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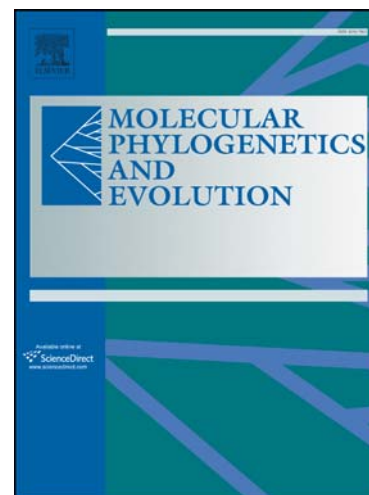Please cite this article as: Laver, R.J., Nielsen, S.V., Rosauer, D.F., Oliver, P.M., Trans-biome diversity in Australian grass-specialist lizards (Diplodactylidae: *Strophurus*), *Molecular Phylogenetics and Evolution* (2017), doi: http://dx.doi.org/10.1016/j.ympev.2017.07.015

Original Article

Trans-biome diversity in Australian grass-specialist lizards (Diplodactylidae: *Strophurus*)

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ABSTRACT

Comparisons of biodiversity patterns within lineages that occur across major climate gradients and biomes, can provide insights into the relative roles that lineage history, landscape and climatic variation, and environmental change have played in shaping regional biotas. In Australia, while there has been extensive research into the origins and patterns of diversity in the Australian Arid Zone (AAZ), how diversity is distributed across this biome and the Australian Monsoonal Tropics (AMT) to the north, has been less studied. We compared the timing and patterns of diversification across this broad aridity gradient in a clade of lizards (*Strophurus*: phasmid geckos) that only occur in association with a unique Australian radiation of sclerophyllous grasses (*Triodia*: spinifex). Our results indicate that overall genetic diversity is much higher, older and more finely geographically structured within the AMT, including distantly related clades endemic to the sandstone escarpments of the Kimberley and Arnhem Plateau. Niche modelling analyses also suggest that the distribution of taxa in the AMT is more strongly correlated with variation in topographic relief than in the AAZ. The two broad patterns that we recovered – i) lineage endemism increases as latitude decreases, and ii) endemism is tightly correlated to rocky regions – parallel and corroborate other recent studies of habitat generalists and specialised saxicoline lineages occurring across these same regions. Early Miocene diversification estimates also suggest that, soon after *Triodia* grasses colonised Australia and began to diversify in the Miocene, phasmid geckos with Gondwanan ancestry shifted into these grasses, and have subsequently remained closely associated with this unique vegetation type.

Keywords: Australian Arid Zone, Australian Monsoonal Tropics, cryptic diversity, northern deserts, spinifex, vegetative change

1. Introduction

The interaction between local climatic change and topographic variation plays a pervasive role in shaping the contemporary distribution of biological diversity (Carnaval et al., 2009; Hewitt, 1996; Qiu et al., 2011). During the Neogene, the vast Australian continent experienced a profound spread and intensification of aridity, while remaining relatively geologically and topographically stable (Bowman et al., 2010; Byrne et al., 2011; Byrne et al., 2008). Comparative phylogenetic and phylogeographic analyses of lineages that occur across environmentally contrasting biomes provide valuable opportunities to understand how patterns of biodiversity may have been shaped by this combination of changing climate and stable topography (Byrne et al., 2011; Byrne et al., 2008; Crisp et al., 2009; Oliver et al., 2014a; 2014c).

The two largest Australian biomes are the Australian Arid Zone (AAZ) and the Monsoonal Tropics (AMT), which share an extensive border spanning the north of the continent (Bowman et al., 2010; Byrne et al., 2008; Fig. 1). There has been a longstanding tendency in Australian biogeography to consider AAZ lineages derived from mesic ancestry (Chapple and Keogh, 2004; Ladiges et al., 2011; Toon et al., 2012; Williams et al., 2010), and many studies have found signatures of younger diversity and range-expansion within this region (Fujita et al., 2010; Jennings et al., 2003; Kuch et al., 2005; Marin et al., 2013). However, despite good evidence that many contemporary deserts have formed fairly recently (<4 Ma; Fujioka et al., 2009), significant genetic structure (especially in rocky ranges) and divergent lineages raise the possibility that arid and semi-arid habitats have had a much longer history (Kear et al., 2016; Maryan et al., 2007; Melville et al., 2011; Oliver and McDonald, 2016; Shoo et al., 2008).

The history and biogeography of the AMT remains more poorly resolved. Genetic insights into patterns of diversity within this biome, and its interrelationships with other biomes such

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as the AAZ, are only just beginning to emerge. Work to date has indicated two broad themes. First, lineages in arid areas tend to have wider distributions and lower genetic diversity, both along aridity gradients within the AMT, and also between the AMT and AAZ (Fujita et al., 2010; Kuch et al., 2005; Laver et al., in review; Oliver et al., 2014c; Pepper et al., 2011b). Second, more specialised taxa (particularly saxicoline taxa) tend to show much higher levels of localised endemism, especially in the AMT, but also to lesser extent in the AAZ (Laver et al., in review; Pepper et al., 2011a; Potter et al., 2012; Rosauer et al., 2016). Evidence also suggests a potentially complex history of transitions between the AAZ and AMT biomes (e.g. Catullo and Keogh, 2014; Nielsen et al., 2016; Oliver et al., 2014a; Toon et al., 2015). To date, phylogeographic and phylogenetic work has focused on widespread terrestrial/generalist vertebrate taxa, or specialist saxicoline taxa. Studies of additional taxa with a range of ecological associations are needed to refine understanding of patterns of diversity and distribution.

A globally unique feature of the Australian environment is the abundant, drought-tolerant, sclerophyllous grasses of the genus *Triodia* (spinifex). Since initial colonisation of the continent in the Miocene, this lineage has radiated to become a dominant habitat component across ~30% of Australia, especially on disjunct rocky plateaux of the AMT, and over much larger expanses of the AAZ (Bowman et al., 2010; Crisp and Cook, 2013). Spinifex provides food, shelter, and a thermally-buffered microhabitat for a diverse associated biotic community (Pianka, 1981; Wilson, 2012), including apparently specialised lineages (e.g. birds, (Christidis et al., 2010); mammals, (Haythornthwaite and Dickman, 2006); reptiles, (Gordon et al., 2010); and invertebrates (Dessen, 2008)). Many Australian lizard taxa are particularly closely associated with spinifex (Rabosky et al., 2007b; Wilson and Swan, 2013; Wilson, 2012).

One of the most diverse lineages of specialised spinifex-dwelling (graminicolous) lizards are the colloquially named 'phasmid' geckos (5 species) within the genus *Strophurus* (19 species). These geckos only occur on spinifex habitat (King and Horner, 1993; Storr, 1978; Wilson and Swan, 2013; Wilson, 2012) and have a distinctive morphology including small to very small size (44–56mm SVL; see Appendix: Fig. A.1), an elongate body-form, and, most distinctively, a 'pin-striped' dorsal, and sometimes ventral, colour pattern (Nielsen et al., 2016; Wilson and Swan, 2013; Wilson, 2012). The phasmid *Strophurus* include five recognised species distributed across the AMT and AAZ: i) *Strophurus jeanae* – distributed throughout the northern deserts of the AAZ, from the Pilbara craton to the central ranges; ii) *S. mcmillani* – west Kimberley, iii) *S. robinsoni* – east Kimberley (Ord Region), iv) *S. horneri* – Arnhem Land (Top End), and v) *S. taeniatus* – Northern Deserts Region from the southwest Kimberley to the Selwyn Ranges (Fig. 1). Many of these taxa are difficult to distinguish (e.g. *S. jeanae* and *S. taeniatus* were synonymous for most of the last century; Storr, 1988). They are also relatively rarely collected, and both previously unknown populations (Vanderduys et al., 2012) and new species continue to be discovered (Oliver and Parkin, 2014). This suggests further diversity remains unrecognised, a pattern that has emerged from many other taxa within the AMT (e.g. Moritz et al., 2015; Oliver et al., 2016; Oliver et al., 2012).

The broader genus *Strophurus*, in which the phasmid geckos are placed, is part of a Gondwanan radiation with a history pre-dating the isolation of Australia from Antarctica (Oliver et al., 2009; Oliver and Sanders, 2009). Recent work by Nielsen *et al.* (2016) suggested that *Strophurus*, including the phasmid group, may have a long history in the AAZ. Phylogenetic analyses of spinifex have also suggested that while this lineage colonised Australia post the final break-up of East Gondwana (Miocene), it too initially diversified in what is now the arid zone, with subsequent colonisation of the AMT (Toon et al., 2015).

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Given the potential origins and long histories of both spinifex and spinifex-dwelling phasmid geckos in the AAZ, it could be predicted that phasmid geckos may show higher genetic structuring within this biome. Alternatively, based on recent analyses of other vertebrate groups, the more topographically complex and wetter landscapes of the north could be predicted to hold higher genetic structure.

Using newly generated genetic data, we compared lineage diversity and distributions of phasmid geckos across the broad aridity gradient and regions of the AMT and AAZ. Using all available tissues, we assembled a multi-locus dataset and used phylogenetic and species delimitation analyses to assess and compare levels and patterns of genetic diversity. We implemented dating estimation methods to further compare the timescales of diversification across the group. Finally, we performed species distribution modelling to assess if the environmental variables that best correlated with the distributions of phasmid *Strophurus* lineages varied between regions and biomes.

2. Materials and methods

2.1. Sampling

Tissues used in genetic analyses are listed in Table A.1. Our sampling included all tissues registered in Australian museums (47 individuals) across all five currently recognised species of phasmid *Strophurus* geckos (Fig. 1, Table A.1a). Sequence data for an additional 57 outgroup taxa (22 from *Strophurus*) were included to provide calibration nodes for dating analyses (Table A.1b). We sequenced a portion of the mitochondrial NADH dehydrogenase subunit 2 (*ND2*) locus for all samples, and three nuclear loci – phosducin (*PDC*), the prolactin receptor (*PRLR*), and the recombination-activating gene-1 (*RAG-1*) – for majority of individuals (including outgroups). Details and characteristics of genetic data are listed in Table A.2. Primer sequences and amplification protocols are provided in Table A.3.

Genomic DNA was extracted from liver or tail tip samples using a Qiagen DNeasy extraction kit or a Qiaxtractor (Qiagen, Valencia, CA). PCR products were purified using 1uL of a 20% dilution of ExoSAP-IT (US78201, Amersham Biosciences, Piscataway, NJ), incubated at 37°C for 30 min, followed by 80°C for 15 min. Clean products were then sent to genetic services companies (Macrogen, Seoul, South Korea and DNASU, Arizona State University, USA) with amplicons sequenced in both directions. Gene sequences were assembled and edited using GENEIOUS v.6.1.7 (Drummond et al., 2008), and alignments were visually examined and translated into amino acids to confirm correct reading frames and full translation. Previously published sequences were also used in analyses and all new sequences were deposited to GenBank (Table A.1).

2.2. Nucleotide data and phylogenetic analyses

Congruence between mitochondrial and nuclear data was assessed by estimating phylogenies for i) single loci, ii) a concatenated nuclear dataset, and iii) a combined four locus dataset (mtDNA + nuDNA). Models of nucleotide substitution and partitioning strategies (Table A.4) were selected for each locus in PARTITIONFINDER v1.1.1 (Lanfear et al., 2012) using the Bayesian information criterion (BIC). Phylogenetic relationships were estimated using Maximum-Likelihood (RAXML v8.0.24; Stamatakis, 2006; Stamatakis et al., 2008) and Bayesian (MRBAYES v3.2.2; Ronquist and Huelsenbeck, 2003) analyses implemented through the CIPRES Science Gateway 3.1 for online phylogenetic analysis (Miller et al., 2010). Default RAXML settings were used in CIPRES following selected partition strategies. Bayesian analyses in MRBAYES also used selected models and partitions, running four independent Markov Chain Monte Carlo (MCMC) chains with 4 x 10 million generations sampling every 1000. Convergence and stability of log likelihoods were confirmed in TRACER v1.6 (Rambaut et al., 2014) and ARE WE THERE YET (AWTY;

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Wilgenbusch et al., 2004), then Maximum Clade Credibility (MCC) trees were constructed after 20% of samples were discarded as burn-in.

To assess and compare diversity levels across regions and biomes average, minimum, and maximum genetic distances (Tamura Nei [TN]; Tamura and Nei, 1993) within and between major mtDNA (genetic divergence ≧ 8%) lineages within the phasmid geckos were calculated in MEGA v6.06 (Tamura et al., 2013). An 8% divergence threshold was chosen as it is equal to the divergence between other currently recognised Diplodactylid species, *Oedura gemmata* and *O. marmorata*, (Oliver et al., 2014c). This threshold is not considered as evidence of species status, rather to identify potentially divergent lineages which we then went on to test for evolutionary independence (see below). Phylogenetic tree methods can be problematic at the level of population genetics or lower diversity where reticulation and recombination can lower resolution and increase uncertainty of relationship estimation (Posada and Crandall, 2002; Vriesendorp and Bakker, 2005). For this reason, lineage relationships were also visualized by generating phylogenetic networks of mtDNA and concatenated nuDNA separately, using the Neighbor-Net algorithm (Bryant and Moulton, 2004) in SplitsTree v4.10 (Huson and Bryant, 2006). As this method only compares the pairwise divergences between sequences rather than estimating evolutionary history, concatenating the nuclear loci is not an issue. We assessed support for inferred splits with 1000 bootstrap pseudoreplicates.

To determine if demographic histories varied in different biomes we also tested for signatures of range expansion in mtDNA using Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) neutrality tests calculated in DNASP v5.10.01 (Librado and Rozas, 2009) for the major lineages identified (see below).

For accurate comparison of diversity patterns between regions it is important to assess if genetic structure observed is not a signal artefact of historical structure persisting in certain

genetic markers. We therefore tested the independence of divergent mtDNA lineages detected using coalescent species delimitation with the program Bayesian Phylogenetics and Phylogeography (BP&P) v2.1 (Yang, 2015), which provides evidence for population structure if not species (Sukumaran and Knowles, 2017). We conducted multiple Bayesian species delimitation analyses using a nuDNA dataset (three loci) we had phased with the PHASE v2.1 program (Stephens and Donnelly, 2003; Stephens et al., 2001) and SeqPHASE online (Flot, 2010). The starting tree topology used matched the mtDNA tree and we explicitly tested for the distinctness of nine major lineages (see Results; Figs. 2 and 3, and Table A.5). We ran multiple BP&P analyses varying population size parameters (θs) and divergence time the species tree root (τ_0). Gamma priors were assigned as i) both θs and τ_0 equal to G(1, 10), mean = 0.1; ii) both θs and $\tau 0$ equal to G(2,2000), mean = 0.001; iii) θs – G(1,10), τ_0 – G(2,2000); and iv) θs – G(2,2000), τ_0 – G(1,10). Other divergence time parameters were assigned the Dirichlet prior (Yang and Rannala, 2010]: equation 2). Species delimitation rjMCMC algorithm 0 with finetune $\varepsilon = 2$ was used with species model prior set to 0 so as not to favour symmetric trees. Analyses were run for 100,000 generations, sampling every two, with a burn-in of 8,000; and two independent runs were conducted with different starting seeds to ensure consistency of results.

2.3. Phylogenetic dating

To understand the evolutionary history of the phasmid geckos within different biomes we estimated timescales of diversification using StarBEAST2 v0.13.5 (Ogilvie et al., 2017). We wanted to build upon and improve the robustness of the phylogenetic dating analysis conducted in previous work by Nielsen (2016) by including an additional nuclear locus and using a multi-species coalescent estimation method. We analysed the combined four locus mitochondrial and nuclear dataset, with simplified substitution model and partitioning

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strategies (Table A.4), and a strict clock for each locus, with an overall Birth-Death prior. In addition, we included a single secondary constraint on the species-tree root height – a normal prior with mean = 46.9 Ma, standard deviation = 2.4 – derived from the age of the family Diplodactylidae as estimated by previous fossil calibrated gecko phylogenies (Skipwith et al., 2016). Two independent analyses were run for 20 million generations, sampling every 1000. Convergence and stability were assessed in TRACER v1.6 (Rambaut et al., 2014) and AWTY (Wilgenbusch et al., 2004), and we ensured adequate ESS values for all parameters (ESS > 200). After 20% of samples were discarded as burn-in from each analysis, remaining MCC trees were combined and summarised with TREEANNOTATOR v1.8.0 (Drummond et al., 2012).

2.4. Distribution modelling

In addition to comparing genetic diversity patterns across biomes, we wanted to assess if distributions of phasmid *Strophurus* lineages in different regions were closely correlated with the same or different ecological variables. The distribution of spinifex varies in the AMT – where it tends to be patchy and associated with oligotrophic soils in areas such as escarpments and surrounds (Bowman et al., 2010), compared to the AAZ – where it is more continuous and widespread (Crisp et al., 2004; Crisp and Cook, 2013). With this in mind, we predicted that environmental variables associated with topographic complexity (rock escarpments) might correlate more strongly with distributions of phasmid geckos in the AMT than the AAZ. We used MaxEnt v3.3.3 (Phillips and Dudík, 2008) to estimate species distribution models (SDMs) for the three major clades of phasmid *Strophurus* (see Results) and to compare the correlation of climate, geology and vegetation features with distributions of different taxa between biomes. Analyses were conducted at major clade level (i.e. Kimberley, Top End, and AAZ) because species and lineage level would have resulted in

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sample sizes too small to accurately model distributions (particularly within the Kimberley region). We chose eleven environmental variables (see Table A.6a) considered possible determinants of distributions for these geckos to compare in the SDMs. Modelling was conducted using all available museum records, which comprised 66 records for Kimberley, 80 for Top End and 255 records for the AAZ, using a regularisation multiplier of 1 and sampling 10,000 background points from a 2.5 degree radius around presence locations. For each lineage the model was repeated 20 times using the MaxEnt bootstrapping option, with the median result retained. Model performance was evaluated using the sample withheld for testing in each replicate.

3. Results

3.1. *Phylogenetic relationships and lineage diversity*

Monophyly of the clade comprising the five recognised phasmid gecko species relative to other *Strophurus* was strongly supported in all analyses with concatenated datasets (i.e. nuDNA only vs. all loci [mtDNA + nuDNA]; Figs. A.2–3). The monophyly of this clade was similarly supported in individual locus analyses for two loci (*PRLR*, *RAG1*). Though the remaining two loci (*ND2*, *PDC*) did not recover strong support (i.e. <75 Maximum-Likelihood bootstraps, <0.90 Bayesian posterior probabilities) for this node there were also no strongly supported incongruent relationships. Within the phasmid *Strophurus* clade, three geographically cohesive lineages were also strongly supported in all analyses: two clades in the AMT – i) *mcmillani/robinsoni* (Kimberley/Ord Region), and ii) *horneri/taeniatus* (Arnhem Land/Northern Deserts); and a single lineage from northern AAZ iii) *jeanae* (Figs. A.2–7). The pattern of relationships between these three lineages was not resolved.

We recovered deep mtDNA structure within all nominal species except *S. jeanae*. The *mcmillani/robinsoni* clade comprised five lineages with mean Tamura-Nei (TN) mtDNA

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divergences ranging from 10.5–14.4% (Figs. 2a and 3, lineages 1–5; Table A.5). The distribution and relationships of these five lineages were inconsistent with current taxonomy, and samples identified as *S. robinsoni* render *S. mcmillani* paraphyletic. Within the Arnhem Land/Northern Deserts clade the two currently recognised species (*S. horneri* and *S. taeniatus*) formed two discrete mtDNA lineages, also showing further divergences (5.6–9.2%; Figs. 2a and 3). Mitochondrial divergences within the relatively restricted *S. horneri* from the Arnhem Plateau were comparable to or deeper than those within the much more widely distributed Northern Deserts taxon *S. taeniatus* (*S. taeniatus* 'west' and *S. taeniatus* 'east', Fig. 2a, Table A.5). In contrast to high mtDNA diversity in the AMT, diversity within *S. jeanae* occupying the AAZ is comparatively shallow (0–4.7%) despite the fact this taxon has the widest distribution.

Analyses based on nuDNA (*PDC*, *PRLR* and *RAG1*) provided no evidence of further subdivisions or recognition of distinct taxa within the Kimberley/Ord Region and Arnhem Land/Northern Deserts clades (Fig. 2b). However, these loci typically used in phylogenetic studies across highly divergent genera often lack sufficient resolution for phylogeographic studies.

Tajima's D tests for range expansion within all three clades of phasmid geckos were not significant: Kimberley/Ord Region (D = 0.010, *P* = 0.545); Arnhem Land/Northern Deserts (D = 0.196, *P* = 0.623); and AAZ (D = -0.700, *P* = 0.258). The Fu's Fs statistic for the AAZ *jeanae* lineage however, was significant (Fs = -6.448, *P* = 0.009*), unlike those of the AMT clades (*mcmillani/robinsoni* – Fs = 1.338, *P* = 0.661, *horneri/taeniatus* – Fs = -0.225, *P* = 0.274), implying a signature of demographic expansion within the AAZ.

Species delimitation tests in Bayesian Phylogenetics and Phylogeography (BP&P) consistently supported the distinctness of the nine divergent mtDNA lineages in the

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Kimberley and Top End (≧0.98 Posterior Probability), despite limited recovery in nuDNA-based phylogenetic analyses.

3.2. Divergence dates

Age estimates from the multi-species coalescent analysis indicated the crown radiation of the phasmid geckos dates to the early Miocene (21.6 Ma, 95% Highest Posterior Density [HPD] 17.3–25.9 Ma; Table 1). Estimated crown ages for the AMT clades tended to date to the late-Miocene or early-Pliocene (6.6–7.9 Ma, HPD 3.6–10.1 Ma; Table 1), while diversity within the single AAZ lineage is comparatively young (1–3 Ma; from preliminary analyses which included intra-specific sampling not shown here).

3.3. Distribution models

The environmental variables that contributed most to the species distribution models (SDMs; Fig. A.8) differed between the three clades of phasmid *Strophurus* (see Table A.6b), although total annual precipitation contributed the highest percentage to all models. Climatic factors such as precipitation and radiation accounted for 88% of the distribution of the *jeanae* lineage in the AAZ. Lineages in the AMT showed a stronger signal from physical habitat variables; most notably slope (related to local topographic relief) contributed ~19% of the SDMs for both AMT lineages, as opposed to <3% for the AAZ lineage. Within the AMT lineages further differentiation was evident. Climatic (precipitation) variables contributed ~50%, and physical habitat (topographic and vegetation) variables contributed ~50% to prediction of the *horneri/taeniatus* distribution in the Top End/Northern Deserts. While in comparison, climatic variables, especially those associated with precipitation (including rainfall seasonality), explained almost 80% of the distribution of *mcmillani/robinsoni* within

the Kimberley. Performance for the AAZ, Top End/Northern Deserts and Kimberley models, according to the area under the curve (AUC) was 0.80, 0.90, and 0.94 respectively.

4. Discussion

We present a detailed phylogeographic and phylogenetic study of phasmid *Strophurus*, a lineage of spinifex-specialised geckos from the Australian Monsoonal Tropics (AMT) and Arid Zone (AAZ) biomes. Our analyses strongly support the monophyly of the phasmid geckos (concordant with previous work; Nielsen et al., 2016) and further support three major clades: i) *jeanae* – a single species widespread in the AAZ; ii) *horneri/taeniatus* – two species occurring in the semi-arid Northern Deserts (widespread) and Monsoonal Tropics (AMT) (restricted); and iii) *mcmillani/robinsoni* complex – entirely restricted to the AMT. Though not overt from the slowly evolving nuDNA loci sequenced here, the latter includes at least five deeply divergent mtDNA lineages consistently supported as evolutionarily distinct by BP&P. Further work is required to clarify the evolutionary significance of these lineages, but these results suggest the possible presence of additional cryptic candidate taxa within both the Kimberley and Top End regions. Relationships between the three major clades are not resolved.

4.1. Contrasting distributions across an aridity gradient

An abundance of deeply divergent, highly geographically-structured, and taxonomically unrecognised lineages is a striking, and until recently overlooked, pattern emerging from multiple phylogeographic analyses of vertebrates from the AMT (e.g. Moritz et al., 2015; Oliver et al., 2014c; Potter et al., 2016; Rosauer et al., 2016). Geographic structuring appears particularly pronounced in topographically complex escarpments of the Kimberley (Doughty, 2011; Oliver et al., 2010; 2014b; Potter et al., 2012) and Arnhem Land (Catullo et al., 2014b;

Cracraft, 1991; Ladiges et al., 2003; Oliver and Parkin, 2014). The phasmid geckos conform to these patterns, despite having an ecology not linked (at least not directly) to rocks. Five divergent mitochondrial lineages (*mcmillani/robinsoni* complex) were identified in the Kimberley/Ord Region (divergences deeper or close to species-level divergences in other Diplodactylid geckos [e.g. 8–16%; Oliver and Sanders, 2009; Oliver et al., 2014c]; Fig. 3, lineages 1–5; Table A.5), and at least two (within *S. horneri*) on the Arnhem Plateau. Most of these mtDNA lineages were not recovered by slowly-evolving nuclear loci used in this study, however analyses with rapidly-evolving nuclear exons and greater sampling are required to further test their evolutionary significance (see Potter et al., 2016). In contrast, just one lineage with low genetic diversity (*S. jeanae*), also showing evidence of recent range expansion, is widespread across much of the AAZ – again a pattern replicated in many taxa (Chapple and Keogh, 2004; Fujita et al., 2010; Jennings et al., 2003; Oliver et al., 2014c). Although some groups, including spinifex grasses, show evidence of genetic structure within rocky isolates in the AAZ, especially the Pilbara (Anderson et al., 2016; Maryan et al., 2007; Melville et al., 2011; Pepper et al., 2008; 2011a), phasmid geckos show no evidence of this.

These contrasting patterns across aridity gradients in Australia could be attributed to multiple factors: differing biome age (i.e. time for speciation; Fujita et al., 2010; Pepper et al., 2011b) and/or differing net diversification (speciation minus extinction) rates in different regions (Hutter et al., 2013; Rabosky et al., 2007a). Given the small number of lineages involved and associated uncertainty in ancestral state estimates, our data provide no strong evidence phasmid geckos have a longer history in the AMT. However, in conjunction with spatial modelling, they do suggest topographic complexity is an important correlate with lineages in the AMT, but not in the AAZ. Empirically, the most geographically restricted lineages are associated with regions of geologically stable, ancient, and exposed escarpment in the Kimberley and Arnhem Land. Unlike many other highly geographically-structured taxa

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of this region, phasmid geckos are not rock specialists (saxicoline). However, in heavily burnt and wetter areas of the northern AMT, large areas of (typically) fire-sensitive spinifex are closely associated with stable rocky outcrops and often isolated by grassy savannah woodlands or black-soil plains (Bowman et al., 2010). In the AAZ, spinifex is more widespread, occurring across many different substrates (Crisp and Cook, 2013) and there is also strong evidence desert habitats have been highly mobile and changed significantly throughout the Plio-Pleistocene (Fujioka et al., 2009). Our findings would suggest that varying effects of underlying topography and climatic variation on the distribution of spinifex in different biomes may have also shaped contrasting patterns of phylogeographic diversity in phasmid geckos.

4.2. *Definition and evolutionary history of Australian biomes*

The distributions of phasmid gecko clades broadly correspond with and support the boundaries of two major Australian biomes and bioregions. One is restricted to the arid biome (*jeanae*), one to the monsoonal tropics (*mcmillani/robinsoni*), whilst the third (*horneri/taeniatus*) includes sub-lineages associated with the Arnhem Plateau of the AMT (*S. horneri*) and the seasonally wet semi-arid zone (Northern Deserts) along the northern edge of the AAZ (*S. taeniatus*). The latter region has long been recognised as a broad interzone between biomes (Cracraft, 1991; Nix, 1982) and under different definitions could be viewed as either part of the AAZ (e.g. aridity index < 0.5) or AMT (> 85% of rainfall concentrated in summer; Bowman et al., 2010). Our findings complement a growing number of phylogenetic studies indicating the Northern Deserts region has a unique biota (Catullo et al., 2014a; 2014b; Fujita et al., 2010; Ladiges et al., 2011; Melville et al., 2011; Smith et al., 2011).

There is also a general and longstanding trend to place the trajectory of evolution in the Australian continent into a framework of a broad directional transition from ancestral mesic

environments into derived and younger arid environments (Byrne et al., 2011; Byrne et al., 2008; Crisp et al., 2004). This framework is generally supported when comparing the central arid and mesothermal biomes along the east coast (Byrne et al., 2011; Byrne et al., 2008; Chapple and Keogh, 2004; Hugall et al., 2008; Oliver and Bauer, 2011). However, there is potential for much nuance to this overall theme, and the history and patterns of biotic interchange between seasonally mesic or arid areas (especially the AMT and AAZ) remain rather poorly understood (Crisp and Cook, 2013; Jabaily et al., 2014; Toon et al., 2015).

Previous work on *Strophurus* suggested that the arid biome may be ancestral (Nielsen et al., 2016); a pattern matching results for *Triodia* (Toon et al., 2015). The depth of divergences (early- to mid-Miocene) between the three major clades of phasmid geckos, and late Miocene diversification in the AMT clades, further emphasises that the seasonally arid or arid biomes to which this lineage is now restricted date back well into the Miocene. This concurs with a growing number of other paleo-ecological and phylogenetic datasets suggesting at least seasonally arid biomes – and/or fire-affected biomes – may have a long history on the Australian continent (Crisp and Cook, 2013; Kear et al., 2016; Oliver et al., 2010; Oliver and Bauer, 2011; Toon et al., 2015).

This result also adds to studies indicating a potentially long history of biotic interchange between the AMT and AAZ (Catullo and Keogh, 2014; Fujita et al., 2010; Oliver et al., 2014a; Toon et al., 2015). Interestingly, however, the lack of genetic structure, and possible signatures of recent range expansion, within the phasmid gecko lineage (*S. jeanae*) in the AAZ is at odds with an inferred long history within this biome (Nielsen et al., 2016). This is particularly true when contrasted with the patterns observed in *Triodia* of elevated structure across the Pilbara (Anderson et al., 2016), as well as in other gecko taxa distributed across the AAZ (Pepper et al., 2008; 2013). Considering evidence for a long history of *Strophurus* in arid biomes, we argue that the comparative patterns of genetic diversity in phasmid geckos

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between the AMT and AAZ biomes cannot necessarily be explained by differences in age of the two biomes (Nielsen et al., 2016). In particular, the age of the AMT is poorly resolved, but mounting evidence for relatively young (mid- to late-Miocene) crown-ages of vertebrate lineages within the Kimberley and Top End regions suggests endemic diversity within the Monsoonal Tropics biome is likely no older than many arid zone radiations (Laver et al., in review). These findings and observations lead us to suggest that the heterogeneity of the landscape, and potentially the associated differences in distribution of spinifex within the AMT, has played an important role in facilitating diversification and persistence of short-range endemic lineages within the more topographically complex escarpment regions. Thus the low divergence in *S. jeanae* may reflect long-term connectivity of this widespread population, perhaps facilitated by a more continuous distribution of spinifex habitat within this biome.

4.3. An evolutionary response to vegetative change?

The vegetation of the Australian continent has changed profoundly over the last 20 million years (Crisp and Cook, 2013). However compelling demonstrations of how this has affected the long-term evolutionary trajectory of the Australian biota are relatively few. Phasmid geckos are only known to occur on spinifex habitat (King and Horner, 1993; Storr, 1978; Wilson and Swan, 2013; Wilson, 2012) and show a number of traits that appear to associate with this ecology. In particular, amongst gekkotans, thin, longitudinal and highly contrasting stripes are particularly rare (< 30 examples in over 1,500 species; Appendix: Table A.7) and often associated with utilisation of longitudinally-oriented vegetation (e.g. bamboo, sedges, grasses, etc.) suggesting a specialised disruptive or camouflage function (Losos, 2009). However, despite the ecological dominance and importance of spinifex within the AMT and AAZ biomes, recent studies have suggested that the sub-tribe Triodiinae only colonised

Australia around the mid-Miocene (Toon et al., 2015). In contrast, phasmid *Strophurus* are members of a near endemic Australian family of lizards with Gondwanan origins. Our early Miocene estimate for the crown radiation of phasmid *Strophurus* somewhat pre-dates current estimates for the initial radiation of spinifex. Conservatively, this temporal overlap suggests that phasmid *Strophurus* shifted into spinifex close to when it initially began to radiate, and have subsequently persisted with relatively little outward ecological diversification (little evidence of sympatry and deeply divergent taxa such as *S. jeanae* and *S. taeniatus* differ only in subtle scale characters). A less conservative interpretation is that these lineages shifted into this niche independently. More work is required to refine estimates of how and when other lizards (including other lineages of *Strophurus*) may have specialised to use spinifex. However, these data all point towards a potentially strong example of ecological shift in a pre-existing faunal lineage following invasion and radiation of a grass that is now a dominant component of a continental vegetation.

4.4. *Conclusions*

Spinifex-specialised phasmid *Strophurus* geckos exhibit higher and older genetic structuring associated with rock regions in the AMT and wider distributions with shallower divergences in the AAZ, which do not reflect the inferred long history within this arid biome. Despite shallow present structure in the AAZ, the old crown age of the phasmid geckos, along with old diversity within the AMT support the growing body of data indicating that at least seasonally-arid environments may have a long history within Australia dating back well into the Miocene. Finally, this study emphasizes that biome age or history of occupancy alone cannot explain the especially high genetic diversity being uncovered in the AMT compared to the AAZ, and implies an important role of the topographically variable landscape in shaping and preserving diversity within the AMT.

Acknowledgements

Appendix A. Supplementary tables and figures

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Table 1 Prior and posterior distributions for root and crown-age estimates of major lineages within the Australian phasmid *Strophurus* complex and related taxa based. Results are derived from a multi-species coalescent analysis conducted in StarBEAST2 on the combined (mtDNA+nuDNA) dataset, with a secondary calibration set for the species-tree root height. Values in brackets represent standard deviation around the mean for the prior, and 95% Highest Posterior Densities (HPD) for the posteriors.

	Combined (mtDNA+nuDNA)
Prior	
Root (Diplodactylidae; Normal)	46.9 (42.2–51.6)
Posteriors (crown ages)	
Root (Diplodactylidae)	46.7 (41.9–51.3)
Australian Diplodactylidae	37.2 (31.3–43.0)
Strophurus	25.0 (20.8–29.4)
Spiny-tail *Strophurus* clade	10.8 (8.7–12.9)
Phasmid *Strophurus* complex	21.6 (17.3–25.9)
1) Arid Zone – *Strophurus jeanae*	n.a.
2) Kimberley/Ord – *Strophurus mcmillani/robinsoni*	7.9 (5.6–10.1)
3) Arnhem/N Deserts – *Strophurus horneri/taeniatus*	6.6 (3.6–9.4)

n.a., no estimate available

Figure captions

Figure 1 Distribution of all phasmid gecko specimen records in Australian museums, acquired from the Atlas of Living Australia online (ALA; http://ozcam.ala.org.au/). *Strophurus jeanae* (pink) – Arid Zone; *S. mcmillani* (blue) – Kimberley, *S. robinsoni* (yellow) – Ord Region; and *S. horneri* (purple) – Arnhem Land, *S. taeniatus* (green) – Northern Deserts. Approximate boundary of the Australian Arid Zone (AAZ) is indicated by the dotted line as modified from Byrne et al., (2008), whilst the Australian Monsoonal Tropics (AMT) spans the region to the north. Brown dashed regions indicate the boundaries of the Kimberley Plateau and Arnhem escarpment, whilst the blue dashed line surrounds the approximate Ord Region as modified from Catullo et al., (2014b). Photographs courtesy of Henry Cook, Ryan Francis, Stephen Richards, Brendan Schembri, and Stephen Zozaya.

Figure 2 SplitsTree networks of phasmid *Strophurus* geckos for **a)** mitochondrial (*ND2*), and **b)** concatenated nuclear (*PDC, PRLR, RAG1*) datasets. Number symbols indicate lineages within the *mcmillani/robinsoni* clade, circles are taxa identified as *S. mcmillani*, whilst squares are identified as *S. robinsoni*. Triangle symbols indicate lineages within the *horneri/taeniatus* clade (*S. horneri* – purple, *S. taeniatus* – green). Red ellipse in **b)** highlights samples of *S. horneri* nested within *S. taeniatus*. Maps display geographic distributions of the three major phasmid gecko lineages.

Figure 3 Chronogram of divergence dates between lineages of the Australian phasmid *Strophurus* complex and congeners estimated with StarBEAST2 using the combined mitochondrial (*ND2*) and nuclear (*PDC, PRLR, RAG1*) dataset with a secondary calibration on species-tree root height. Major genetically divergent clades (≧8% Tamura-Nei divergence) within the phasmid geckos are highlighted, black circles on key nodes indicate

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posterior probability support values of $\geqq 0.95$. Red shaded rectangles over key nodes indicate the 95% Highest Posterior Densities (HPD) for key nodes. Green column indicates range from minimum to maximum of 95% HPD estimates for the crown age of *Triodia* within Australia (Toon et al., 2015). Taxa labels in brown indicate lineages within the phasmid *Strophurus* clade.

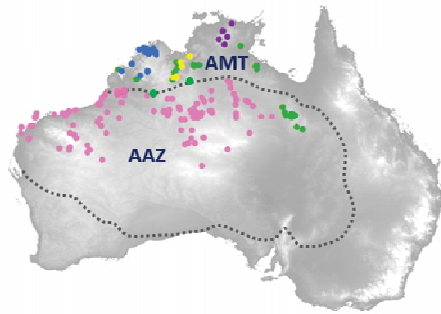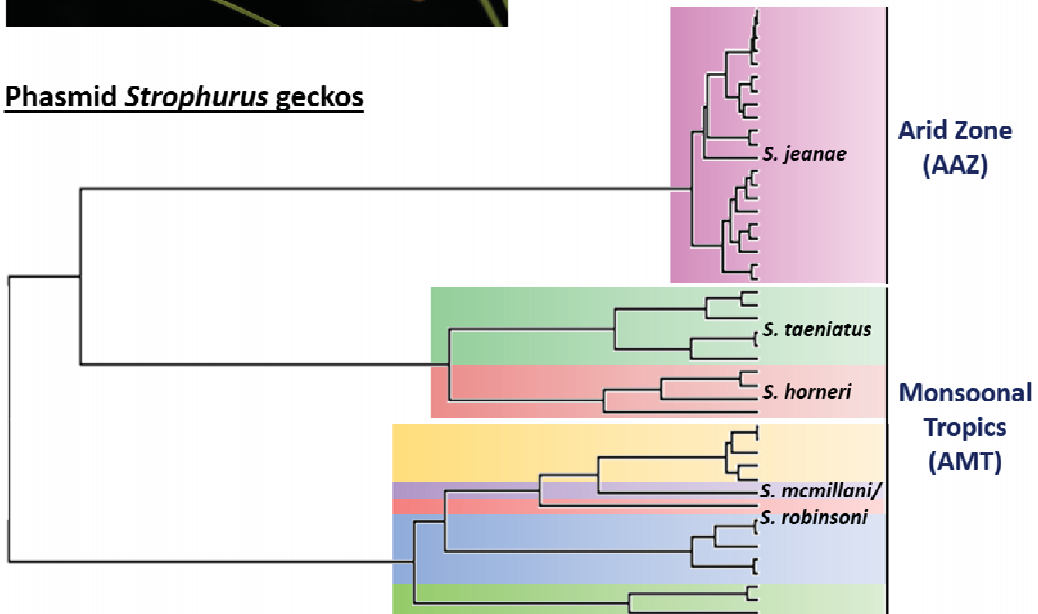a) Mitochondrial

b) Nuclear
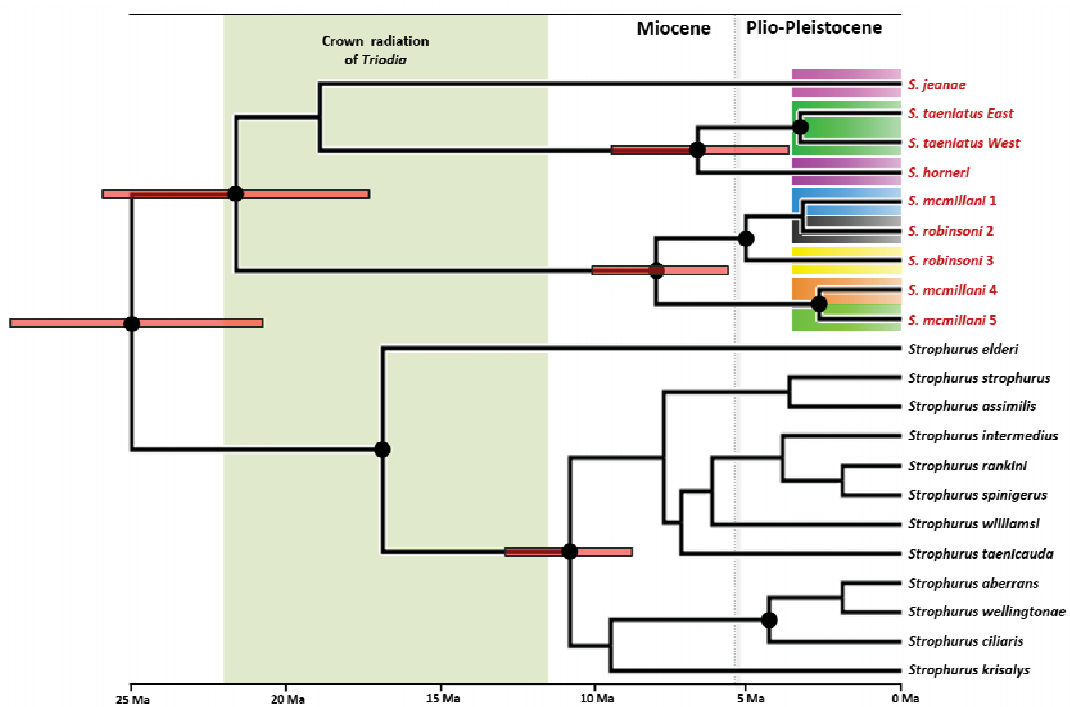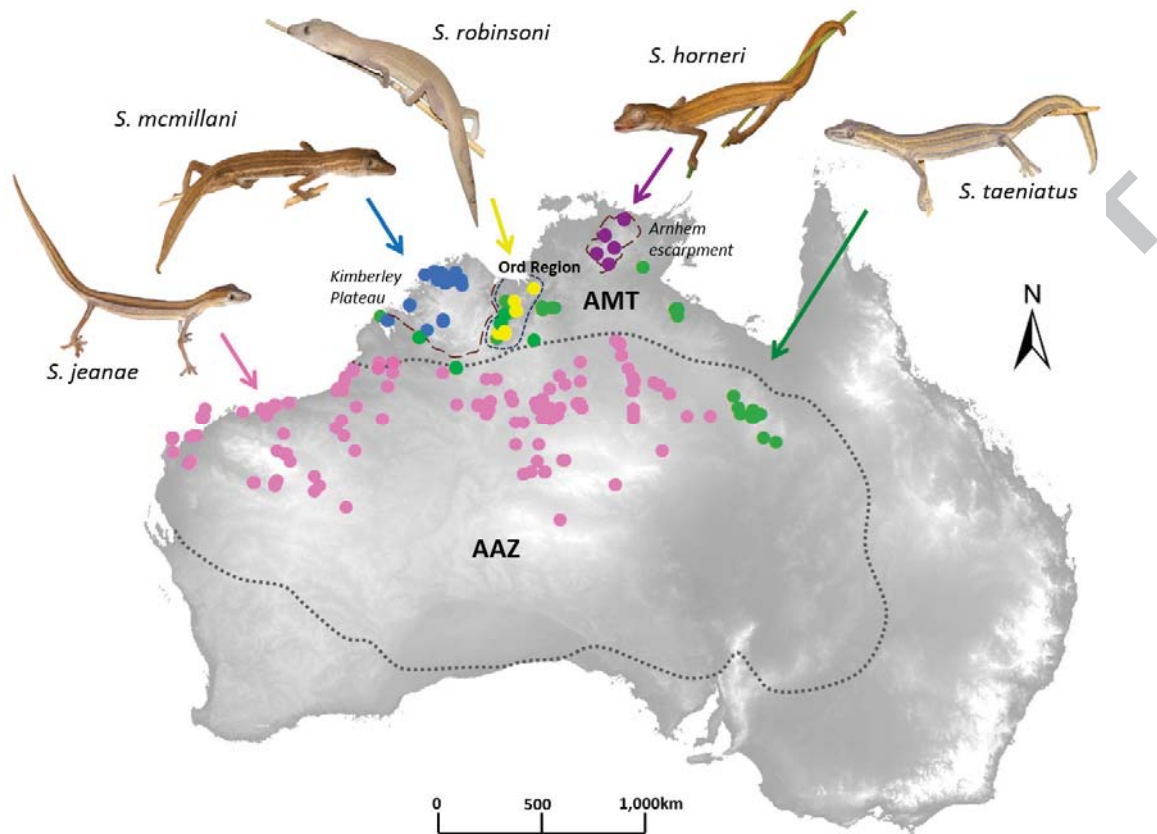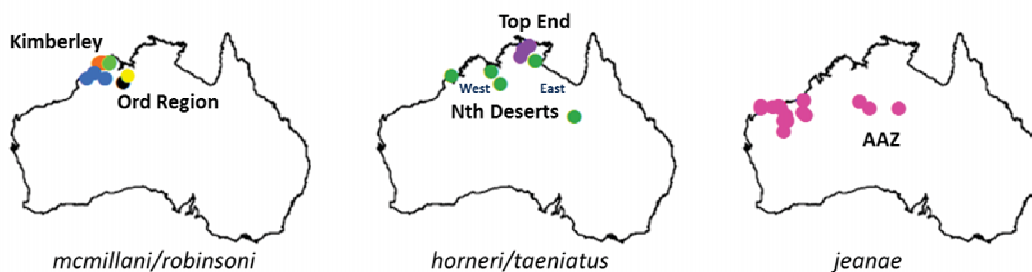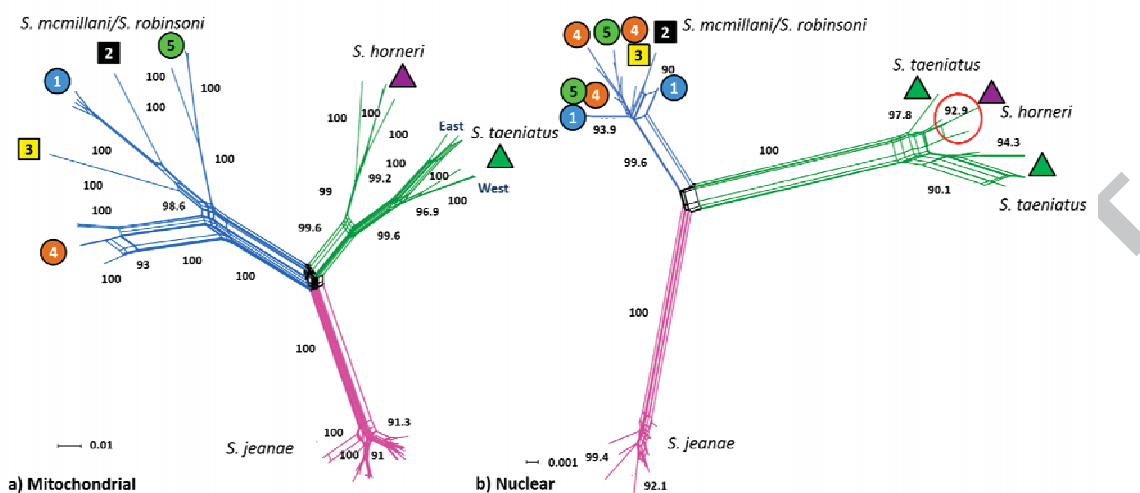
Trans-biome diversity in specialised Australian lizards (Diplodactylidae: *Strophurus*)

Rebecca Laver, Stuart Nielsen, Dan Rosauer, Paul Oliver

Highlights:

- Cryptic diversity in the Australian Monsoonal Tropics.
- Lineage diversity and endemism decreases over an increasing aridity.
- Lineage endemism correlated with topographic complexity in the monsoonal biome.
- Early ecological shift with subsequent stasis following Miocene vegetative change.

Graphical abstract

Phasmid _Strophurus_ geckos