5 family members, OPN4x, PIN, PP, PRT, VAOP (and TMT in eutherians). Crocodiles have retained OPN4x, OPN3, OPN5, RRH, RGR, and lost OPN4m, PIN, NEUR2, PRT and PP (Wall 1942; Emerling, 2017a). Studies of squamate reptiles have also found multiple, independent losses of NVOs in snakes and geckos, and both of these groups are expected to have undergone ancestral low light bottlenecks in parallel with mammals and crocodiles (snakes: Walls, 1942; Simões et al, 2015; geckos: Pinto et al, 2019). In snakes, previous studies have found losses of PIN, PP, PRT, NEUR2, NEUR3, TMT2, TMTa and OPN4m (Emerling, 2017b; Schott et al, 2017), of which PIN, PP, PRT, NEUR2 were also lost in mammals (Gerkema et al, 2013). Geckos are also known to have lost NEUR2, OPN4m, PP and PRT (Emerling, 2017b).

A conspicuous feature of the evolution of non-visual photoreception during nocturnal bottlenecks is the regression of the pineal organ and parietal eye, and the concomitant loss of NVOs associated with this anatomy (PIN, PRT and PP). In mammals, substantial regression of brain photoreception and the movement of the pineal organ to the deep brain (Hankins et al, 2014), was accompanied by losses of the pineal associated PIN gene, and the additional loss of VAOP (vertebrate ancient opsin, which has been linked to photoperiodic response in birds: García-Fernández et al, 2015). Previous work on snake pineal gland cytology has drawn comparisons to the cellular morphology of mammal pineals, with both snakes and mammals lacking photoreceptive type cells (Petit, 1971; Ekström and Meissl, 2003). Biochemical studies have also failed to find immunoreactive opsins within the snake pineal gland (Kalsow et al, 1991; Fejér et al, 1997). Interestingly, some commonalities to these regressed clades (snakes and mammals) can also be found in the skink pineal. *Tiliqua rugosa* possesses modified photoreceptor cells, like lacertid lizards and unlike snakes, but these photoreceptor cells do have regressed outer sections, or no outer section at all (Teo, 1997). Photoreception generally occurs in the outer section of the photoreceptor (Kolb, 2012), so this could potentially indicate lack of photoreception in the pinealocytes of *Tiliqua rugosa*.

Walls (1942) noted a thinning of the ganglion cell layer of the retinae of nocturnal vertebrates which receive visual and non visual information from photoreceptors, so we may expect to see the loss or reduction in function of the ganglion cell layer, and perhaps the loss or alteration of genes expressed in this tissue, such as OPN4m (Provencio et al, 2000), NEUR1, OPN3, RGR (Nieto et al, 2011) and VAOP (Jenkins et al, 2003).

Similarly to the pineal organ, the parietal eye shows regressed morphology and losses of associated genes (PRT and PP) in several independent lineages (mammals, archosaurs, turtles, snakes and geckos: Gerkema et al, 2013; Emerling, 2017a&b), and in all of these clades has been associated with ancestral periods of nocturnality (Kielan-Jaworowska et al, 2005; Heesy and Hall, 2010; Anderson et al, 2017). However, regression of the parietal eye has also been linked to transitions to elevated metabolic rates in birds (Borges et al, 2015), crocodiles (Emerling, 2017a), and mammals (Gerkema et al, 2013; Benoit et al, 2016; Borges et al, 2018), and to fossoriality and burrowing in stem turtles (Lyson et al, 2016; Emerling, 2017a). In addition to snakes and geckos, many lizards that are nocturnal, fossorial, and occupy lower latitudes have also lost the parietal eye, but very few of these taxa have been included in previous analyses of NVO evolution (Su et al, 2006; Wada et al, 2012). Based on the apparent correlation between parietal eye loss and loss of PP and PRT, we should also expect to see the loss of NVOs in clades such as chameleons and teiids where the parietal eye is lost or has a regressed morphology (Gundy & Wurst, 1976a&b; Hernández Morales et al, 2019). Conversely, we would predict the retention of these genes in clades in which the majority of species possess this structure such as Lacertidae, Iguania (other than chameleons), Anguimorpha and Scincomorpha (Gundy and Wurst, 1976a&b, Su et al, 2006; Wada et al, 2012).

This paper aimed to address a conspicuous gap in our knowledge of the evolutionary history of non-visual opsins within the ecologically and taxonomically diverse Squamata. To do this, we provide the first comprehensive analysis of fifteen non-visual opsin genes across 70 species spanning all major groups of snakes and lizards and their sister taxon *Sphenodon punctatus*. Our sampling included several key species and clades that had been neglected in previous studies of NVO genes, most notably the species rich skinks (Scincomorpha). By examining clade-wide patterns of gene presence, absence, and pseudogenisation in these sequences, we infer macroevolutionary transitions in the VNO complements of squamates and discuss the implications of these findings for understanding the evolution of non-visual photoreception during ecological transitions in ectotherms and other vertebrates.

Methods

Sequence Generation

Taxon sampling and DNA Extraction

Tissue samples from 127 species in 23 genera of skink (Squamata: Scincidae) were obtained via fieldwork (35 samples) and from The Australian Biological Tissue Collection

(ABTC) of the South Australia Museum (92 samples) (SI, Table 2.1). We also collected the eyes of 13 species for transcriptome sequencing (SI, Table 2.1). Specimens were collected under permits from the Animal Ethics Committee of the University of Adelaide (21877) and from the Government of South Australia (Q26642-5) and Queensland (WA0009193). Opsin sequences were downloaded from GenBank for: *Bos taurus*, *Anolis carolinensis*, *Sphenodon punctatus*, and *Python bivittatus* from GenBank (SI Table 2.1). DNeasy Blood and Tissue kits (Qiagen, Chadstone, VIC, Australia) were used to extract DNA from liver and muscle following the manufacturer protocols. Samples were quantified using a QuBit™ 2.0 (ThermoFisher Scientific, Waltham, MA, USA).

Library preparation and sequencing

DNA was sheared to ~400-500bp with a Bioruptor Pico Sonicator (Diagenode, Denville, NJ, USA). Ampure XP (Beckman Coulter Inc, Brea, CA, USA) with 8.5% PEG for bead clean-up (Li et al, 2013) was used to select fragments over 200bp. Protocols from Meyer and Kircher (2010) with the on-bead method of Fisher et al (2011) and Li et al (2013) were used to prepare DNA for Illumina sequencing. The DNA library then went through blunt end repair followed by adaptor ligation and ligation fill-in and the samples were double indexed by PCR amplification (Meyer and Kircher, 2010). Equimolar amounts of each library were combined in pools of five samples each and then centrivapped down to 7µl. Which were then hybridised to custom RNA baits (Daicel Arbor Biosciences, Ann Arbor, Michigan, USA). MyBaits targeted capture protocols version v4 (http://www.arborbiosci.com/mybaits-manual/) were used with baits designed with the *Anolis*, *Pogona*, *Python* and *Gekko* genomes. After capture with streptavidin beads (Dynabeads, ThermoFisher), targeted non-visual genes were PCR-amplified in 16 cycles and the resulting pools were all checked for concentration with a Qubit. Fragment size distribution was measured using a HS D1000 ScreenTape run on a Tapestation 2200 (Agilent Technologies, Santa Clara, CA, USA). Equimolar amounts of the capture libraries were combined to give a single sample at 10 nM DNA with an average fragment size of 400 bp. Sequencing was performed by Australian Genomics Research Facility (AGRF) using Illumina NovaSeq SP sequencing with 50bp paired end 100 cycle runs (single lane).

RNA extraction, library preparation and assembly

Both eyes from 11 specimens of skinks were used for RNA analyses. These were frozen at -80ºC in RNAlater (see SI Table 2.1). They were then macerated in TRIzol. The RNA was purified using the PureLink™ RNA Mini Kit (ThermoFisher Scientific, Waltham, MA, USA) using the manufacturer's protocol. The mRNA-Seq library was double-indexed and prepared with the mRNA-Seq Library Prep Kit v2 (Lexogen, Vienna, Austria) following the manufacturer's protocol. It was sequenced with 56 other libraries in equimolar concentrations in one lane of an Illumina NovaSeq S4. Trimmomatic (Bolger et al, 2014) was used to identify and remove low quality reads. The remaining reads were assembled and scaffolded using Trinity (Grabherr et al, 2011).

Sequence assembly and annotation

Eleven RNA-seq samples were selected to provide reference sequences that span the phylogenetic breath of the gene capture samples (SI Table 2.1). Trinity v2.10.0 (Grabherr et al, 2011) was used to assemble the RNA-seq data into transcripts, with the Trimmomatic (Bolger et al, 2014) argument specified to trim sequences prior to transcriptome assembly. TransDecoder v5.5.0 (github.com/transdecoder) was then used to predict putative coding regions within the transcripts for each assembly. TransDecoder.LongOrfs was used to predict candidate coding sequences. These were then screened against the Uniprot/SwissProt curated protein dataset using BLAST, along with the PFAM-A protein domain database using HAMMER, to obtain homology information to improve overall coding sequence prediction. The final high-quality coding sequence predictions were generated with TransDecoder.Predict. BLAST was used to search for 15 genes of interest from the coding sequences for each assembly. A multi-fasta file containing the genes of interest was manually compiled by searching the *Alligator mississippiensis*, *Alligator sinensis*, *Protobothrops mucrosquamatus*, *Gallus gallus*, *Chrysemys picta*, *Anolis carolinensis*, *Python bivittatus*, *Pantherophis guttatus*, *Gekko japonicus*, *Columba livia*, *Serinus canaria*, *Aquila chrysaetos*, *Pseudopodoces humilis*, and *Cuculus canorus* (SI Table 2.6) gene annotations for the genes of interest. BLAST hits were filtered for the best hit using bitscore, while also ensuring that the subject and target sequence lengths were comparable. For each transcriptomic reference, the best hit genes were extracted into a multi-fasta file. These then formed the reference files for the gene capture data.

A custom consensus pipeline based on the methods from Schott et. al. (2017) was used to process the gene capture data.Firstly, FastQC v0.11.9 (FastQC, 2015) was used to assess the quality of each gene-capture sample. The data was then quality trimmed using trimmomatic to remove adapter sequences and any low quality bases from the ends of reads. Gene capture samples were then aligned to one of the eleven reference files based on their evolutionary samples to their respective reference files with parameters -B 2 to reduce the mismatch penalty (default value 4) and -M to mark split alignments as secondary. SAMtools (Li et al, 2009; Danacek et al, 2021) was then used to remove the unmapped reads from the alignment files. Genotyping was performed using the SAMtools/BCFtools mpileup and call pipeline (Li, 2011). SAMtools mpileup was run with parameters -d 5000 to increase the maximum depth, -Q 20 to control the mapping quality and -q 20 to control the base quality. BCFtools call was used to convert genotype calls to variant calls. BCFtools norm was used to normalise variants, with -m +any being used to join multiallelic SNPs into single records. Finally, consensus sequences for each gene-capture sample relative to the reference genes of interest were generated using BCFtools consensus with argument -H 1 to use the first allele from the GT field.

Targeted capture of non-visual opsins was done as in Chapter 2 of this thesis. The same sources were also used for skink transcriptomes. Sequences were also drawn from previous works, crocodilians, testudines and avians from Emerling et al (2017a), caenophidian snakes from Schott et al (2017), and geckos from Pinto et al (2019). As many squamate sequences as were available were also collected from NCBI, and any available squamate genomes and/or transcriptomes were collected and BLASTed against closest

known high quality sequences for non visual opsin exons. These were then concatenated together to form a full coding sequence and added to this dataset (SI Table 2.1). Only +80% similarity matches were considered, and all BLASTed sequences were aligned with known, high quality sequences (eg *Anolis carolinensis*) to ensure homology, and most similar sequences were selected. Mammal sequences were also derived from NCBI.

We were also provided with BLASTs on the *Tiliqua rugosa* genome, to supplement the skink transcriptome sequences. Sequences were classified as follows: Present – full or near complete sequences; Pseudo – full or partial sequences with premature stop codons or frameshift mutations; Partial – incomplete sequences with no premature stop codons; Absent – BLAST yielded no results, sequence could not be found.

Alignment and Analyses

Sequences were aligned by gene using Geneious Prime (Version 2021.2.2) using Clustal Omega and finished manually. Sequences were first aligned with those of the same gene, before the alignments were combined into an alignment of all sequences. Firstly, a protein evolution model was selected using ModelTest-NG (Darriba et al, 2020) in raxmlGUI 2.0 (Edler et al, 2020). The chosen evolutionary model (JTT+G4) was then used to construct a tree with RAxML-NG, also using raxmlGUI 2.0 (Edler et al, 2020). The MTNR1A gene was also identified using BLAST and used as an outgroup to root the tree. This tree was then checked visually, with the placement of genes within clades inspected to support their likely identity.

Results

Phylogenetic analysis of NVOs in Squamata

The maximum likelihood tree constructed with these sequences showed relationships among these gene clusters consistent with previous molecular phylogenies for VNO gene families (Figure 1). The tree recovered a clade of OPN1 genes that comprised four subclades of the Vertebrate Ancient opsin (VAOP), Pinopsin (PIN), Parapinopsin (PP), Parietopsin (PRT) genes. Also recovered was a clade of OPN3 genes that comprised three subclades of Encephalopsin (OPN3), Teleost Multiple Tissue opsin (TMT) and its paralog Teleost Multiple Tissue opsin A (TMTa). A clade of OPN4 genes was also recovered that comprised two subclades, the paralogs Melanopsin Mammal-like (OPN4m) and Melanopsin Xenopus-like (OPN4x). A clade of Neuropsin genes was recovered, which comprised four subclades of Neuropsin (also known as OPN5), Neuropsin-like 1 (also known as OPN5L1 or OPN6 (Davies et al, 2015; Beaudry et al, 2017)), Neuropsin-like 2 (also known as cOPN5L2 (Ohuchi et al, 2012) or OPN8) and Neuropsin-like 3 (also known as OPN7 (Davies et al, 2015; Beaudry et al, 2017)). The nomenclature of Davies et al (2015) was used for its simplicity. Outside of these clades, the Retinal G-protein-coupled Receptor (RGR) and Peropsin (also known as Retinal pigment epithelium-derived Rhodopsin Homolog, here RRH). Finally the MTNR1A gene was used as an outgroup (See Fig. 1).

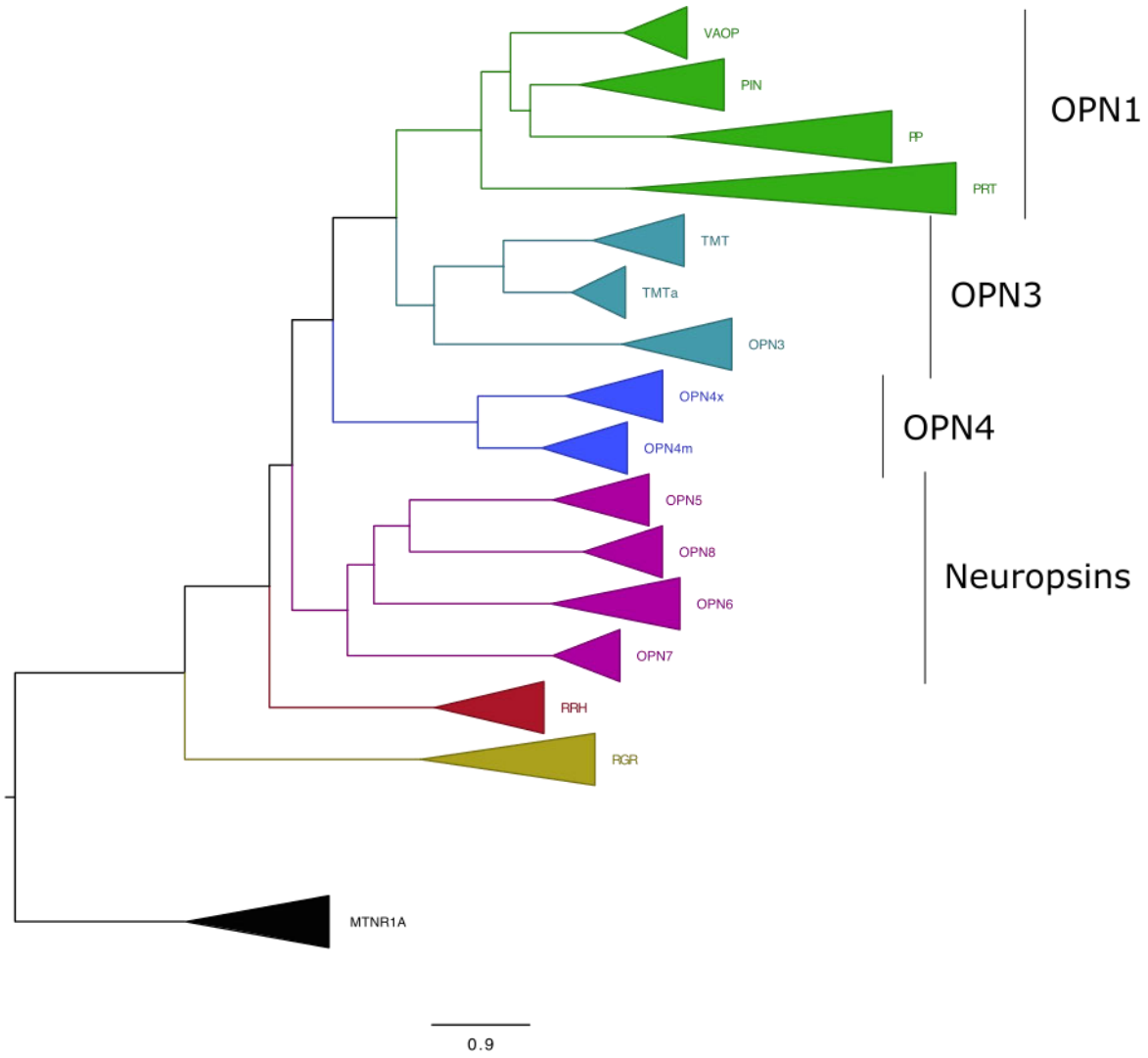

Figure 1: Maximum-Likelihood phylogenetic tree of non-visual opsins. RAxML tree was created using JTT+G4 model. Scale is substitutions per site. This analysis involved 714 sequences and 608 amino acids.

Distributions of NVO sequences are described below for each squamate clade. Where genes are said to be 'present', full or near complete sequences were identified. If full or partial sequences with premature stop codons or frameshift mutations were found, these are considered to be 'pseudogenes'. 'Partial' and 'partially found' genes are incomplete sequences with no premature stop codons. 'Absences' are reported for genes with no BLAST matches.

In all, 714 sequences were recovered, either fully or partially (Fig. 2).

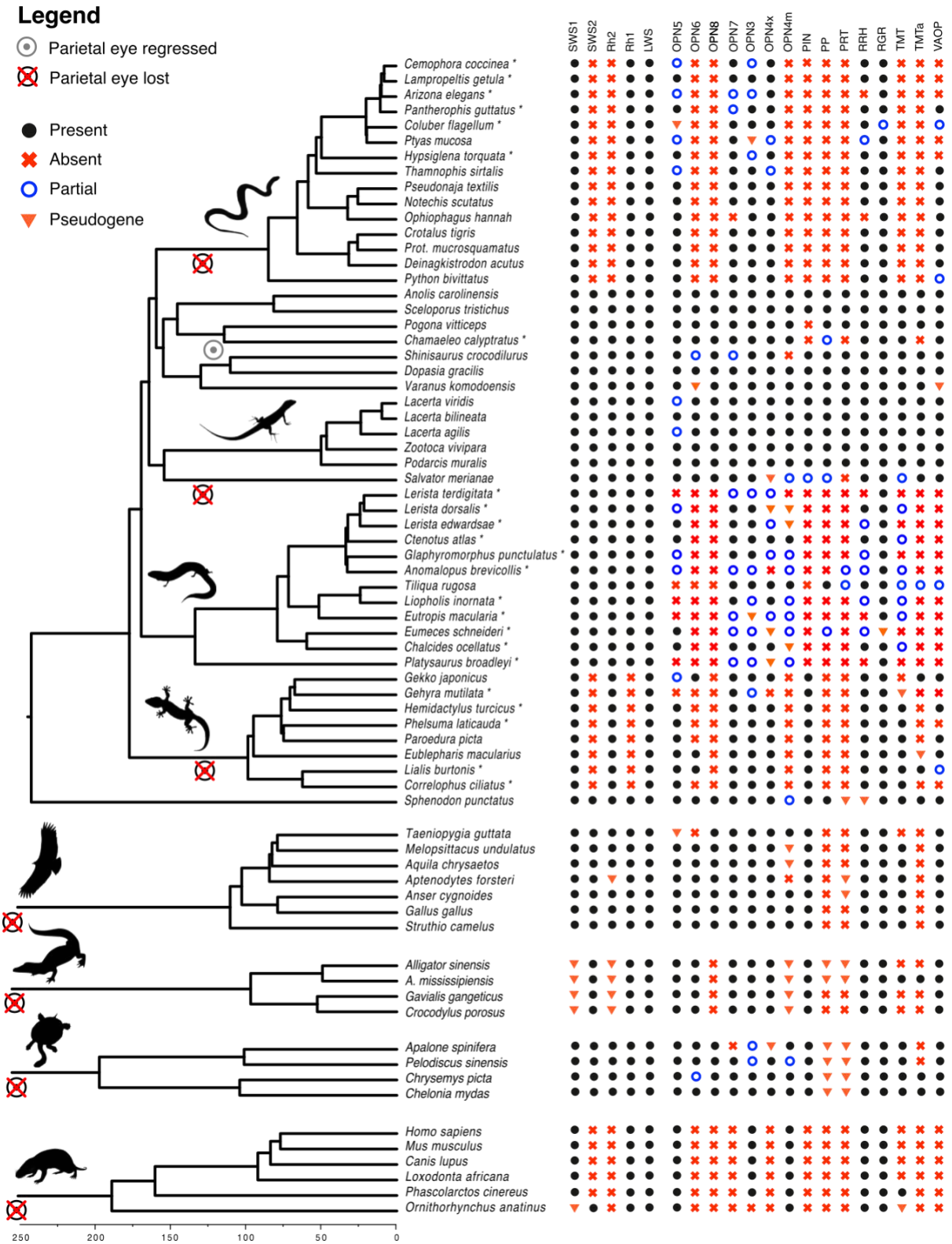

Figure 2: Presence and absence of non-visual opsins across Squamata. All asterisks are species whose sequences are based on transcriptomes.

Snakes

The following non-visual opsins were absent in snakes; OPN4m, OPN6, OPN8, PIN PP, PRT, TMT and TMTa. VAOP was not found in *Lampropeltis getula, Cemophora coccinea, Arizona elegans, Coluber flagellum, Hypsiglena torquata, Ophiophagus hannah*, and partial sequences were recovered in *Ptyas mucosa* and *Python bivittatus*. RRH was found in all snakes other than *Arizona elegans,* but in all of these species it is 7 amino acids shorter at the 5' end than other squamates.

Geckos

All sampled geckos have lost four non-visual opsins; OPN4m, OPN8, PP and PRT. OPN6 was only recovered fully in *Gekko japonicus* and *Lialis burtonis,* and partially in *Eublepharis macularius*. TMT was not found in *Gekko japonicus* and was pseudogenised in *Gehyra mutilata*. TMTa was not found in *Hemidactylus turcicus, Phelsuma laticauda* and *Paroedura picta,* and pseudogenised in *Eublepharis macularius*. VAOP was not found in *Gehyra mutilata, Phelsuma laticauda* and *Correlophus ciliatus*, and only partially found in *Lialis burtonis*.

Skinks

Five non-visual opsins (OPN6, OPN8, PIN, TMTa and VAOP) were absent in all twelve skink species sampled (However partial TMTa and VAOP were recovered in the *T.rugosa* genome). Further, Parapinopsin and Parietopsin were detected only as partial fragments in single species; Parapinopsin was present as a short section of exon 1 in *Eumeces schneideri*, and a partial sequence of Parietopsin was recovered in *Anomalopus brevicollis*. OPN4m was present only in *Ctenotus atlas*, and absent or partial in all other species. Only partial sequences of TMT were recovered in *Lerista dorsalis*, *Ctenotus atlas*, *Anomalopus brevicollis*, *Liopholis inornata, Tiliqua rugosa*, *Eutropis macularia*, *Chalcides ocellatus,* and this gene was absent in the five other sampled skinks.

No NVO was found present in all skinks sampled, but OPN7, OPN4x, OPN3, RRH and RGR were all found partial or present in more than half of the skink species. It is worth noting that for pseudogenised genes, no two skinks share the same location for the stop codon, and there are many closely related skinks that do not have stop codons in those positions. *Lerista dorsalis* and *Liopholis inornata* both have stop codons in OPN4x, but the sequences both continue, seemingly conserved, in the case of *Lerista dorsalis* to the 'true' stop codon shared with all other species. Therefore we strongly suspect these stop codons to be erroneous.

Gene captured skinks reported partial OPN4M, OPN4x, OPN3, PIN, OPN5, OPN7, RGR and RRH, and near complete OPN4m in all species sampled (SI Table 1). OPN4m, OPN4x, OPN5, OPN7, RGR all aligned well with transcriptome and NCBI BLASTed sequences, adding support for their presence in Scincomorpha. OPN3 partially aligned with the transcriptome and NCBI BLASTed sequences, however has some repeat sections inserted in the sequence that do not align with the other sequences. This could be due to poor coverage or assembly by the gene captures. Otherwise the sequences align well and seem

conserved, adding support for its presence in Scincomorpha. RRH was recovered partially in 24 gene captures. OPN6, OPN8, PIN, PRT, PP, TMT and TMTa were not recovered in gene captures. Finally, VAOP was captured in only two species, and they do not align with other squamate VAOP, and can therefore be discounted.
BLAST searches on the *Tiliqua rugosa* genome recovered full OPN7, OPN3, PP and PRT sequences, with near complete OPN4m. OPN7, OPN4m, OPN4x, OPN3, PP were all recovered almost fully and align well with transcriptome and NCBI BLASTed sequences. Only exon 1 of PRT was recovered, with exons 1 and 2 of TMTa and TMT, and exon 3 of VAOP, but again all align well with transcriptome and NCBI BLASTed sequences. When TMT from Tiliqua is taken with sequences from gene captures, a near full sequence is recovered. OPN4x was recovered near complete, but with several missense deletions and exon 8 missing. RGR was recovered near complete, but with exon 1 missing and several missense deletions. RRH was recovered near complete, but with exon 1 and 7 missing and several missense deletions. PIN, OPN5, OPN6 and OPN8 were absent from the *Tiliqua rugosa* genome.

Lacertids, Anguimorphs and *Sphenodon*

The anguimorphs, iguanids and lacertids are successive sister groups to the snakes (Pyron et al, 2013). All NVO genes were present in the 6 of the 13 species sampled in these groups. PP and PRT were partially found and absent respectively in both *Chamaeleo calyptratus* and *Salvator merianae*. However PP and PRT were previously found in *Chamaeleo calyptratus* (Schott et al, 2017). PIN was also found absent in *Chamaeleo calyptratus*, consistent with previous findings (Schott et al, 2017).

Twelve NVOs were present in *Sphenodon*, and two appeared to be pseudogenised: Parietopsin (premature stop codon at 240 [number in Bos RH1]), and RGR (premature stop codon exon 2). However, these inferences of pseudogenisation are questionable given that the rest of the gene seems conserved and aligns well with other lepidosaurs. OPN4m was also only found partially, however only exon 3 was missing, with the rest of the gene appearing conserved and with no premature stop codons or missense deletions.

Discussion

This is the first comprehensive study of retention and loss of non-visual opsins during squamate evolution. We examined all 15 NVO genes for 70 species, representing all major lineages of squamates and their sister taxon (*Sphenodon*). By integrating the results with findings from other tetrapods, we were able to identify several major macroevolutionary patterns. However, it is important to note that many of the reported absences (and detections of partial and pseudogenic fragments) may not indicate true evolutionary losses, but might instead be due to limited genome coverage and sequencing accuracy. However, at the clade level, high quality genomes support inferences of gene retention and loss in all major groups of squamates other than skinks. In skinks, three different methods of sequence discovery were used; genome BLAST, transcriptome analysis, and genomic gene capture. Limited reliability of the skink gene captures was indicated by the recovery of many partial genes, pseudogenisations, and absences in the same species where whole genome sequencing has

detected full, functional sequences (See Figure 2). Our transcriptome data are also limited because these were sequenced only from eye tissues, and many NVOs are known not to be expressed in the retina (e.g. Parapinopsin, Parietopsin). For these reasons, gene losses in skinks are inferred only where these are indicated by the reference quality *Tiliqua* genome. Moreover, due to this variation in data quality, we emphasise that our results should be interpreted in the context of clade-wide (not lineage-specific) distributions of gene presence or absence, and taking into account all methods of data gathering especially in the case of skinks.

The discussion below focuses on patterns of evidence for repeated independent losses of non-visual opsins across squamates, and implications for nocturnal bottlenecks in those clades which have lost these genes, largely following the mammalian pattern.

Nocturnal bottleneck hypothesis: Parallel losses of NVOs in snakes, geckos, mammals and crocodiles

In parallel to mammals and crocodilians, snakes and geckos are understood to have independently undergone nocturnal bottlenecks in their early ancestry which shaped many morphological and molecular aspects of the visual systems of these groups. Previous studies have shown losses of two visual opsins (sws2 and rh2) and eight non-visual opsins (OPN4m, PIN, PP, PRT, OPN6, OPN8, TMT, TMTa) in snakes (Emerling, 2017b; Schott et al, 2017); and losses of two visual opsins (sws2 and rh1) and five non-visual opsins (OPN6, OPN8, OPN4m, PP, PRT) in geckos (Emerling, 2017b; Schott et al, 2017). The present paper substantially expands on the genomic and phylogenetic scope of these previous findings. In geckos, our sampling spans the ancient division between Pygopodoidea and Gekkonoidea, and shows that two additional NVOs, TMTa and OPN6, are retained in *Lialis burtonis* and *Correlophus ciliatus*, and *Lialis burtonis* and *Eublepharis macularius,* respectively. We also confirmed the loss of TMTa in *Phelsuma* and its presence in *Gekko* as in Schott et al (2017). However, our expanded taxon sampling showed for the first time that the loss of TMTa is lineage specific, i.e. lost in all Gekkonidae except for *Gekko japonicus*, pseudogenised in *Eublepharis macularius,* and present in Pygopodoidea. The full sequence of TMTa was found in *Eublepharis*, but it possessed premature stop codons, one in exon 3 and two in exon 5. Interestingly, we found that PIN seems to be retained in geckos, even though it is lost in other low light bottleneck clades, but this does fit with previous findings of expression in the retina of *Phelsuma madagascariensis* (Taniguchi et al, 2001; Schott et al, 2017).

In snakes, our sampling spans the species rich Alethinophidia, in which we confirm the loss of eight NVOs and the presence of six NVOs. There is mixed evidence of losses of VAOP in some geckos and snakes. In the case of snakes, our results may be misleading, as the presence of VAOP was confirmed using gene capture of *Lampropeltis getula*, *Hypsiglena torquata*, *Cemophora coccinea* and *Arizona elegans* previously (Schott et al, 2017). The gecko *Phelsuma laticauda* was also reported to have retained VAOP (Schott et al, 2017). Other than this, the pattern of retention and losses of NVOs in snakes follows that of Schott et al (2017) and corroborates previous works that showed snakes to have retained OPN4x but lost OPN4m (Castoe et al, 2013; Emerling, 2017b; Schott et al, 2017; Hauzmann et al, 2019) and PIN, PRT, PP, OPN6 (Castoe et al, 2013; Emerling et al, 2017b; Schott et al, 2017).

In striking contrast to snakes and geckos, we identify a full complement of 15 NVOs retained in both of the crown groups of lacertid and anguimorph lizards. However, two losses in this group in particular seem worth noting. First, Salvator merianae appears to have lost PRT, and only partial sequences were found in OPN4m, PIN PP, and TMT, with PIN absent and OPN4x found to have a premature stop codon. This stop codon is in the 3' cytoplasmic region however and therefore shouldnt impede function, which may explain why the gene is otherwise conserved. Of more importance are the other partial sequences and losses. Of these PP, PRT and PIN are associated with the pineal gland and the parietal eye (Su et al, 2006; Wada et al, 2012). The loss of the parietal genes were predicted (see introduction), however the loss of PIN was not. The implications of pineal opsin loss will be discussed below. Hence, snakes and geckos share a more similar NVO complement with mammals and crocodiles than with other squamates. Given that many NVOs are linked to circadian photoentrainment, it is plausible that convergent NVO losses in mammals, crocodiles, snakes and geckos indicate a shared simplification of these groups' circadian systems. This is consistent with observations that gene losses in many of these taxa are accompanied by morphological and physiological regression of the parietal-pineal system. Mammals, crocodiles, snakes, geckos, teiids and chameleons have all either lost or regressed the parietal eye (Eakin, 1973; Gundy and Wurst, 1976; Quay, 1979; Emerling, 2017b). The pineal gland is also reduced in turtles, mammals and geckos, and is lost in crocodiles (Tosini, 1997; Ekström and Meissl, 2003; Hankins et al, 2014), and the main cells within the pineal gland (pinealocytes) show degenerate morphology (disorganised membrane whorls) in snakes and birds and are almost entirely lost in mammals (Hankins et al, 2014). In contrast, many lacertid and anguimorph lizards have complex inputs from multiple extraocular (especially parietal) photoreceptors involved in circadian regulation (Tosini, 1997; Bellingham et al, 2006; Su et al, 2006; Wada et al, 2012; Sapède and Cau, 2013; Hankins et al, 2014). This complementary molecular and morphological evidence suggests that during low-light bottlenecks the functions of the pineal gland and parietal eye cease to be maintained by ecological selection pressures. Certainly, the function of the parietal organ as a detector of the polarisation of light from the sun (Freake, 1999; 2001) would be of reduced fitness value in nocturnal species.

Convergent NVO losses in skinks - a cryptic mesopic bottleneck?

A novel finding of this study is the loss of NVOs in skinks. This clade has been neglected in previous studies of NVO evolution, but based on our analyses appears to have lost at least four NVOs, of which three are shared with geckos and all four are shared with snakes. Pinopsin, and OPNs 6 and 8, were absent in all three of our data sets (genome, gene capture and transcriptome), whereas parietopsin, TMTa and VAOP were only partially found in the *Tiliqua rugosa* genome (Figure 2) and were absent in the gene capture and transcriptomes. The observation that skinks have shared losses with snakes and geckos, in contrast to the other predominantly diurnal anguimorphs and lacertids, could be taken as evidence that skinks underwent a dim-light bottleneck. However, while there is some evidence to support an extended dim-light period in the skink ancestor, this is much less consistent than the evidence for dim-light bottlenecks in snakes, geckos, mammals and crocodiles.

An important difference in the visual evolution of skinks (versus snakes, geckos, mammals and crocodiles) is their retention of all five ancestrally present visual opsins (Chapter 2, this thesis). Retention of visual opsins is typical of ancestrally diurnal squamate groups such as lacerids and anguimorphs, thus might be interpreted as evidence against a low-light bottleneck in skinks. However, the highly fossorial amphisbaenians have also retained a full complement of visual opsins (Simões et al, 2015) and have more recent fossorial origins (approximately 65 million years ago: Standhardt, 1986; Sullivan and Lucas, 2000; Simões et al, 2015) compared to the common ancestor of skinks (which is dated 109 million years old; Skinner et al, 2011) (see Chapter 2, this thesis). Consistent with the retained visual function of skinks, most extant species in this group are diurnal (Cogger, 2014; Meiri, 2018). However, phylogenetic analyses provide evidence of shifts in activity times across the skink clade, and support a non-diurnal skink ancestor, with strict diurnality evolving more recently within the crown group (Slavenko et al, 2022).

Support from ocular morphology for a dim-light adapted ancestor of skinks is limited. There is evidence of visual regression and secondary evolution of diurnal traits (similarly to geckos: Röll, 2001b) (Slavenko et al, 2022), but these are distributed across skink phylogeny. For example, relaxed selection on the long wavelength sensitive opsin was detected in derived lineages of limb reduced *Lerista* skinks (compared to less fossorially adapted congeners (Chapter 2, this thesis). Eye anatomy, photoreceptor complements, and the presence of oil droplets also show substantial variation across the skink tree. Like many geckos, skinks mostly possess only cone-like (New et al, 2012; Röll, 2001a) photoreceptor morphology, with some evidence of 'rod-like' photoreceptors in some species (Canei et al, 2020). This suggests possible cases of transmutation among the Scincomorpha. Transmutation was suggested by Walls (1942) to explain cases of transitions between classes of photoreceptors normally associated with secondary ecological transitions. Transitions to diurnality such as seen in some snakes and geckos (Gamble et al., 2015; Simoes et al., 2016, Schott et al., 2016, Pinto et al, 2019) are normally associated with rods being converted in rod-like cones whereas transitions to nocturnality may transition cones into rod-like cones (Simoes et al., 2016, Underwood, 1967).

Detailed studies of ocular anatomy are available for the diurnal skink, *Cryptoblepharus boutonii*, which possesses immovable eyelids with a spectacle (like most geckos: Röll, 2001a) and has retina consisting of only cones, with yellow oil droplets, and a single clearly defined fovea unlike most other lizards and birds, which possess two foveas (Röll, 2001a). In some respects, this is remarkably similar to the gecko eye, which possesses a transmuted all rod-like retina, yet expresses cone opsins in these rod-like photoreceptors, having lost RH1 (Pinto et al, 2019). The *C. boutonii* eye is otherwise quite typical of any diurnal lizard. *Tiliqua rugosa*, another diurnal skink, has no discernable fovea and a retina composed of cone-like photoreceptors, but does express RH1 in a subset of cones (New et al, 2012). Yet Canei et al (2020) found rod-like photoreceptors that express RH1 in the retina of two psammophilic Scincinae (*Eumeces schneideri* and *Scincus scincus*). In summary, while some skink species show scotopic associated eye morphologies, there is little morphological evidence shared by all skinks of a nocturnal bottleneck or secondarily evolved diurnality as in geckos (Fleming 2022).

Loss of pinopsin in skinks and snakes - implications for pineal photoreception

Our study shows that PIN is convergently lost in snakes and skinks. Interestingly, these losses are accompanied by regression of the pineal morphology in both groups. In snakes, pinealocytes show among-species variation (Naz et al, 1999) but typically share a regressive morphology with the non-sensory cell types found in mammals (Petit, 1971). Teo (1997) provided an exhaustive description of the pineal and parietal organs of the skink *Tiliqua rugosa*. This showed that *Tiliqua* pinealocytes share some of the degenerate traits of snake pinealocytes. For example, the outer segments contain no stacked discs, a morphology which Collin and Oksche (1981) considered regressed or rudimentary. Consistent with a reduced photoreceptive capacity of the pineal in *Tiliqua*, Ellis et al (2010) noted that thermocycles are the primary cues (zeitgeber) to circadian rhythms in *Tiliqua rugosa*, with only small adjustments in response to light cycles, and a full circadian rhythm being formed even in the absence of light. To date, *Tiliqua rugosa* is the only skink species that has been used in investigations of the pineal function and circadian rhythms. Our molecular study used the *Tiliqua rugosa* genome to provide the first evidence that pinopsin has been lost in this species, and our gene capture and transcriptome results provided evidence that this gene is lost across the whole skink clade. Hence, the secondary losses of pinopsin shown here for skinks and snakes, together with previous reports of regressed pineal morphology in these taxa, indicates that both skinks and snakes may have lost pineal photoreception, resulting in a dulling of photoentrainment to light compared to other squamates. This would imply that the pineal organs of skinks and snakes have lost opsin activity and maintain only a secretory role, in parallel with mammals (Foster, 2003).

Among the many functions of the pineal gland, the secretion of melatonin, which plays a critical role in the sleep cycle, is among the most important (Sapède and Cau, 2013). Previous work in skinks has shown melatonin-associated enzyme activity in the pineal and retina of the skink *Lampropholis guichenoti* in response to changing light levels (Joss, 1978). This is interesting as pinopsin is normally associated with melatonin suppression (Okano et al, 1997; Csesmus et al, 1999), so while pinopsin may not be necessary for melatonin production and secretion, without this gene it is unclear how melatonin would be regulated. Mammals also do not express pinopsin, yet expression of rhodopsin and phototransduction enzymes has been described in the developing pineal organ (Blackshaw and Snyder, 1997; Sapède and Cau, 2013). It would be interesting to investigate whether convergent mechanisms of melatonin control are found in skinks, snakes and mammals. Perhaps, as Ellis (2010) suggests, skinks have evolved a way of mediating circadian rhythm independent of traditional photoentrainment.

Other squamate groups show variable patterns of pinopsin retention and expression. Geckos, tegus (lacertids) and chameleons (iguanians) have all retained pinopsin despite having degenerate parietal eyes (Gundy and Wurst, 1976). Within Iguania, expression studies found pinopsin in the parietal eye of *Uta* (Su et al, 2006), but not *Iguana* (Wada et al, 2012). Pineal photoreceptors with pinopsin immunoreactivity have been demonstrated in the lacertid *Lacerta* and the gecko *Phelsuma* (Vigh et al, 1998). Birds have also retained pinopsin, despite having a similar regression of pinealocyte morphology to snakes (Hankins et al, 2014). The photoreceptor morphology in Tiliqua is similar to that of the agamid *Uromastyx* lizards (Teo, 1997). While they were not present in this study, their agamid relative *Pogona*

vitticeps was, as was their close relative *Chamaeleo calyptratus*, and pinopsin was the only non-visual opsin not found in this species. The tegu *Salvator merianae* may have lost pinopsin too, as only a short fragment of the gene was found (Figure 2). It would be interesting to examine the expression of opsins, if any, in the pineal of these species and other agamids, and see if the comparison to the pineal of *Tiliqua rugosa* extends to loss of pinopsin also. Detailed comparative genomic studies are needed to understand how losses of the mutually interacting genes such as OPN5, pinopsin and parietopsin, impact circadian regulation in squamates.

Selective retention and redundancy of Melanopsin (Opn4) orthologs

While parallel patterns of genomic and morphological degeneration in these groups suggest a shared response to ecological transitions, we also identified significant among-group differences in patterns of loss and retention. An interesting difference among the groups analysed here is the opposite patterns of loss and retention of melanopsin orthologs in reptiles versus mammals: OPN4m is lost and OPN4x is retained in snakes, geckos and crocodiles (independently), while OPN4x is lost and OPN4m is retained in mammals. In mammals, Davies et al (2012) proposed that the loss of OPN4x was due to potential redundancy between OPN4m and the two visual opsins that were also lost in mammals, RH2 and SWS2. OPN4m is lost alongside SWS2 and RH2 in snakes, alongside RH1 and SWS2 in geckos (Simöes et al, 2015), and alongside SWS1 and RH2 in crocodilians (Emerling 2017a). Of additional interest is that skinks are the only clade within Squamata suspected of a mesopic or nocturnal bottleneck that have retained both melanopsins. Hence there is no consistent pattern among species with respect to loss and retention of melanopsin and these other genes. We interpret this as support for a previous hypothesis that the two OPN4 forms are functionally redundant, and therefore either could be lost in response to low-light bottlenecks (Emerling, 2017a).

It is plausible that functional redundancy of orthologs also explains the idiosyncratic patterns of loss of Neuropsin genes (OPN5-8). Whereas mammals have lost all but OPN5, snakes and geckos seem to have retained OPN5 in addition to OPN7. Most Scincomorpha seem to have lost all Neuropsin genes other than OPN7. However, OPN5 was found in some skink transcriptomes such as *Ctenotus atlas*, but was absent in the *Tiliqua rugosa* genome, and only partial fragments of this gene were found in the transcriptomes of many other skinks.

Conclusion

Our near-comprehensive sampling of non-visual opsins across Squamata has revealed previously unknown parallel opsin losses in mammal, snake, gecko and skink evolution, which adds further support to the hypothesis that these lineages have undergone scotopic bottlenecks in their early ancestry. More unexpectedly, we have for the first time elucidated the non-visual opsin complement of skinks, and uncovered parallels between the and the previously mentioned historically scotopic clades, perhaps hinting at a scotopic bottleneck in their evolutionary history. Further comparative research on the photosensitive tissues of Squamata, especially the retina, pineal gland and parietal eye, and research into the

behaviours of these animals may explain why certain clades have retained opsins that others have lost.

Supplementary Materials

Table 3.1

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Chapter 4: Shifting evolutionary constraints on non-visual opsins in squamates suggest divergent selective pressures on skinks

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Abstract

A large repertoire of photosensitive opsin genes has been linked to non-visual functions such as the circadian entrainment of sleep-wake cycles and the timing of thermoregulatory and reproductive behaviours. These non-visual opsins (NVOs) show striking patterns of loss and retention across the vertebrate tree of life. Recently, it was found that the Scincimorpha (scincid lizards) have lost some NVO genes in parallel with geckos, snakes and mammals, all of which have experienced scotopic bottlenecks, raising the possibility of a mesopic bottleneck in skinks. This study aims to further develop our understanding of NVO evolution in Lepidosauria by testing selection pressures on the retained genes and comparing them across the group. We found evidence of differential selection among the major clades of squamates, with geckos and snakes having increased relaxation across most NVOs. Interestingly, skinks showed signals of purifying or positive selection, indicating strong functional constraints, on five genes: OPN5, OPN3, OPN4m, OPN4x and RRH. We tentatively interpret this as evidence of secondarily evolved diurnality from a low-light bottleneck in the skink ancestor. We further suggest that the NVO losses and shifting selection pressures detected in skinks indicate divergent evolution of non-visual photoreception in this taxon. In particular, while selection pressures (possibly linked to homing behaviours) have maintained the parietal eye and its opsins, pinopsin has been inactivated, with PRT and PP becoming the most likely regulators of melatonin. Behavioural and gene expression studies will ultimately be needed to test these hypotheses.

Introduction

Non-visual opsins (NVOs) comprise a large protein family that mediates non-image forming vision in functions such as the setting of circadian clocks (Nakamura, 1999; Hankins et al, 2008; Peirson et al, 2009; Guido et al, 2020), pupillary constriction (Lucas et al, 2001; Lucas et al, 2003), and skin colour change associated with camouflage (Bertolesi and McFarlane, 2018). The genes encoding NVOs are comparable to the visual opsin genes of the retina in having undergone replicate losses in dim-light adapted lineages. Among vertebrates, convergent losses of multiple NVOs have been linked to nocturnal (or fossorial) bottlenecks

in the early ancestry of mammals (Gerkema et al, 2013; Borges et al, 2018), snakes (Walls, 1942; Simões et al, 2015; Emerling, 2017b; Perry et al, 2018; Schott et al, 2018), geckos (Gamble et al, 2015; Pinto et al, 2019), and crocodiles (Emerling, 2017a). In contrast, groups that have diurnal ancestry possess large complements of up to 15 NVOs that were retained from a vertebrate ancestor. For example, 15 NVOs were found to be present in most lacertids (wall lizards) and anguimorphs (e.g. monitor lizards) (Chapter 3, this thesis). The tuatara, which forms the sister lineage to squamates, possesses at least 15 NVOs (Gemmell et al, 2020). Amphibians have been found to have at least 18 NVOs (Provencio et al, 1998; Bellingham et al, 2006; Currie et al, 2016; Davies et al, 2015, Beaudry et al, 2017). The Scincomorpha (skink lizards) were recently found to have retained most NVOs present in the Squamate ancestor, but showed evidence for losses of at least four and perhaps as many as six NVOs: Pinopsin (PIN), Parietopsin (PRT), Teleost Multiple Tissue opsin a (TMTa), Vertebrate Ancient opsin (VAOP), Neuropsin like-1 (OPN6) and Neuropsin like-2 (OPN8) (Chapter 3, this thesis).

The NVOs inferred to be lost in skinks are associated with the regulation of circadian, thermoregulatory and reproductive patterns. Hence, their loss in skinks was unexpected because molecular evidence suggests that all skinks have retained the full complement of five visual opsins present in ancestral squamates (Chapter 2, this thesis), and most modern skinks are highly diurnal (Meiri, 2018; Slavenko et al, 2022). However, phylogenetic analyses provide evidence of a non-diurnal skink ancestor (Slavenko et al, 2022), and several aspects of ocular morphology further support the possibility of a dim-light phase in the early evolution of the clade. In particular, some skink species possess immovable eyelids with a spectacle, which is a trait associated with dim-like ancestry in snakes and geckos (Walls, 1942; Röll, 2000; Röll, 2001a; Van Doorn and Sivak; 2014). Others, such as *Tiliqua rugosa*, have no discernable fovea and a retina composed of cone-like photoreceptors, features which are also shared with many geckos (Teo, 1997; Röll, 2001b; New et al, 2012). Also consistent with an ancestral bottleneck is a finding of relaxed selection on the long wavelength sensitive opsin of limb reduced *Lerista* skinks (compared to less fossorially adapted congeners (Chapter 2, this thesis).

Complementary evidence for, or against, the hypothesis of a mesopic bottleneck in skinks might be gained by comparing signals of selection on the NVO genes retained in skinks and other lepidosaurs. The few previous studies that have investigated constraint and selection on non-visual opsins have found increased relaxation of functional constraints on lineages that have undergone visual gene losses associated with scotopic bottlenecks. For example, Hauzman et al (2019) found that in snakes, OPN4x has evolved under lower constraint than OPN4x and OPN4m from other vertebrates. In mammals, Borges et al (2018) found conserved OPN4m and RRH genes that had strong functional constraints in platypus. Upton et al (2019) found strong functional constraints on OPN3, OPN5 and RRH but increased mutational rates indicative of relaxed selection pressures in RGR and OPN4m across mammals. However, RRH in particular had decreased constraint in scotopic species (Upton et al, 2019). Together these studies suggest a link between ecological transitions and molecular signals of selection pressures on NVO sequences.

There are also many known amino acid sites in opsins that affect their functional abilities. For example, Yamashita et al (2014) used mutagenesis experiments to investigate a

change at site 168 in OPN5, which they found to be responsible for affinity to all-trans retinal. Eutherian mammals have undergone a change at this site that eliminates the affinity of the opsin to bind all-trans retinal, making it a specialist UV sensitive bleaching opsin similar to visual opsins (Yamashita et al, 2014; Yamashita, 2020). The schiff base linkage at site 296 is usually stabilised by a negative charge at site 113. The E/DRY motif is a feature of all GPCRs, including non-opsins, and is thought to be crucial for maintaining signalling ability, especially the negatively charged amino acid that begins the motif (Rovati et al, 2007; Terakita et al, 2012; Yamashita, 2020). Many bistable pigments possess a negative counter-ion at site 181 instead of at 113, and the transition of the counter-ion to site 113 in the evolution of vertebrate photopigments is thought to be responsible for bleaching behaviour and loss of bistability, and to allow for greater conformational change and perhaps higher photosensitivity (Terakita et al, 2012).

In this paper, we aimed to shed light on the visual ecology of ancestral skinks by comparing selection pressures on NVO genes in this lineage to those of other squamates, particularly groups that have undergone convergent gene losses associated with dim-light bottlenecks. Very few previous studies have analysed selection pressures on NVOs in any clade. Hence, our work aims to provide significant new knowledge. If scotopic bottlenecks have reduced the functional importance of all or most NVOs, we would expect to see relaxed selection on retained NVOs in snakes and geckos compared to the ancestrally diurnal lacertids and anguimorphs. Alternatively, we might expect stronger functional constraints on retained genes in lineages that have lost other NVOs, due to either compensation for gene losses or co-option for alternative photoreceptive functions. It is also possible that divergent patterns of NVO evolution characterise different squamate lineages, reflecting lineage-specific selection pressures on these genes.

Methods

Sequence Generation

Targeted capture of non-visual opsins was done as in Chapter 2 of this thesis (See Chapter 2 and 3). The same sources were also used for skink transcriptomes. Sequences were also drawn from previous works, crocodilians, testudines and avians from Emerling et al (2017a), caenophidian snakes from Schott et al (2018), and geckos from Pinto et al (2019). As many squamate sequences as were available were also collected from NCBI, and any available squamate genomes and/or transcriptomes were collected, BLASTed against closest known high quality sequences, and added to this dataset (SI Table 3.1). Only +80% similarity matches were considered, and all BLASTed sequences were concatenated and aligned with known, high quality full sequences (eg *Anolis carolinensis*) to ensure homology, and most similar sequences were selected. Mammal sequences were also derived from NCBI. Sequences were classified as follows: Present – full or near complete sequences; Pseudo – full or partial sequences with premature stop codons or frameshift mutations; Partial – incomplete sequences with no premature stop codons; Absent – BLAST yielded no results, sequence could not be found.

Alignment and Analyses

Sequences were aligned by gene using Clustal Omega in Geneious Prime (Version 2021.2.2) and finished manually. These alignments were then combined into one, which was analysed with ModelTest-NG (Darriba et al, 2020) in raxmlGUI 2.0 (Edler et al, 2020) to determine the correct model (JTT+G4) to use to create a tree of all sequences using RAxML-NG, also in raxmlGUI 2.0 (Edler et al, 2020). These were then aligned to known and annotated sequences from *Anolis* and *Python* to check the sequence identities and identify important sites. Even so, most non-visual opsin genes are incompletely annotated with few identified features. Therefore, gene alignments were then aligned to *Bos taurus* RH1 in order to identify other potentially important sites.

Molecular Evolution

These sequences were analysed for selected sites using PAML version 4.8a (July 2014) through ete3 (Huerta-Cepas et al, 2016). Sequences were analysed for sites under selection, branches under selection, variance at sites on different branches, and clade selection. Site selection tests were in two parts, M1a (null) vs M2a (test) and M7 β (null) vs M8 β&ω (test). Branch tests used M0 (null) vs two ratio model (test). Branch-site tests used model A1 (null) vs model A (test). Clade tests used M2a_rel (null) vs CmC (test). Branches and clades were divided into groups which were as follows: Nocturnal, Diurnal (test 1); snakes, lizards, background (test 2); geckos, snakes, skinks, background (test 3); geckos, snakes, other squamates, background (test 4); Lacertoidea vs other lepidosaurs (test 5); and Toxicofera vs other lepidosaurs (test 6). Where the term 'background' is used, it refers to all other lepidosaurs not in the test group/groups. Not all these groups possess all genes analysed and so were omitted (SI Tables 4.2, 4.3, 4.4). These groups were analysed for branch, branch-site and clade analyses. Selected sites found with site and branch site analyses will be reported based on the bovine RH1 residue number. These genes also underwent analysis specifically for relaxed/intensified selection using the RELAX algorithm (Wertheim et al, 2015). RELAX requires the use of only one test group compared to one reference group, thus it was necessary to choose different groups in these analyses. These groups were as follows: Geckos vs other squamates; Snakes vs other squamates; Skinks vs other squamates; Geckos + snakes + skinks vs other squamates; and nocturnal vs diurnal.

Results

In all, 714 sequences were recovered, either fully or partially (SI Table 3.1).
RAxML GUI Model Selector (Edler et al, 2021) chose model JTT+G4 with BIC, AIC and AICc. RAxML was then also used to build a tree to check the relationships between genes and the species within these gene clades. RAxML produced a maximum likelihood tree with correctly grouped taxa in all instances except two (*Apalone spinifera* PRT and *Pelodiscus sinensis* PRT). These were removed from the alignment and analyses were rerun with no grouping errors found. However, since these two sequences were generated by (Emerling, 2017a), they were not discounted as false or misidentified when reporting on their presence and properties. Selection tests were then performed using a different tree, constructed using the data from Pyron et al (2013).

Due to the high number of tests and results, only significant findings will be mentioned here. Full PAML results are shown in supplementary tables 4.1-4.4.

Selection Analyses

Melanopsin Mammal-like (OPN4m)

M8 β&ω was not a significantly better fit than its null model, M7 β, and no significantly selected sites were found (SI Table 4.1). Branch models show that nocturnal (ω=1.576) lepidosaurs show evidence of positive selection when compared to diurnal lepidosaurs (ω=0.225). In test 3, skinks (ω=0.353) showed lower constraint than background species (ω=0.206). Both of these models were a significantly better fit than M0 (ω=0.232)(Fig. 3; SI Table 4.2). In branch-site analyses, site 279 shows positive selection in nocturnal branches when compared to diurnal branches (ω=38.662). In skinks, sites 281, 306, 337, 348, 353, 396, 406, 463, 464, 481, 482 all showed evidence of positive selection compared to other Lepidosauria (ω=3.169)(SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=5.08) show evidence of positive divergent selection when compared to diurnal lepidosaurs (ω=0.319). In test 3, skinks (ω=0.408) show more constraint than background animals (ω=1.21), which show evidence of positive divergent selection. These tests were a significantly better fit than M2a_rel (ω=0.382)(Fig. 2; SI Table 4.4). RELAX results show that skinks (k=10.2) have strong intensification compared to other lepidosaurs. Nocturnal lepidosaurs (k=1.01) did not show significant differences to diurnal lepidosaurs.

Melanopsin Xenopus-like (OPN4x)

Neither model M2a nor M8 β&ω were significantly better fits than their null models M1a and M7 β respectively, therefore we cannot reject the hypothesis that there are no selected sites in OPN4x (SI Table 4.1). Branch models show that nocturnal (ω=0.239) lepidosaurs show slightly stronger constraint than diurnal lepidosaurs (ω=0.304). In test 2, snakes (ω=0.390) showed weaker constraint than Lizards (ω= 0.266) and the tuatara (ω=0.297). In test 3, Snakes (ω=0.390) and skinks (ω=0.399) both showed weaker constraint than geckos (ω=0.234) and background species (ω=0.244). In test 4, Snakes (ω=0.390) show weaker constraint than geckos (ω=0.234), other squamates (ω=0.278) and the tuatara (ω=0.295). All these models were a significantly better fit than M0 (ω=0.292)(Fig. 3; SI Table 4.2). In branch-site analyses, snakes show significant positive selection at 13, 21, 27, 228, 329, 336, 353, 377, 480 when compared to other Lepidosauria (ω=1.448). Skinks show positive selection at 71, 88, 90, 92, 94, 95, 97, 215, 216, 217, 218, 219, 221, 239, 267, 268, 269, 340 when compared to other Lepidosauria (ω=2.166)(SI Table 4.3). In clade analyses in test 2, lizards (ω=0.225) showed higher constraint than snakes (ω=0.439) and the tuatara (ω=0.315). In test 3, skinks (ω=0.557) showed weaker constraint than snakes (ω=0.413), which in turn showed weaker constraint than geckos (ω=0.177) and background animals (ω=0.167). In test 4, snakes (ω=0.446) showed weaker constraint than geckos (ω=0.183), squamates (ω=0.244), and the tuatara (ω=0.323). All these models were significantly better fit than M2a_rel (ω=0.281)(Fig. 2; SI Table 4.4). In RELAX tests, geckos (k=0) show significant, strong relaxation when compared to other lepidosaurs. Snakes also show significant relaxation when compared to other lepidosaurs (k=0.84). Skinks (k=1.95) showed intensification when compared to other lepidosaurs. Snakes, geckos and skinks combined showed intensification

when compared to other lepidosaurs (k=2.07). Nocturnal lepidosaurs (k=0.75) were significantly more relaxed than diurnal lepidosaurs.

Neuropsin (OPN5)

M8 β&ω is a significantly better fit than its null model, M7 β. According to M8 β&ω however, there were no significantly selected sites (SI Table 4.1). Branch models show that in test 2, Snakes (ω=0.324) showed weaker constraint than Lizards (ω= 0.193) and the tuatara (ω=0.127). In test 3, Snakes (ω=0.325) and skinks (ω=0.269) showed higher constraint than geckos (ω=0.173) and background (ω=0.181). In test 4, Snakes (ω=0.324) show weaker constraint than other squamates (ω=0.210) which in turn show weaker constraint than geckos (ω=0.174) and the tuatara (ω=0.127). In test 6, Toxicofera (ω=0.252) show lower constraint than other lepidosaurs (ω=0.180). All these models were a significantly better fit than M0 (ω=0.207)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=0.416) showed weaker constraint than diurnal lepidosaurs (ω=0.259). Snakes (ω=0.654) showed much weaker constraint than lizards (ω=0.193) and the tuatara (ω=0.199). In test 3, skinks (ω=0.126), geckos (ω=0.252), and background animals (ω=0.140) showed much higher constraint than snakes (ω=0.679). In test 4, geckos (ω=0.667) showed weaker constraint than snakes (ω=0.250), which in turn were more relaxed than squamates (ω=0.126), and the tuatara (ω=0.189). All these models were significantly better fit than M2a_rel (ω=0.337)(Fig. 2; SI Table 4.4). RELAX analyses show that geckos are significantly more relaxed than other lepidosaurs (k=0.08). Skinks (k=3.78) show intensification of selection compared to other lepidosaurs. Nocturnal lepidosaurs (k=39.05) show significant intensification of selection compared to diurnal lepidosaurs.

Neuropsin-like 1 (OPN6)

Neither M2a nor M8 β&ω were significantly better fits than their null models M1a and M7 β, therefore we cannot reject the hypothesis that there are no selected sites in OPN6 (SI Table 4.1). Branch models show that nocturnal (ω=0.119) lepidosaurs show stronger constraint than diurnal lepidosaurs (ω=0.229). In test 3, Geckos (ω=0.107) show higher constraint than background (ω=0.235). In test 4, Geckos (ω=0.107) also show higher constraint than other squamates (ω=0.242) and the tuatara (ω=0.179). In test 6, Toxicofera (ω=0.242) shows lower constraint than background (ω=0.177). All these models were a significantly better fit than M0 (ω=0.209)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=0.102) showed more constraint than diurnal lepidosaurs (ω=0.281). Test 3 showed geckos (ω=0.105) showing more constraint than background animals (ω=0.260). In test 4, again geckos (ω=0.105) have higher constraint than squamates (ω=0.274) and the tuatara (ω=0.194). These tests were a significantly better fit than M2a_rel (ω=0.232)(Fig. 2; SI Table 4.4). RELAX analyses showed that Geckos (k=1.57) show significant intensification of selection compared to other lepidosaurs.

Neuropsin-like 2 (OPN8)

Neither M2a nor M8 β&ω were significantly better fits than their null models M1a and M7 β, therefore we cannot reject the hypothesis that there are no selected sites in OPN8 (SI Table 4.1). No branch models were a significantly better fit than M0 (ω=0.233)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). No clade models were a significantly better fit to data than M2a_rel (ω=0.362)(Fig. 2; SI Table 4.4). RELAX also showed no significant results.

Neuropsin-like 3 (OPN7)

M8 β&ω was a significantly better fit than its null model, M7 β. However, according to M8 β&ω, there were no positively selected sites (SI Table 4.1). Branch models show that nocturnal (ω=0.270) lepidosaurs show weaker constraint than diurnal lepidosaurs (ω=0.135). In test 2, Snakes (ω=0.244) showed weaker constraint than Lizards (ω= 0.138) and the tuatara (ω=0.081). In test 3, Snakes (ω=0.246) and geckos (ω=0.217) both showed weaker constraint than skinks (ω=0.191) and background species (ω=0.078). In test 4, Snakes (ω=0.246) and geckos (ω=0.217) show weaker constraint than the tuatara (ω=0.106) and background (ω=0.080). In test 5, Lacertoidea (ω=0.103) showed higher constraint than background (ω=0.159). All these models were a significantly better fit than M0 (ω=0.152)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=0.400) were significantly more relaxed than diurnal lepidosaurs (ω=0.203). Lizards (ω=0.182) and the tuatara (ω=0.098) showed higher constraint than snakes (ω=0.405). In test 3, snakes (ω=0.460) and geckos (ω=0.373) showed lower constraint than skinks (ω=0.252) and background animals (ω=0.106). In test 4, again snakes (ω=0.460) were more relaxed than geckos (ω=0.371) and other squamates (ω=0.371), which had lower constraint than the tuatara (ω=0.103). All these models were significantly better fit than M2a_rel (ω=0.217)(Fig. 2; SI Table 4.4). RELAX analyses show that geckos (k=0.86) show significant relaxation compared to other lepidosaurs. Snakes (k=0.52) also show significant relaxation compared to other lepidosaurs. Snakes, geckos and skinks combined (k=0.68) show significant relaxation compared to other lepidosaurs.

Encephalopsin (OPN3)

M8 β&ω was a significantly better fit than its null model, M7 β. According to M8 β&ω, there were no positively selected sites (SI Table 4.1). Branch models show that nocturnal (ω=0.236) lepidosaurs show weaker constraint than diurnal lepidosaurs (ω=0.155). In test 2, Snakes (ω=0.237) both showed weaker constraint than Lizards (ω= 0.155) and the tuatara (ω=0.126). In test 3, Snakes (ω=0.237) showed lower constraint than skinks (ω=0.195), background (ω=0.139) and geckos (ω=0.155). In test 4, Snakes (ω=0.237) show weaker constraint than geckos (ω=0.155), the tuatara (ω=0.133) and other squamates (ω=0.153). In test 5, Lacertoidea (ω=0.119) showed higher constraint than background (ω=0.174). All these models were a significantly better fit than M0 (ω=0.167)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=0.395) showed significantly weaker constraint than diurnal lepidosaurs (ω=0.273). Snakes (ω=0.440) showed weaker constraint than lizards (ω=0.267) and the tuatara (ω=0.203). In test 3, snakes (ω=0.427) showed lower constraint

than skinks (ω=0.337) which showed lower constraint than geckos (ω=0.250) and background animals (ω=0.239). In test 4, snakes (ω=0.427) showed lower constraint than other squamates (ω=0.337), which showed lower constraint than the tuatara (ω=0.239) and geckos (ω=0.250). All these models were significantly better fit than M2a_rel (ω=0.303)(Fig. 2; SI Table 4.4). RELAX results show that geckos (k=1.03) do not differ significantly from other lepidosaurs. Snakes (k=0.39) show strong significant relaxation compared to other lepidosaurs. Skinks (k=3.56) show intensification of selection compared to other lepidosaurs. Combined geckos, snakes and skinks (k=0.77) show relaxation of selection compared to other lepidosaurs.

Parapinopsin (PP)
M8 β&ω was a significantly better fit than its null model, M7 β. According to M8 β&ω, there were no positively selected sites (SI Table 4.1). None of the branch tests were a significantly better fit to the data than M0 (ω=0.227)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=1.307) showed positive selection, whereas diurnal lepidosaurs (ω=0.084) showed strong constraint. In test 4, the tuatara (ω=1.307) showed positive selection, whereas squamates (ω=0.084) showed strong constraint. Both these models were significantly better fit than M2a_rel (ω=0.170)(Fig. 2; SI Table 4.4). RELAX analyses show that nocturnal lepidosaurs (k=0) have significant relaxation of selection when compared to diurnal lepidosaurs.

Parietopsin (PRT)
Neither M2a nor M8 β&ω were a significantly better fit than their null models, M1a and M7 β respectively. Therefore we may not reject the null hypothesis that there are no selected sites (SI Table 4.1). Branch models show that no tests were a significantly better fit than M0 (ω=0.151)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). No clade analyses were a significantly better fit to data than M2a_rel (ω=0.320)(Fig. 2; SI Table 4.4). RELAX results show that nocturnal (k=0.81) lepidosaurs show no significant differences between any group.

Retinal Pigment Epithelium-derived Rhodopsin Homologue (RRH)
M8 β&ω was a significantly better fit than its null model, M7 β. According to M8 β&ω, site 155 was positively selected for (SI Table 4.1). Branch models show that nocturnal (ω=0.208) lepidosaurs show weaker constraint than diurnal lepidosaurs (ω=0.147). In test 2, Snakes (ω=0.246) showed weaker constraint than Lizards (ω= 0.150) and the tuatara (ω=0.067). In test 3, Snakes (ω=0.246) showed lower constraint than geckos (ω=0.183), skinks (ω=0.122) and background (ω=0.125). In test 4, snakes (ω=0.246) show lower constraint than geckos (ω=0.184), other squamates (ω=0.135), and the tuatara (ω=0.067). In test 5, Lacertoidea (ω=0.069) showed higher constraint than background (ω=0.171). In test 6, Toxicofera (ω=0.205) showed lower constraint than background (ω=0.126). All these models were a significantly better fit than M0 (ω=0.157)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=0.402) showed lower constraint than diurnal lepidosaurs (ω=0.261). Lizards

(ω=0.275) showed higher constraint than snakes (ω=0.465), but lower constraint than the tuatara (ω=0.091). In test 3, snakes (ω=0.461) showed lower constraint than lizards (ω=0.366), which in turn had higher relaxation than skinks (ω=0.222), and background animals (ω=0.201). In test 4, again snakes (ω=0.483) showed lower constraint than geckos (ω=0.388), squamates (ω=0.237), and the tuatara (ω=0.094). All these models were significantly better fit than M2a_rel (ω=0.281)(Fig. 2; SI Table 4.4). RELAX results showed that geckos (k=0.57) were significantly more relaxed than other lepidosaurs. Snakes (k=0.24) also showed significant relaxation when compared to other lepidosaurs. Skinks (k=44.03) showed significant strong intensification of selection compared to other lepidosaurs. Snakes, geckos and skinks combined (k=1.69) showed milder intensification compared to other lepidosaurs.

Pinopsin (PIN)

M8 β&ω was a significantly better fit than its null model, M7 β. According to M8 β&ω, site 11 is positively selected for. This could be adjacent to or within an N-linked glycosylation site (SI Table 4.1). Branch models show that nocturnal (ω=0.209) lepidosaurs show weaker constraint than diurnal lepidosaurs (ω=0.152). In test 3, Geckos (ω=0.166) showed lower constraint than background (ω=0.145). In test 4, geckos (ω=0.169) show similar constraint to other squamates (ω=0.165) but lower than the tuatara (ω=0.065). All of these models were a significantly better fit than M0 (ω=0.150)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses no model was a significantly better fit to data than M2a_rel (ω=0.230)(Fig. 2; SI Table 4.4). RELAX also shows that no tested group differs significantly from another.

Retinal G-protein-coupled Receptor (RGR)

Neither M2a nor M8 β&ω were significantly better fits than their null models M1a and M7 β, therefore we cannot reject the hypothesis that there are no selected sites in RGR (SI, Table 4.1). Branch models show that nocturnal (ω=0.209) lepidosaurs show weaker constraint than diurnal lepidosaurs (ω=0.152). In test 2, Snakes (ω=0.232) showed weaker constraint than Lizards (ω= 0.146) and the tuatara (ω=0.121). In test 3, geckos (ω=0.175) and skinks (ω=0.159) showed higher constraint than Snakes (ω=0.232), but lower than background (ω=0.123). In test 4, geckos (ω=0.175) showed higher constraint than Snakes (ω=0.232), but lower than the tuatara (ω=0.121) and other squamates (ω=0.134). In test 5, Lacertoidea (ω=0.119) showed higher constraint than background (ω=0.187). All these models were a significantly better fit than M0 (ω=0.163)(Fig. 3; SI Table 4.2). In branch-site analyses, only snakes show positive selection at sites 30, 37, 38, 317 when compared to other lepidosauria (ω=2.729)(SI, Table 4.3). In clade analyses, no models were a significantly better fit to data than M2a_rel (ω=0.261)(Fig. 2; SI Table 4.4). RELAX results show that snakes (k=0.82) show significant relaxation compared to other lepidosaurs. Snakes, geckos and skinks combined (k=0.8) show relaxation compared to other lepidosaurs, but neither skinks nor geckos are significantly different as separate groups.

Teleost Multiple Tissue opsin (TMT)

Neither M2a nor M8 β&ω were significantly better fits than their null models M1a and M7 β, therefore we cannot reject the hypothesis that there are no selected sites in TMT (SI Table 4.1). Branch models show that nocturnal (ω=0.354) lepidosaurs show weaker constraint than diurnal lepidosaurs (ω=0.183). In test 3, geckos (ω=0.323) showed weaker constraint than skinks (ω=0.233) and background (ω=0.148). In test 4, geckos (ω=0.323) show weaker constraint than other squamates (ω=0.164) and the tuatara (ω=0.124). In test 5, Lacertoidea (ω=0.139) showed higher constraint than background (ω=0.216). In test 6, Toxicofera (ω=0.154) showed higher constraint than background (ω=0.229). All these models were a significantly better fit than M0 (ω=0.202)(Fig. 3; SI Table 4.2). In branch-site analyses, nocturnal lepidosaurs show significant positive selection at sites 222, 300, 301, 302, 303, 305, 310, 311, 314, 320 when compared to diurnal lepidosaurs (ω=3.056)(SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=0.827) were less constrained than diurnal lepidosaurs (ω=0.268). In test 3, geckos (ω=0.790) showed lower constraint than skinks (ω=0.313), which in turn were less constrained than background animals (ω=0.129). In test 4, geckos (ω=0.769) were less constrained than squamates (ω=0.178), which were less constrained than the tuatara (ω=0.077). All these models were significantly better fit than M2a_rel (ω=0.272)(Fig. 2; SI Table 4.4). RELAX results show that geckos (k=0.72) are significantly more relaxed than other lepidosaurs. Geckos and skinks together (k=0.22) show significant relaxation compared to other lepidosaurs. Nocturnal lepidosaurs (k=17.27) show significant intensification of selection compared to diurnal lepidosaurs.

Teleost Multiple Tissue opsin a (TMTa)

Neither M2a nor M8 β&ω were significantly better fits than their null models M1a and M7 β, therefore we cannot reject the hypothesis that there are no selected sites in TMT (SI Table 4.1). Branch models show that nocturnal (ω=0.302) lepidosaurs show weaker constraint than diurnal lepidosaurs (ω=0.177). In test 3, geckos (ω=0.265) showed lower constraint than background (ω=0.167). In test 4, other squamates (ω=0.184) show higher constraint than geckos (ω=0.265), but weaker constraint than the tuatara (ω=0.095). All these tests were a significantly better fit than M0 (ω=0.190)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses, nocturnal lepidosaurs (ω=0.714) showed weaker constraint than diurnal lepidosaurs (ω=0.392). In test 3, geckos (ω=0.591) showed weaker constraint than background animals (ω=0.349). In test 4, geckos (ω=0.647) showed weaker constraint than squamates (ω=0.482) and the tuatara (ω=0.182). All these models were significantly better fit than M2a_rel (ω=0.438)(Fig. 2; SI Table 4.4). RELAX analyses show that geckos are significantly more relaxed than other lepidosaurs (k=0.76).

Vertebrate Ancient Opsin (VAOP)

M8 β&ω is a better fit than its null model, M7 β. According to M8 β&ω site 329 is positively selected for (SI Table 4.1). Branch models show no test models were a significantly better fit than M0 (ω=0.191)(Fig. 3; SI Table 4.2). In branch-site analyses, there was no support for significant positive selection (SI Table 4.3). In clade analyses, no model provided a significantly better fit to data than M2a_rel (ω=0.279) (Fig. 2; SI Table 4.4). RELAX analyses show that geckos show intensification of selection compared to other lepidosaurs

(k=4.29). Geckos and snakes combined (k=12.92) show significant intensification of selection compared to other lepidosaurs.

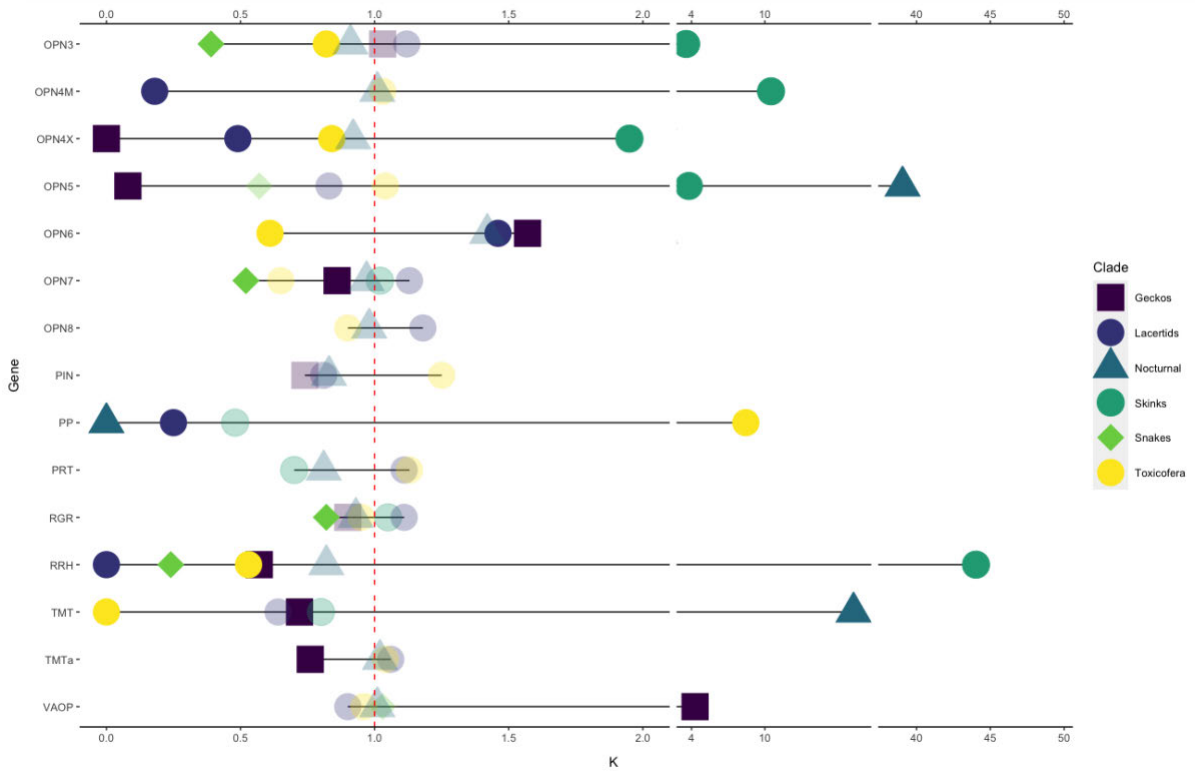

Figure 1: Relaxation and intensification on different clades according to RELAX analysis showing RELAX parameter k, with k>1 indicating intensified selection and k<1 indicating relaxed 5 of 15 genes show intensified selection in skinks versus other squamate clades. Faded points indicate non significant results.

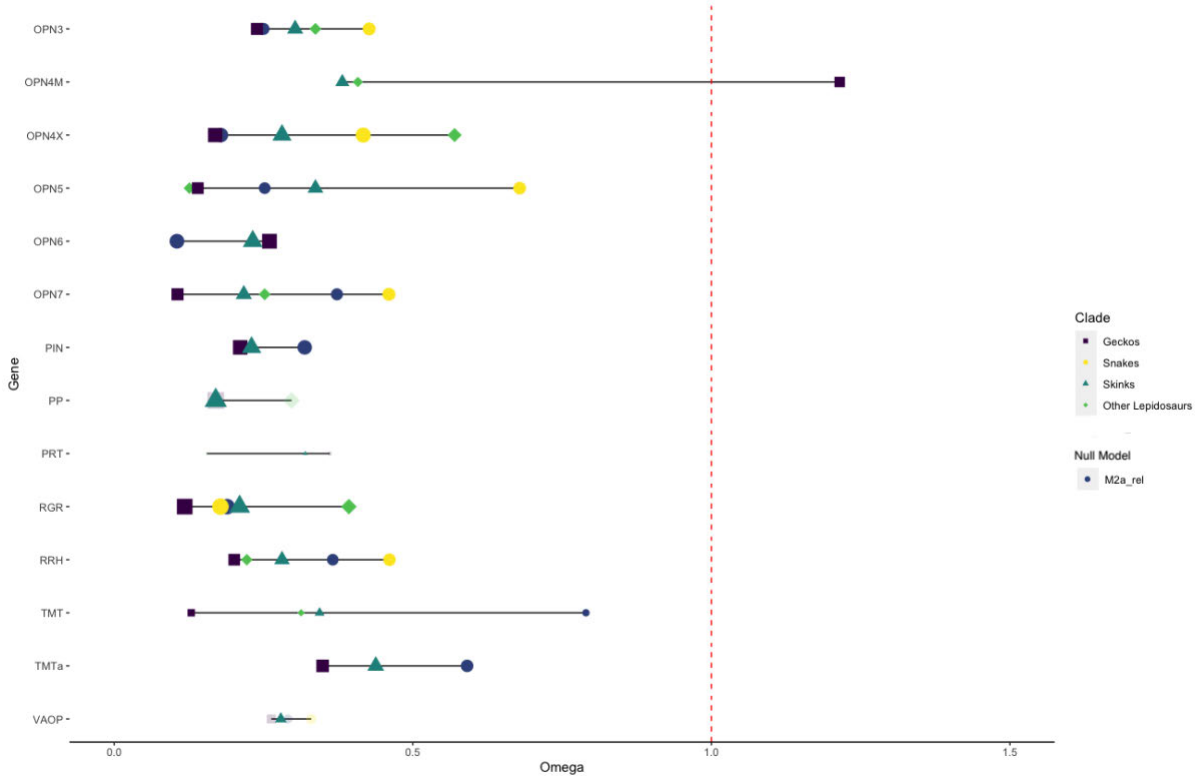

Figure 2: Site specific selection within clades, as determined by CmC test 3 (Geckos + Snakes + Skinks vs background). The size of the points is proportional to the proportion of sites in site class ω2. Points with alpha=0.2 are not significant. (SI Table 3). M2a_rel was also included as a group, as this is the null model in this test.

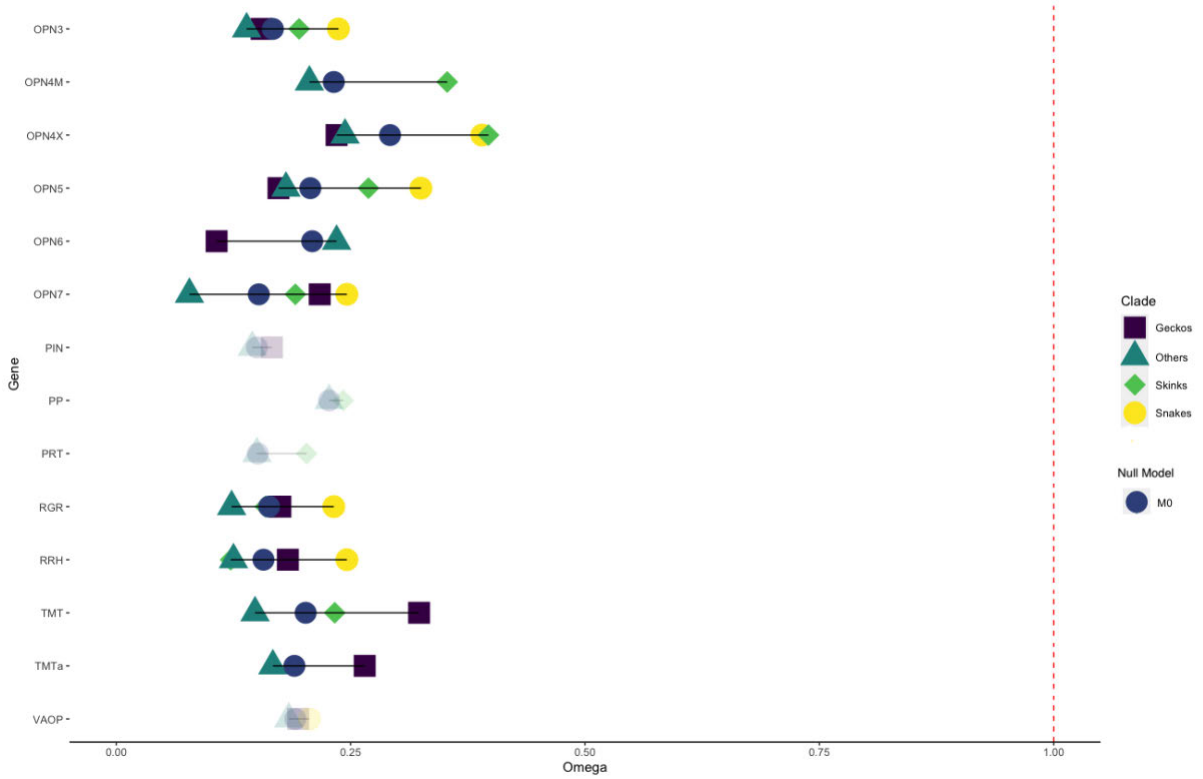

Figure 3: Selection on different clades, as determined by one and two branch models in PAML, test 3 (Geckos vs Snakes vs Skinks vs background). Faded points are not significant. (SI Table 2). M0 is also included as a group as this is the null model in this test.

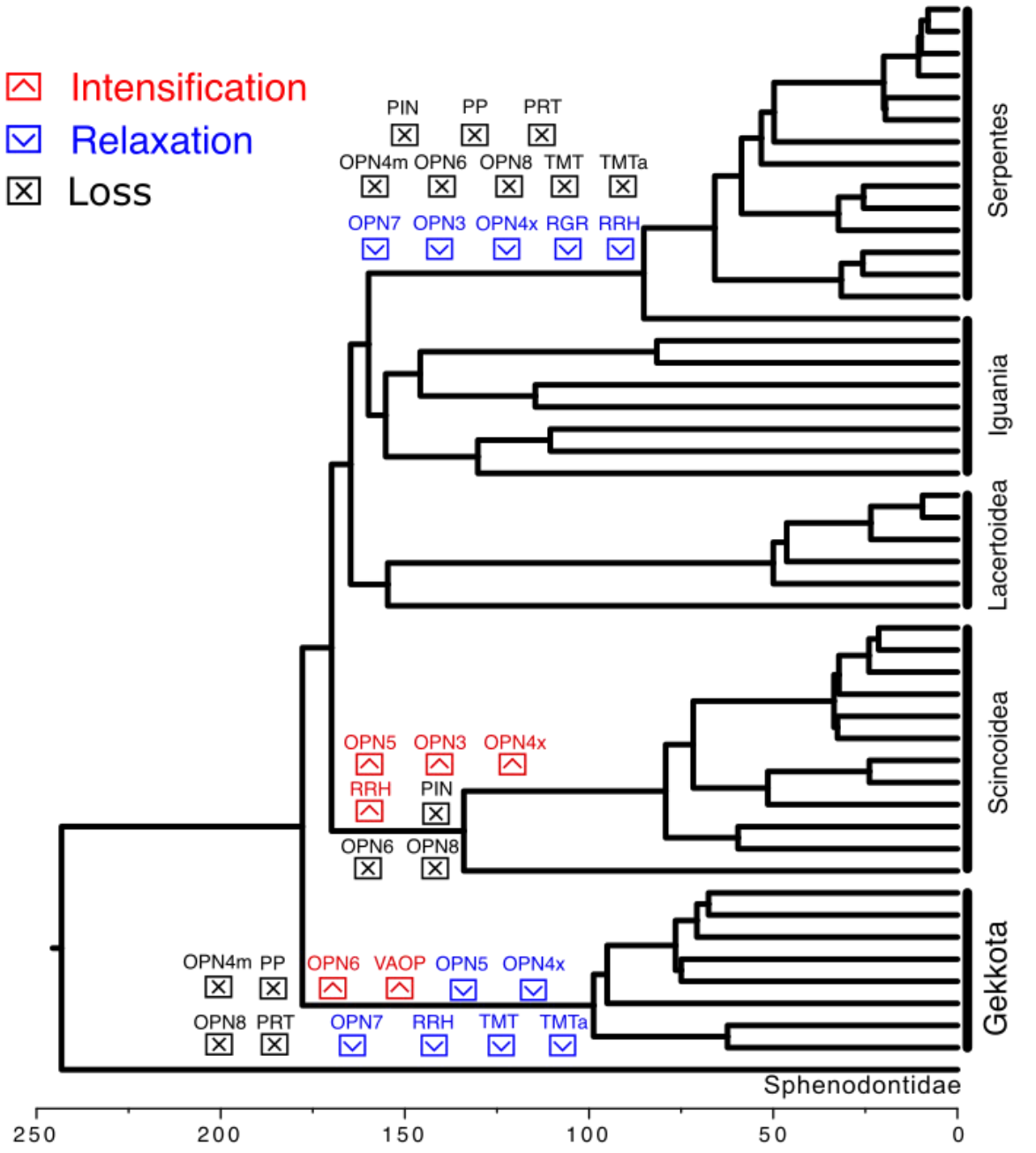

Figure 4: Selection on clades of Squamata, showing intensification and relaxation of selection, according to RELAX analyses.

Skinks

The LRT statistic of tests 3 and 4, with skinks and non snake and gecko squamate foreground branches respectively, of the branch and clade analyses (SI Tables 4.2, 4.4) shows that the geckos + skinks + snakes vs other squamates partition explains more variation in the data than the geckos, snakes and other squamates partition (SI Tables 4.2, 4.4). This is true in OPN7, OPN4x, OPN3, TMT and RGR but not in OPN5 and RRH for both analyses. However in both cases, both test 3 and 4 were significantly better fit than the null model. Thus we can see that separation of skinks from other lepidosaurs is justified. Branch tests show that RRH in skinks is more constrained than snakes and geckos, and even slightly more

than other lepidosaurs. Constraint is also higher in skink RGR than in snakes and geckos, and they have higher constraint than is average across lepidosaurs. In CmC tests between clades, site specific differential selection within clades is widespread, an expected result considering the diverse nature of each of these clades. For skinks, CmC results largely agree with RELAX results and show similarity with nocturnal results, with consistently higher levels of site specific constraint than M2a_rel across OPN5, RGR, RRH, TMT, most NVOs. RELAX results are the most surprising, with skinks showing high constraint on OPN5, OPN3, OPN4x and RRH. High constraint on OPN5 was also shared by nocturnal squamates.

Snakes

The partitioning of Toxicofera versus other lepidosaurs recovered fewer significant differences than the (paraphyletic) partition of snakes versus lizards, and where results were significant the 2Δ (ln L) statistic was consistently lower in the Toxicofera analysis compared to the snakes vs lizards analysis, thus the snakes versus lizards analysis is consistently a better fit to data than the Toxicofera versus other lepidosaur analysis. Branch tests show significant relaxation of selection in snake NVOs: OPN4x, OPN5, OPN7, OPN3, RRH, and RGR. Snakes show site specific relaxation of selection in most NVOs, apart from RGR where they have positive divergent selection according to CmC and branch site tests. Branch-site tests also found various selected sites in snake OPN4x and RGR (SI Table 4.3). The significance of these sites are unknown. According to RELAX testing, snakes show significant relaxation compared to other lepidosaurs in OPN7, OPN3, OPN4x, RGR and RRH.

Geckos

Branch results in geckos show relaxed constraint compared to all lepidosaurs in OPN4, RRH, PIN, RGR and both TMTs. Geckos are more constrained than the average lepidosaur in OPN4x, OPN5 and OPN6 (however there is only one partial sequence here). In CmC tests, geckos have more site specific selection than lepidosaurs on average in OPN7, PIN, RRH and both TMTs, but more site specific constraint on OPN5, OPN6 (see above), OPN3, RGR. Branch-site tests show that no sites in geckos are positively selected for compared to other lepidosaurs. RELAX analyses reveal that geckos show relaxation of selection in OPN5, OPN7, OPN4x, RRH and TMTs, with relaxation significantly higher in OPN5, OPN4x and TMTa than other clades. Geckos also show intensification of selection in OPN6 and VAOP.

Discussion

Compared with the visual opsins that mediate image formation, the eco-physiological role and evolution of non-visual opsins are poorly understood. This is particularly the case in squamates, where in a previous study (Chapter 3, this thesis) we found broadscale losses of NVOs in geckos and snakes, and parallel losses of six non-visual opsins in skinks. However, the selective pressures driving the losses of non-visual opsins of squamates are still unknown, including whether transitions to low-light environments such as nocturnality and fossoriality are accompanied by relaxation of selection on these genes. To answer this, we assembled the most comprehensive dataset of non-visual opsins in squamates so far and analysed this under

branch, site, branch-site and clade models. Our site model tests, which analysed the NVOs across their codon structure, suggested that of the many significant amino acid sites that affect function of the opsins, none show consistent positive selection all across Squamata. This perhaps indicates selection or relaxation over the whole gene rather than tuning of specific sites, or that site selection occurs only in specific clades. Our branch and branch-site model analysis, however, revealed contrasting selection pressures among several phylogenetic and ecological divisions. These results are discussed below.

Contrasting selection pressures on NVOs during evolutionary transitions in squamates

Ten out of fifteen NVO genes showed significant variation in selection pressures among major phylogenetic and ecological divisions of squamates (SI, Tables 4.2, 4.2, 4.4). In particular, the branch models with the primarily nocturnal versus primarily diurnal/crepuscular partition reveal higher constraint on OPN4x, OPN6 and PIN in nocturnal species, with a more relaxed functional constraint on OPN7, OPN3, RRH, RGR, TMT and TMTa in these species. A relaxation of functional constraint on these non-visual opsins in nocturnal species fits with prior expectations, as many nocturnal clades of have lost TMT and TMTa, and lower constraint on nocturnal species has previously been noted in RGR and RRH in mammals (Upton et al, 2021). Following the patterm reported in mammals (Upton et al 2021), we suggest that primarily diurnal squamates, being reliant on visual cues, keep photoisomerases under higher constraint on order to ensure the recovery of 11-cis-retinal for visual opsins (Bailey and Cassone, 2004; Nagata et al, 2010; Mordeshian et al, 2019).

For geckos, three non-visual (OPN7, OPN4x and RRH) opsins appear to have a similar fate to snakes where relaxation appears to act in a similar way. Two of those NVO's (OPN4x and RRH) appear to be relaxed in skinks as well. This suggests that these species converged in their relaxative pressures in their non-visual opsin system. Interestingly, RRH appears to be also relaxed across nocturnal mammals (Upton et al, 2021) suggesting further convergence across vertebrate lineages. This makes sense as RRH is a photoisomerase whose primary function is to convert all-trans retinal to 11-cis retinal (Koyanagi et al, 2002; Nagata et al, 2010). Nocturnal species may have relaxed selection on their visual system, and so the activity of visual opsins doesn't exert as large a selective pressure in nocturnal species as it does in diurnal species. Relaxed selection on RRH has been found in nocturnal clades of mammals (Upton et al, 2021). OPN4 has been linked to numerous functions, including light controlled skin pigmentation (Bertolesi and McFarlane, 2017), circadian rhythm mediation (Nakamura, 1999; Hankins et al, 2008; Peirson et al, 2009; Guido et al, 2020) and pupillary light reflex (Lucas et al, 2001; Lucas et al, 2003), and was found to have high sequence diversity in mammals (Upton et al, 2012), paralleled here. The reason for this however is as yet unknown.

Neuropsin genes appear to be the only squamate NVOs where the DRY motif is commonly altered. A common feature among the nocturnally adapted geckos, snakes and mammals seems to be the losses of OPN6 and OPN8 (Beaudry et al, 2017, Emerling, 2017b; Schott et al, 2017; See Chapter 2, Fig. 2, this thesis). Little is known of the functions of these two genes; however, in birds OPN8 is known to be primarily expressed in the adrenal glands, and may have a chemosensory function (Ohuchi et al, 2012). It was postulated that the opsin may be able to activate the G protein cascade even in the absence of light, as well as by light

irradiation (Ohuchi et al, 2012). However, the apparent relaxation of selection pressure and loss of this gene in all clades suspected of a nocturnal or even mesopic bottleneck leads us to suggest that the primary function of OPN8 involves photosensitivity. OPN8 on the other hand is a light inactivated opsin, only binding to all-trans retinal (Sato et al, 2018), and as reported previously in the chicken, we can confirm a cysteine residue at site 188 that allows the opsin to form a stabilised resting inactive state induced by light, the opposite of visual opsins (Sato et al, 2018). In view of this, lack of functional value during the dim-light bottleneck of snakes and geckos provides a highly plausible explanation for the loss of this particular opsin in these taxa.

Our results show that, in all squamates, OPN6 and OPN8 seem to have lost the DRY/ionic lock region entirely, while OPN7 seems to have undergone a D134T (numbering based on *Bos taurus* RH1) mutation. This replacement of a negatively charged amino acid with a neutral one at this site may disrupt activation of the opsin, prohibiting signalling, as with mammal RGR (Vogel et al, 2008; Upton et al, 2021). Previously in OPN8, an IRF motif has been found in birds, yet the opsin still functions as a signalling protein (Ohuchi et al 2012). We have found that this motif is shared in all reptiles that possess OPN6, with only a few exceptions. Therefore we must surmise that it is a signalling protein in squamates too, despite having lost the motif that is thought to be crucial to signalling potential (Yamashita et al, 2000).

Two additional results should be treated with caution due to the low number of lineages included in the nocturnal partition. Firstly in OPN6, only three lineages were included in the nocturnal partition - close relatives *Eublepharis macularius* and *Gekko japonicus*, plus *Sphenodon punctatus*, which is only generally nocturnal, and can also be day active (Saint Girons et al, 1980). Hence, the finding of selection on OPN6 in nocturnal species could be due to the low number of nocturnal species to have retained this gene (three), and their close phylogenetic relationships (*Eublepharis macularius* and *Gekko japonicus* in particular). *Sphenodon punctatus* is also only generally nocturnal, and can also be day active (Saint Girons et al, 1980) and thus there may be evolutionary benefit to the retention of this gene in this species, even if there is little evidence of its presence in other nocturnal species.

The finding of lower selective constraints on RGR and RRH in nocturnal squamates suggests convergent evolution with mammals (Upton et al, 2021), which also shows a relaxation of these genes that has been linked to nocturnality (Upton et al, 2021). These genes have been retained in squamates (Chapter 3, this study) as well as eutherians and monotremes (but not marsupials: Upton et al, 2021). However, Upton (2021) notes that due to mutations in the ionic lock region of RGR, this gene may no longer function as a signalling GPCR. In squamate RGR, the DRY motif is conserved in all studied species with the exception of *Anolis carolinensis* and *Sceloporus tristichus* (two closely related iguanids), and *Hemidactylus turcicus* and *Correlophus ciliatus* (distantly related geckos). Given that these four taxa are not closest relatives (based on molecular phylogenies: Pyron et al, 2013), it appears that they have all convergently gained the same DRH mutation from an ancestral DRY motif. This DRH motif is known outside of opsins in V2 vasotocin receptors (Rawat et al, 2015). Mutation of the tyrosine in the DRY motif often has no or little effect on receptor

function (Rovati et al, 2007). However in most squamates, the DRY motif is conserved, indicating that they still have the potential to signal with retinal (Vogel et al, 2008).

Lacertids and anguimorphs, placed within the same partition in our analysis, generally do not show higher functional constraint on OPN4m than other clades. Yet, within the group there is evidence of site specific selection (Fig. 2). This may reflect the diversity within this paraphyletic partition, as this is a grouping of ecologically highly diverse clades, and selection tests conducted on the NVOs of Lacertoidea separately from Anguimorpha indicate that they have higher constraint than other lepidosaurs (SI Table 4.4).

OPN4m is the only gene found to have undergone positive selection in the primarily diurnal versus nocturnal partition division. However, as with OPN6, we have low confidence in this result because some squamate lineages such as skinks, lacertids and iguanids have a very small number of nocturnal species. *Anomalopus brevicollis* is the only low-light sampled Scincomorpha present in the OPN4m analysis. This sequence is only partial, and the only positive selected site is the last one captured. Which results in this finding being regarded with some lack of confidence.

Also notable is the lack of seemingly any significant selection between clades or within Squamata on VAOP. This may be seen as surprising, as skinks show evidence of losses of VAOP, with only a partial sequence recovered in the *Tiliqua rugosa* genome (See Chapter 3, this thesis), and this gene is lost in the nocturnally adapted mammals (Gerkema et al, 2013). Snakes and geckos, however, seem to have retained VAOP (Schott et al, 2017; See Chapter 3, this thesis), so perhaps the loss of this gene is not explained by nocturnal bottlenecks. VAOP has been reported to be strongly expressed in the hypothalamus of birds, and is suspected to allow photoperiod controlled reproductive changes (Davies et al, 2012). The reproductive timing of skinks has been associated with exposure to solar radiation (Clerke and Alford, 1993), although some skinks are also capable of year round reproduction (Wilhoft, 1963) so perhaps selective pressures on photoperiod detection maintain this gene in skinks, and the inability of our study to find a full skink VAOP sequence is not indicative of loss in the clade. Further research is needed to confirm its loss or presence.

It must also be noted that in skinks, PP, PRT, TMT are all partial sequences. However the relaxed selection normally observed in pseudogenes is not observed in these fragmented genes, suggesting that these might still be functional. In fact many of these genes thought to be lost appear to have vastly increased constraint on skinks, according to RELAX tests (see Fig. 1), adding to evidence of their retention. These genes were not found to have any premature stop codons or missense mutations, and in previous work (See Chapter 3, this thesis), other methods recovered gene fragments, which together with those from gene captures combine to give a full sequence for TMT. Though conclusive evidence is still needed, we heavily suspect that PP, PRT and TMT are intact genes in Scincomorpha.

Implications of NVO evolution for understanding the ancestral ecology of skinks

A key aim of this study was to shed light on the visual ecology of ancestral skinks by comparing selection pressures on the NVO genes of this lineage to those of other squamates. We found clear among-clade differences in selection pressures on NVOs. Groups that have undergone convergent gene losses associated with dim-light bottlenecks, i.e. geckos and snakes, show signals relaxation of selection on NVOs that clearly contrast the signals of

conservation and intensification seen in the VNOs of ancestrally diurnal lacertids and anguimorphs. These results are consistent with expectations that scotopic bottlenecks have reduced the functional importance of all or most NVOs.

In skinks, however, our results paint a surprising picture. While skinks show convergent NVO losses with snakes and geckos, they show intensification of positive selection on remaining NVOs (in contrast to the further convergence in relaxed selection pressures on snake and gecko NVOs) (Figs. 1, 2 and 3; SI Tables 4.2, 4.3, and 4.4). Similarly in CmC tests, skinks showed higher levels of site specific functional constraint than geckos or snakes (Fig. 2). This is also backed up by branch site results, with the only genes with selected sites in skinks being the same genes that CmC found the highest amount of variation in, OPN4x and OPN4m. Yet their gene losses show many parallels with snakes and geckos (Chapter 3, this thesis). From this we can infer that the NVOs of skinks have undergone divergent selection histories with respect to not only geckos and snakes, but the rest of Squamata.

Further indication of differences in the evolution of non-visual photoreception in skinks is their pattern of losses and retention. Skinks have retained a pineal gland and parietal eye, yet seem to have lost two photopigments that are associated with these organs (pinopsin in the pineal gland, and parietopsin in the parietal eye). A known function of the parietal eye in skinks involves sensitivity to polarised light to facilitate homing behaviours. Freake (1999; 2001) showed that many skink species, including *Tiliqua rugosa*, maintain the ability to detect the polarisation of light via the the parietal eye; covering this organ in *Tiliqua*, resulted in impairment of homing activity, indicating that visual cues are used for orientation in the outward journey (and for reverse orientation during the return journey) (Freake 2001). Skinks are known to regulate melatonin using the parietal eye (Firth and Kennaway, 1980). Behavioural studies that removed parietal organs in *Sceloporus* showed an impairment of thermoregulatory behaviours in these taxa, but the effect here seems to be not as great as with the removal of the lateral eye in these species (Bethea and Walker, 1978), providing evidence of a thermic regulatory role for the parietal eye. Parietalectomised *Anolis* lizards, on the other hand, showed no effect to their pineal melatonin rhythm, and there was no effect even when the lateral eye was removed (Underwood and Calaban, 1987).

There is evidently great variation among lizards in the mode of melatonin regulation. Perhaps the circadian rhythm functions normally performed by pinopsin in the pineal gland in lacertids and other lizards that have maintained pinopsin are performed by parapinopsin or parietopsin in the parietal eye in skinks. Further, if the ancestral skink regulated melatonin with a combination of parietal eye opsins and pinopsin, as we infer to be the case in *Sceloporus*, the mesopic bottleneck we suspect skinks experienced may have relaxed selection on pinopsin, while the role in homing behaviours maintained selection on the parietal eye and its opsins, allowing pinopsin to be inactivated and PRT and PP to become the primary regulators of melatonin.

Both OPN4 genes appear to have both high selective pressures in skinks, compared to other clades, which contrasts with the findings of Hauzman et al (2019) in snakes, which had relatively lower constraint compared to other vertebrate groups. Skinks show remarkably higher levels of selective constraint on both OPN4 genes and other opsins, than all other squamate groups, and the implications of this are as yet unclear.

Behavioural studies using a broader phylogenetic sampling of skinks will ultimately be needed to resolve the functional significance of the parietal eye (and pineal organ) in these lizards. Gene expression studies are also needed to understand the genomic basis of photoreception in the extraocular photoreceptor organs of skinks. Given that genes associated with the parietal-associated genes such as PIN and VAOP have been lost in skinks (Chapter 2, this thesis), genes may have been co-opted from other photoreceptor pathways to serve a novel function in the skink pineal gland.

Conclusions

We tentatively suggest that our results provide new evidence of widespread secondarily evolved diurnality from a low light bottleneck (Slavenko et al, 2022) at the origin of skinks. From the retention of a greater number of non-visual opsins in Scincomorpha, and the less conclusive morphological evidence from the lateral eye and retention of a functional parietal eye (Freake, 1999; 2001), we can infer that skinks underwent a different, potentially milder form of low light bottleneck than in other clades such as snakes, geckos and mammals. This study suggests skinks have enhanced functional constraint on many non-visual opsins, in contrast with findings in snakes, geckos and mammals, which we may interpret as reapplication of selection on retained non-visual opsins upon the emergence of skinks from their mesopic bottleneck to their current, primarily diurnal state.

Supplementary Materials

Table 4.1: Site CodeML results

Table 4.2. Branch CodeML results

Table 4.3. Branch-site CodeML results

Table 4.4. Clade CodeML results

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Chapter 5: Discussion

Thesis Overview

Scinciformata are a conspicuously understudied clade in terms of visual evolution. Many studies have been conducted into the visual opsin complement of geckos, snakes, crocodiles, testudines, and mammals, but these have included only one skink (Simões et al, 2015). The absorption spectra of the *Platysaurus broadleyi* (Cordylidae) i(Fleishman et al, 2011) is the only member of Scinciformata studied in that regard. There exist few studies into the ocular and retinal morphology of skinks (Röll, 2001; New et al, 2012; Canei et al, 2020; Zhao et al, 2020; Fleming, 2022). The comparative lack of attention this clade has previously attracted is surprising, as skinks are the most species-rich lineage in Squamata, occupying a diverse range of habitats, activity habits, mating and pairing behaviours. This thesis has attempted to address this comparative lack of research in the clade and examine the visual and non-visual opsins of skinks and compare them to the opsins known to be retained in Squamata (Beaudry et al, 2017), as well as previously described losses in snakes and geckos (Emerling, 2017; Schott et al, 2017). I investigated both one particular clade of Scincidae (*Lerista*) for evidence of visual evolution due to fossorial adaptation, and Scinciformata as a whole with respect to the rest of Squamata.

In this final chapter, I will summarise the findings of the previous chapters and their implications for understanding the visual evolution of the clade of Scinciformata, for Squamata as a whole, and visual evolution as an area of research. I will outline future directions of research to improve resolution on losses and retention in the various clades of Squamata. Once these losses are defined, reasons why and inferences around the effect on circadian rhythm, thermoregulation, and homing behaviours can be made.

Visual Opsins and Fossorial Adaptation

In chapter 2 of this thesis I investigated visual opsin evolution in *Lerista*, a clade of scincid lizards endemic to Australia, that show a spectrum of fossorial adaptation across species. I conducted gene captures on 86 members of *Lerista* and their sister clade *Ctenotus*. This revealed that they maintain all 5 ancestral visual opsins. Selection tests on each opsin uncovered relaxed selection on LWS in fossorially adapted species of *Lerista*. We also uncovered a potential site change in RH1 which could have implications for the stability and therefore sensitivity, spectral tuning and retinal reset ability of the opsin. We surmised that though there may be substantial physical adaptation to a fossorial lifestyle in some *Lerista* species, colour vision maintains selective advantage. All species of *Lerista* maintain surface activity, no matter how fossorially adapted they may be, and therefore there may be substantive selective pressure to maintain colour vision and therefore visual opsins. The reduction in eye size and other regressive phenotypes we see in the more fossorial *Lerista*

may not alter their ability to perceive colour, only their focal distance, which as a small skink moving primarily through sand, leaf litter, and interstitial spaces, may not be of much concern. We did however find that selection on LWS was reduced in species with digit and limb loss. The retention of LWS could also be due to the relatively short length of time *Lerista* have been fossorial, which may be not enough time for loss of gene function due to relaxation of selection (See Chapter 2, this thesis). The lack of gene loss in *Lerista* shows that adaptations to ecological impeta are not uniform, there is much diversity and studies into adaptation should be taken on a clade by clade basis. Though snakes, geckos, many clades of mammals, even individual cichlid species have all lost opsins in response to low light environments, *Lerista* have taken a different path. This highlights that every clade undergoing a particular adaptive pressure may not reply to it in the same way, and that combinations of selection pressures will be exactly the same for any two species. Not many examples of loss of the LWS gene exist in the animal kingdom (Jacobs et al, 2013; Meredith et al, 2013), and from these previous losses, there are no obvious parallels with *Lerista* that would inform us of the reasons for this relaxation.

Lerista is a good candidate clade for investigation of fossorial adaptation, but by no means uniquely so. Many other skink clades also have fossorially adapted species; for example, genera such as *Anomalopus* and *Chalcides* contain species that show fossorial adaptation of different extents within the clade (Caputo et al, 1995; Hutchinson et al, 2021). Genera such as *Feylinia* and *Acontias* are also fully limbless and highly fossorially adapted (Whiting et al, 2003; see also Camaiti et al, 2022). These clades would be excellent candidates for further investigation of potential relaxation on LWS in other fossorial clades of skink. It is possible that clades with older transitions to fossoriality than *Lerista* or ones that are all fully limbless and fossorial have had longer time to adapt and undergo changes due to relaxation of selection. It is known from Simões et al (2015) that *Feylinia* have retained all visual opsins, but this study did not elaborate on selection. We may find here that genera such as *Feylinia* are more visually adapted than they first appear. Microspectrophotometry and mutagenesis experiments in skinks and investigations into the sensitivity of the RH1 gene may give further insight into the alteration of the zinc binding site and its potential impact on RH1, as well as the selected sites we uncovered whose effects on spectral tuning are currently unknown.

Retentions and Losses of Non-Visual Opsins in Squamata

Squamates are perhaps some of the most eco-physiologically diverse vertebrates (Vitt et al, 2003). Already examples from sex chromosome evolution and sexual determination (Mezzasalma et al, 2021), ovi- and viviparity (Blackburn, 2006) and limb losses (Camaiti et al, 2021). Other clades like mammals and birds are much more uniform in life traits but squamates show remarkable diversity. This makes squamates a prime clade for understanding the effects of these various ecological and physiological transitions on visual evolution. Throughout Squamata we see multiple apparent nocturnal bottlenecks, loss of visual genes (Emerling, 2017; Schott et al, 2017), and as I have uncovered, loss of NVOs. We confirmed many losses that were previously described in other studies (See Chapter 3, this thesis). We

also fulfilled our prediction of the loss of parietal associated non-visual opsins in the tegu, a species that has lost the parietal eye. Previous investigation into the visual opsins of a gymnopthalmid, *Bachia flavescens*, revealed the loss of RH2 (Simões et al, 2015).

Finally, previously unknown loss of pinopsin in the agamid *Pogona vitticeps* was described (See Chapter 3, this thesis). As the pinealocytes of the agamid *Uromastyx* have previously been described as regressive, and loss has been found in its close relative *Pogona vitticeps*, as well as *Chamaeleo calyptratus*, and Chamaeleonidae is a sister group to Agamidae, this may be seen as a confirmation of loss of pineal photoreception in an agamid, and reveals the potential for loss across Acrodonta.

Multiple losses in diverse clades with very different habits calls into question some previous explanations of why such losses occur, for example loss of parietal eye in turtles due to burrowing (Lyson et al, 2016). Within Scinciformata, there are many examples of burrowing behaviour, many more obligate and frequent than exhibited by any turtle, yet all skinks maintain the parietal eye (Gundy and Wurst, 1976). In mammals and archosaurs thermoregulation and a transition to endothermic lifestyle has been forwarded as an explanation of the loss of the parietal eye (Emerling, 2017), yet snakes and geckos still ectothermal and have lost it too. Some of these explanations may be true but again as with *Lerista*, need to look on a clade by clade basis, each clade may at first look to have a similar impetus for gene losses, but time and again they have different reasons for apparent convergent adaptations or losses. We found intact TMTa in the pygopod *Lialis burtonis*. Previously, retention of TMTa in Sphaerodactylidae, and the *Gekko* genus, was also described (Schott et al, 2017). Functions of TMTa are mostly unknown as of yet, so the reason for this curious pattern of retention cannot be speculated on.

Unfortunately only one member of Gymnopthalmoidea was included in this study, so there is no indication of whether the loss of parietal genes is restricted to *Salvator merianae*, Teiidae or all of Gymnopthalmoidea (Alopoglossidae and Gymnopthalmidae). Full genomes are not yet available for any member of Gymnopthalmoidea other than *S. merianae*, therefore the gene capture techniques first set out by Schott et al (2017) and used throughout this thesis (See Chapters 2, 3 and 4, this thesis), could be used to assess the non-visual opsin complement across this clade, and give further evidence to the loss of parietal genes. So too should losses of pinopsin in agamids and chameleons be investigated. For this transcriptome data from the pineal gland and retina of agamids and chameleons could be investigated, and see if pinopsin is present in the pineal, as in *Uta stansburiana*, *Anolis* and others, in the retina, as in the diurnal gecko *Phelsuma laticauda*, or not at all. These studies could also include dating analysis to discover whether the loss of PIN in Gymnopthalmoidea and Acrodonta, along with other clades such as Scincomorpha and Ophidia, occurred at similar times, and could be attributed the same event, for example climatic conditions of the era, or reveal separate loss events, perhaps for different reasons in each clade. Gecko TMTa retention should also be investigated, it is a very curious pattern of retention. Greater sampling of geckos would uncover precise divisions in losses and retention, and perhaps uncover reasons why.

Novel losses of NVOs in Scincomorpha

This thesis looked at losses of NVO's losses under a phylogenetic framework in Scincomorpha and analysed the selection pressures acting on NVOs. Has been done before in geckos, snakes, crocs, mammals, turtles (Emerling, 2017a&b; Upton et al, 2021), but many squamate clades have previously been ignored. Our research uncovered six potential losses in the clade Scincoidea; OPN6, OPN8, PIN, PRT, TMTa and VAOP. In particular, no sequence, not even a partial sequence, was recovered in OPN6, OPN8 or PIN, giving significant support for the loss of these opsins. There has been some previous notion of mesopic bottlenecks in skinks (Slavenko et al, 2022), and this thesis adds considerable evidence to this hypothesis by highlighting the convergent losses of non-visual opsins in skinks with snakes, geckos and mammals (Gerkema et al, 2013; Schott et al, 2017), though in skinks the losses are not as extensive (See Chapter 3, Fig. 2). We also examined the selection pressures on the genes present and investigated any selective differences between or within clades in Lepidosauria. We found many similarities between geckos and snakes, with generally lower constraint on opsins, but interestingly we found high levels of constraint on the same opsins in skinks, often even greater than in lacertids and iguanids, that have retained function in all non-visual opsins. We hypothesise that this is due to a mesopic bottleneck, with early skinks having undergone either a cathemeral or crepuscular ancestral period. This would explain the loss of some opsins, such as OPN6 and OPN8 while retaining others that more nocturnally adapted clades have lost, such as PP and PRT. Many skinks exhibit burrowing behaviours, which has been suggested as a factor in the loss of the parietal eye in turtles (Lyson et al, 2016). The skull morphology of *Tiliqua rugosa* makes it clear that this animal has gone to great lengths to conserve the function of this organ however, with an obvious parietal foramen that penetrates a thick skull and tough scales that coat it. Seems like there is strong selection to maintain the parietal in skinks, even though we could not recover full sequences of parietopsin or parapinopsin in any skink using gene capture, and therefore could not assess selection.

Oddest of all perhaps is the apparent loss of pineal photoreception in skinks. All signs point to the loss of pinopsin, and in *Tiliqua rugosa*, the only skink examined thus far, pineal photoreceptors show a regressed morphology similar to that of *Uromastyx*, and snakes (See Chapter 4, this thesis). We also see in *Tiliqua rugosa* that the parietal eye, and by inference the opsins parapinopsin and parietopsin expressed within, can fulfil the function of regulation of melanopsin (Firth and Kennaway, 1980) that is normally fulfilled by native pineal photoreception with pinopsin (Csernus et al, 1999). This is in contrast to other clades of lizard who do not depend on the parietal eye for melatonin regulation (Bethea and Walker, 1978; Underwood and Calaban, 1987). Geckos have retained pinopsin across the clade, and do express it in the retina (Taniguchi et al, 2001), and have lost the parietal eye (Gundy and Wurst, 1976), so presumably they still maintain pinopsin as the melatonin controlling photoreceptor. Therefore there could be a novel mode of melatonin regulation in skinks.

The presence of parapinopsin and parietopsin in the parietal eye of skinks needs to be verified with transcriptome evidence or immunohisochemistry. Transcriptome data would also allow selection tests, with which we could compare the strength of constraint on these opsins to constraint in lacertids and iguanids, and other clades where the parietal eye and its

genes are conserved. Parapinopsin and parietopsin are thought to give the parietal eye UV and green wavelength sensitivity, and the eye has been linked to detection of polarised light, allowing lizards to navigate and exhibit homing behaviour using the third eye. However no test has examined the relevance of each gene to this function, and how the detection of the e vector of polarised light is performed, and how this is used by the skink as a cue to orient itself. Therefore, an examination of the morphology and behaviour of the parietal eye, including the transmission of light through the parietal scale, lens and cornea, with special attention to the polarisation of transmitted light would begin to draw links between function and opsins. Also, experiments into the function of the parietal eye in skinks on melatonin regulation and circadian rhythm should now be re-examined, since most of the research in this area was conducted in a time before knowledge of opsins and the molecular mechanisms underlying photoentrainment and circadian rhythm control. New experiments into the excitation of parapinopsin and parietopsin in the parietal eye, and the effect this has on melatonin and circadian rhythm could give us much greater understanding of this seemingly unique mode of regulation in skinks.

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Supplementary Material

Supplementary material for Chapter 2

Table 2.1. List of specimens with SA Museum ABTC numbers, NCBI numbers, type of sequence and presence of each visual opsin

Species	ABTC Number	NCBI number	Type of Sequence	LWS	RH1	RH2	SWS1	SWS2
Chalcides ocellatus	NHM_E36		Gene captures	✓	✓	✓	✓	✓
Corucia zebrata	50360		Gene captures	✓	✓	✓	✓	✓
Ctenotus pantherinus	35092		Gene captures	✓	✓	✓	✓	✓
Ctenotus spaldingi	57114		Gene captures	✓	✓	✓	✓	✓
Cyclodomorphus gerrardii	11496		Gene captures	✓	✓	✓	✓	✓
Egernia striolata	96398		Gene captures	✓	✓	✓	✓	✓
Emoia acrocostata	50439		Gene captures	✓	✓	✓	✓	✓
Eugongylus rufescens	98675		Gene captures	✓	✓	✓	✓	✓
Eulamprus quoyii	85427		Gene captures	✓	✓	✓	✓	✓
Lampropholis guichenoti	23334		Gene captures	✓	✓	✓	✓	✓
Lerista arenicola	40786		Gene captures	✓	✓	✓	✓	✓
Lerista bougainvillii	94931		Gene captures	✓	✓	✓	✓	✓
Lerista desertorum	94330		Gene captures	✓	✓	✓	✓	✓
Lerista dorsalis	106178		Gene captures	✓	✓	✓	✓	✓
Lerista edwardsae	139775		Gene captures	✓	✓	✓	✓	✓
Lerista ips	91595		Gene captures	✓	✓	✓	✓	✓
Lerista timida	39962		Gene captures	✓	✓	✓	✓	✓
Liopholis inornata	100983		Gene captures	✓	✓	✓	✓	✓
Liopholis whitii	68829		Gene captures	✓	✓	✓	✓	✓

Liopholis striata	91720	Gene captures	✓	✓	✓	✓	✓
Ophioscincus ophioscincus	32202	Gene captures	✓	✓	✓	✓	✓
Plestiodon fasciatus	12334	Gene captures		✓	✓	✓	✓
Pseudemoia entrecasteauxii	23134	Gene captures	✓	✓	✓	✓	✓
Saiphos equalis	14195	Gene captures	✓	✓	✓	✓	✓
Saproscincus challengeri	11015	Gene captures	✓	✓	✓	✓	✓
Silvascincus silvascincus	12360	Gene captures	✓	✓	✓	✓	✓
Sphenodon puncatatus	32244	Gene captures	✓	✓	✓	✓	✓
Tiliqua scincoides	57724	Gene captures	✓	✓	✓	✓	✓
Anomalopus brevicollis	10849	Gene captures	✓	✓	✓	✓	✓
Anomalopus gowi	10861	Gene captures	✓	✓	✓	✓	✓
Anomalopus leuckartii	53604	Gene captures	✓	✓	✓	✓	✓
Anomalopus pluto	10961	Gene captures	✓	✓	✓	✓	✓
Anomalopus swansoni	6970	Gene captures	✓	✓	✓	✓	✓
Anomalopus verreauxii	138983	Gene captures	✓	✓	✓	✓	✓
Ctenotus agrestis	113815	Gene captures	✓	✓	✓	✓	✓
Ctenotus allotropis	8980	Gene captures	✓	✓	✓	✓	✓
Ctenotus arcanus	137937	Gene captures	✓	✓	✓	✓	✓
Ctenotus ariadnae	706	Gene captures	✓	✓	✓	✓	✓
Ctenotus atlas	10488	Gene captures	✓	✓	✓	✓	✓
Ctenotus brevipes	72811	Gene captures	✓	✓	✓	✓	✓
Ctenotus brooksi	133010	Gene captures	✓	✓	✓	✓	✓
Ctenotus burbidgei	105767	Gene captures	✓	✓	✓	✓	✓
Ctenotus capricorni	105818	Gene captures	✓	✓	✓	✓	✓
Ctenotus coggeri	30198	Gene captures	✓	✓	✓	✓	✓

Ctenotus decaneurus	29731	Gene captures	✔	✔	✔	✔	✔
Ctenotus dux	91591	Gene captures	✔	✔	✔	✔	✔
Ctenotus essingtonii	29141	Gene captures	✔	✔	✔	✔	✔
Ctenotus euclae	118396	Gene captures	✔	✔	✔	✔	✔
Ctenotus eutaenius	77199	Gene captures	✔	✔	✔	✔	✔
Ctenotus gagudju	29095	Gene captures	✔	✔	✔	✔	✔
Ctenotus gemmula	62119	Gene captures	✔	✔	✔	✔	✔
Ctenotus grandis	14303	Gene captures	✔	✔	✔	✔	✔
Ctenotus greeri	91836	Gene captures	✔	✔	✔	✔	✔
Ctenotus hebetior	9065	Gene captures	✔	✔	✔	✔	✔
Ctenotus impar	53530	Gene captures	✔	✔	✔	✔	✔
Ctenotus ingrami	32120	Gene captures	✔	✔	✔	✔	✔
Ctenotus joanae	56695	Gene captures	✔	✔	✔	✔	✔
Ctenotus labillardieri	58013	Gene captures	✔	✔	✔	✔	✔
Ctenotus lancelini	63038	Gene captures	✔	✔	✔	✔	✔
Ctenotus leonhardii	10011	Gene captures	✔	✔	✔	✔	✔
Ctenotus militaris	29855	Gene captures	✔	✔	✔	✔	✔
Ctenotus mimetes	82597	Gene captures	✔	✔	✔	✔	✔
Ctenotus monticola	105816	Gene captures	✔	✔	✔	✔	✔
Ctenotus olympicus	35032	Gene captures	✔	✔	✔	✔	✔
Ctenotus orientalis	119442	Gene captures	✔	✔	✔	✔	✔
Ctenotus pantherinus	137975	Gene captures	✔	✔	✔	✔	✔
Ctenotus piankai	23830	Gene captures	✔	✔	✔	✔	✔
Ctenotus quinkan	105817	Gene captures	✔	✔	✔	✔	✔
Ctenotus regius	137945	Gene captures	✔	✔	✔	✔	✔
Ctenotus rimacolus	30342	Gene captures	✔	✔	✔	✔	✔

Ctenotus rubicundus	128170	Gene captures	✓	✓	✓	✓	✓
Ctenotus septenarius	88059	Gene captures	✓	✓	✓	✓	✓
Ctenotus spaldingi	70692	Gene captures	✓	✓	✓	✓	✓
Ctenotus strauchii	9042	Gene captures	✓	✓	✓	✓	✓
Ctenotus striaticeps	30403	Gene captures	✓	✓	✓	✓	✓
Ctenotus taeniatus	37907	Gene captures	✓	✓	✓	✓	✓
Ctenotus taeniolatus	16644	Gene captures	✓	✓	✓	✓	✓
Ctenotus vertebralis	28180	Gene captures	✓	✓	✓	✓	✓
Lampropholis delicata	93599	Gene captures	✓	✓	✓	✓	✓
Lerista aericeps	73312	Gene captures	✓	✓	✓	✓	✓
Lerista ameles	77171	Gene captures	✓	✓	✓	✓	✓
Lerista arenicola	140003	Gene captures	✓	✓	✓	✓	✓
Lerista axillaris	63826	Gene captures	✓	✓	✓	✓	✓
Lerista baynesi	133331	Gene captures	✓	✓	✓	✓	✓
Lerista bipes	60733	Gene captures	✓	✓	✓	✓	✓
Lerista borealis	63741	Gene captures	✓	✓	✓	✓	✓
Lerista bougainvillii	11151	Gene captures	✓	✓	✓	✓	✓
Lerista carpentariae	41192	Gene captures	✓	✓	✓	✓	✓
Lerista chalybura	62967	Gene captures					
Lerista chordae	77000	Gene captures	✓	✓	✓	✓	✓
Lerista cinerea	72914	Gene captures	✓	✓	✓	✓	✓
Lerista connivens	59766	Gene captures	✓	✓	✓	✓	✓
Lerista desertorum	12580	Gene captures	✓	✓	✓	✓	✓
Lerista dorsalis	85818	Gene captures	✓	✓	✓	✓	✓
Lerista edwardsae	108252	Gene captures	✓	✓	✓	✓	✓
Lerista elegans	63791	Gene captures	✓	✓	✓	✓	✓

Lerista elongata	57634		Gene captures	✓	✓	✓	✓	✓
Lerista emmotti	32033		Gene captures	✓	✓	✓	✓	✓
Lerista fragilis	102718		Gene captures	✓	✓	✓	✓	✓
Lerista frosti	24169		Gene captures	✓	✓	✓	✓	✓
Lerista gascoynensis	138913		Gene captures	✓	✓	✓	✓	✓
Lerista gerrardii	63760		Gene captures	✓	✓	✓	✓	✓
Lerista greeri	63744		Gene captures	✓	✓	✓	✓	✓
Lerista ips	91444		Gene captures	✓	✓	✓	✓	✓
Lerista karschmidti	30712		Gene captures	✓	✓	✓	✓	✓
Lerista kennedyensis	63954		Gene captures	✓	✓	✓	✓	✓
Lerista lineopunctulata	72594		Gene captures	✓	✓	✓	✓	✓
Lerista microtis	63807		Gene captures	✓	✓	✓	✓	✓
Lerista neander	63650		Gene captures	✓	✓	✓	✓	✓
Lerista nichollsi	59789		Gene captures	✓	✓	✓	✓	✓
Lerista orientalis	29482		Gene captures	✓	✓	✓	✓	✓
Lerista picturata	23720		Gene captures	✓	✓	✓	✓	✓
Lerista planiventralis	109119		Gene captures	✓	✓	✓	✓	✓
Lerista praepedita	62971		Gene captures	✓	✓	✓	✓	✓
Lerista terdigitata	56833		Gene captures	✓	✓	✓	✓	✓
Lerista timida	74055		Gene captures					
Lerista uniduo	63645		Gene captures	✓	✓	✓	✓	✓
Lerista varia	138923		Gene captures	✓	✓	✓	✓	✓
Lerista walkeri	63823		Gene captures	✓	✓	✓	✓	✓
Lerista wilkinsi	76998		Gene captures	✓	✓	✓	✓	✓
Anolis carolinensis		XP_008102123.1, AF134189/90/91, AH007735.2, AH007736.2, AF133907.1	NCBI	✓	✓	✓	✓	✓
Bos taurus		NM_174566, NM_0010148, NM_174567	NCBI	✓	✓		✓	

Carlia fusca	AY508937	NCBI			✓		
Carlia rhomboidalis	AY508933	NCBI			✓		
Carlia rostralis	AY508939	NCBI			✓		
Carlia rubrigularis	AY508932	NCBI			✓		
Carlia rufilatus	AY508938	NCBI			✓		
Carlia timlowi	AY508935	NCBI			✓		
Carlia vivax	AY508934	NCBI			✓		
Feylinia sp	KR336714, KR336742, KR336754, KR336717, KR336751	NCBI	✓	✓	✓	✓	✓
Lampropholis coggeri	AY508941	NCBI			✓		
Melanoseps occidentalis	KR336713.1, KR336743.1, KR336718.1, KR336750	NCBI	✓	✓		✓	✓
Python bivittatus	LOC103048730, XP_007423324.1, XP_007441698.1	NCBI	✓	✓		✓	
Saproscincus basiliscus	AY508940	NCBI			✓		
Sphenodon punctatus		NCBI	✓	✓	✓	✓	✓
Anomalopus brevicollis		transcriptomes	✓				
Chalcides ocellatus		transcriptomes	✓	✓	✓	✓	
Ctenotus atlas		transcriptomes	✓	✓	✓	✓	✓
Eumeces schneideri		transcriptomes	✓	✓	✓	✓	✓
Eutropis macularia		transcriptomes	✓		✓	✓	
Glaphyromorphus punctulatus		transcriptomes	✓	✓	✓	✓	✓
Lerista dorsalis		transcriptomes	✓	✓	✓	✓	✓
Lerista edwardsae		transcriptomes	✓	✓	✓	✓	✓
Lerista terdigitata		transcriptomes	✓	✓	✓	✓	✓
Liopholis inornata		transcriptomes	✓	✓	✓	✓	✓
Melanoseps occidentalis		transcriptomes	✓				✓
Platysaurus broadleyi		transcriptomes	✓	✓	✓	✓	✓

Tiliqua rugosa transcriptomes ✔

Table 2.2. Site CodeML results

Models	Parameters	D.F.	Models Compared	2Δ (ln L)	P	
1. *lws* opsin gene						
A. M1a	ω0=0.031, ω1=1, p0=0.924, p1=0.076					
B. M2a	ω0=0.035, ω1=1, ω2=3.753, p0=0.925, p1=0.055, p2=0.020	2	B vs. A	57.398	0	**
C. M7	p=0.025, q=0.133					
D. M8 (β&ω)	p0=0.977, p1=0.023, p=0.103, q=1.007, ω=3.482	2	D vs. C	64.489	0	**
2. *sws1* opsin gene						
E. M1a	ω0=0.022, ω1=1, p0=0.948, p1=0.052					
F. M2a	ω0=0.022, ω1=1, ω2=15.951, p0=0.948, p1=0.052, p2=0.000	2	F vs. E	2.60E-05	1	
G. M7	p=0.084, q=0.854					
H. M8 (β&ω)	p0=0.955, p1=0.045, p=0.334, q=11.794, ω=1.000	2	H vs G	22.05	0.000016	**
3. *rh1* rhodopsin gene						
I. M1a	ω0=0.024, ω1=1, p0=0.848, p1=0.152					
J. M2a	ω0=0.024, ω1=1, ω2=1, p0=0.849, p1=0.102, p2=0.049	2	J vs. I	0.881	0.644	
K. M7β	p=0.071, q=0.387					
L. M8 (β&ω)	p0=0.945, p1=0.055, p=0.105, q=0.1.033, ω=1.436	2	L vs. K	5.017	0.081	
3. *rh2* rhodopsin gene						
M. M1a	ω0=0.015, ω1=1, p0=0.855, p1=0.145					
N. M2a	ω0=0.016, ω1=1, ω2=1.587, p0=0.861, p1=0.104, p2=0.035	2	N vs. M	7.432	0.024	*
O. M7β	p=0.019, q=0.097					
P. M8 (β&ω)	p0=0.980, p1=0.020, p=0.028, q=0.160, ω=1.828	2	P vs. O	8.128	0.017	*
3. *sws2* rhodopsin gene						

Q. M1a	ω0=0.026, ω1=1, p0=0.812, p1=0.188				
R. M2a	ω0=0.029, ω1=1, ω2=2.733, p0=0.816, p1=0.143, p2=0.040	2	R vs. Q	27.862	0.000001 **
S. M7β	p=0.027, q=0.139				
T. M8 (β&ω)	p0=0.950, p1=0.050, p=0.085, q=0.520, ω=2.482	2	T vs. S	36.696	0 **

Table 2.3. Branch CodeML results

Models	ω(d_N/d_S)	D.F.	Models Compared	2Δ (in *L*)	P
1. *sws1* opsin gene					
A. All branches have one ω	ω=0.0475	-	-	-	-
B. Temperate, ω1; Arid, ω2	ω1=0.0582, ω2=0.0402	1	B vs. A	0.291	0.589
C. Mobile eyelids, ω1; Fused Lower Eyelid, ω2	ω1=0.0001, ω2=0.0565	1	C vs. A	1.104	0.293
D. Reduced limbs, ω1; Non reduced limbs with intermediates, ω2	ω1=0.0280, ω2=0.0677	1	D vs. A	0.773	0.214
E. Reduced limbs with intermediates, ω1; Non reduced limbs, ω2	ω1=0.0561, ω2=0.0201	1	E vs. A	0.969	1
F. Each branch has its own ω	Variable by branch	38	F vs. A	30.73	0.999
2. *lws* opsin gene					
G. All branches have one ω	ω=0.120	-	-	-	-
H. Temperate, ω1; Arid, ω2	ω1=0.110, ω2=0.132	1	H vs. G	0.269	0.604
I. Mobile eyelids, ω1; Fused Lower Eyelid, ω2	ω1=0.0723, ω2=0.140	1	I vs. G	2.49	0.115
J. Reduced limbs, ω1; Non reduced limbs with intermediates, ω2	ω1=0.146, ω2=0.0883	1	J vs. G	2.003	0.157
K. Reduced limbs with intermediates, ω1; Non reduced limbs, ω2	ω1=0.151, ω2=0.0664	1	K vs. G	4.249	0.0393
L. Each branch has its own ω	Variable by branch	38	L vs. G	46.412	0.996
3. *rh1* rhodopsin gene					
M. All branches have one ω	ω=0.125	-	-	-	-

N. Temperate, ω1; Arid, ω2	ω1=0.147, ω2=0.104	1	N vs. M	2.703	0.1
O. Mobile eyelids, ω1; Fused Lower Eyelid, ω2	ω1=0.124, ω2=0.125	1	O vs. M	0.00231	0.962
P. Reduced limbs, ω1; Non reduced limbs with intermediates, ω2	ω1=0.127, ω2=0.123	1	P vs. M	0.019	0.891
Q. Reduced limbs with intermediates, ω1; Non reduced limbs, ω2	ω1=0.119, ω2=0.139	1	Q vs. M	0.48	0.489
R. Each branch has its own ω	Variable by branch	38	R vs. M	23.935	1
3. *rh2* rhodopsin gene					
S. All branches have one ω	ω=0.464	-	-	-	-
T. Temperate, ω1; Arid, ω2	ω1=0.668, ω2=0.344	1	T vs. S	2.1	0.147
U. Mobile eyelids, ω1; Fused Lower Eyelid, ω2	ω1=0.360, ω2=0.482	1	U vs. S	0.182	0.67
V. Reduced limbs, ω1; Non reduced limbs with intermediates, ω2	ω1=0.452, ω2=0.475	1	V vs. S	0.0115	0.914
W. Reduced limbs with intermediates, ω1; Non reduced limbs, ω2	ω1=0.437, ω2=0.524	1	W vs. S	0.143	0.705
X. Each branch has its own ω	Variable by branch	38	X vs. S	38.444	0.999
3. *sws2* rhodopsin gene					
Y. All branches have one ω	ω=0.260	-	-	-	-
Z. Temperate, ω1; Arid, ω2	ω1=0.258, ω2=0.262	1	Z vs. Y	0.00293	0.957
O. Mobile eyelids, ω1; Fused Lower Eyelid, ω2	ω1=0.234, ω2=0.267	1		0.15	0.698
P. Reduced limbs, ω1; Non reduced limbs with intermediates, ω2	ω1=0.245, ω2=0.274	1		2.848	1
Q. Reduced limbs with intermediates, ω1; Non reduced limbs, ω2	ω1=0.248, ω2=0.288	1		0.273	0.601
R. Each branch has its own ω	Variable by branch	38		47.696	0.994

Table 2.4. Branch site CodeML results

Gene	Foreground branch	Sites under positive selection	2Δl	P Value
***lws* opsin gene**	S10A Arid	112	10.91	**0.000956**
	S10B Fixed Lower Eyelid	None	0.000066	**0.994**
	S10C Reduced Limbs	112 - 270	27.05	**0**

	S10D Reduced Limbs and Intermediates	112- 270	47.853	0
***sws1* opsin gene**	S10A Arid	None	2.07	**1**
	S10B Fixed Lower Eyelid	None	0.465	**1**
	S10C Reduced Limbs	None	2.0262	**0.155**
	S10D Reduced Limbs and Intermediates	None	0.229	1
***rh1* rhodopsin gene**	S10A Arid	None	0.0448	0.832
	S10B Fixed Lower Eyelid	None	0.0145	**0.904**
	S10C Reduced Limbs	None	0	**1**
	S10D Reduced Limbs and Intermediates	None	0.0195	1
***rh2* rhodopsin gene**	S10A Arid	72 - 79 - 96 - 113	29.073	0
	S10B Fixed Lower Eyelid	None	0	**1**
	S10C Reduced Limbs	72 - 79	21.99	**0.000003**
	S10D Reduced Limbs and Intermediates	72 - 79 - 96	44.954	0
***sws2* opsin gene**	S10A Arid	2 - 108	16.532	0.000048
	S10B Fixed Lower Eyelid	None	0	**1**
	S10C Reduced Limbs	11 - 89 - 108	25.921	**0**
	S10D Reduced Limbs and Intermediates	2 - 11 - 50 - 88 - 89 - 108	57.734	0

Table 2.5. Clade CodeML results

lws opsin genes

Partition	Model	NP	ln*L*	AIC	*k*	Parameters	Null	2Δl	df	P

						ω0	ω1	ω2				
—	M2a_rel	81	-2534.343169	5230.686338	1.82447	0.00466 (0.95942)	1 (0.03240)	15.24374 (0.00818)	—	—	—	—
S10A Arid	CmC	82	-2569.302	5302.604	1.70265	0 (0)	1 (0.03931)	0.00876 (0.96069)	M2a_rel	69.917662	1	**6.18E-17**
S10B Tropical/ Temperate	CmC	82	-2569.302	5302.604	1.70265	0 (0)	1 (0.03931)	0 (0.96069)	M2a_rel	69.917662	1	**6.18E-17**
S10C Fused Eyelids	CmC	82	-2569.327422	5302.654844	1.69896	0 (0.0001)	1 (0.0381)	0 (0.9619)	M2a_rel	69.968506	1	**6.03E-17**
S10D Unfused Eyelids	CmC	82	-2569.327422	5302.654844	1.69896	0 (0.0001)	1 (0.0381)	0.00732 (0.9619)	M2a_rel	69.968506	1	**6.03E-17**
S10E Reduced Limbs	CmC	82	-2569.222311	5302.444622	1.7004	0 (0.00002)	1 (0.03871)	0.00875 (0.96127)	M2a_rel	69.758284	1	**6.70E-17**
S10F Background	CmC	82	-2569.222311	5302.444622	1.7004	0 (0.00002)	1 (0.03871)	0 (0.96127)	M2a_rel	69.758284	1	**6.70E-17**
S10G Reduced Limbs With intermediates	CmC	82	-2569.126441	5302.252882	1.69608	0 (0)	1 (0.03649)	0.00921 (0.96351)	M2a_rel	69.566544	1	**7.39E-17**
S10H Background	CmC	82	-2569.126441	5302.252882	1.69608	0 (0)	1 (0.03649)	0 (0.96351)	M2a_rel	69.566544	1	**7.39E-17**

RH1 rhodopsin gene

Partition	Model	NP	lnL	AIC	k	Parameters			Null	2Δl	df	P
						ω0	ω1	ω2				
—	M2a_rel	81	-2827.813523	5817.627046	3.91093	0.00598 (0.90112)	1 (0.06925)	2.32614 (0.02963)	—	—	—	—

S10A Arid	CmC	82	-2831.171943	5826.343886	3.64271	0.00413 (0.89120)	1 (0.073)	0.77616 (0.0358)	M2a_rel	6.71684	1	**9.55E-03**
S10B Tropical/ Temperate	CmC	82	-2831.171943	5826.343886	3.64271	0.00413 (0.89120)	1 (0.073)	0.10366 (0.03580)	M2a_rel	6.71684	1	**9.55E-03**
S10C Fused Eyelids	CmC	82	-2837.809655	5839.61931	3.91063	0.00598 (0.90113)	1 (0.06905)	2.40908 (0.02981)	M2a_rel	19.992264	1	**7.78E-06**
S10D Unfused Eyelids	CmC	82	-2837.809655	5839.61931	3.91063	0.00598 (0.90113)	1 (0.06905)	2.30557 (0.02981)	M2a_rel	19.992264	1	**7.78E-06**
S10E Reduced LImbs	CmC	82	-2827.69058	5819.38116	3.90596	0.00597 (0.90108)	1 (0.07029)	2.5808 (0.02864)	M2a_rel	0.245886	1	**6.20E-01**
S10F Background	CmC	82	-2827.69058	5819.38116	3.90596	0.00597 (0.90108)	1 (0.07029)	2.14986 (0.02864)	M2a_rel	0.245886	1	**6.20E-01**
S10G Reduced Limbs With intermediates	CmC	82	-2833.762294	5831.524588	3.70533	0.03671 (0.11346)	1 (0.09715)	0 (0.78938)	M2a_rel	11.897542	1	**5.62E-04**
S10H Background	CmC	82	-2833.762294	5831.524588	3.70533	0.03671 (0.11346)	1 (0.09715)	0.00602 (0.78938)	M2a_rel	11.897542	1	**5.62E-04**

***RH2* opsin gene**

Partition	**Model**	**NP**	**lnL**	**AIC**	**k**	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2**				
—	M2a_rel	81	-1095.233723	2352.467446	2.32473	0.03085 (0.93225)	1 (0.04225)	15.40160 (0.0255)	—	—	—	—
S10A Arid	CmC	82	-1139.549363	2443.098726	1.77554	0 (0)	1 (0.0808)	0.01804 (0.9192)	M2a_rel	88.63128	1	**4.76E-21**

S10B Tropical/ Temperate	CmC	82	-1139.549363	2443.098726	1.77554	0 (0)	1 (0.0808)	0 (0.9192)	M2a_rel	88.63128	1	**4.76E-21**
S10C Fused Eyelids	CmC	82	-1139.784896	2443.569792	1.76989	0 (0)	1 (0.08132)	0 (0.91868)	M2a_rel	89.102346	1	**3.75E-21**
S10D Unfused Eyelids	CmC	82	-1139.784896	2443.569792	1.76989	0 (0)	1 (0.08132)	0.00909 (0.91868)	M2a_rel	89.102346	1	**3.75E-21**
S10E Reduced Limbs	CmC	82	-1139.75586	2443.51172	1.77879	0 (0)	1 (0.08468)	0 (0.91532)	M2a_rel	89.044274	1	**3.86E-21**
S10F Background	CmC	82	-1139.75586	2443.51172	1.77879	0 (0)	1 (0.08468)	0.01085 (0.91532)	M2a_rel	89.044274	1	**3.86E-21**
S10G Reduced Limbs With intermediates	CmC	82	-1138.274821	2440.549642	1.81701	0.00538 (0.91632)	1 (0.0762)	0 (0.00748)	M2a_rel	86.082196	1	**1.73E-20**
S10H Background	CmC	82	-1138.274821	2440.549642	1.81701	0.00538 (0.91632)	1 (0.0762)	8.05017 (0.00748)	M2a_rel	86.082196	1	**1.73E-20**

SWS1 opsin gene

Partition	**Model**	**NP**	**lnL**	**AIC**	**k**	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2**				
—	M2a_rel	81	-1835.272374	3832.544748	9.1945	0.00005 (0.98586)	1 (0.00110)	4.45544 (0.01304)	—	—	—	—
S10A Arid	CmC	82	-1838.917434	3841.834868	8.7285	0 (0.8719)	1 (0.02554)	0 (0.10256)	M2a_rel	7.29012	1	**6.93E-03**
S10B Tropical/ Temperate	CmC	82	-1838.917434	3841.834868	8.7285	0 (0.8719)	1 (0.02554)	0 (0.10256)	M2a_rel	7.29012	1	**6.93E-03**

S10C Fused Eyelids	CmC	82	-1840.283982	3844.567964	8.72495	0 (0.87727)	1 (0.02555)	0 (.09719)	M2a_rel	10.023216	1	**1.55E-03**
S10D Unfused Eyelids	CmC	82	-1840.283982	3844.567964	8.72495	0 (0.87727)	1 (0.02555)	0 (.09719)	M2a_rel	10.023216	1	**1.55E-03**
S10E Reduced Limbs	CmC	82	-1839.952647	3843.905294	8.72504	0 (0.90819)	1 (0.02554)	0 (0.06627)	M2a_rel	9.360546	1	**2.22E-03**
S10F Background	CmC	82	-1839.952647	3843.905294	8.72504	0 (0.90819)	1 (0.02554)	0 (0.06627)	M2a_rel	9.360546	1	**2.22E-03**
S10G Reduced Limbs With intermediates	CmC	82	-1838.917434	3841.834868	8.72848	0 (0.83713)	1 (0.02554)	0 (0.13733)	M2a_rel	7.29012	1	**6.93E-03**
S10H Background	CmC	82	-1838.917434	3841.834868	8.72848	0 (0.83713)	1 (0.02554)	0 (0.13733)	M2a_rel	7.29012	1	**6.93E-03**

***SWS2* opsin gene**

Partition	**Model**	**NP**	**lnL**	**AIC**	**k**	**Parameters**			**Null**	**2Δl**	**df**	**p**
						ω0	**ω1**	**ω2**				
—	M2a_rel	81	-1723.931778	3609.863556	7.37316	0.01186 (0.91176)	1 (0.04098)	6.58461 (0.04727)	—	—	—	—
S10A Arid	CmC	82	-1779.143238	3722.286476	5.43399	0 (0.85413)	1 (0.08704)	0.5061 (0.05883)	M2a_rel	110.42292	1	**7.92E-26**
S10B Tropical/ Temperate	CmC	82	-1779.143238	3722.286476	5.43399	0 (0.85413)	1 (0.08704)	0 (0.05883)	M2a_rel	110.42292	1	**7.92E-26**
S10C Fused Eyelids	CmC	82	-1780.517123	3725.034246	5.39046	0 (0)	1 (0.08803)	0 (0.91197)	M2a_rel	113.17069	1	**1.98E-26**

S10D Unfused Eyelids	CmC	82	-1780.517123	3725.034246	5.39046	0 (0)	1 (0.08803)	0.00992 (0.91197)	M2a_rel	113.17069	1	**1.98E-26**
S10E Reduced Limbs	CmC	82	-1780.853319	3725.706638	5.42242	0.00729 (0)	1 (0.08927)	0.01089 (0.91073)	M2a_rel	113.843082	1	**1.41E-26**
S10F Background	CmC	82	-1780.853319	3725.706638	5.42242	0.00729 (0)	1 (0.08927)	0.00462 (0.91073)	M2a_rel	113.843082	1	**1.41E-26**
S10G Reduced Limbs With Intermediates	CmC	82	-1781.311377	3726.622754	5.41024	0.00579 (0.00001)	1 (0.09)	0.00834 (0.90999)	M2a_rel	114.759198	1	**8.89E-27**
S10H Background	CmC	82	-1781.311377	3726.622754	5.41024	0.00579 (0.00001)	1 (0.09)	0.00555 (0.90999)	M2a_rel	114.759198	1	**8.89E-27**

Table 2.6. Genes Sampled with Probes, with Probe species used

Gene symbol	Description	Function	Probe Sequence Species	Number of Probe Species	BC/BS	Size (bp) in *Anolis*
LWS (OPN1LW)	Long wavelength sensitive opsin	Visual Opsin	*Anolis, Chrysemys, Gallus, Gecko, Python, Thamnophis*	6	BC/BS	1110
RH1 (RHO)	Rhodopsin 1	Visual Opsin	*Alligator, Anolis, Gallus, Pelodiscus, Thamnophis, Uta*	6	BC/BS	1059
RH2	Rhodopsin 2	Visual Opsin	*Anolis, Chrysemys, Gallus, Gekko, Pelodiscus, Uta*	6	BC/BS	1068
SWS1 (OPN1SW)	Short-wave sensitive opsin 1	Visual Opsin	*Anolis, Chrysemys, Gallus, Gecko, Python, Thamnophis, Uta*	7	BC/BS	1041
SWS2	Short-wave sensitive opsin 2	Visual Opsin	*Alligator, Anolis, Chrysemys, Gallus, Uta*	5	BC/BS	1092
OPN5	Neuropsin	Non-visual opsin	*Anolis, Gallus, Pelodiscus, Python*	4	BC/BS	1044
OPN6	Neuropsin 2	Non-visual opsin	*Anolis, Gallus, Gekko*	3	BC/BS	1044
OPN8	Neuropsin 3	Non-visual opsin	*Anolis, Gallus, Gekko*	3	BC/BS	1044
OPN7	Neuropsin 4	Non-visual opsin	*Anolis, Gallus*	2	BC/BS	1044
OPN9	Neuropsin 5	Non-visual opsin	*Anolis*	1	BC/BS	1044
OPN3 (ENC)	Encephalopsin	Non-visual opsin	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1224
OPN4m (MEL1)	Melanopsin mammal-like	Non-visual opsin	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1644
OPN4x (MEL2)	Melanopsin Xenopus-like	Non-visual opsin	*Anolis, Gallus, Gekko, Python*	4	BC/BS	1644
Parapinopsin	Parapinopsin	Non-visual opsin	*Anolis*	1	BC/BS	1038

Parietopsin	Parietopsin	Non-visual opsin	*Anolis*	1	BC/BS	1038
Pinopsin	Pinopsin	Non-visual opsin	*Anolis, Gallus, Chrysemys, Uta*	4	BC/BS	1245
TMT2	Teleost multiple tissue opsin 2	Non-visual opsin	*Anolis, Gallus*	2	BC/BS	1222
VAOP	Vertebrate ancient opsin	Non-visual opsin	*Anolis, Gallus, Gekko*	3	BC/BS	1167
ABCA4	ABC transporter (ABCR or Rim Protein)	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	6978
AIPL1	Aryl Hydrocarbon Receptor Interacting Protein-like 1	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1287
ARR3	Arrestin 3, retinal, X-arrestin, cone	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1185
ARRB2	Beta-arrestin-2	Phototransduction & Visual Cycle	*Pelodiscus, Gekko, Python*	3	BC/BS	1455
CALM1	Calmodulin	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	450
CNGA1	Cyclic nucleotide-gated cation channel alpha 1, rod	Phototransduction & Visual Cycle	*Gallus, Chysemys, Pelodiscus, Gekko, Python*	5	BC/BS	2184
CNGA3	Cyclic nucleotide-gated cation channel alpha 3, cone	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	2025
CNGB1	Cyclic nucleotide-gated cation channel beta 1, rod	Phototransduction & Visual Cycle	*Gallus, Pelodiscus, Gekko, Python*	4	BC/BS	2411
CNGB3	Cyclic nucleotide-gated cation channel beta 3, cone	Phototransduction & Visual Cycle	*Anolis, Gallus, Chysemys, Pelodiscus, Gekko, Python*	6	BC/BS	2244
CRABP1	Cellular retinoic acid binding protein 1	Phototransduction & Visual Cycle	*Gallus, Chrysemys, Python*	3	BC/BS	414
GNAT1	Guanine nucleotide-binding protein alpha-1 (Transducin, rod)	Phototransduction & Visual Cycle	*Anolis, Gallus, Chrysemys, Gekko, Python*	5	BC/BS	1053
GNAT2	Guanine nucleotide-binding protein alpha-2 (Transducin, cone)	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1065

GNB1	Guanine nucleotide-binding protein beta 1, rod	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1023
GNB2	Guanine nucleotide-binding protein beta 2	Phototransduction & Visual Cycle	*Anolis, Pelodiscus, Gekko, Python*	4	BC/BS	1023
GNB3	Guanine nucleotide-binding protein beta 3, cone	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1023
GNB5	Guanine nucleotide-binding protein beta 5, RGS9 beta subunit	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1062
GNGT2	Guanine nucleotide-binding protein gamma-T2, cone	Phototransduction & Visual Cycle	*Anolis, Pelodiscus, Gekko, Python*	4	BC/BS	210
GRK1	G protein-coupled receptor kinase 1, Rhodopsin kinase (RHOK)	Phototransduction & Visual Cycle	*Anolis, Pelodiscus, Gekko*	3	BC/BS	1686
GRK7	G protein-coupled receptor kinase 7	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1638
GUCA1A	guanylate cyclase activator 1A (retina)	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	597
GUCA1B	guanylate cyclase activator 1B (retina)	Phototransduction & Visual Cycle	*Anolis, Gallus, Chrysemys, Gekko, Python*	5	BC/BS	597
GUCY1A3	Guanylate cyclase 1, soluble, alpha 3	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	2064
GUCY1B3	Guanylate cyclase 1, soluble, beta 3	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1860
GUCY2D	Retinal guanylyl cyclase 1	Phototransduction & Visual Cycle	*Anolis, Pelodiscus, Python*	3	BC/BS	3357
GUCY2F	Retinal guanylyl cyclase 2	Phototransduction & Visual Cycle	*Anolis, Gallus, Gekko, Python*	4	BC/BS	3057
LRAT	Lecithin retinol acyltransferase	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	696
PDC	Phosducin	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	756
PDE6B	Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta precursor	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	2571

PDE6C	Cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha'	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus*	3	BC/BS	2532
PDE6D	phosphodiesterase 6D, cGMP-specific, rod, delta	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	396
PDE6G	phosphodiesterase 6D, cGMP-specific, rod, gamma	Phototransduction & Visual Cycle	*Anolis, Gallus, Gekko, Python*	4	BC/BS	264
PDE6H	Retinal cone rhodopsin-sensitive cGMP 3',5'-cyclic phosphodiesterase subunit gamma	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	264
PGK1	Phosphoglycerate kinase 1	Phototransduction & Visual Cycle	*Gallus, Chrysemys*	2	BC/BS	1254
PPP2R1A	Serine/threonine-protein phosphatase 2A	Phototransduction & Visual Cycle	*Anolis, Gekko, Python*	3	BC/BS	1770
PRPH2	Periphin 2, Retinal Degeneration Slow (RDS)	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1065
RARA	Retinoic acid receptor, alpha	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1551
RARB	Retinoic acid receptor, beta	Phototransduction & Visual Cycle	*Anolis, Gallus, Gekko, Python*	4	BC/BS	1521
RARG	Retinoic acid receptor, gamma	Phototransduction & Visual Cycle	*Anolis, Gekko, Python*	3	BC/BS	1458
RBP1	Retinol binding protein 1 // Crystallin, iota	Phototransduction & Visual & Cycle Lens Crystallins	*Anolis, Gallus, Pelodiscus, Python*	4	BC/BS	408
RBP3	Retinol binding protein 3, interstitial	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	3693
RBP4	Retinol-binding protein 4, plasma	Phototransduction & Visual Cycle	*Anolis, Gallus, Gekko, Python*	4	BC/BS	606
RCVRN	Recoverin, RCV1	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	582
RDH12	Retinol dehydrogenase 12	Phototransduction & Visual Cycle & Carotenoid Oil droplet	*Anolis, Gallus, Gekko*	3	BC/BS	963
RDH14	Retinol dehydrogenase 14	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	993

RDH5	Retinol dehydrogenase 5 (11-cis and 9-cis)	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	960
RDH8	Retinol dehydrogenase 8 (Photoreceptor outer segment all-trans)	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	951
RGR	Retinal G protein-coupled receptor	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	888
RGS9	Regulator of G-protein signaling 9	Phototransduction & Visual Cycle	*Anolis, Gallus, Gekko, Python*	4	BC/BS	1152
RGS9BP	Regulator of G protein signaling 9-binding protein (R9AP)	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	2064
RLBP1	Retinaldehyde-binding protein 1, cellular	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	954
RPE65	Retinal pigment epithelium-specific 65 kDa protein	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1602
RRH	Retinal Pigment Epithemlium-derived Rhodopsin Homolog, Peropsin	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1002
SAG	S-arrestin	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1548
STRA6	Stimulated by retinoic acid gene 6	Phototransduction & Visual Cycle	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1995
CRYAA	Crystallin, alpha A	Lens Crystallins	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	519
CRYAB	Crystallin, alpha B	Lens Crystallins	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	528
CRYBA1	Crystallin, beta A1	Lens Crystallins	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	648
CRYBA2	Crystallin, beta A2	Lens Crystallins	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	591
CRYBA4	Crystallin, beta A4	Lens Crystallins	*Gallus, Gekko, Python*	3	BC/BS	591
CRYBB1	Crystallin, beta B1	Lens Crystallins	*Gallus, Pelodiscus, Gekko*	3	BC/BS	591

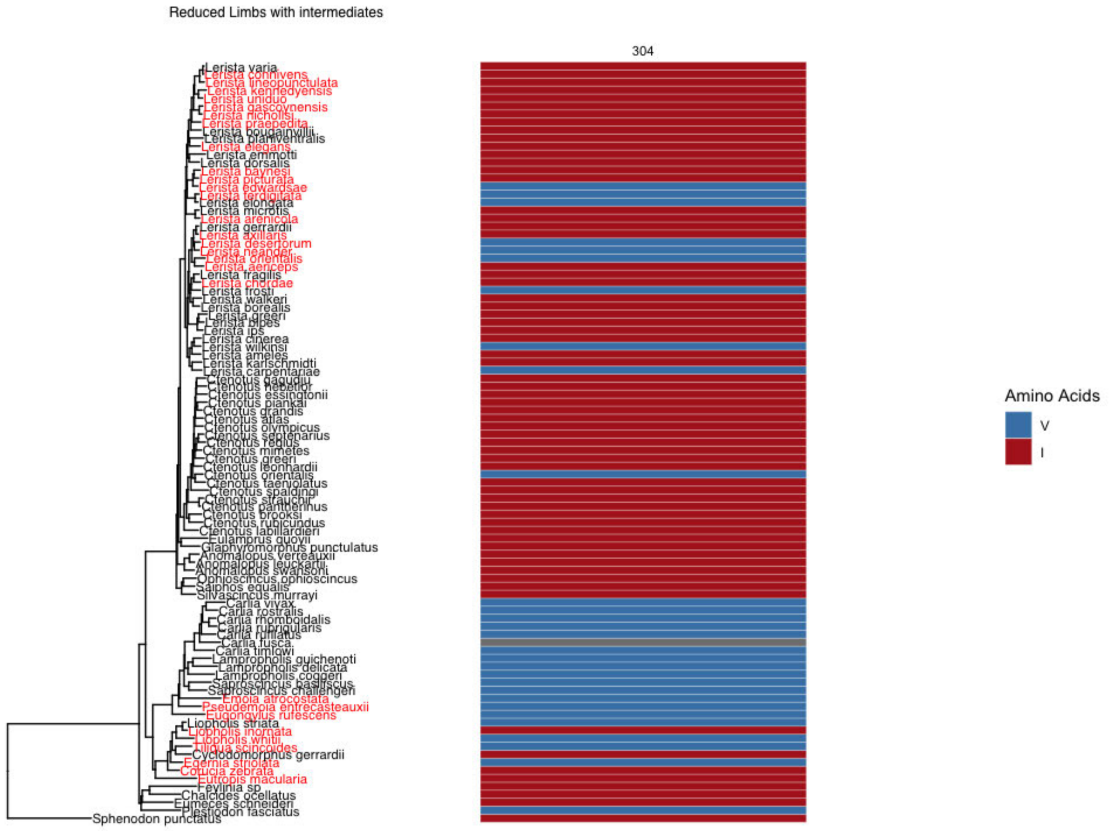
CRYBB2	Crystallin, beta B2	Lens Crystallins	*Gallus, Gekko, Python*	3	BC/BS	717
CRYBB3	Crystallin, beta B3	Lens Crystallins	*Gallus, Pelodiscus*	2	BC/BS	780
CRYD1	Crystallin, delta 1 // ASL1	Lens Crystallins	*Gallus*	1	BC/BS	1401
CRYD2	Crystallin, delta 2 // ASL2	Lens Crystallins	*Gallus*	1	BC/BS	1401
CRYGN	Crystallin, gamma N	Lens Crystallins	*Anolis, Gallus, Pelodiscus*	3	BC/BS	612
CRYGS	Crystallin, gamma S	Lens Crystallins	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	537
CRYL1	Crystallin, lambda 1	Lens Crystallins	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1056
CRYM	Crystallin, mu	Lens Crystallins	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	942
CRYZ	Crystallin, zeta // NADPH (quinone oxidoreductase)	Lens Crystallins	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	993
CRYZL1	Crystallin, zeta-like 1	Lens Crystallins	*Gallus, Pelodiscus, Gekko, Python*	4	BC/BS	1044
ENO1	Crystallin, tau // Enolase 1, (alpha)	Lens Crystallins & Phylogenetic Marker	*Alligator, Gallus, Pelodiscus, Sceloporus, Gekko, Python*	6	BC/BS	1305
GAPDH	Crystallin, pi // Glyceraldehyde-3-phosphate dehydrogenase	Lens Crystallins & Housekeeping	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1002
LIM2	Lens intrinsic membrane protein 2	Lens Optics	*Anolis, Gekko, Python, Gallus, Chrysemys, Alligator*	6	BS	1923
BFSP2	Beaded Filament Structural Protein 2	Lens Optics	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	1326
CRX (OTX3)	Cone-rod homeobox	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	873
NOTCH1	NOTCH family 1	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	6003

NR2E3	Nuclear receptor subfamily 2, group E, member 3	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1257
NRL	Neural retina leucine zipper	Photoreceptor Development	*Anolis, Gekko, Python*	3	BC/BS	780
OTX2	Orthodenticle homeobox 2	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	870
PCDH15 (CDHR1)	Protocadherin-related 15	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	5817
PROM1	Prominin 1	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	2646
RORB	RAR-related orphan receptor B	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1743
RS1	Retinoschisin 1	Photoreceptor Development	*Anolis, Gallus, Pelodiscus*	3	BC/BS	897
TRB2	Tribbles homolog 2	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1071
USH1C	Usher syndrome 1C	Photoreceptor Development	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC/BS	1707
VSX1	Visual System Homeobox 1	Photoreceptor Development	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	1113
HIPK2	Homeodomain Interacting Protein Kinase 2	Retina Formation	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	3715
RAX	Retina And Anterior Neural Fold Homeobox	Retina Formation	*Anolis, Gekko, Pyhton, Gavialis, Gallus, Chrysemys*	6	BS	1038
SIX3	Sine Oculis Homeobox Homolog 3	Retina Formation	*Anolis, Gekko, Python, Gallus Pelodiscus, Alligator*	6	BS	1011
PAX6	Paired Box 6	Retina Formation	*Anolis, Gekko, Python, Gallus Pelodiscus, Alligator*	6	BS	1317
ZNF513	Zinc Finger Protein 513	Retina Formation	*Gekko, Python, Gallus*	3	BS	1206
PCDH21	Cadherin-related family member 1 (Photoreceptor cadherin, prCAD)	Structural	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC	2604

RP1	Retinitis pigmentosa 1, autosomal dominant (Oxygen-regulated protein 1)	Structural	*Anolis, Gallus, Pelodiscus*	3	BC	8561
RP1L1	Retinitis pigmentosa 1-like 1	Structural	*Anolis, Gallus, Pelodiscus*	3	BC	6003
ANXA2	Annexin A2	Photoreceptor Phagocytosis	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC	1020
CD36	Cluster of differentiation 36, thrombospondin receptor	Photoreceptor Phagocytosis	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC	1419
GAS6	Growth arrest-specific 6	Photoreceptor Phagocytosis	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC	2025
MERTK	c-mer proto-oncogene tyrosine kinase	Photoreceptor Phagocytosis	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC	2997
MFGE8	Milk fat globule-EGF factor 8	Photoreceptor Phagocytosis	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC	1425
PTK2 (FAK)	Protein tyrosine kinase 2 (Focal adhesion kinase)	Photoreceptor Phagocytosis	*Anolis, Gallus, Pelodiscus, Gekko, Python*	5	BC	3342
TRPA1	Transient receptor potential channel 1	Infrared detection	*Anolis, Gallus, Pantherophis, Pelodiscus, Python*	5	BC	3339
EEVS	2-Epi-5-Epi-Valiolone Synthase	Ultraviolet Protection	*Anolis, Gallus, Chrysemys*	3	BS	1386
MT-Ox	MT-Ox protein	Ultraviolet Protection	*Anolis, Gallus, Pelodiscus*	3	BS	1311
MDFIC	MyoD Family Inhibitor Domain Containing	Ultraviolet Protection	*Anolis, Gekko, Protobothrops, Gallus, Pelodiscus, Alligator*	5	BS	750
CYP27C1	Cytochrome P450 Family 27 Subfamily C Member 1	Chromophore formation	*Alligator, Gallus, Bitis, Ophiophagus*	4	BS	1410
PER1	Circadian Clock Protein PERIOD 1	Circadian Clock	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	3948
PER2	Circadian Clock Protein PERIOD 2	Circadian Clock	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	3855
ARNTL	Aryl Hydrocarbon Receptor Nuclear Translocator Like	Circadian Clock	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	1902

CLOCK	Clock Circadian Regulator	Circadian Clock	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	2664
CRY1	Cryptochrome Circadian Clock 1	Circadian Clock	*Anolis, Gekko, Python, Gallus, Chrysemys, Alligator*	6	BS	1866
CRY2	Cryptochrome Circadian Clock 2	Circadian Clock	*Anolis, Gekko, Python, Gallus, Chrysemys, Alligator*	6	BS	1761
TIMELESS	Timeless Circadian Clock	Circadian Clock	*Anolis, Gekko, Python, Gallus, Alligator*	5	BS	3615
NR1D1	Nuclear Receptor Subfamily 1 Group D Member 1	Circadian Clock	*Anolis, Gekko, Python, Gallus, Chrysemys, Alligator*	6	BS	1836
RORA	Retinoid-Related Orphan Receptor-Alpha	Circadian Clock	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	1437
BHLHE40	Basic Helix-Loop-Helix Family Member E40	Circadian Clock	*Anolis, Gekko, Python, Columba, Pelodiscus*	6	BS	1278
BHLHE41	Basic Helix-Loop-Helix Family Member E41	Circadian Clock	*Anolis, Gekko, Python, Gallus, Pelodiscus, Gavialis*	6	BS	1269
EREG	Epiregulin	Eyelids open at birth	*Anolis, Deinagkristodon, Pelodiscus, Alligator*	4	BS	498
CECR2	CECR2, Histone Acetyl-Lysine Reader	Eyelids open at birth	*Gekko, Python, Gallus, Alligator*	4	BS	4392
EXT1	Exostosin Glycosyltransferase 1	Eyelids open at birth	*Anolis, Python, Serinus, Pelodiscus, Alligator*	5	BS	2262
EGF	Epidermal Growth Factor	Eyelids open at birth	*Anolis, Gekko, Python, Gallus, Alligator*	5	BS	3168
TGFA	Transforming Growth Factor Alpha	Eyelids open at birth	*Gekko, Python, Pelodiscus, Alligator*	4	BS	483
BCO2	β-Carotene Oxygenase 2	Carotenoid Methabolism - Oil droplet	*Anolis, Gekko, Python, Columba, Pelodiscus*	6	BS	1095
RETSAT	Retinol Saturase	Carotenoid Methabolism - Oil droplet	*Anolis, Gekko, Gallus, Pelodiscus, Alligator*	5	BS	1530
CYP2J19	Cytochrome P450 2J2-like	Carotenoid Methabolism - Oil droplet	*Anolis, Gekko, Crysemys, Serinus*	4	BS	1572

STAR	Steroidogenic Acute Regulatory Protein	Carotenoid Methabolism	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	802
BCO1	β-Carotene Oxygenase 1	Carotenoid Methabolism	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	1095
SCARB1 (SR-BI)	Scavenger Receptor Class B Member 1	Carotenoid Methabolism & Chromophore formation	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	1641
SCARB2	Scavenger Receptor Class B Member 2	Carotenoid Methabolism	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	1449
KCNV2	Potassium Channel, Subfamily V, Member 2	Retinal Dystrophy	*Anolis, Gekko, Python, Gallus, Alligator*	5	BS	1668
BEST1	Bestrophin 1	Retinal Dystrophy	*Anolis, Gekko, Python, Gallus, Alligator*	5	BS	2298
ELOVL4	ELOVL Fatty Acid Elongase 4	Retinal Dystrophy	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	894
RPGRIP1	Retinitis Pigmentosa GTPase Regulator Interacting Protein 1	Retinal Dystrophy	*Anolis, Gekko, Python, Pelodiscus, Alligator*	5	BS	3675
TIMP3	TIMP Metallopeptidase Inhibitor 3	Retinal Dystrophy	*Anolis, Gekko, Python, Gallus, Pelodiscus, Alligator*	6	BS	639
CACNA1F	Calcium Voltage-Gated Channel Subunit Alpha1 F	Retinal Dystrophy	*Anolis, Chrysemys, Python, Alligator*	4	BS	460
EFEMP1	EGF Containing Fibulin Like Extracellular Matrix Protein 1	Retinal Dystrophy	*Anolis, Gekko, Python, Gallus, Alligator*	5	BS	1344
ROM1	Retinal Outer Segment Membrane Protein 1	Retinal Dystrophy	*Gallus, Alligator*	2	BS	1098
CHM	Choroideremia (Rab Escort Protein 1)	Retinal Dystrophy	*Anolis, Gekko, Protobothrops, Gallus, Pelodiscus, Alligator*	6	BS	2031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Lerista bougainvillii
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Lerista edwardsae
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Lerista fragilis
Lerista chordae
Lerista frosti
Lerista walkeri
Lerista borealis
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Lerista bipes
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Lerista cinerea
Lerista wilkinsi
Lerista ameles
Lerista karlschmidti
Lerista carpentariae
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Ctenotus hebetior
Ctenotus essingtonii
Ctenotus piankai
Ctenotus grandis
Ctenotus atlas
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Ctenotus mimetes
Ctenotus greeri
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Ctenotus orientalis
Ctenotus taeniolatus
Ctenotus spaldingi
Ctenotus strauchii
Ctenotus pantherinus
Ctenotus brooksi
Ctenotus rubicundus
Ctenotus labillardieri
Eulamprus quoyii
Glaphyromorphus punctulatus
Anomalopus verreauxii
Anomalopus leuckartii
Anomalopus swansoni
Ophioscincus ophioscincus
Saiphos equalis
Silvascincus murrayi
Carlia vivax
Carlia rostralis
Carlia rhomboidalis
Carlia rubrigularis
Carlia rufilatus
Carlia fusca
Carlia timlowi
Lampropholis guichenoti
Lampropholis delicata
Lampropholis coggeri
Saproscincus basiliscus
Saproscincus challengeri
Emoia atrocostata
Pseudemoia entrecasteauxii
Eugongylus rufescens
Liopholis striata
Liopholis inornata
Liopholis whitii
Tiliqua scincoides
Cyclodomorphus gerrardii
Egernia striolata
Corucia zebrata
Eutropis macularia
Feylinia sp
Chalcides ocellatus
Eumeces schneideri
Plestiodon fasciatus
Sphenodon punctatus
Amino Acids
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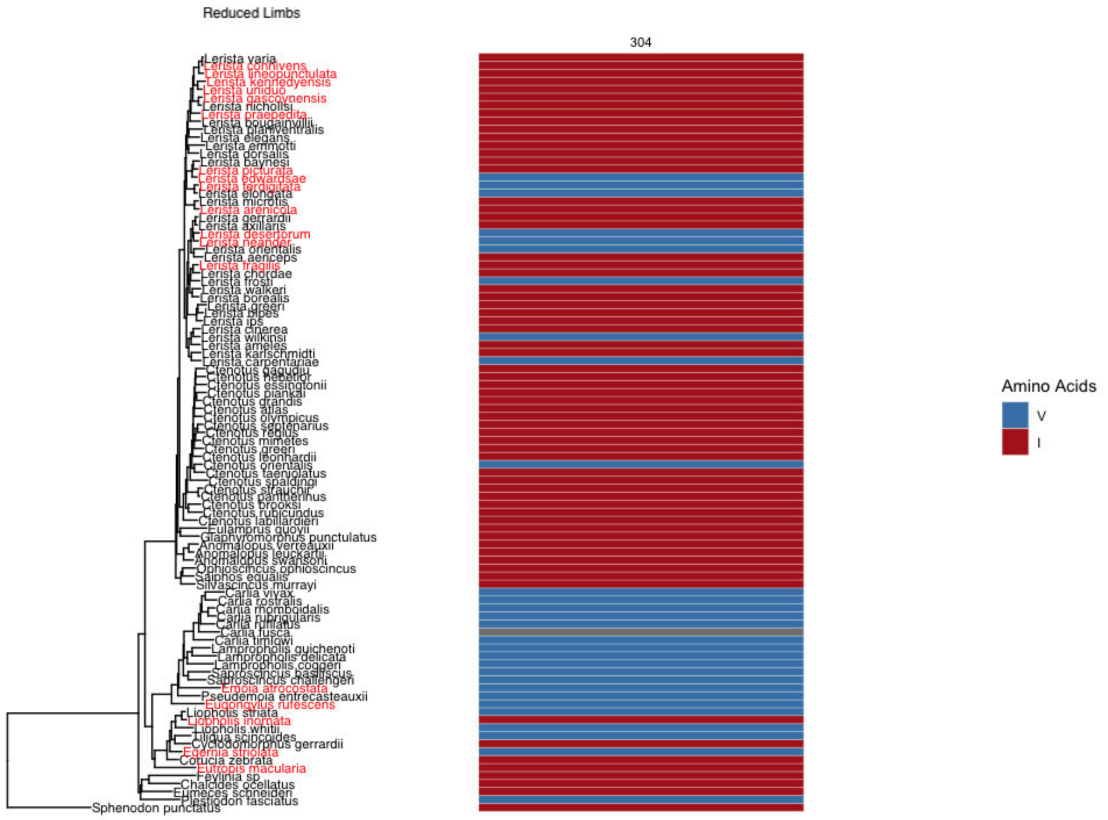
Reduced Limbs
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Ctenotus mimetes
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Ctenotus orientalis
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Ctenotus spaldingi
Ctenotus strauchii
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Egernia striolata
Corucia zebrata
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Chalcides ocellatus
Eumeces schneideri
Plestiodon fasciatus
Sphenodon punctatus
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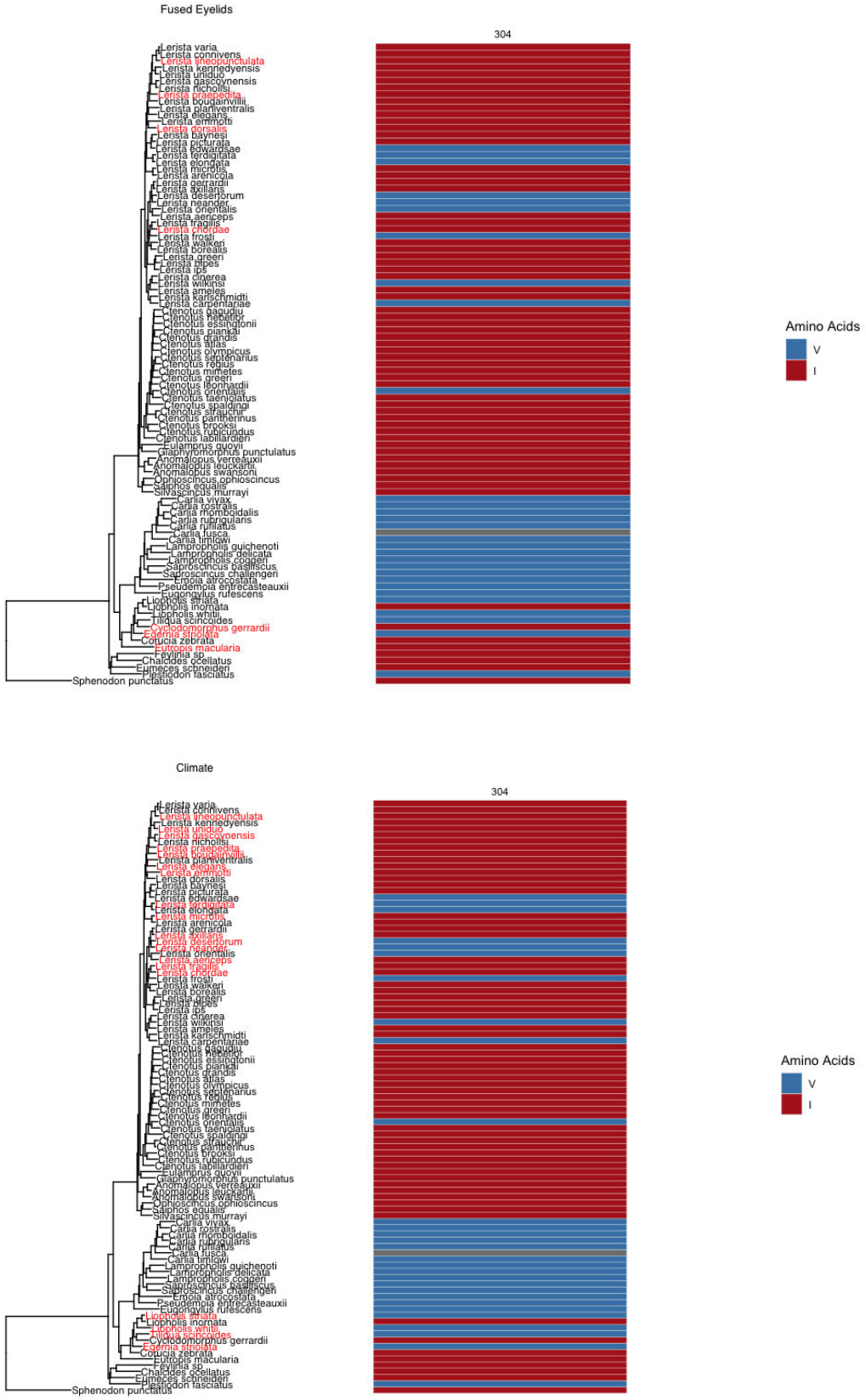
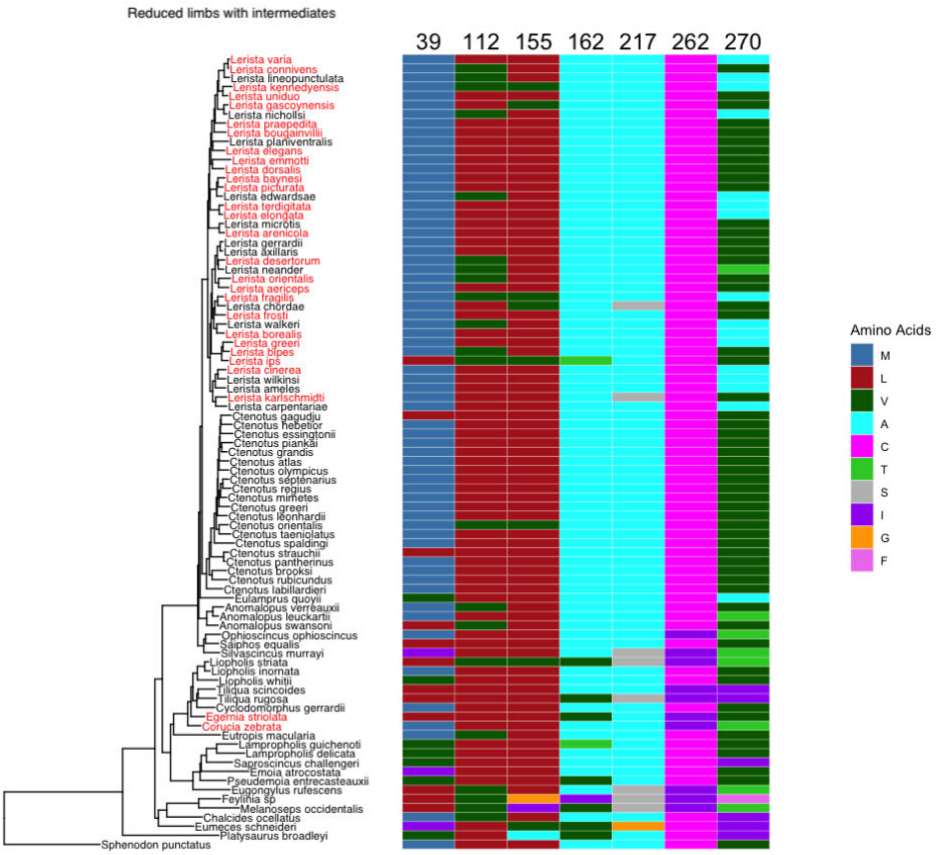


Figure 2.1: Phylogenetic trees showing ecological and phylogenetic partitions as labelled, and selected sites in RH2 with corresponding amino acids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Lerista gascoynensis
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Lerista wilkinsi
Lerista ameles
Lerista karlschmidti
Lerista carpentariae
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Ctenotus hebetior
Ctenotus essingtonii
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Ctenotus strauchii
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Ctenotus brooksi
Ctenotus rubicundus
Ctenotus labillardieri
Eulamprus quoyii
Anomalopus verreauxii
Anomalopus leuckartii
Anomalopus swansoni
Ophioscincus ophioscincus
Saiphos equalis
Silvascincus murrayi
Liopholis striata
Liopholis inornata
Liopholis whitii
Tiliqua scincoides
Tiliqua rugosa
Cyclodomorphus gerrardii
Egernia striolata
Corucia zebrata
Eutropis macularia
Lampropholis guichenoti
Lampropholis delicata
Saproscincus challengeri
Emoia atrocostata
Pseudemoia entrecasteauxii
Eugongylus rufescens
Feylinia sp
Melanoseps occidentalis
Chalcides ocellatus
Eumeces schneideri
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Sphenodon punctatus
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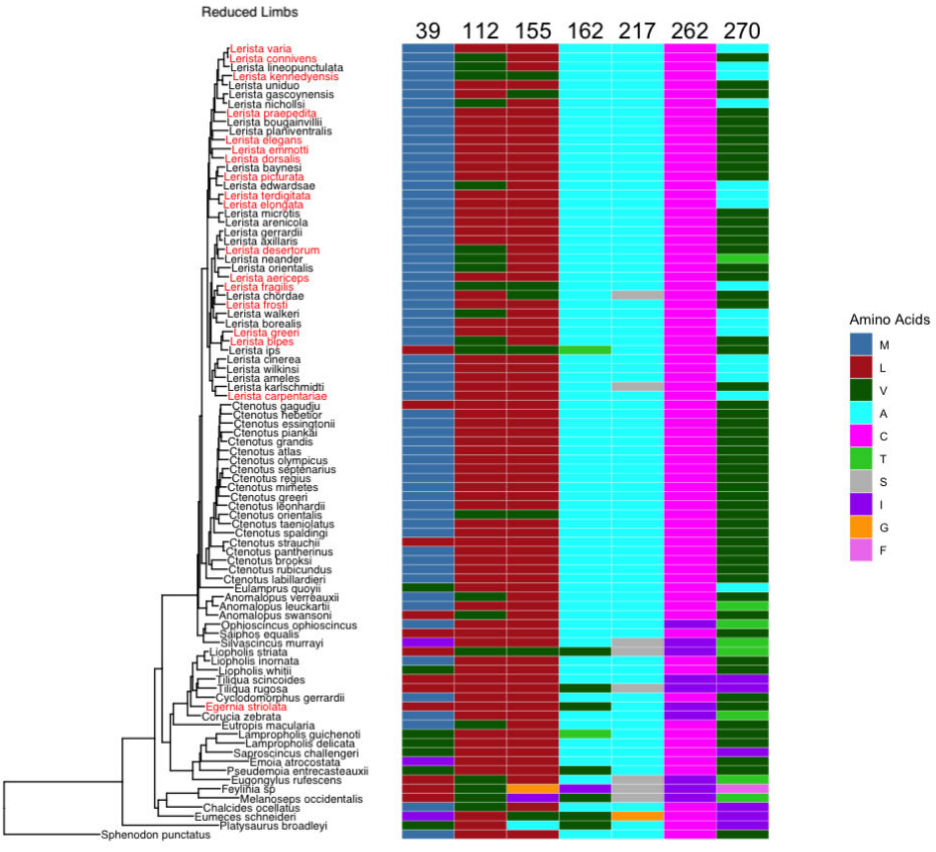
Reduced Limbs
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Lerista gascoynensis
Lerista nichollsi
Lerista praepedita
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Lerista dorsalis
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Lerista terdigitata
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Ctenotus essingtonii
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Ctenotus grandis
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Ctenotus mimetes
Ctenotus greeri
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Anomalopus swansoni
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Liopholis whitii
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Tiliqua rugosa
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Emoia atrocostata
Pseudemoia entrecasteauxii
Eugongylus rufescens
Feylinia sp
Melanoseps occidentalis
Chalcides ocellatus
Eumeces schneideri
Platysaurus broadleyi
Sphenodon punctatus
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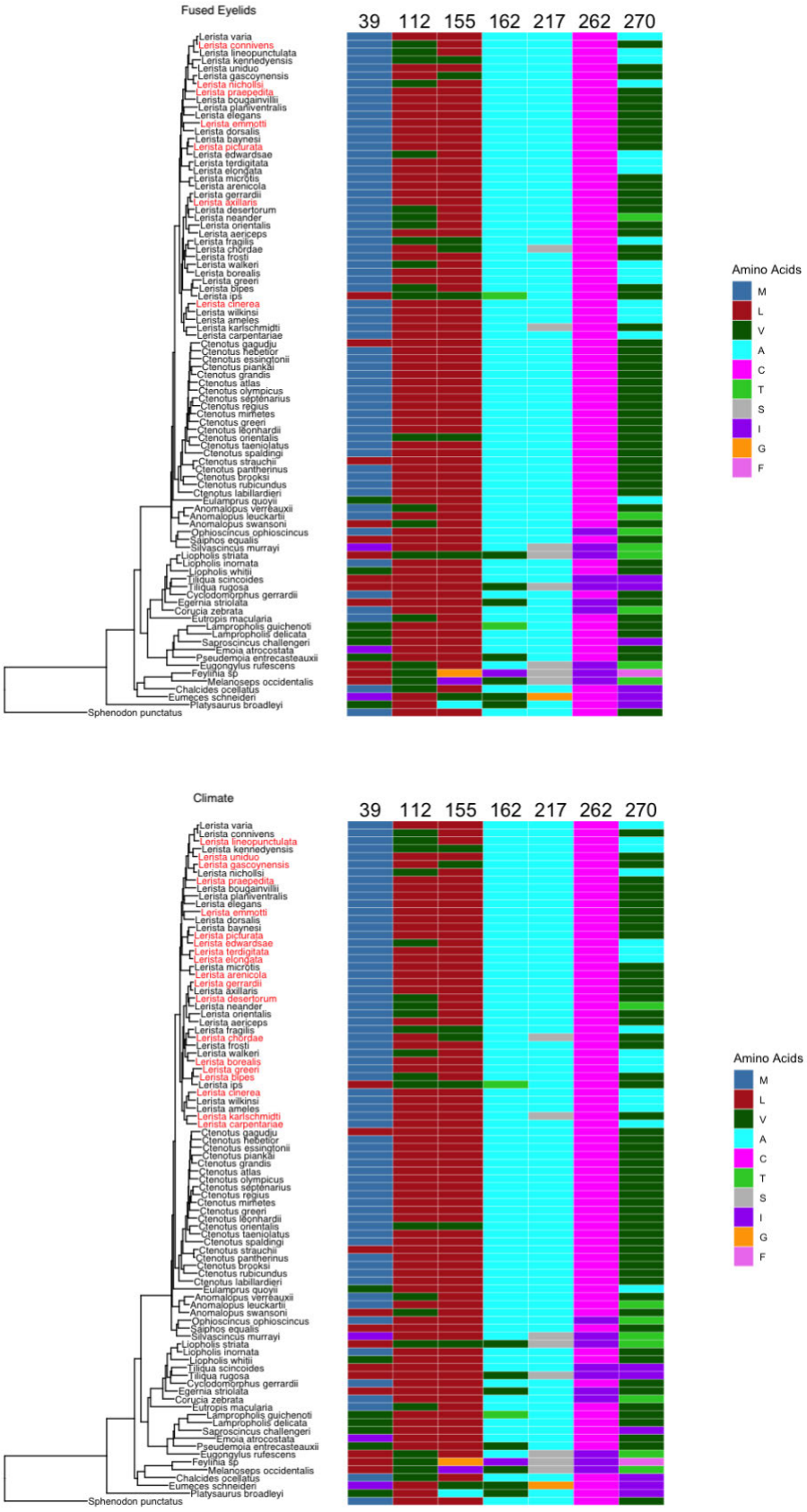
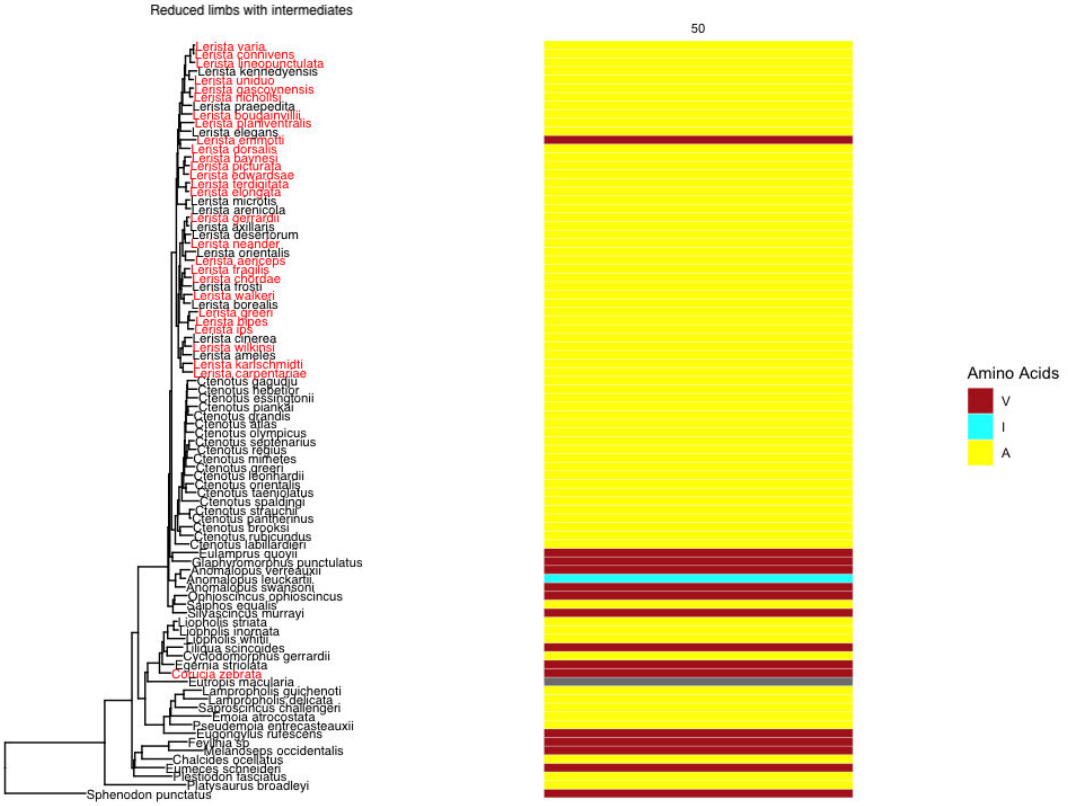


Figure 2.2: Phylogenetic tree showing group partitions as labelled, and selected sites in LWS with corresponding amino acids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Lerista kennedyensis
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Lerista gascoynensis
Lerista nichollsi
Lerista praepedita
Lerista boulainvillii
Lerista planiventralis
Lerista elegans
Lerista emmotti
Lerista dorsalis
Lerista baynesi
Lerista picturata
Lerista edwardsae
Lerista terdigitata
Lerista elongata
Lerista microtis
Lerista arenicola
Lerista gerrardii
Lerista axillaris
Lerista desertorum
Lerista neander
Lerista orientalis
Lerista aericeps
Lerista fragilis
Lerista chordae
Lerista frosti
Lerista walkeri
Lerista borealis
Lerista greeri
Lerista bipes
Lerista ips
Lerista cinerea
Lerista wilkinsi
Lerista ameles
Lerista karlschmidti
Lerista carpentariae
Ctenotus gagudju
Ctenotus hebetior
Ctenotus essingtonii
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Ctenotus grandis
Ctenotus atlas
Ctenotus olympicus
Ctenotus septenarius
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Ctenotus greeri
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Ctenotus taeniolatus
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Saiphos equalis
Silvascincus murrayi
Liopholis striata
Liopholis inornata
Liopholis whitii
Tiliqua scincoides
Cyclodomorphus gerrardii
Egernia striolata
Coeucia zebrata
Eutropis macularia
Lampropholis guichenoti
Lampropholis delicata
Saproscincus challengeri
Emoia atrocostata
Pseudemoia entrecasteauxii
Eugongylus rufescens
Feylinia sp.
Melanoseps occidentalis
Chalcides ocellatus
Eumeces schneideri
Plestiodon fasciatus
Platysaurus broadleyi
Sphenodon punctatus
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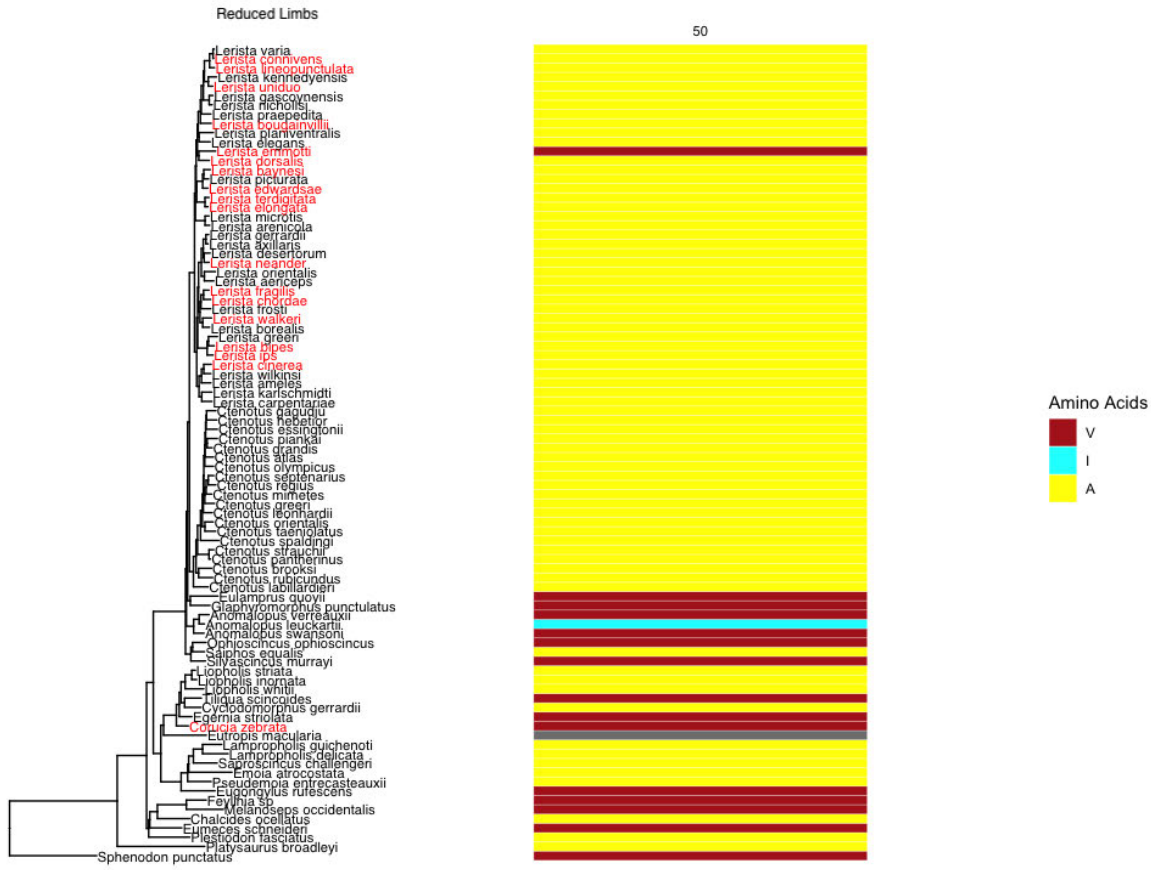
Reduced Limbs
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Lerista ameles
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Lerista carpentariae
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Ctenotus orientalis
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Eugongylus rufescens
Feylinia sp.
Melanoseps occidentalis
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Plestiodon fasciatus
Platysaurus broadleyi
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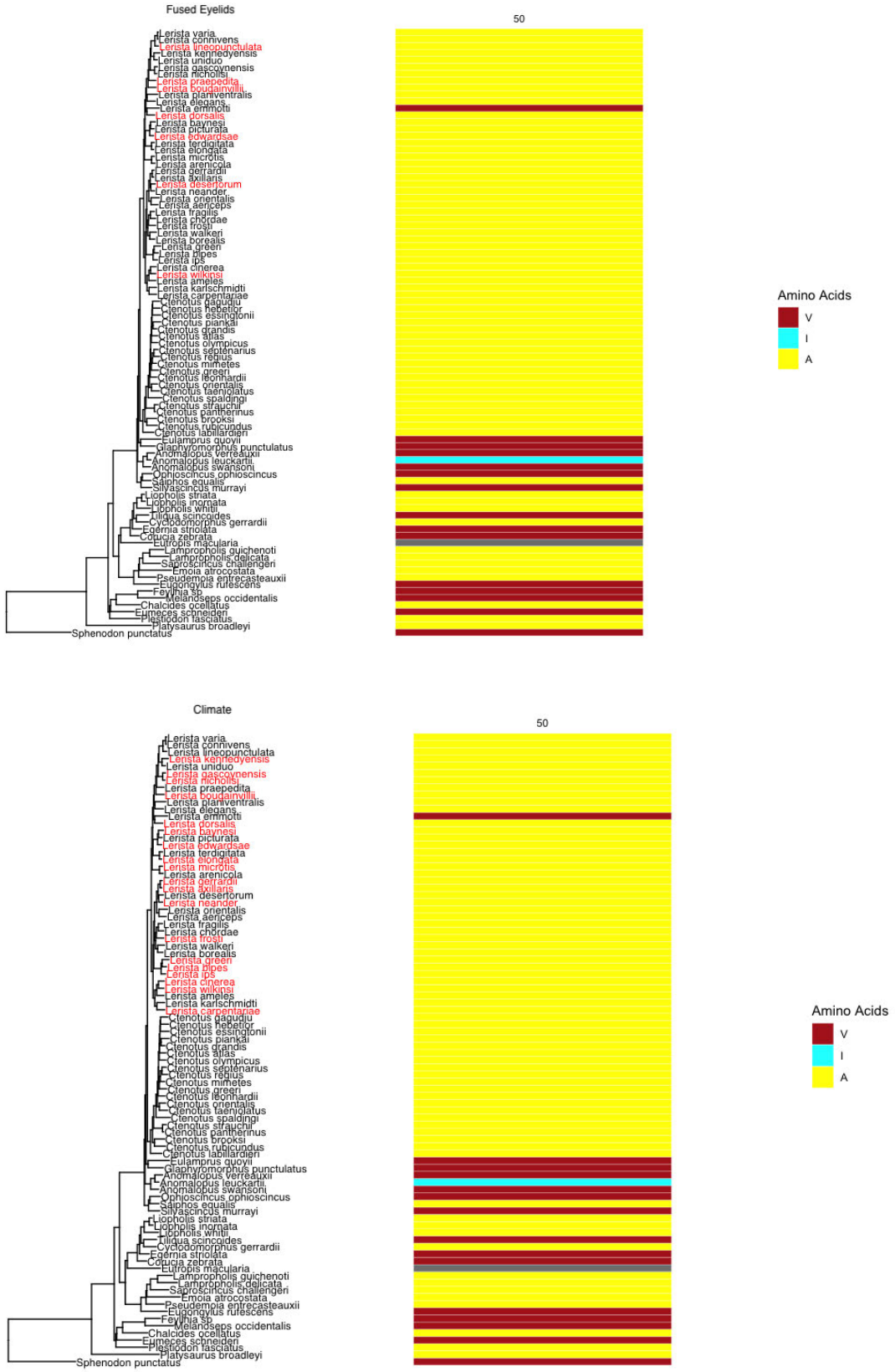
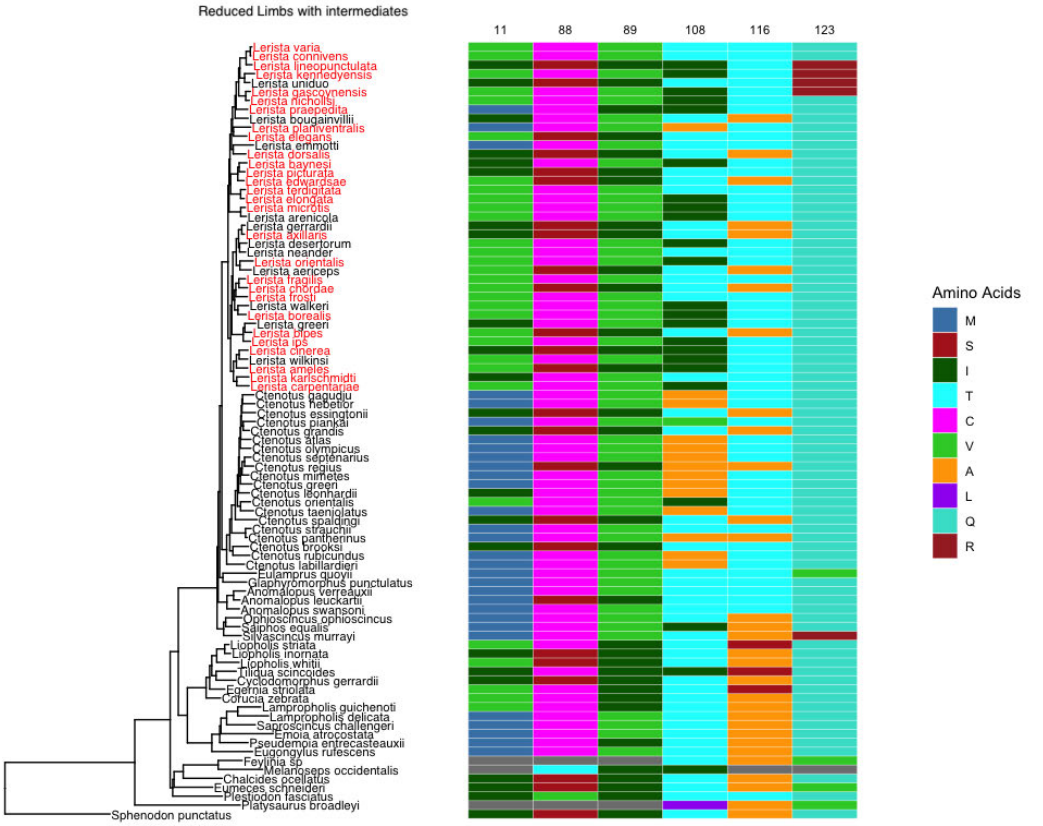


Figure 2.3. Phylogenetic tree showing group partitions as labelled, and selected sites in SWS1 with corresponding amino acids


Reduced Limbs with intermediates
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88
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123
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Lerista connivens
Lerista lineopunctulata
Lerista kendedyensis
Lerista uniduo
Lerista gascoynensis
Lerista nichollsi
Lerista praepedita
Lerista bougainvillii
Lerista planiventralis
Lerista elegans
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Lerista walkeri
Lerista borealis
Lerista greeri
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Lerista ips
Lerista cinerea
Lerista wilkinsi
Lerista ameles
Lerista karlschmidti
Lerista carpentariae
Ctenotus pagudu
Ctenotus hebetior
Ctenotus essingtonii
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Ctenotus grandis
Ctenotus atlas
Ctenotus olympicus
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Ctenotus regius
Ctenotus mimetes
Ctenotus greeri
Ctenotus leonhardii
Ctenotus orientalis
Ctenotus taeniolatus
Ctenotus spaldingi
Ctenotus strauchii
Ctenotus pantherinus
Ctenotus brooksi
Ctenotus rubicundus
Ctenotus labillardieri
Eulamprus quoyii
Glaphyromorphus punctulatus
Anomalopus verreauxii
Anomalopus leuckartii
Anomalopus swansoni
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Saiphos equalis
Silvascincus murrayi
Liopholis striata
Liopholis inornata
Liopholis whitii
Tiliqua scincoides
Cyclodomorphus gerrardii
Egernia striolata
Corucia zebrata
Lampropholis guichenoti
Lampropholis delicata
Saproscincus challengeri
Emoia atrocostata
Pseudemoia entrecasteauxii
Eugongylus rufescens
Feylinia sp
Melanoseps occidentalis
Chalcides ocellatus
Eumeces schneideri
Plestiodon fasciatus
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Sphenodon punctatus
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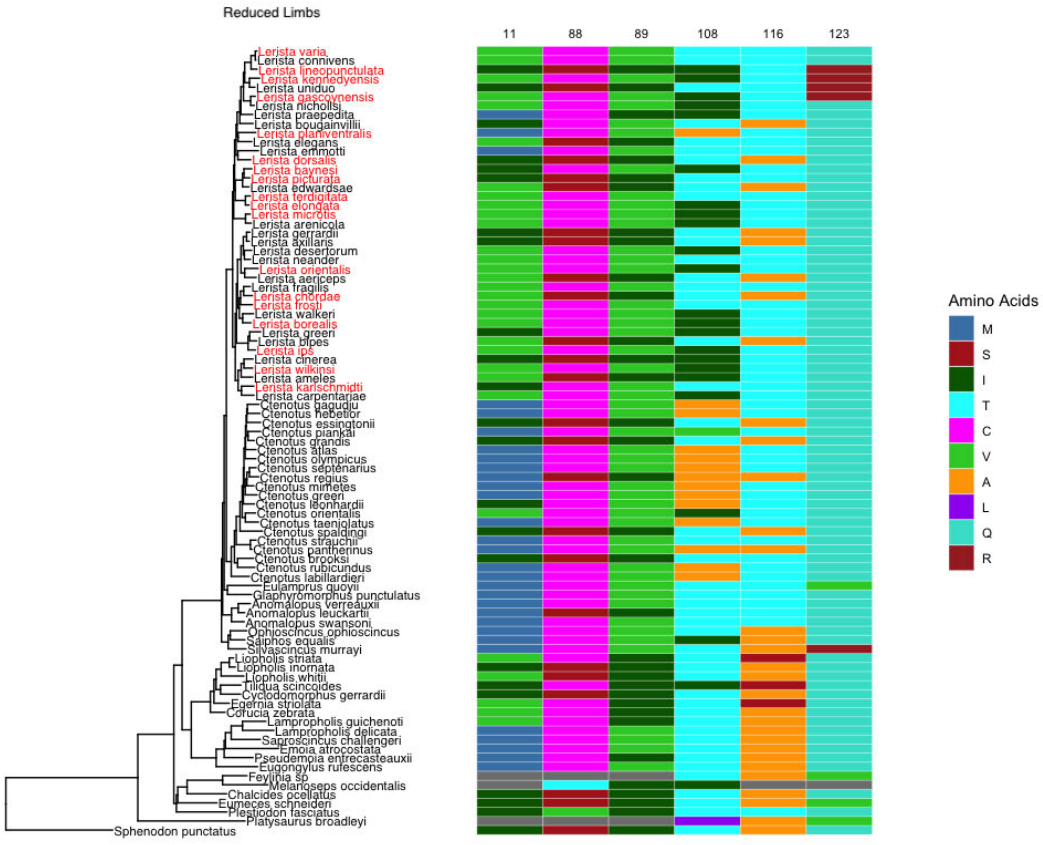
Reduced Limbs
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Lerista varia
Lerista connivens
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Lerista kendedyensis
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Lerista ameles
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Lerista carpentariae
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Ctenotus septenarius
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Melanoseps occidentalis
Chalcides ocellatus
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Plestiodon fasciatus
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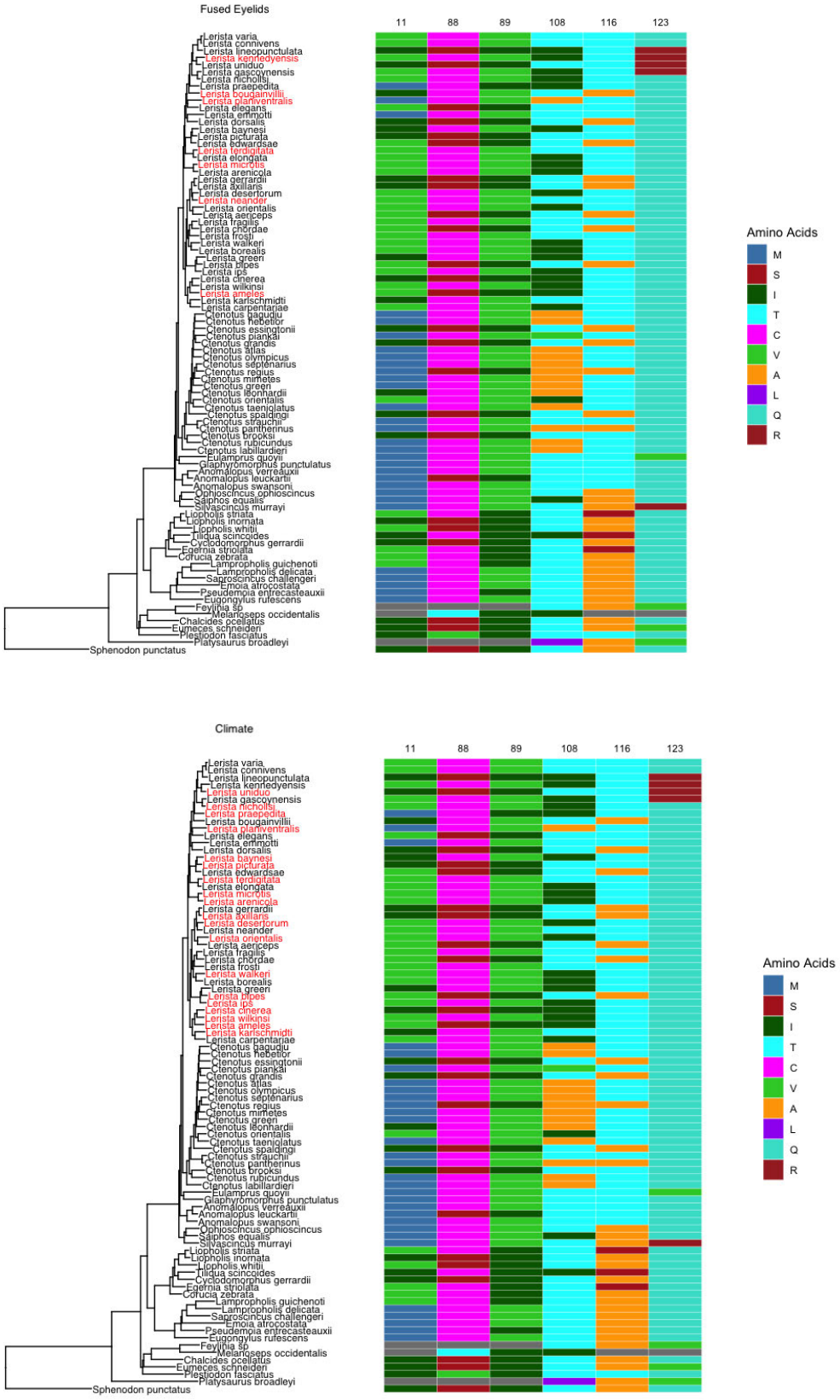

Figure 2.4. Phylogenetic tree showing group partitions as labelled, and selected sites in SWS2 with corresponding amino acid

Supplementary material for Chapter 3

Table 3.1. List of genes used in this study: where NCBI or other provenance is not given, genes were BLASTed from the genome quoted by NCBI genome ID

Species	Provenance	MTNR1A	OPN4m	OPN4x	OPN5	OPN6	OPN8	OPN7	OPN3	PP	PRT	RRH	PIN	RGR	TMT	TMTa	VAOP
Alligator mississippiensis	Genome ID:13409			XM_014599949	XM_014601607	XM_006276156.3		XM_019500545.1				XM_006264079		XM_006270171	XM_019478701.1		XM_006272964
Alligator sinensis	Genome ID:22419			XM_006020048	XM_006027399.1	XM_006036562.3		XM_025197612.1					XM_006016114	XM_006029497			XM_006027015
Anolis carolinensis	Genome ID:708			XM_008119109.2	XM_008116579	XM_003223528.3	XM_003215369.3	XM_003215935.2	XM_008123887.2	NM_001293131.1		XM_008112114.2	XM_016997727.1	XM_016995001.1	XM_008106312.2	XM_008107067.1	GQ280392
Anomalopus brevicollis	Transcriptome (Chapter 2, this thesis)																
Anser cygnoides	Genome ID:31397				XM_013194527.1	XM_013183518.2	XM_013184430.2	XM_013170590.2					XM_013196444.2				XM_013198742.2
Apalone spinifera	Genome ID:15301				KB930558.1												
Aquila chrysaetos	Genome ID:32031				XM_030037957.1	XM_030012199.2	XM_041128832.1	XM_030035897.2					XM_030028164.1			XM_029998897.1	XM_030030613.2
Arizona elegans	Schott et al, 2018																
Cemophora coccinea	Schott et al, 2018																
Chalcides ocellatus	Transcriptome (Chapter 2, this thesis)																
Chamaeleo calyptratus	Pinto et al, 2019b																
Chelonia mydas	Genome ID:13308			XM_007063033	XM_007068312 3	XR_005224340.2	XM_037893900.2	XM_007057945.3				XM_007054417	XM_007057737	XM_007067237	XM_043522965.1	XM_043536384.1	XM_007053023

Chrysemys picta	Genome ID:12107			XM_005278461	XM_005297358 2	XM_042854872.1	XM_005312962.3	XM_005296341.3	XM_005300525		XM_005287919	XM_005298149	XM_005294019	XM_005282491.2	XM_005306967.3	XM_005305330
Correlophus ciliatus	Pinto et al, 2019a															
Crocodylus porosus	Genome ID:13505				XM_019538095.1	XM_019548112.1		XM_019549738.1								XM_019555539.1
Crotalus tigris	Genome ID:97744	XM_039322834.1		XM_039332172				XM_039352295.1	XM_039349111		XM_039338352					XM_039337400
Ctenotus atlas	Transcriptome (Chapter 2, this thesis)															
Deinagkistrodon acutus	Yin et al, 2016 DOI:10.5524/100196															
Eublepharis macularius	Xiong et al, 2016 DOI:10.5524/100246															
Eumeces schneideri	Transcriptome (Chapter 2, this thesis)															
Eutropis macularia	Transcriptome (Chapter 2, this thesis)															
Gallus gallus	Genome ID:111		NM_204625	NM_204625	NM_001130743	NM_001310056.3	NM_001162892	XM_040668797.2	XM_426139		NM_001079759	GGU15762	NM_001031216		NM_001318431.2	NM_001310089
Gavialis gangeticus	Genome ID:14671				XM_019520694.1	XM_019510257.1		XM_019527699.1								XM_019502211.1
Gehyra mutilata	Pinto et al, 2019a															
Gekko japonicus	Genome ID:40475	XM_015417572.1		XM_015418685	XM_015413052.1	XM_015414046.1		XM_015423210.1				XM_015421659.1		XM_015411222.1		XM_015426886
Glaphyromorphus punctulatus	Transcriptome (Chapter 2, this thesis)															
Hemidactylus	Pinto et al,															

turcicus	2019a													
Homo sapiens	Genome ID:51	NM_005958	AF147788.1		AY377391				AF303588			NM_006583	AH005747	
Hypsiglena torquata	Schott et al, 2018													
Lacerta agilis	Genome ID:18390	XM_033160356.1							XM_033144352	XM_033142567		XM_033160502		
Lacerta bilineata	Genome ID:41613													
Lacerta viridis	Genome ID:69465													
Lampropeltis getula	Schott et al, 2018													
Lerista dorsalis	Transcriptome (Chapter 2, this thesis)													
Lerista edwardsae	Transcriptome (Chapter 2, this thesis)													
Lerista terdigitata	Transcriptome (Chapter 2, this thesis)													
Lialis burtonis	Pinto et al, 2019a													
Liopholis inornata	Transcriptome (Chapter 2, this thesis)													
Masticophis flagellum	Schott et al, 2018													
Melopsittacus undulatus	Genome ID:10765	AGA 01067676.1		XM_013128944.3	XM_005146432 2	XM_005153537.2	XM_005146417.3	XM_013128056.2	XM_005146009.4		XM_005148624.3	XM_005153631.2	XM_013129619.3	XM_005144165.3
Mus musculus	Genome ID:52		NM_013887.2						NM_010098		NM_009102	AF076930		
Notechis scutatus	Genome ID:14408	XM_026666983.1		XM_026672626				XM_026669312.1						XM_026670667

Ophiophagus hannah	Genome ID:10842												AZIM01000864.1			
Ophisaurus gracilis	Song et al, 2015 DOI:10.5524/100119															
Pantherophis guttatus	Schott et al, 2018		XM_034440490	XM_034426817				XM_034428267		XM_034442371					XM_034436530	
Paroedura picta	Genome ID:41304															
Pelodiscus sinensis	Genome ID:14578		XM_006111948	XM_006120685	XM_025180000.1	XM_006120664.3	XM_006131291.3	XM_014570659		XM_006119280	XM_006138728	XM_006112389		XM_006114560.3	XM_006136623	
Phelsuma laticauda	Pinto et al, 2019a															
Platysaurus broadleyi	Transcriptome (Chapter 2, this thesis)															
Podarcis muralis	Genome ID:8765	XM_028744209.1		XM_028721679					XM_028721041	XM_028744046		XM_028729238.1			XM_028729857	
Pogona vitticeps	Genome ID:7589							XM_020788365	XM_028721041 XM_028048200.1			XM_020796842.1			XM_020795006	
Protobothrops mucrosquamatus	Genome ID:18192	XM_015831814.1	XM_029284085					XM_015816690		XM_015829314		XM_029285569.1			XM_015813972	
Pseudonaja textilis	Genome ID:72610	XM_026697799.1	XM_026697858							XM_026698217		XM_026714548.1			XM_026710510	
Ptyas mucosa	Genome ID:44753															
Python bivittatus	Genome ID:17893	XM_007444881 2		XM_015889325				XM_007440093				XM_007442104 2				
Salvator merianae	Genome ID:72628															
Sceloporus tristichus	Genome ID:98233															

Shinisaurus crocodilurus	Genome ID:7383																
Sphenodon punctatus	Genome ID:7296																
Struthio camelus	Genome ID:122	XM_009665889.1			XM_009667941	XM_009683051.1	XM_009667980.1	XM_009686644.1	XM_009681115		XM_009665408				LOC104138067	XM_009670510.1	
Taeniopygia guttata	Genome ID:367				XM_002189219	XM_002193694.5	XM_002189830.4	XM_002193694.5			XM_002195191	XM_002198907	XM_002193290			NM_001279265	
Thamnophis sirtalis	Schott et al, 2018			XM_014063405							XM_032224421					XM_032232002	
Tiliqua rugosa	Transcriptome (Chapter 2, this thesis)																
Varanus komodoensis	Genome ID:79093																
Zootoca vivipara	Genome ID:37102								XM_035110527	XM_035116352	XM_035113908					XM_035137803	
Aptenodytes forsteri	Genome ID:32061	XM_009277383	XM_009272386	XM_009273539.2	XP_009287241.1	XM_009285624.1	XM_009288966	XM_009283794.1	XM_009276182		XM_009273583		XM_009282910	XM_019471220.1	LOC109279376	XM_019471670.1	
Canis lupus familiaris	Genome ID:85	XM_038690395	XM_022273694		XP_038538775.1				XM_038671508		XM_038444375		XM_038663105				
Ornithorhynchus anatinus	Genome ID:110	XM_001519082	XP_039767258.1_1		XP_028928374.1						XM_001506366		XM_029061296.1	XP_039770534.1			
Phascolarctos cinereus	Genome ID:23339	XM_020968493	XM_020994724.1		XP_020849609.1				XP_020837717.1		XM_020857087.1_1				XP_020843599.1		
Loxodonta africana	Genome ID:224	XM_003415638.1	XP_023414389.1		XP_023398756.1_1				XM_003410915		XM_003410397		XM_003418559				

Supplementary material for Chapter 4

Table 4.1: Site CodeML results

Models	Sites Under Positive Selection (BEB)
3. OPN4X	
E. M2a	-
F. M8 (β&ω)	432
4. PIN	
G. M2a	-
H. M8 (β&ω)	8
5. RRH	
I. M2a	-
J. M8 (β&ω)	143
6. VAOP	
K. M2a	-
L. M8 (β&ω)	329

Table 4.2. Branch CodeML results

Models	$\omega(d_N/d_S)$	D.F.	Models Compared	2Δ (ln *L*)	P
2. *OPN4m*					
G. All branches have one ω	ω=0.232	-	-		
H. Nocturnal, ω1; Diurnal, ω2	ω1=1.576, ω2=0.225	1	H vs. G	11.45972	0.000711
I. Snakes, ω1; Lizards, ω2; background, ω3	/	/	/		/
J. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω3=0.353, ω4=0.206	1	J vs. G	18.17285	0.00002
K. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω3=0.232, ω4=0.563	1	K vs. G	0.000018	1
K. Lacertoidea, ω1; background ω2	ω1=0.205, ω2=0.239	1		1.374498	0.241041

K. Toxicofera, ω1; background, ω2	ω1=0.210, ω2=0.250	1		2.751318	0.097174
L. Each branch has its own ω	Variable by branch	45	L vs. G	87.9846	0.000134
3. *OPN4x*					
M. All branches have one ω	ω=0.292	-	-		
N. Nocturnal, ω1; Diurnal, ω2	ω1=0.239, ω2=0.304	1	N vs. M	5.232962	0.022163
O. Snakes, ω1; Lizards, ω2; background, ω3	ω1=0.390, ω2=0.266, ω3=0.297	2	O vs. M	20.59361	0.000034
P. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.235, ω2=0.390, ω3=0.397, ω4=0.244	3	P vs. M	46.19442	0
Q. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.234, ω2=0.390, ω3=0.278, ω4=0.295	3	Q vs. M	23.73356	0.000028
R. Lacertoidea, ω1; background, ω2	ω1=0.249, ω2=0.299	1	Q vs. M	2.809894	0.093685
R. Toxicofera, ω1; background, ω2	ω1=0.299, ω2=0.286	1	Q vs. M	0.387058	0.53385
R. Each branch has its own ω	Variable by branch	91	R vs. M	292.4613	0
4. *OPN5*					
S. All branches have one ω	ω=0.207	-	-		
T. Nocturnal, ω1; Diurnal, ω2	ω1=0.220, ω2=0.204	1	T vs. S	0.405008	0.524514
U. Snakes, ω1; Lizards, ω2; background, ω3	ω1=0.324, ω2=0.193, ω3=0.127	2	U vs. S	29.27792	0
V. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.173, ω2=0.325, ω3=0.269, ω4=0.181	3	V vs. S	31.00762	0.000001
W. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.174, ω2=0.324, ω3=0.210, ω4=0.127	3	W vs. S	32.26204	0
X. Lacertoidea, ω1; background, ω2	ω1=0.182, ω2=0.210	1	X vs. S	0.865198	0.352288
Y. Toxicofera, ω1; background, ω2	ω1=0.252, ω2=0.180	1	X vs. S	13.38232	0.000254
X. Each branch has its own ω	Variable by branch	83	X vs. S	220.2031	0
5. *OPN6*					
Y. All branches have one ω	ω=0.209	-	-		
Z. Nocturnal, ω1; Diurnal, ω2	ω1=0.119, ω2=0.229	1	Z vs. Y	9.63044	0.00191
AA. Snakes, ω1; Lizards, ω2; background, ω3	/	/	/	/	/
AB. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.107, ω4=0.235	1	AB vs. Y	19.7966	0.000009

AC. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.107, ω3=0.242, ω4=0.179	2	AC vs. Y	20.93585	0.000028
A. Lacertoidea, ω1; background, ω2	ω1=0.224, ω2=0.205	1	AD vs. Y	0.359958	0.54853
A. Toxicofera, ω1, background, ω2	ω1=0.252, ω2=0.177	1	AD vs. Y	8.44795	0.003655
AD. Each branch has its own ω	Variable by branch	31	AD vs. Y	107.4249	0
6. *OPN8*					
AE. All branches have one ω	ω=0.233	-	-		
AF. Nocturnal, ω1; Diurnal, ω2	ω1=0.824, ω2=0.233	1	AF vs. AE	6.60E-05	1
AG. Snakes, ω1; Lizards, ω2; background, ω3	/	/	/		/
AH. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	/	/	/		/
AI. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω3=0.239, ω4=0.062	1	AI vs. AE	0.874884	0.349607
A. Lacertoidea, ω1; background, ω2	ω1=0.247, ω2=0.195	1	AD vs. Y	2.214194	0.1367465
A. Toxicofera, ω1, background, ω2	ω1=0.260, ω2=0.199	1	AD vs. Y	3.743536	0.053012
AJ. Each branch has its own ω	Variable by branch	25	AJ vs. AE	36.00727	0.07149
7. *OPN7*					
AJ. All branches have one ω	ω=0.152	-	-		
AK. Nocturnal, ω1; Diurnal, ω2	ω1=0.270, ω2=0.135	1	AK vs. AJ	25.60122	0
AL. Snakes, ω1; Lizards, ω2; background, ω3	ω1=0.244, ω2=0.138, ω3=0.081	2	AL vs. AJ	29.72583	0.00E+00
AM. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.217, ω2=0.246, ω3=0.191, ω4=0.078	3	AM vs. AJ	93.68588	0
AN. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.217, ω2=0.246, ω3=0.106, ω4=0.080	3	AN vs. AJ	64.79505	0
AN. Lacertoidea, ω1; background, ω2	ω1=0.103, ω2=0.159	1	AO vs. AJ	6.082188	0.013655
Toxicofera, ω1; background	ω1=0.144, ω2=0.158	1		0.884842	0.3468789
AO. Each branch has its own ω	Variable by branch	91	AO vs. AJ	193.129	0
8. *OPN3*					
AP. All branches have one ω	ω=0.167	-	-		
AQ. Nocturnal, ω1; Diurnal, ω2	ω1=0.236, ω2=0.155	1	AQ vs. AP	8.600772	0.00336

AR. Snakes, ω1; Lizards, ω2; background, ω3	ω1=0.237, ω2=0.155, ω3=0.126	2	AR vs. AP	13.12024	0.001416
AS. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.155, ω2=0.237, ω3=0.195, ω4=0.139	3	AS vs. AP	16.36588	0.000954
AT. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.155, ω2=0.237, ω3=0.153, ω4=0.133	3	AT vs. AP	12.6371	0.005491
AU. Lacertoidea, ω1; background, ω2	ω1=0.119, ω2=0.174	1	AU vs. AP	5.526586	0.01873
AV. Toxicofera, ω1; background, ω2	ω1=0.187, ω2=0.152	1	AU vs. AP	3.726992	0.05354
AU. Each branch has its own ω	Variable by branch	91	AU vs. AP	180.3277	0
9. *PP*					
AV. All branches have one ω	ω=0.227	-	-		
AW. Nocturnal, ω1; Diurnal, ω2	ω1=0.303, ω2=0.211	1	AW vs. AV	3.4534	0.063122
AX. Snakes, ω1; Lizards, ω2; background, ω3	/	/	/		/
AY. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω3=0.242, ω4=0.227	1	AY vs. AV	0.001966	0.964634
AZ. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω3=0.211, ω4=0.303	1	AZ vs. AV	3.4534	0.063122
AU. Lacertoidea, ω1; background, ω2	ω1=0.223, ω2=0.239	1	AU vs. AP	0.13936	0.7089181
AV. Toxicofera, ω1; background, ω2	ω1=0.200, ω2=0.266	1	AU vs. AP	3.302416	0.069178
BA. Each branch has its own ω	Variable by branch	27	BA vs. AV	68.83611	0.000016
10. *PRT*					
BB. All branches have one ω	ω=0.151	-	-		
BC. Nocturnal, ω1; Diurnal, ω2	ω1=0.202, ω2=0.149	1	BC vs. BB	0.283094	0.59468
BD. Snakes, ω1; Lizards, ω2; background, ω3	/	/	/		/
BE. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω3=0.203, ω4=0.150	1	BE vs. BB	0.2639	0.607453
BF. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω3=0.151, ω4=0.844	1	BF vs. BB	0.00289	0.957127
AU. Lacertoidea, ω1; background, ω2	ω1=0.142, ω2=0.153	1	AU vs. AP	0.106808	0.743808
AV. Toxicofera, ω1; background, ω2	ω1=0.150, ω2=0.154	1	AU vs. AP	0.020488	0.886183
BG. Each branch has its own ω	Variable by branch	23	BG vs. BB	25.62312	0.318978
11. *RRH*					

BH. All branches have one ω	ω=0.157	-	-		
BI. Nocturnal, ω1; Diurnal, ω2	ω1=0.208, ω2=0.147	1	BI vs. BH	5.542704	0.018558
BJ. Snakes, ω1; Lizards, ω2; background, ω3	ω1=0.246, ω2=0.150, ω3=0.067	2	BJ vs. BH	26.27883	0.000002
BK. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.183, ω2=0.246, ω3=0.122, ω4=0.125	3	BK vs. BH	24.06402	0.000024
BL. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.184, ω2=0.246, ω3=0.135, ω4=0.067	3	BL vs. BH	31.56189	0.000001
BN. Lacertoidea, ω1; background, ω2	ω1=0.069, ω2=0.171	1	BM vs. BH	21.06274	0.000004
B. Toxicofera, ω1; background, ω2	ω1=0.205, ω2=0.126	1	BM vs. BH	18.83365	0.000014
BM. Each branch has its own ω	Variable by branch	85	BM vs. BH	227.4965	0
12. *PIN*					
BN. All branches have one ω	ω=0.150	-	-		
BO. Nocturnal, ω1; Diurnal, ω2	ω1=0.1, ω2=0.167	1	BO vs. BN	9.13382	0.002509
BP. Snakes, ω1; Lizards, ω2; background, ω3	/	/	/		/
BQ. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.166, ω4=0.145	1	BQ vs. BN	0.765106	0.341735
BR. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.169, ω3=0.165, ω4=0.065	2	BR vs. BN	18.80639	0.000082
BS. Lacertoidea, ω1; background, ω2	ω1=0.151, ω2=0.149	1	BS vs. BN	0.002446	0.960555
BT. Toxicofera, ω1; background, ω2	ω1=0.172, ω2=0.134	1	BS vs. BN	3.578474	0.058533
BS. Each branch has its own ω	Variable by branch	27	BS vs. BN	99.99997	0
13. *RGR*					
BT. All branches have one ω	ω=0.163	-	-		
BU. Nocturnal, ω1; Diurnal, ω2	ω1=0.209, ω2=0.152	1	BU vs. BT	6.473856	0.010947
BV. Snakes, ω1; Lizards, ω2; background, ω3	ω1=0.232, ω2=0.146, ω3=0.121	2	BV vs. BT	18.30613	0.000106
BW. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.175, ω2=0.232, ω3=0.159, ω4=0.123	3	BW vs. BT	25.0946	0.000015
BX. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.175, ω2=0.232, ω3=0.134, ω4=0.121	3	BX vs. BT	22.69071	0.000047
BY. Lacertoidea, ω1; baackground, ω2	ω1=0.119, ω2=0.187	1	BY vs. BT	5.75647	0.016428
B. Toxicofera, ω1; background, ω2	ω1=0.177, ω2=0.151	1	BY vs. BT	2.575042	0.108561

BY. Each branch has its own ω	Variable by branch	95	BY vs. BT	192.4844	0
14. *TMT*					
BZ. All branches have one ω	ω=0.202	-	-		
CA. Nocturnal, ω1; Diurnal, ω2	ω1=0.354, ω2=0.183	1	CA vs. BX	18.50493	0.000017
CB. Snakes, ω1; Lizards, ω2; background, ω3	/	/	/		/
CC. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.323, ω3=0.233, ω4=0.148	2	CC vs. BX	45.47287	0
CD. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.323, ω3=0.164, ω4=0.124	2	CD vs. BX	41.96061	0
CE. Lacertoidea, ω1; background, ω2	ω1=0.139, ω2=0.216	1	CE vs. BX	8.052474	0.004544
C. Toxicofera, ω1; background, ω2	ω1=0.154, ω2=0.229	1	CE vs. BX	11.37735	0.000743
CE. Each branch has its own ω	Variable by branch	53	CE vs. BX	116.9634	0.000001
15. *TMTa*					
CF. All branches have one ω	ω=0.190	-	-		
CG. Nocturnal, ω1; Diurnal, ω2	ω1=0.302, ω2=0.177	1	CG vs. CF	7.909054	0.004919
CH. Snakes, ω1; Lizards, ω2; background, ω3	/	/	/		/
CI. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.265, ω4=0.167	1	CI vs. CF	10.60978	0.001125
CJ. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.265, ω3=0.184, ω4=0.095	2	CJ vs. CF	19.90291	0.000048
CI. Lacertoidea, ω1; background, ω2	ω1=0.159, ω2=0.198	1	CK vs. CF	1.91656	0.166236
C. Toxicofera, ω1; background, ω2	ω1=0.197, ω2=0.1851	1	CK vs. CF	0.251158	0.616261
CK. Each branch has its own ω	Variable by branch	29	CK vs. CF	60.33087	0.000562
16. *VAOP*					
CL. All branches have one ω	ω=0.191	-	-		
CM. Nocturnal, ω1; Diurnal, ω2	ω1=0.174, ω2=0.195	1	CM vs. CF	0.800516	0.370939
CN. Snakes, ω1; Lizards, ω2; background, ω3	ω1=0.206, ω2=0.190, ω3=0.161	2	CN vs. CF	1.481594	0.476734
CO. Geckos, ω1; Snakes, ω2; Skinks, ω3; background, ω4	ω1=0.194, ω2=0.206, ω4=0.184	2	CO vs. CF	0.835172	0.658635
CP. Geckos, ω1; Snakes, ω2; other Squamates, ω3; background,ω4	ω1=0.195, ω2=0.206, ω3=0.188, ω4=0.160	3	CP vs. CF	1.545456	0.67182

CQ. Lacertoidea, ω1; background, ω2	ω1=0.190, ω2=0.191	1	CQ vs. CF	0.002254	0.962134
C. Toxicofera, ω1; background, ω2	ω1=0.195, ω2=0.185	1	CQ vs. CF	0.281506	0.595716
CQ. Each branch has its own ω	Variable by branch	53	CQ vs. CF	52.93482	0.476682

Table 4.3. Branch-site CodeML results

Gene	Foreground branch	Sites under positive selection	Branch	ω				2ΔI	P Value
				0	1	2a	2b		
OPN5	S10A Nocturnal vs Diurnal	168 - 180 - 258	Nocturnal	0.07592 (0.64268)	1 (0.21017)	1 (0.11089)	1 (0.03626)	0.018146	**0.892843**
			Diurnal	0.07592 (0.64268)	1 (0.21017)	0.07592 (0.11089)	1 (0.03626)		
	S10B Geckos vs rest	/	Geckos	0.08409 (0.71045)	1 (0.21391)	1 (0.05814)	1 (0.01750)	8.00E-06	**0.997743**
			Rest	0.08409 (0.71045)	1 (0.21391)	0.08409 (0.05814)	1 (0.01750)		
	S10C Snakes vs rest	66 - 98 - 142 - 148 - 162 - 201 - 211 - 228 - 235 - 236 - 238 - 241 - 245 - 249 - 251 - 258 - 259 - 261	Snakes	0.06536 (0.55247)	1 (0.17498)	1 (0.20699)	1 (0.06556)	2.00E-06	**1**
			Rest	0.06536 (0.55247)	1 (0.17498)	0.06536 (0.20699)	1 (0.06556)		
	S10D Skinks vs rest	/	Skinks	0.09168 (0.73896)	1 (0.23922)	3.41225 (0.01649)	3.41225 (0.00534)	2.72058	**0.099062**
			Rest	0.09168 (0.73896)	1 (0.23922)	0.09168 (0.01649)	1 (0.00534)		
	Snakes Geckos Skinks vs rest	/	Snakes, Geckos, Skinks	0.07232 (0.68084)	1 (0.18052)	1 (0.10958)	1 (0.02906)	2.20E-05	0.996258
			Rest	0.07232 (0.68084)	1 (0.18052)	0.07232 (0.10958)	1 (0.02906)		
	Lacertoidea vs rest	/	Lacertoidea	0.09235 (0.75147)	1 (0.24383)	4.83715 (0.00355)	4.83715 (0.00115)	1.97439	0.159983

			Rest	0.09235 (0.75147)	1 (0.24383)	0.09235 (0.00355)	1 (0.00115)		
	Toxicofera vs rest	/	Toxicofera	0.08489 (0.70952)	1 (0.20887)	1 (0.06305)	1 (0.01856)	1.60E-05	0.996808
			Rest	0.08489 (0.70952)	1 (0.20887)	0.08489 (0.06305)	1 (0.01856)		
	S10A Nocturnal vs Diurnal	/	Nocturnal	0.06693 (0.60271)	1 (0.39729)	1 (0)	1 (0)	0.021998	**0.882092**
			Diurnal	0.06693 (0.60271)	1 (0.39729)	0.06693 (0)	1 (0)		
	S10B Geckos vs rest	/	Geckos	0.06693 (0.60270)	1 (0.39730)	1 (0)	1 (0)	1.20E-05	**1**
			Rest	0.06693 (0.60270)	1 (0.39730)	0.06693 (0)	1 (0)		
	S10C Snakes vs rest	/	Snakes	/	/	/	/	/	**/**
			Rest						
	S10D Skinks vs rest	/	Skinks	/	/	/	/	/	/
OPN6			Rest						
	Snakes Geckos Skinks vs rest	/	Snakes, Geckos, Skinks	0.06693 (0.60270)	1 (0.39730)	1 (0)	1 (0)	1.20E-05	1
			Rest	0.06693 (0.60270)	1 (0.39730)	0.06693 (0)	1 (0)		
	Lacertoidea vs rest	94	Lacertoidea	0.06008 (0.58157)	1 (0.38590)	1.98754 (0.01955)	1.98754 (0.01298)	0.59097	0.442044
			Rest	0.06008 (0.58157)	1 (0.38590)	0.06008 (0.01955)	1 (0.01298)		
	Toxicofera vs rest	/	Toxicofera	0.06693 (0.60272)	1 (0.39728)	1 (0)	1 (0)	6.20E-05	1
			Rest	0.06693 (0.60272)	1 (0.39728)	0.06693 (0)	1 (0)		
OPN8	S10A Nocturnal vs Diurnal	/	Nocturnal	0.11051 (0.64487)	1 (0.18960)	2.77306 (0.12792)	2.77306 (0.03761)	1.60E-05	**1**

			Diurnal	0.11051 (0.64487)	1 (0.18960)	0.11051 (0.12792)	1 (0.03761)		
	S10B Geckos vs rest	/	Geckos	/	/	/	/	/	/
			Rest						
	S10C Snakes vs rest	/	Snakes	/	/	/	/	/	/
			Rest						
	S10D Skinks vs rest	/	Skinks	/	/	/	/	/	/
			Rest						
	Snakes Geckos Skinks vs rest	/	Snakes, Geckos, Skinks	/	/	/	/	/	/
			Rest						
	Lacertoidea vs rest	/	Lacertoidea	0.11092 (0.77382)	1 (0.22439)	3.87342 (0.00138)	3.87342 (0.00040)	0.158844	0.690223
			Rest	0.11092 (0.77382)	1 (0.22439)	0.11092 (0.00138)	1 (0.00040)		
	Toxicofera vs rest	148 - 336	Toxicofera	0.10755 (0.76429)	1 (0.20340)	1 (0.02552)	1 (0.00679)	0.00E+00	1
			Rest	0.10755 (0.76429)	1 (0.20340)	0.10755 (0.02552)	1 (0.00679)		
	S10A Nocturnal vs Diurnal	39 - 359	Nocturnal	0.07256 (0.80959)	1 (0.11698)	1.20543 (0.06416)	1.20543 (0.00927)	0.267352	**0.605114**
			Diurnal	0.07256 (0.80959)	1 (0.11698)	0.07256 (0.06416)	1 (0.00927)		
OPN7	S10B Geckos vs rest	4 - 6 - 81 - 195	Geckos	0.07162 (0.81788)	1 (0.11263)	1.24570 (0.06108)	1.24570 (0.00841)	0.73762	**0.390424**
			Rest	0.07162 (0.81788)	1 (0.11263)	0.07162 (0.06108)	1 (0.00841)		
	S10C Snakes vs rest	25 - 39 - 148 - 361 - 375 - 379 - 381	Snakes	0.06563 (0.78344)	1 (0.10988)	1.26007 (0.09356)	1.26007 (0.01312)	0.978554	0.322556
			Rest	0.06563 (0.78344)	1 (0.10988)	0.06563 (0.09356)	1 (0.01312)		

	S10D Skinks vs rest	/	Skinks	0.07557 (0.83033)	1 (0.12761)	1.05585 (0.03645)	1.05585 (0.00560)	0.00636	0.936436
			Rest	0.07557 (0.83033)	1 (0.12761)	0.07557 (0.03645)	1 (0.00560)		
	Snakes Geckos Skinks vs rest	4 - 6 - 25 - 81 - 110 - 141 - 148 - 160 - 161 - 195 - 277 - 348 - 352 - 353 - 379	Snakes, Geckos, Skinks	0.05524 (0.76333)	1 (0.06680)	1 (0.15620)	1 (0.01367)	0	1
			Rest	0.05524 (0.76333)	1 (0.06680)	0.05524 (0.15620)	1 (0.01367)		
	Lacertoidea vs rest	/	Lacertoidea	0.07900 (0.86163)	1 (0.13470)	2.2184 (0.00318)	2.2184 (0.00050)	0.150654	0.697911
			Rest	0.07900 (0.86163)	1 (0.13470)	0.07900 (0.00318)	1 (0.00050)		
	Toxicofera vs rest	148 - 336	Toxicofera	0.07009 (0.82656)	1 (0.12419)	1 (0.04281)	1 (0.00643)	2.00E-06	1
			Rest	0.07009 (0.82656)	1 (0.12419)	0.07009 (0.04281)	1 (0.00643)		
	S10A Nocturnal vs Diurnal	/	Nocturnal	0.09369 (0.79094)	1 (0.12681)	1 (0.07088)	1 (0.01137)	2.00E-06	**1**
			Diurnal	0.09369 (0.79094)	1 (0.12681)	0.09369 (0.07088)	1 (0.01137)		
	S10B Geckos vs rest	/	Geckos	0.09410 (0.81938)	1 (0.13735)	1.20905 (0.03706)	1.20905 (0.00621)	0.24348	**0.621704**
			Rest	0.09410 (0.81938)	1 (0.13735)	0.09410 (0.03706)	1 (0.00621)		
OPN3	S10C Snakes vs rest	8 - 31 - 100 - 184 - 272 - 290 - 360	Snakes	0.08839 (0.74753)	1 (0.12219)	1 (0.11198)	1 (0.01830)	0	**1**
			Rest	0.08839 (0.74753)	1 (0.12219)	0.08839 (0.11198)	1 (0.01830)		
	S10D Skinks vs rest	132	Skinks	0.09431 (0.79645)	1 (0.13463)	1.32625 (0.05895)	1.32625 (0.00997)	0.37453	0.540545
			Rest	0.09431 (0.79645)	1 (0.13463)	0.09431 (0.05895)	1 (0.00997)		
	Snakes Geckos Skinks vs rest	/	Snakes, Geckos, Skinks	0.09210 (0.81488)	1 (0.11393)	1 (0.06246)	1 (0.00873)	0	1

			Rest	0.09210 (0.81488)	1 (0.11393)	0.09210 (0.06246)	1 (0.00873)		
	Lacertoidea vs rest	/	Lacertoidea	0.10144 (0.85292)	1 (0.14708)	1 (0)	1 (0)	0	1
			Rest	0.10144 (0.85292)	1 (0.14708)	0.10144 (0)	1 (0)		
	Toxicofera vs rest	/	Toxicofera	0.08496 (0.77627)	1 (0.12405)	1 (0.08594)	1 (0.01373)	0	1
			Rest	0.08496 (0.77627)	1 (0.12405)	0.08496 (0.08594)	1 (0.01373)		
	S10A Nocturnal vs Diurnal	279	Nocturnal	0.10082 (0.70990)	1 (0.24266)	38.66169 (0.03536)	38.66169 (0.01208)	3.845374	**0.049883**
			Diurnal	0.10082 (0.70990)	1 (0.24266)	0.10082 (0.03536)	1 (0.01208)		
	S10B Geckos vs rest	/	Geckos	/	/	/	/	/	**/**
			Rest						
	S10C Snakes vs rest	/	Snakes	/	/	/	/	/	**/**
			Rest						
OPN4M	S10D Skinks vs rest	281 - 306 - 337 - 348 - 352 - 396 - 406 - 463 - 464 - 481 - 482	Skinks	0.09078 (0.67863)	1 (0.20941)	3.16900 (0.08555)	3.16900 (0.02640)	19.48978	0.00001
			Rest	0.09078 (0.67863)	1 (0.20941)	0.09078 (0.08555)	1 (0.02640)		
	Snakes Geckos Skinks vs rest	281 - 306 - 337 - 348 - 352 - 396 - 406 - 463 - 464 - 481 - 482	Snakes, Geckos, Skinks	0.09078 (0.67863)	1 (0.20941)	3.16900 (0.08555)	3.16900 (0.02640)	19.48978	0.00001
			Rest	0.09078 (0.67863)	1 (0.20941)	0.09078 (0.08555)	1 (0.02640)		
	Lacertoidea vs rest	/	Lacertoidea	0.13641 (0.68788)	1 (0.30886)	1.10765 (0.00225)	1.10765 (0.00101)	0.002158	0.962948
			Rest	0.13641 (0.68788)	1 (0.30886)	0.13641 (0.00225)	1 (0.00101)		
	Toxicofera vs rest	/	Toxicofera	0.10325 (0.73431)	1 (0.26569)	1 (0)	1 (0)	0	1

			Rest	0.10325 (0.73431)	1 (0.26569)	0.10325 (0)	1 (0)		
	S10A Nocturnal vs Diurnal	/	Nocturnal	0.13316 (0.66179)	1 (0.29363)	1 (0.03088)	1 (0.01370)	1.302176	**1**
			Diurnal	0.13316 (0.66179)	1 (0.29363)	0.13316 (0.03088)	1 (0.01370)		
	S10B Geckos vs rest	/	Geckos	0.13575 (0.68566)	1 (0.29829)	1.75339 (0.01118)	1.75339 (0.00487)	0.41491	**0.519488**
			Rest	0.13575 (0.68566)	1 (0.29829)	0.13575 (0.01118)	1 (0.00487)		
	S10C Snakes vs rest	13 - 21 - 27 - 228 - 329 - 336 - 353 - 377 - 480	Snakes	0.11796 (0.60823)	1 (0.24939)	1.44754 (0.10098)	1.44754 (0.04140)	3.762218	**0.052423**
			Rest	0.11796 (0.60823)	1 (0.24939)	0.11796 (0.10098)	1 (0.04140)		
OPN4X	S10D Skinks vs rest	71 - 88 - 90 - 92 - 94 - 95 - 97 - 215 - 216 - 217 - 218 - 219 - 221 - 239 - 267 - 268 - 269 - 270 - 340	Skinks	0.10997 (0.58672)	1 (0.25151)	2.16608 (0.11323)	2.16608 (0.04854)	24.50236	0
			Rest	0.10997 (0.58672)	1 (0.25151)	0.11323 (0.11323)	1 (0.04854)		
	Snakes Geckos Skinks vs rest	224	Snakes, Geckos, Skinks	0.11385 (0.61634)	1 (0.21539)	1 (0.12470)	1 (0.04358)	2.00E-06	1
			Rest	0.11385 (0.61634)	1 (0.21539)	0.11385 (0.12470)	1 (0.04358)		
	Lacertoidea vs rest	/	Lacertoidea	0.13641 (0.68788)	1 (0.30886)	1.10765 (0.00225)	1.10765 (0.00101)	0.002158	0.962948
			Rest	0.13641 (0.68788)	1 (0.30886)	0.13641 (0.00225)	1 (0.00101)		
	Toxicofera vs rest	34 - 329	Toxicofera	0.12373 (0.64917)	1 (0.25329)	1 (0.07016)	1 (0.02737)	2.00E-06	1
			Rest	0.12373 (0.64917)	1 (0.25329)	0.12373 (0.07016)	1 (0.02737)		
PIN	S10A Nocturnal vs Diurnal	/	Nocturnal	0.08486 (0.76459)	1 (0.23541)	1 (0)	1 (0)	0	**1**

		Diurnal	0.08486 (0.76459)	1 (0.23541)	0.08486 (0)	1 (0)		
S10B Geckos vs rest	45	Geckos	0.08101 (0.74890)	1 (0.21022)	1.37597 (0.03192)	1.37597 (0.00896)	0.259504	**0.610461**
		Rest	0.08101 (0.74890)	1 (0.21022)	0.08101 (0.03192)	1 (0.00896)		
S10C Snakes vs rest	/	Snakes	/	/	/	/	/	**/**
		Rest						
S10D Skinks vs rest	/	Skinks	/	/	/	/	/	/
		Rest						
Snakes Geckos Skinks vs rest	45	Snakes, Geckos, Skinks	0.08101 (0.74890)	1 (0.21022)	1.37597 (0.03192)	1.37597 (0.00896)	0.259504	**0.610461**
		Rest	0.08101 (0.74890)	1 (0.21022)	0.08101 (0.03192)	1 (0.00896)		
Lacertoidea vs rest	/	Lacertoidea	0.08114 (0.73627)	1 (0.23183)	1 (0.02427)	1 (0.00764)	0	1
		Rest	0.08114 (0.73627)	1 (0.23183)	0.08114 (0.02427)	1 (0.00764)		
Toxicofera vs rest	1	Toxicofera	0.07706 (0.71863)	1 (0.15783)	1 (0.10130)	1 (0.02225)	0	1
		Rest	0.07706 (0.71863)	1 (0.15783)	0.07706 (0.10130)	1 (0.02225)		
S10A Nocturnal vs Diurnal	/	Nocturnal	0.09005 (0.66812)	1 (0.17737)	1.36190 (0.12209)	1.36190 (0.03241)	0.172048	**0.678298**
		Diurnal	0.09005 (0.66812)	1 (0.17737)	0.09005 (0.12209)	1 (0.03241)		
S10B Geckos vs rest	/	Geckos	/	/	/	/	/	/
		Rest						
S10C Snakes vs rest	/	Snakes	/	/	/	/	/	**/**
		Rest						

PP

S10D Skinks vs rest	/	Skinks	0.11143 (0.78208)	1 (0.20775)	15.23374 (0.00803)	15.23374 (0.00213)	0.036342	0.848811
		Rest	0.11143 (0.78208)	1 (0.20775)	0.11143 (0.00803)	1 (0.00213)		
Snakes Geckos Skinks vs rest	/	Snakes, Geckos, Skinks	0.11143 (0.78208)	1 (0.20775)	15.23374 (0.00803)	15.23374 (0.00213)	0.036342	0.848811
		Rest	0.11143 (0.78208)	1 (0.20775)	0.11143 (0.00803)	1 (0.00213)		
Lacertoidea vs rest	/	Lacertoidea	0.13641 (0.68788)	1 (0.30886)	1.10765 (0.00225)	1.10765 (0.00101)	0.002158	0.962948
		Rest	0.13641 (0.68788)	1 (0.30886)	0.13641 (0.00225)	1 (0.00101)		
Toxicofera vs rest	/	Toxicofera	0.11022 (0.78766)	1 (0.18725)	1.40044 (0.02028)	1.40044 (0.00482)	0.309094	0.578237
		Rest	0.11022 (0.78766)	1 (0.18725)	0.11022 (0.02028)	1 (0.00482)		
S10A Nocturnal vs Diurnal	/	Nocturnal	0.07270 (0.78513)	1 (0.11574)	1 (0.08639)	1 (0.01274)	0	**1**
		Diurnal	0.07270 (0.78513)	1 (0.11574)	0.07270 (0.08639)	1 (0.01274)		
S10B Geckos vs rest	/	Geckos	/	/	/	/	/	**/**
		Rest						
S10C Snakes vs rest		Snakes	/	/	/	/	/	**/**
		Rest						
S10D Skinks vs rest	/	Skinks	0.08220 (0.79559)	1 (0.11769)	1 (0.07555)	1 (0.01118)	0	1
		Rest	0.08220 (0.79559)	1 (0.11769)	0.08220 (0.07555)	1 (0.01118)		
Snakes Geckos Skinks vs rest	/	Snakes, Geckos, Skinks	0.08220 (0.79559)	1 (0.11769)	1 (0.07555)	1 (0.01118)	0	1
		Rest	0.08220 (0.79559)	1 (0.11769)	0.08220 (0.07555)	1 (0.01118)		

PRT

	Lacertoidea vs rest	/	Lacertoidea	0.13641 (0.68788)	1 (0.30886)	1.10765 (0.00225)	1.10765 (0.00101)	0.002158	0.962948
			Rest	0.13641 (0.68788)	1 (0.30886)	0.13641 (0.00225)	1 (0.00101)		
	Toxicofera vs rest	/	Toxicofera	0.08434 (0.87543)	1 (0.10819)	1.74175 (0.01458)	1.74175 (0.00180)	0.673598	0.4118
			Rest	0.08434 (0.87543)	1 (0.10819)	0.08434 (0.01458)	1 (0.00180)		
	S10A Nocturnal vs Diurnal	102 - 107 - 129	Nocturnal	0.08175 (0.80702)	1 (0.14622)	1 (0.03959)	1 (0.00717)	0	**1**
			Diurnal	0.08175 (0.80702)	1 (0.14622)	0.08175 (0.03959)	1 (0.00717)		
	S10B Geckos vs rest	91 - 99 - 100 - 174	Geckos	0.08029 (0.79966)	1 (0.14291)	1 (0.04873)	1 (0.00871)	0	**1**
			Rest	0.08029 (0.79966)	1 (0.14291)	0.08029 (0.04873)	1 (0.00871)		
	S10C Snakes vs rest	02/03/2018	Snakes	0.11366 (0.84529)	1 (0.12976)	4.17337 (0.02163)	4.17337 (0.00332)	14.70965	**0.000125403**
			Rest	0.11366 (0.84529)	1 (0.12976)	0.11366 (0.02163)	1 (0.00332)		
RGR	S10D Skinks vs rest	132	Skinks	0.08440 (0.81661)	1 (0.14881)	1 (0.02924)	1 (0.00533)	0	1
			Rest	0.08440 (0.81661)	1 (0.14881)	0.08440 (0.02924)	1 (0.00533)		
	Snakes Geckos Skinks vs rest	7 - 18	Snakes, Geckos, Skinks	0.08117 (0.82190)	1 (0.10751)	1 (0.06243)	1 (0.00817)	0	1
			Rest	0.08117 (0.82190)	1 (0.10751)	0.08117 (0.06243)	1 (0.00817)		
	Lacertoidea vs rest	47	Lacertoidea	0.11224 (0.85408)	1 (0.14207)	6.22472 (0.00330)	6.22472 (0.00055)	4.48025	0.034289
			Rest	0.11224 (0.85408)	1 (0.14207)	0.11224 (0.00330)	1 (0.00055)		
	Toxicofera vs rest	/	Toxicofera	0.10835 (**0.84043**)	1 (0.12904)	1 (0.02646)	1 (0.00406)	2.00E-06	0.998872

			Rest	0.10835 (**0.84043)**	1 (0.12904)	0.10835 (0.02646)	1 (0.00406)		
	S10A Nocturnal vs Diurnal	160	Nocturnal	0.06373 (0.75460)	1 (0.16746)	1 (0.06379)	1 (0.01416)	0	**1**
			Diurnal	0.06373 (0.75460)	1 (0.16746)	0.06373 (0.06379)	1 (0.01416)		
	S10B Geckos vs rest	319	Geckos	0.06564 (0.77081)	1 (0.16237)	1.00485 (0.05519)	1.00485 (0.01163)	0.000202	**0.98866**
			Rest	0.06564 (0.77081)	1 (0.16237)	0.06564 (0.05519)	1 (0.01163)		
	S10C Snakes vs rest	242	Snakes	0.05888 (0.73208)	1 (0.18694)	1 (0.05888)	1 (0.01647)	0	**1**
			Rest	0.05888 (0.73208)	1 (0.18694)	1 (0.05888)	1 (0.01647)		
RRH	S10D Skinks vs rest	130	Skinks	0.06523 (0.77924)	1 (0.19194)	1.30666 (0.02313)	1.30666 (0.00570)	0.165552	0.684096
			Rest	0.06523 (0.77924)	1 (0.19194)	0.06523 (0.02313)	1 (0.00570)		
	Snakes Geckos Skinks vs rest	230 - 233 - 273	Snakes, Geckos, Skinks	0.06032 (0.76718)	1 (0.11288)	1 (0.10456)	1 (0.01538)	0	1
			Rest	0.06032 (0.76718)	1 (0.11288)	0.06032 (0.10456)	1 (0.01538)		
	Lacertoidea vs rest	/	Lacertoidea	0.06843 (0.79921)	1 (0.20079)	1 (0)	1 (0)	2.00E-06	0.998872
			Rest	0.06843 (0.79921)	1 (0.20079)	0.06843 (0)	1 (0)		
	Toxicofera vs rest	8 - 10 - 23 - 241 - 242	Toxicofera	0.05306 (0.72966)	1 (0.15501)	1 (0.09512)	1 (0.02021)	0	1
			Rest	0.05306 (0.72966)	1 (0.15501)	0.05306 (0.09512)	1 (0.02021)		
TMT	S10A Nocturnal vs Diurnal	222 - 300 - 301 - 302 - 303 - 305 -	Nocturnal	0.06972 (0.69907)	1 (0.21529)	3.05635 (0.06548)	3.05635 (0.02017)	12.66927	**0.000372**

		310 - 311 - 314 - 320	Diurnal	0.06972 (0.69907)	1 (0.21529)	0.06972 (0.06548)	1 (0.02017)		
	S10B Geckos vs rest	73 - 157 - 198 - 221 - 246 - 265 - 271 - 302 - 303 - 310 - 344 - 348 - 358 - 362 - 378	Geckos	0.05779 (0.64501)	1 (0.16668)	1.08310 (0.14964)	1.0831 (0.03867)	0.12075	**0.728222**
			Rest	0.05779 (0.64501)	1 (0.16668)	0.05779 (0.14964)	1 (0.03867)		
	S10C Snakes vs rest	/	Snakes	/	/	/	/	/	**/**
			Rest						
	S10D Skinks vs rest	216	Skinks	0.07902 (0.70521)	1 (0.22817)	1.01077 (0.05033)	1.01077 (0.01629)	0.000212	0.988383
			Rest	0.07902 (0.70521)	1 (0.22817)	0.07902 (0.05033)	1 (0.01629)		
	Snakes Geckos Skinks vs rest	73 - 153 - 185 - 198 - 246 - 265 - 271 - 302 - 303 - 310 - 332 - 358 - 362 - 378	Snakes, Geckos, Skinks	0.05496 (0.64536)	1 (0.15712)	1 (0.15885)	1 (0.03867)	0	1
			Rest	0.05496 (0.64536)	1 (0.15712)	0.05496 (0.15855)	1 (0.03867)		
	Lacertoidea vs rest	/	Lacertoidea	0.08291 (0.75318)	1 (0.24682)	1 (0)	1 (0)	0	1
			Rest	0.08291 (0.75318)	1 (0.24682)	0.08291 (0)	1 (0)		
	Toxicofera vs rest	/	Toxicofera	0.08291 (0.75318)	1 (0.24682)	1 (0)	1 (0)	0	1
			Rest	0.08291 (0.75318)	1 (0.24682)	0.08291 (0)	1 (0)		
TMTa	S10A Nocturnal vs Diurnal	241	Nocturnal	0.08114 (0.73917)	1 (0.21937)	1.97950 (0.03197)	1.97950 (0.00949)	0.230736	**0.630979**

			Diurnal	0.08114 (0.73917)	1 (0.21937)	0.08114 (0.03197)	1 (0.00949)		
	S10B Geckos vs rest	/	Geckos	0.076710 (0.70603)	1 (0.19814)	1 (0.07483)	1 (0.02100)	0	**1**
			Rest	0.076710 (0.70603)	1 (0.19814)	0.07610 (0.07483)	1 (0.02100)		
	S10C Snakes vs rest	/	Snakes	/	/	/	/	/	**/**
			Rest						
	S10D Skinks vs rest	/	Skinks	/	/	/	/	/	/
			Rest						
	Snakes Geckos Skinks vs rest	/	Snakes, Geckos, Skinks	0.07610 (0.70603)	1 (0.19814)	1 (0.07483)	1 (0.02100)	0	1
			Rest	0.07610 (0.70603)	1 (0.19814)	0.07610 (0.07483)	1 (0.02100)		
	Lacertoidea vs rest	/	Lacertoidea	0.08475 (0.76511)	1 (0.23489)	1 (0)	1 (0)	0	1
			Rest	0.08475 (0.76511)	1 (0.23489)	0.08475 (0)	1 (0)		
	Toxicofera vs rest	/	Toxicofera	0.0807 (0.74698)	1 (0.20973)	1 (0.03380)	1 (0.00949)	0	1
			Rest	0.0807 (0.74698)	1 (0.20973)	0.0807 (0.03380)	1 (0.00949)		
VAOP	S10A Nocturnal vs Diurnal	/	Nocturnal	0.07022 (0.75798)	1 (0.21888)	1 (0.01796)	1 (0.00519)	0	**1**
			Diurnal	0.07022 (0.75798)	1 (0.21888)	0.07022 (0.01796)	1 (0.00519)		
	S10B Geckos vs rest	14	Geckos	0.07001 (0.76342)	1 (0.21634)	1.39277 (0.01577)	1.39277 (0.00447)	0.18774	**0.664804**

		Rest	0.07001 (0.76342)	1 (0.21634)	0.07001 (0.01577)	1 (0.00447)		
S10C Snakes vs rest	266	Snakes	0.07003 (0.76187)	1 (0.21314)	1.15114 (0.01953)	1.15114 (0.00546)	0.019496	**0.888954**
		Rest	0.07003 (0.76187)	1 (0.21314)	0.07003 (0.01953)	1 (0.00546)		
S10D Skinks vs rest	/	Skinks	/	/	/	/	/	/
		Rest						
Snakes Geckos Skinks vs rest	148	Snakes, Geckos, Skinks	0.06815 (0.75878)	1 (0.20250)	1 (0.03056)	1 (0.00816)	0	1
		Rest	0.06815 (0.75878)	1 (0.20250)	0.06815 (0.03056)	1 (0.00816)		
Lacertoidea vs rest	/	Lacertoidea	0.07188 (0.77447)	1 (0.22553)	1 (0)	1 (0)	0	1
		Rest	0.07188 (0.77447)	1 (0.22553)	0.07188 (0)	1 (0)		
Toxicofera vs rest	/	Toxicofera	0.06728 (0.75689)	1 (0.20347)	1 (0.03124)	1 (0.00840)	0	1
		Rest	0.06728 (0.75689)	1 (0.20347)	0.06728 (0.03124)	1 (0.00840)		

Table 4.4. Clade CodeML results

Partition	Model	NP	ln*L*	AIC	*k*	Parameters			Null	2Δl	df	P
						ω0	ω1	ω2/ωd				
OPN5												
—	M2a_rel	89	-11202.46347	22582.92695	2.17792	0.03361 (0.55525)	1 (0.11003)	0.33680 (0.33472)				

Nocturnal	CmC	90	-11199.36954	22578.73909	2.19015	0.02516 (0.50085)	1 (0.13356)	0.41644 (0.36559)	M2a_rel	6.187858	1	**0.013**
Diurnal	CmC	90	-11199.36954	22578.73909	2.19015	0.02516 (0.50085)	1 (0.13356)	0.25873 (0.36559)	M2a_rel	6.187858	1	**0.013**
Snakes	CmC	91	-11175.69195	22533.38389	2.20869	0.01887 (0.45730)	1 (0.15328)	0.65404 (0.38942)	M2a_rel	53.543054	2	**0.000**
Lizards	CmC	91	-11175.69195	22533.38389	2.20869	0.01887 (0.45730)	1 (0.15328)	0.19343 (0.38942)	M2a_rel	53.543054	2	**0.000**
background	CmC	91	-11175.69195	22533.38389	2.20869	0.01887 (0.45730)	1 (0.15328)	0.19889 (0.38942)	M2a_rel	53.543054	2	**0.000**
Geckoes	CmC	92	-11169.94437	22523.88874	2.22348	0.01853 (0.45003)	1 (0.16910)	0.25238 (0.38087)	M2a_rel	65.038204	3	**0.000**
Snakes	CmC	92	-11169.94437	22523.88874	2.22348	0.01853 (0.45003)	1 (0.16910)	0.67862 (0.38087)	M2a_rel	65.038204	3	**0.000**
Skinks	CmC	92	-11169.94437	22523.88874	2.22348	0.01853 (0.45003)	1 (0.16910)	0.12639 (0.38087)	M2a_rel	65.038204	3	**0.000**

background	CmC	92	-11169.94437	22523.88874	2.22348	0.01853 (0.45003)	1 (0.16910)	0.14015 (0.38087)	M2a_rel	65.038204	3	**0.000**
Geckoes	CmC	92	-11169.17103	22522.34207	2.22485	0.01742 (0.44387)	1 (0.17050)	0.24972 (0.38563)	M2a_rel	66.58488	3	**0.000**
Snakes	CmC	92	-11169.17103	22522.34207	2.22485	0.01742 (0.44387)	1 (0.17050)	0.66607 (0.38563)	M2a_rel	66.58488	3	**0.000**
Squamates	CmC	92	-11169.17103	22522.34207	2.22485	0.01742 (0.44387)	1 (0.17050)	0.12637 (0.38563)	M2a_rel	66.58488	3	**0.000**
background	CmC	92	-11169.17103	22522.34207	2.22485	0.01742 (0.44387)	1 (0.17050)	0.18946 (0.38563)	M2a_rel	66.58488	3	**0.000**
Lacertoidea	CmC	90	-11201.59561	22583.19122	2.17905	0.03168 (0.54354)	1 (0.11384)	0.24277 (0.34261)	M2a_rel	1.735722	1	**0.188**
background	CmC	90	-11201.59561	22583.19122	2.17905	0.03168 (0.54354)	1 (0.11384)	0.33548 (0.34261)	M2a_rel	1.735722	1	**0.188**
Toxicofera	CmC	90	-11199.54649	22579.09297	2.1804	0.02984 (0.53329)	1 (0.11468)	0.38478 (0.35203)	M2a_rel	5.833976	1	**0.016**

background	CmC	90	-11199.54649	22579.09297	2.1804	0.02984 (0.53329)	1 (0.11468)	0.27652 (0.35203)	M2a_rel	5.833976	1	**0.016**

OPN6

Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2/ωd**				
—	M2a_rel	37	-9521.144538	19116.28908	2.97565	0.00072 (0.34407)	1 (0.16185)	0.23212 (0.49408)				
Nocturnal	CmC	38	-9515.981479	19107.96296	2.93681	0.00174 (0.35248)	1 (0.14857)	0.10247 (0.49895)	M2a_rel	10.326118	1	**0.001**
Diurnal	CmC	38	-9515.981479	19107.96296	2.93681	0.00174 (0.35248)	1 (0.14857)	0.28134 (0.49895)	M2a_rel	10.326118	1	**0.001**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Lizards	CmC	/	/	/	/	/	/	/	/	/	/	/
Geckoes	CmC	38	-9515.7679	19107.5358	2.97734	0 (0.33816)	1 (0.15383)	0.10500 (0.50801)	M2a_rel	10.753276	1	**0.001**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**

Skinks	CmC	/	/	/	/	/	/	/	/	/	/	**/**
background	CmC	38	-9515.7679	19107.5358	2.97734	0 (0.33816)	1 (0.15383)	0.26018 (0.50801)	M2a_rel	10.753276	1	**0.001**
Geckoes	CmC	39	-9515.047846	19108.09569	2.97082	0 (0.33837)	1 (0.15151)	0.10458 (0.51012)	M2a_rel	12.193384	2	**0.002**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Squamates	CmC	39	-9515.047846	19108.09569	2.97082	0 (0.33837)	1 (0.15151)	0.27388 (0.51012)	M2a_rel	12.193384	2	**0.002**
background	CmC	39	-9515.047846	19108.09569	2.97082	0 (0.33837)	1 (0.15151)	0.19397 (0.51012)	M2a_rel	12.193384	2	**0.002**
Lacertoidea	CmC	38	-9521.118568	19118.23714	2.97616	0.00065 (0.34349)	1 (0.16141)	0.24123 (0.49510)	M2a_rel	0.05194	1	**0.820**
background	CmC	38	-9521.118568	19118.23714	2.97616	0.00065 (0.34349)	1 (0.16141)	0.22939 (0.49510)	M2a_rel	0.05194	1	**0.820**

Toxicofera	CmC	38	-9518.082822	19112.16564	2.96988	0.00103 (0.34722)	1 (0.15769)	0.29116 (0.49510)	M2a_rel	6.123432	1	**0.013**
background	CmC	38	-9518.082822	19112.16564	2.96988	0.00103 (0.34722)	1 (0.15769)	0.19192 (0.49510)	M2a_rel	6.123432	1	**0.013**

OPN8

Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2/ωd**				
—	M2a_rel	31	-5844.66794	11751.33588	2.96548	0.03720 (0.51877)	1 (0.10520)	0.36150 (0.37603)				
Nocturnal	CmC	32	-5844.66794	11753.33588	2.96548	0.03720 (0.51877)	1 (0.10520)	0.37358 (0.37603)	M2a_rel	0	1	**1.000**
Diurnal	CmC	32	-5844.66794	11753.33588	2.96548	0.03720 (0.51877)	1 (0.10520)	0.36150 (0.37603)	M2a_rel	0	1	**1.000**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Lizards	CmC	/	/	/	/	/	/	/	/	/	/	/
Geckoes	CmC	/	/	/	/	/	/	/	/	/	/	**/**

Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Skinks	CmC	/	/	/	/	/	/	/	/	/	/	/
Geckoes	CmC	/	/	/	/	/	/	/	/	/	/	/
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Squamates	CmC	32	-5844.663764	11753.32753	2.96569	0.03711 (0.51844)	1 (0.10498)	0.36292 (0.37658)	M2a_rel	0.008352	1	**0.927**
background	CmC	32	-5844.663764	11753.32753	2.96569	0.03711 (0.51844)	1 (0.10498)	0 (0.37658)	M2a_rel	0.008352	1	**0.927**
Lacertoidea	CmC	32	-5844.106377	11752.21275	2.96209	0.03749 (0.52114)	1 (0.10239)	0.30092 (0.37647)	M2a_rel	1.123126	1	**0.289**
background	CmC	32	-5844.106377	11752.21275	2.96209	0.03749 (0.52114)	1 (0.10239)	0.38821 (0.37647)	M2a_rel	1.123126	1	**0.289**
Toxicofera	CmC	32	-5844.309814	11752.61963	2.96461	0.03655 (0.51712)	1 (0.10210)	0.38920 (0.38079)	M2a_rel	0.716252	1	**0.397**

background	CmC	32	-5844.309814	11752.61963	2.96461	0.03655 (0.51712)	1 (0.10210)	0.32863 (0.38079)	M2a_rel	0.716252	1	**0.397**

OPN7

Partition	Model	NP	ln*L*	AIC	*k*	Parameters			Null	2Δl	df	P
						ω0	ω1	ω2/ωd				
—	M2a_rel	99	-10642.07869	21482.15738	3.00804	0.00678 (0.51810)	1 (0.08817)	0.21736 (0.39373)				
Nocturnal	CmC	100	-10635.24195	21470.4839	3.00738	0.00838 (0.53405)	1 (0.08125)	0.40023 (0.38469)	M2a_rel	13.673472	1	**0.000**
Diurnal	CmC	100	-10635.24195	21470.4839	3.00738	0.00838 (0.53405)	1 (0.08125)	0.20294 (0.38469)	M2a_rel	13.673472	1	**0.000**
Snakes	CmC	101	-10625.66878	21453.33757	3.01014	0.00531 (0.50889)	1 (0.08551)	0.40488 (0.40563)	M2a_rel	32.81981	2	**0.000**
Lizards	CmC	101	-10625.66878	21453.33757	3.01014	0.00531 (0.50889)	1 (0.08551)	0.18198 (0.40563)	M2a_rel	32.81981	2	**0.000**
background	CmC	101	-10625.66878	21453.33757	3.01014	0.00531 (0.50889)	1 (0.08551)	0.09782 (0.40563)	M2a_rel	32.81981	2	**0.000**

Geckoes	CmC	102	-10605.65502	21415.31003	3.00107	0.00811 (0.53963)	1 (0.06772)	0.37290 (0.39265)	M2a_rel	72.847342	3	**0.000**
Snakes	CmC	102	-10605.65502	21415.31003	3.00107	0.00811 (0.53963)	1 (0.06772)	0.45957 (0.39265)	M2a_rel	72.847342	3	**0.000**
Skinks	CmC	102	-10605.65502	21415.31003	3.00107	0.00811 (0.53963)	1 (0.06772)	0.25220 (0.39265)	M2a_rel	72.847342	3	**0.000**
background	CmC	102	-10605.65502	21415.31003	3.00107	0.00811 (0.53963)	1 (0.06772)	0.10607 (0.39265)	M2a_rel	72.847342	3	**0.000**
Geckoes	CmC	102	-10612.10143	21428.20287	3.00932	0.00879 (0.54178)	1 (0.07242)	0.37063 (0.38580)	M2a_rel	59.954508	3	**0.000**
Snakes	CmC	102	-10612.10143	21428.20287	3.00932	0.00879 (0.54178)	1 (0.07242)	0.45971 (0.38580)	M2a_rel	59.954508	3	**0.000**
Squamates	CmC	102	-10612.10143	21428.20287	3.00932	0.00879 (0.54178)	1 (0.07242)	0.37063 (0.38580)	M2a_rel	59.954508	3	**0.000**
background	CmC	102	-10612.10143	21428.20287	3.00932	0.00879 (0.54178)	1 (0.07242)	0.10267 (0.38576)	M2a_rel	59.954508	3	**0.000**

Lacertoidea	CmC	100	-10640.02572	21480.05143	3.01062	0.00721 (0.52173)	1 (0.08761)	0.13999 (0.39066)	M2a_rel	4.105942	1	**0.043**
background	CmC	100	-10640.02572	21480.05143	3.01062	0.00721 (0.52173)	1 (0.08761)	0.22929 (0.39066)	M2a_rel	4.105942	1	**0.043**
Toxicofera	CmC	100	-10641.8308	21483.66159	3.01145	0.00634 (0.51369)	1 (0.08987)	0.22694 (0.39643)	M2a_rel	0.495784	1	**0.481**
background	CmC	100	-10641.8308	21483.66159	3.01145	0.00634 (0.51369)	1 (0.08987)	0.20510 (0.39643)	M2a_rel	0.495784	1	**0.481**

OPN3

Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2/ωd**				
—	M2a_rel	97	-9963.741274	20121.48255	2.68006	0.03134 (0.58179)	1 (0.05671)	0.30310 (0.36150)				
Nocturnal	CmC	98	-9961.817349	20119.6347	2.68551	0.02854 (0.56323)	1 (0.05885)	0.39458 (0.37793)	M2a_rel	3.84785	1	**0.050**
Diurnal	CmC	98	-9961.817349	20119.6347	2.68551	0.02854 (0.56323)	1 (0.05885)	0.27275 (0.37793)	M2a_rel	3.84785	1	**0.050**
Snakes	CmC	99	-9958.724365	20115.44873	2.67928	0.02845 (0.55900)	1 (0.05749)	0.43961 (0.38352)	M2a_rel	10.033818	2	**0.007**

Lizards	CmC	99	-9958.724365	20115.44873	2.67928	0.02845 (0.55900)	1 (0.05749)	0.26710 (0.38352)	M2a_rel	10.033818	2	**0.007**
background	CmC	99	-9958.724365	20115.44873	2.67928	0.02845 (0.55900)	1 (0.05749)	0.20286 (0.38352)	M2a_rel	10.033818	2	**0.007**
Geckoes	CmC	100	-9957.951576	20115.90315	2.6791	0.02737 (0.55151)	1 (0.05791)	0.25006 (0.39058)	M2a_rel	11.579396	3	**0.009**
Snakes	CmC	100	-9957.951576	20115.90315	2.6791	0.02737 (0.55151)	1 (0.05791)	0.42659 (0.39058)	M2a_rel	11.579396	3	**0.009**
Skinks	CmC	100	-9957.951576	20115.90315	2.6791	0.02737 (0.55151)	1 (0.05791)	0.33659 (0.39058)	M2a_rel	11.579396	3	**0.009**
background	CmC	100	-9957.951576	20115.90315	2.6791	0.02737 (0.55151)	1 (0.05791)	0.23921 (0.39058)	M2a_rel	11.579396	3	**0.009**
Geckoes	CmC	100	-9958.897675	20117.79535	2.67873	0.02853 (0.55948)	1 (0.05748)	0.25006 (0.39058)	M2a_rel	9.687198	3	**0.021**
Snakes	CmC	100	-9958.897675	20117.79535	2.67873	0.02853 (0.55948)	1 (0.05748)	0.42659 (0.39058)	M2a_rel	9.687198	3	**0.021**

Squamates	CmC	100	-9958.897675	20117.79535	2.67873	0.02853 (0.55948)	1 (0.05748)	0.33659 (0.39058)	M2a_rel	9.687198	3	**0.021**
background	CmC	100	-9958.897675	20117.79535	2.67873	0.02853 (0.55948)	1 (0.05748)	0.23921 (0.39058)	M2a_rel	9.687198	3	**0.021**
Lacertoidea	CmC	98	-9961.516091	20119.03218	2.68061	0.03025 (0.57455)	1 (0.05676)	0.20097 (0.36869)	M2a_rel	4.450366	1	**0.035**
background	CmC	98	-9961.516091	20119.03218	2.68061	0.03025 (0.57455)	1 (0.05676)	0.31503 (0.36869)	M2a_rel	4.450366	1	**0.035**
Toxicofera	CmC	98	-9961.642941	20119.28588	2.67846	0.03147 (0.58113)	1 (0.05689)	0.35705 (0.36198)	M2a_rel	4.196666	1	**0.041**
background	CmC	98	-9961.642941	20119.28588	2.67846	0.03147 (0.58113)	1 (0.05689)	0.26529 (0.36198)	M2a_rel	4.196666	1	**0.041**

OPN4M

Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2/ωd**				
—	M2a_rel	51	-9877.541295	19857.08259	2.82536	0.03377 (0.50565)	1 (0.11522)	0.38211 (0.37913)				
Nocturnal	CmC	52	-9871.891967	19847.78393	2.83421	0.02622 (0.46321)	1 (0.13273)	5.08284 (0.40406)	M2a_rel	11.298656	1	**0.001**

Diurnal	CmC	52	-9871.891967	19847.78393	2.83421	0.02622 (0.46321)	1 (0.13273)	0.31892 (0.40406)	M2a_rel	11.298656	1	**0.001**

Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Lizards	CmC	/	/	/	/	/	/	/	/	/	/	**/**

Geckoes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Skinks	CmC	52	-9861.697837	19827.39567	2.84263	0.05190 (0.59428)	1 (0.06659)	0.40840 (0.33913)	M2a_rel	31.686916	1	**0.000**
background	CmC	52	-9861.697837	19827.39567	2.84263	0.05190 (0.59428)	1 (0.06659)	1.21466 (0.33913)	M2a_rel	31.686916	1	**0.000**

Geckoes	CmC	/	/	/	/	/	/	/	/	/	/	/
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/

Squamates	CmC	52	-9877.541298	19859.0826	2.82537	0.03377 (0.50566)	1 (0.11522)	0.38211 (0.37912)	M2a_rel	0.000006	1	**0.998**
background	CmC	52	-9877.541298	19859.0826	2.82537	0.03377 (0.50566)	1 (0.11522)	0.96735 (0.37912)	M2a_rel	0.000006	1	**0.998**
Lacertoidea	CmC	52	-9876.733878	19857.46776	2.82268	0.03289 (0.50167)	1 (0.11572)	0.30687 (0.38261)	M2a_rel	1.614834	1	**0.204**
background	CmC	52	-9876.733878	19857.46776	2.82268	0.03289 (0.50167)	1 (0.11572)	0.39695 (0.38261)	M2a_rel	1.614834	1	**0.204**
Toxicofera	CmC	52	-9874.829889	19853.65978	2.82644	0.03945 (0.53496)	1 (0.10083)	0.33296 (0.36421)	M2a_rel	5.422812	1	**0.020**
background	CmC	52	-9874.829889	19853.65978	2.82644	0.03945 (0.53496)	1 (0.10083)	0.49572 (0.36421)	M2a_rel	5.422812	1	**0.020**

OPN4X

Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2/ωd**				
—	M2a_rel	99	-19174.26177	38546.52353	2.79003	0.02262 (0.30899)	1 (0.21677)	0.28050 (0.47424)				
Nocturnal	CmC	100	-19174.2345	38548.46901	2.78871	0.02600 (0.32336)	1 (0.21331)	0.28023 (0.46332)	M2a_rel	0.054526	1	**0.815**

Diurnal	CmC	100	-19174.2345	38548.46901	2.78871	0.02600 (0.32336)	1 (0.21331)	0.29119 (0.46332)	M2a_rel	0.054526	1	**0.815**
Snakes	CmC	101	-19159.192	38520.384	2.79431	0.01956 (0.29659)	1 (0.22251)	0.44356 (0.48089)	M2a_rel	30.139528	2	**0.000**
Lizards	CmC	101	-19159.192	38520.384	2.79431	0.01956 (0.29659)	1 (0.22251)	0.22636 (0.48089)	M2a_rel	30.139528	2	**0.000**
background	CmC	101	-19159.192	38520.384	2.79431	0.01956 (0.29659)	1 (0.22251)	0.31523 (0.48089)	M2a_rel	30.139528	2	**0.000**
Geckoes	CmC	102	-19128.36415	38460.72831	2.80935	0.01665 (0.28802)	1 (0.22925)	0.17887 (0.48273)	M2a_rel	91.795224	3	**0.000**
Snakes	CmC	102	-19128.36415	38460.72831	2.80935	0.01665 (0.28802)	1 (0.22925)	0.41668 (0.48273)	M2a_rel	91.795224	3	**0.000**
Skinks	CmC	102	-19128.36415	38460.72831	2.80935	0.01665 (0.28802)	1 (0.22925)	0.57028 (0.48273)	M2a_rel	91.795224	3	**0.000**
background	CmC	102	-19128.36415	38460.72831	2.80935	0.01665 (0.28802)	1 (0.22925)	0.16864 (0.48273)	M2a_rel	91.795224	3	**0.000**

Geckoes	CmC	102	-19157.36749	38518.73498	2.79028	0.02023 (0.30062)	1 (0.21806)	0.18422 (0.48133)	M2a_rel	33.78855	3	**0.000**
Snakes	CmC	102	-19157.36749	38518.73498	2.79028	0.02023 (0.30062)	1 (0.21806)	0.45150 (0.48133)	M2a_rel	33.78855	3	**0.000**
Squamates	CmC	102	-19157.36749	38518.73498	2.79028	0.02023 (0.30062)	1 (0.21806)	0.24606 (0.48133)	M2a_rel	33.78855	3	**0.000**
background	CmC	102	-19157.36749	38518.73498	2.79028	0.02023 (0.30062)	1 (0.21806)	0.32374 (0.48133)	M2a_rel	33.78855	3	**0.000**
Lacertoidea	CmC	100	-19173.11092	38546.22184	2.79779	0.01882 (0.29349)	1 (0.22574)	0.21567 (0.48077)	M2a_rel	2.301688	1	**0.129**
background	CmC	100	-19173.11092	38546.22184	2.79779	0.01882 (0.29349)	1 (0.22574)	0.27919 (0.48077)	M2a_rel	2.301688	1	**0.129**
Toxicofera	CmC	100	-19172.53616	38545.07231	2.79132	0.02625 (0.32366)	1 (0.21643)	0.25390 (0.45991)	M2a_rel	3.45122	1	**0.063**
background	CmC	100	-19172.53616	38545.07231	2.79132	0.02625 (0.32366)	1 (0.21643)	0.31682 (0.45991)	M2a_rel	3.45122	1	**0.063**

PIN

Partition	Model	NP	ln*L*	AIC	*k*	Parameters			Null	2Δl	df	P
						ω0	ω1	ω2/ωd				
—	M2a_rel	33	-7508.847283	15083.69457	2.61529	0.01429 (0.42687)	1 (0.07605)	0.22952 (0.49708)				
Nocturnal	CmC	34	-7508.043676	15084.08735	2.62048	0.01432 (0.42919)	1 (0.07379)	0.31753 (0.49702)	M2a_rel	1.607214	1	**0.205**
Diurnal	CmC	34	-7508.043676	15084.08735	2.62048	0.01432 (0.42919)	1 (0.07379)	0.22329 (0.49702)	M2a_rel	1.607214	1	**0.205**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Lizards	CmC	/	/	/	/	/	/	/	/	/	/	/
Geckoes	CmC	34	-7506.25015	15080.5003	2.62642	0.01427 (0.43276)	1 (0.07253)	0.31901 (0.49471)	M2a_rel	5.194266	1	**0.023**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Skinks	CmC	/	/	/	/	/	/	/	/	/	/	**/**

background	CmC	34	-7506.25015	15080.5003	2.62642	0.01427 (0.43276)	1 (0.07253)	0.21050 (0.49471)	M2a_rel	5.194266	1	**0.023**
Geckoes	CmC	35	-7498.393885	15066.78777	2.63762	0.01407 (0.42652)	1 (0.07360)	0.30938 (0.49988)	M2a_rel	20.906796	2	**0.000**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Squamates	CmC	35	-7498.393885	15066.78777	2.63762	0.01407 (0.42652)	1 (0.07360)	0.24468 (0.49988)	M2a_rel	20.906796	2	**0.000**
background	CmC	35	-7498.393885	15066.78777	2.63762	0.01407 (0.42652)	1 (0.07360)	0.08406 (0.49988)	M2a_rel	20.906796	2	**0.000**
Lacertoidea	CmC	34	-7508.54634	15085.09268	2.61148	0.01476 (0.43097)	1 (0.07300)	0.20140 (0.49603)	M2a_rel	0.601886	1	**0.438**
background	CmC	34	-7508.54634	15085.09268	2.61148	0.01476 (0.43097)	1 (0.07300)	0.23850 (0.49603)	M2a_rel	0.601886	1	**0.438**
Toxicofera	CmC	34	-7507.835545	15083.67109	2.61022	0.01467 (0.42622)	1 (0.07621)	0.25958 (0.49757)	M2a_rel	2.023476	1	**0.155**

background	CmC	34	-7507.835545	15083.67109	2.61022	0.01467 (0.42622)	1 (0.07621)	0.20616 (0.49757)	M2a_rel	2.023476	1	**0.155**

PP

Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2/ωd**				
—	M2a_rel	33	-4971.272622	10008.54524	2.91745	0 (0.21337)	1 (0.17801)	0.17043 (0.60862)				
Nocturnal	CmC	34	-4966.617773	10001.23555	2.98667	0.09004 (0.66465)	1 (0.21189)	1.30706 (0.12346)	M2a_rel	9.309698	1	**0.002**
Diurnal	CmC	34	-4966.617773	10001.23555	2.98667	0.09004 (0.66465)	1 (0.21189)	0.08350 (0.12346)	M2a_rel	9.309698	1	**0.002**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Lizards	CmC	/	/	/	/	/	/	/	/	/	/	/
Geckoes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**

Skinks	CmC	34	-4971.236397	10010.47279	2.91663	0 (0.21369)	1 (0.17762)	0.29695 (0.60869)	M2a_rel	0.07245	1	**0.788**
background	CmC	34	-4971.236397	10010.47279	2.91663	0 (0.21369)	1 (0.17762)	0.17028 (0.60869)	M2a_rel	0.07245	1	**0.788**
Geckoes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Squamates	CmC	34	-4966.61773	10001.23546	2.98667	0.09004 (0.66465)	1 (0.21189)	0.08350 (0.12346)	M2a_rel	9.309784	1	**0.002**
background	CmC	34	-4966.61773	10001.23546	2.98667	0.09004 (0.66465)	1 (0.21189)	1.30704 (0.12346)	M2a_rel	9.309784	1	**0.002**
Lacertoidea	CmC	34	-4968.47683	10004.95366	2.96895	0.10439 (0.71515)	1 (0.21323)	0.96073 (0.07162)	M2a_rel	5.591584	1	**0.018**
background	CmC	34	-4968.47683	10004.95366	2.96895	0.10439 (0.71515)	1 (0.21323)	0.00000 (0.07162)	M2a_rel	5.591584	1	**0.018**

Toxicofera	CmC	34	-4959.732226	9987.464452	2.9528	0.08393 (0.60381)	1 (0.20389)	0.00000 (0.19230)	M2a_rel	23.080792	1	**0.000**
background	CmC	34	-4959.732226	9987.464452	2.9528	0.08393 (0.60381)	1 (0.20389)	0.58070 (0.19230)	M2a_rel	23.080792	1	**0.000**

PRT

Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2/ωd**				
—	M2a_rel	29	-4337.230365	8732.46073	2.1556	0.04143 (0.67169)	1 (0.06285)	0.31967 (0.26547)				
Nocturnal	CmC	30	-4335.936596	8731.873192	2.1552	0.01523 (0.47586)	1 (0.08360)	0.30640 (0.44054)	M2a_rel	2.587538	1	**0.108**
Diurnal	CmC	30	-4335.936596	8731.873192	2.1552	0.01523 (0.47586)	1 (0.08360)	0.17939 (0.44054)	M2a_rel	2.587538	1	**0.108**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Lizards	CmC	/	/	/	/	/	/	/	/	/	/	/
Geckoes	CmC	/	/	/	/	/	/	/	/	/	/	**/**

Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Skinks	CmC	30	-4337.010626	8734.021252	2.15553	0.04622 (0.70637)	1 (0.06054)	0.15547 (0.23308)	M2a_rel	0.439478	1	**0.507**
background	CmC	30	-4337.010626	8734.021252	2.15553	0.04622 (0.70637)	1 (0.06054)	0.36100 (0.23308)	M2a_rel	0.439478	1	**0.507**
Geckoes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Squamates	CmC	30	-4335.847645	8731.69529	2.15315	0.03276 (0.60592)	1 (0.07546)	0.23121 (0.31862)	M2a_rel	2.76544	1	**0.096**
background	CmC	30	-4335.847645	8731.69529	2.15315	0.03276 (0.60592)	1 (0.07546)	0.41702 (0.31862)	M2a_rel	2.76544	1	**0.096**
Lacertoidea	CmC	30	-4337.196782	8734.393564	2.15537	0.31977 (0.26509)	1 (0.06286)	0.03559 (0.67205)	M2a_rel	0.067166	1	**0.796**
background	CmC	30	-4337.196782	8734.393564	2.15537	0.31977 (0.26509)	1 (0.06286)	0.04252 (0.67205)	M2a_rel	0.067166	1	**0.796**

Toxicofera	CmC	30	-4336.843359	8733.686718	2.16046	0.01730 (0.48308)	1 (0.08396)	0.17991 (0.43295)	M2a_rel	0.774012	1	**0.379**
background	CmC	30	-4336.843359	8733.686718	2.16046	0.01730 (0.48308)	1 (0.08396)	0.24626 (0.43295)	M2a_rel	0.774012	1	**0.379**

RGR

Partition	Model	NP	ln*L*	AIC	*k*	Parameters			Null	2Δl	df	P
						ω0	ω1	ω2/ωd				
—	M2a_rel	103	-10969.43278	22144.86556	2.3919	0.02349 (0.39881)	1 (0.10331)	0.21015 (0.49788)				
Nocturnal	CmC	104	-10969.25792	22146.51583	2.39206	0.02434 (0.40485)	1 (0.10272)	0.22869 (0.49243)	M2a_rel	0.349728	1	**0.554**
Diurnal	CmC	104	-10969.25792	22146.51583	2.39206	0.02434 (0.40485)	1 (0.09094)	0.20769 (0.49243)	M2a_rel	0.349728	1	**0.554**
Snakes	CmC	105	-10974.36257	22158.72514	2.3587	0.09238 (0.81322)	1 (0.00790)	1.04357 (0.17888)	M2a_rel	9.859574	2	**0.002**
Lizards	CmC	105	-10974.36257	22158.72514	2.3587	0.09238 (0.81322)	1 (0.00790)	0.54430 (0.17888)	M2a_rel	9.859574	2	**0.002**

background	CmC	105	-10974.36257	22158.72514	2.3587	0.09238 (0.81322)	1 (0.00790)	0.18489 (0.17888)	M2a_rel	9.859574	2	**0.002**
Geckoes	CmC	106	-10944.69806	22101.39613	2.39467	0.01396 (0.33020)	1 (0.11838)	0.18903 (0.55142)	M2a_rel	49.469436	3	**0.000**
Snakes	CmC	106	-10944.69806	22101.39613	2.39467	0.01396 (0.33020)	1 (0.11838)	0.17795 (0.55142)	M2a_rel	49.469436	3	**0.000**
Skinks	CmC	106	-10944.69806	22101.39613	2.39467	0.01396 (0.33020)	1 (0.11838)	0.39332 (0.55142)	M2a_rel	49.469436	3	**0.000**
background	CmC	106	-10944.69806	22101.39613	2.39467	0.01396 (0.33020)	1 (0.11838)	0.11803 (0.55142)	M2a_rel	49.469436	3	**0.000**
Geckoes	CmC	106	-10974.225	22160.45	2.36031	0.09214 (0.81236)	1 (0.01010)	0.57404 (0.17754)	M2a_rel	9.58444	3	**0.002**
Snakes	CmC	106	-10974.225	22160.45	2.36031	0.09214 (0.81236)	1 (0.01010)	1.04441 (0.17754)	M2a_rel	9.58444	3	**0.002**
Squamates	CmC	106	-10974.225	22160.45	2.36031	0.09214 (0.81236)	1 (0.01010)	0.52300 (0.17754)	M2a_rel	9.58444	3	**0.002**

background	CmC	106	-10974.225	22160.45	2.36031	0.09214 (0.81236)	1 (0.01010)	0.18113 (0.17754)	M2a_rel	9.58444	3	**0.002**
Lacertoidea	CmC	104	-10969.00017	22146.00035	2.39409	0.02178 (0.38649)	1 (0.10516)	0.16591 (0.50836)	M2a_rel	0.865216	1	**0.352**
background	CmC	104	-10969.00017	22146.00035	2.39409	0.02178 (0.38649)	1 (0.10516)	0.20967 (0.50836)	M2a_rel	0.865216	1	**0.352**
Toxicofera	CmC	104	-10964.43038	22136.86076	2.40375	0.01591 (0.34380)	1 (0.11539)	0.15009 (0.54081)	M2a_rel	10.004802	1	**0.002**
background	CmC	104	-10964.43038	22136.86076	2.40375	0.01591 (0.34380)	1 (0.11539)	0.22772 (0.54081)	M2a_rel	10.004802	1	**0.002**

RRH

Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**Parameters**			**Null**	**2Δl**	**df**	**P**
						ω0	**ω1**	**ω2/ωd**				
—	M2a_rel	93	-8596.16694	17378.33388	2.71483	0.01152 (0.56244)	1 (0.08086)	0.28116 (0.35670)				
Nocturnal	CmC	94	-8593.7173	17375.4346	2.71752	0.01164 (0.56421)	1 (0.07768)	0.40122 (0.35811)	M2a_rel	4.89928	1	**0.027**
Diurnal	CmC	94	-8593.7173	17375.4346	2.71752	0.01164 (0.56421)	1 (0.07768)	0.26081 (0.35811)	M2a_rel	4.89928	1	**0.027**

Snakes	CmC	95	-8586.604853	17363.20971	2.71724	0.01105 (0.56130)	1 (0.07276)	0.46503 (0.36594)	M2a_rel	19.124174	2	**0.000**
Lizards	CmC	95	-8586.604853	17363.20971	2.71724	0.01105 (0.56130)	1 (0.07276)	0.27509 (0.36594)	M2a_rel	19.124174	2	**0.000**
background	CmC	95	-8586.604853	17363.20971	2.71724	0.01105 (0.56130)	1 (0.07276)	0.09105 (0.36594)	M2a_rel	19.124174	2	**0.000**
Geckoes	CmC	96	-8585.792699	17363.5854	2.70783	0.00975 (0.55181)	1 (0.07133)	0.36580 (0.37686)	M2a_rel	20.748482	3	**0.000**
Snakes	CmC	96	-8585.792699	17363.5854	2.70783	0.00975 (0.55181)	1 (0.07133)	0.46090 (0.37686)	M2a_rel	20.748482	3	**0.000**
Skinks	CmC	96	-8585.792699	17363.5854	2.70783	0.00975 (0.55181)	1 (0.07133)	0.22152 (0.37686)	M2a_rel	20.748482	3	**0.000**
background	CmC	96	-8585.792699	17363.5854	2.70783	0.00975 (0.55181)	1 (0.07133)	0.20059 (0.37686)	M2a_rel	20.748482	3	**0.000**
Geckoes	CmC	96	-8582.951284	17357.90257	2.71044	0.01223 (0.57023)	1 (0.06677)	0.38779 (0.36299)	M2a_rel	26.431312	3	**0.000**

Snakes	CmC	96	-8582.951284	17357.90257	2.71044	0.01223 (0.57023)	1 (0.06677)	0.48321 (0.36299)	M2a_rel	26.431312	3	**0.000**
Squamates	CmC	96	-8582.951284	17357.90257	2.71044	0.01223 (0.57023)	1 (0.06677)	0.23717 (0.36299)	M2a_rel	26.431312	3	**0.000**
background	CmC	96	-8582.951284	17357.90257	2.71044	0.01223 (0.57023)	1 (0.06677)	0.09436 (0.36299)	M2a_rel	26.431312	3	**0.000**
Lacertoidea	CmC	94	-8587.173849	17362.3477	2.71685	0.00945 (0.54928)	1 (0.07855)	0.09359 (0.37217)	M2a_rel	17.986182	1	**0.000**
background	CmC	94	-8587.173849	17362.3477	2.71685	0.00945 (0.54928)	1 (0.07855)	0.37217 (0.37217)	M2a_rel	17.986182	1	**0.000**
Toxicofera	CmC	94	-8590.41084	17368.82168	2.72309	0.00973 (0.54891)	1 (0.08646)	0.36027 (0.36463)	M2a_rel	11.5122	1	**0.001**
background	CmC	94	-8590.41084	17368.82168	2.72309	0.00973 (0.54891)	1 (0.08646)	0.21162 (0.36463)	M2a_rel	11.5122	1	**0.001**

TMT

						Parameters						
Partition	**Model**	**NP**	**ln*L***	**AIC**	***k***	**ω0**	**ω1**	**ω2/ωd**	**Null**	**2Δl**	**df**	**P**

—	M2a_rel	59	-8640.322372	17398.64474	3.17546	0.03161 (0.56903)	1 (0.12917)	0.34441 (0.30180)					
Nocturnal	CmC	60	-8629.448972	17378.89794	3.19706	0.03076 (0.55952)	1 (0.14574)	0.82748 (0.29474)	M2a_rel	21.7468	1	**0.000**	
Diurnal	CmC	60	-8629.448972	17378.89794	3.19706	0.03076 (0.55952)	1 (0.14574)	0.26758 (0.29474)	M2a_rel	21.7468	1	**0.000**	
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/	
Lizards	CmC	/	/	/	/	/	/	/	/	/	/	/	
Geckoes	CmC	61	-8609.650515	17341.30103	3.2379	0.03412 (0.56568)	1 (0.16851)	0.78955 (0.26581)	M2a_rel	61.343714	2	**0.000**	
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/	
Skinks	CmC	61	-8609.650515	17341.30103	3.2379	0.03412 (0.56568)	1 (0.16851)	0.31293 (0.26581)	M2a_rel	61.343714	2	**0.000**	
background	CmC	61	-8609.650515	17341.30103	3.2379	0.03412 (0.56568)	1 (0.16851)	0.12867 (0.26581)	M2a_rel	61.343714	2	**0.000**	

Geckoes	CmC	61	-8610.831859	17343.66372	3.2371	0.03155 (0.55614)	1 (0.16164)	0.76880 (0.28222)	M2a_rel	58.981026	2	**0.000**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Squamates	CmC	61	-8610.831859	17343.66372	3.2371	0.03155 (0.55614)	1 (0.16164)	0.17849 (0.28222)	M2a_rel	58.981026	2	**0.000**
background	CmC	61	-8610.831859	17343.66372	3.2371	0.03155 (0.55614)	1 (0.16164)	0.07662 (0.28222)	M2a_rel	58.981026	2	**0.000**
Lacertoidea	CmC	60	-8631.869391	17383.73878	3.2165	0.01127 (0.42375)	1 (0.18087)	0.04685 (0.39538)	M2a_rel	16.905962	1	**0.000**
background	CmC	60	-8631.869391	17383.73878	3.2165	0.01127 (0.42375)	1 (0.18087)	0.25646 (0.39538)	M2a_rel	16.905962	1	**0.000**
Toxicofera	CmC	60	-8634.673769	17389.34754	3.17428	0.03931 (0.61291)	1 (0.11113)	0.25161 (0.27595)	M2a_rel	11.297206	1	**0.001**
background	CmC	60	-8634.673769	17389.34754	3.17428	0.03931 (0.61291)	1 (0.11113)	0.49050 (0.27595)	M2a_rel	11.297206	1	**0.001**

TMTa

Partition	Model	NP	ln*L*	AIC	*k*	Parameters			Null	2Δl	df	P
						ω0	ω1	ω2/ωd				
—	M2a_rel	35	-6804.751703	13679.50341	2.69611	0.02577 (0.58507)	1 (0.01838)	0.43784 (0.39655)				
Nocturnal	CmC	36	-6801.1579	13674.3158	2.70087	0.02400 (0.57540)	1 (0.02302)	0.71364 (0.40158)	M2a_rel	7.187606	1	**0.007**
Diurnal	CmC	36	-6801.1579	13674.3158	2.70087	0.02400 (0.57540)	1 (0.02302)	0.39156 (0.40158)	M2a_rel	7.187606	1	**0.007**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	/
Lizards	CmC	/	/	/	/	/	/	/	/	/	/	/
Geckoes	CmC	36	-6799.98107	13671.96214	2.7041	0.02089 (0.55923)	1 (0.02929)	0.59100 (0.41147)	M2a_rel	9.541266	1	**0.002**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Skinks	CmC	/	/	/	/	/	/	/	/	/	/	**/**

background	CmC	36	-6799.98107	13671.96214	2.7041	0.02089 (0.55923)	1 (0.02929)	0.34924 (0.41147)	M2a_rel	9.541266	1	**0.002**
Geckoes	CmC	37	-6793.87978	13661.75956	2.7001	0.02967 (0.60680)	1 (0)	0.64717 (0.39320)	M2a_rel	21.743846	2	**0.000**
Snakes	CmC	/	/	/	/	/	/	/	/	/	/	**/**
Squamates	CmC	37	-6793.87978	13661.75956	2.7001	0.02967 (0.60680)	1 (0)	0.48182 (0.39320)	M2a_rel	21.743846	2	**0.000**
background	CmC	37	-6793.87978	13661.75956	2.7001	0.02967 (0.60680)	1 (0)	0.18236 (0.39320)	M2a_rel	21.743846	2	**0.000**
Lacertoidea	CmC	36	-6803.614702	13679.2294	2.70022	0.02275 (0.56885)	1 (0.02756)	0.32698 (0.40359)	M2a_rel	2.274002	1	**0.132**
background	CmC	36	-6803.614702	13679.2294	2.70022	0.02275 (0.56885)	1 (0.02756)	0.44041 (0.40359)	M2a_rel	2.274002	1	**0.132**
Toxicofera	CmC	36	-6804.362262	13680.72452	3.17428	0.02676 (0.59045)	1 (0.01524)	0.48302 (0.39431)	M2a_rel	0.778882	1	**0.377**

background	CmC	36	-6804.362262	13680.72452	3.17428	0.02676 (0.59045)	1 (0.01524)	0.42221 (0.39431)	M2a_rel	0.778882	1	**0.377**

VAOP

Partition	Model	NP	ln*L*	AIC	*k*	Parameters			Null	2Δl	df	P
						ω0	ω1	ω2/ωd				
—	M2a_rel	59	-9418.211435	18954.42287	3.0491	0.02132 (0.56026)	1 (0.14074)	0.27850 (0.29900)				
Nocturnal	CmC	60	-9418.195975	18956.39195	3.05011	0.02112 (0.55863)	1 (0.14129)	0.28772 (0.30008)	M2a_rel	0.03092	1	**0.860**
Diurnal	CmC	60	-9418.195975	18956.39195	3.05011	0.02112 (0.55863)	1 (0.14129)	0.27512 (0.30008)	M2a_rel	0.03092	1	**0.860**
Snakes	CmC	61	-9417.603134	18957.20627	3.05211	0.02214 (0.56661)	1 (0.13962)	0.33421 (0.29376)	M2a_rel	1.216602	2	**0.544**
Lizards	CmC	61	-9417.603134	18957.20627	3.05211	0.02214 (0.56661)	1 (0.13962)	0.27620 (0.29376)	M2a_rel	1.216602	2	**0.544**
background	CmC	61	-9417.603134	18957.20627	3.05211	0.02214 (0.56661)	1 (0.13962)	0.23925 (0.29376)	M2a_rel	1.216602	2	**0.544**
Geckoes	CmC	61	-9417.609947	18957.21989	3.05057	0.02191 (0.56472)	1 (0.14022)	0.29070 (0.29506)	M2a_rel	1.202976	2	**0.548**

Snakes	CmC	61	-9417.609947	18957.21989	3.05057	0.02191 (0.56472)	1 (0.14022)	0.32968 (0.29506)	M2a_rel	1.202976	2	**0.548**
Skinks	CmC	/	/	/	/	/	/	/	/	/	/	**/**
background	CmC	61	-9417.609947	18957.21989	3.05057	0.02191 (0.56472)	1 (0.14022)	0.26278 (0.29506)	M2a_rel	1.202976	2	**0.548**
Geckoes	CmC	62	-9417.528346	18959.05669	3.05294	0.02200 (0.56535)	1 (0.14035)	0.29242 (0.29429)	M2a_rel	1.366178	3	**0.713**
Snakes	CmC	62	-9417.528346	18959.05669	3.05294	0.02200 (0.56535)	1 (0.14035)	0.33135 (0.29429)	M2a_rel	1.366178	3	**0.713**
Squamates	CmC	62	-9417.528346	18959.05669	3.05294	0.02200 (0.56535)	1 (0.14035)	0.26821 (0.29429)	M2a_rel	1.366178	3	**0.713**
background	CmC	62	-9417.528346	18959.05669	3.05294	0.02200 (0.56535)	1 (0.14035)	0.23662 (0.29429)	M2a_rel	1.366178	3	**0.713**
Lacertoidea	CmC	60	-9418.19726	18956.39452	3.04921	0.02131 (0.56010)	1 (0.14092)	0.26975 (0.29897)	M2a_rel	0.02835	1	**0.866**

background	CmC	60	-9418.19726	18956.39452	3.04921	0.02131 (0.56010)	1 (0.14092)	0.27988 (0.29897)	M2a_rel	0.02835	1	**0.866**
Toxicofera	CmC	60	-9418.159701	18956.3194	3.0497	0.02146 (0.56130)	1 (0.14063)	0.28647 (0.29807)	M2a_rel	0.103468	1	**0.748**
background	CmC	60	-9418.159701	18956.3194	3.0497	0.02146 (0.56130)	1 (0.14063)	0.27119 (0.29807)	M2a_rel	0.103468	1	**0.748**