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Evolutionary origins and diversification of dragon lizards in Australia's tropical savannas

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ABSTRACT

Australia's monsoonal tropics are dominated by the largest and least modified savanna woodlands in the world, and they are globally significant for their high biodiversity and regional endemism. Despite this, there have been very few molecular studies of the evolutionary origins and diversification of vertebrates in this region. The semi-arboreal dragon lizards of *Lophognathus* and *Amphibolurus* are widely distributed in the savanna and dry sclerophyll woodlands of Australasia, including the monsoon tropics. We sequenced a ∼1400 bp region of mitochondrial DNA and a ∼1400 bp nuclear gene (*RAG1*) to investigate the phylogenetic relationships and phylogeographic structuring of all seven species of *Lophognathus* and *Amphibolurus*. Our analyses show that there is a higher level of species and generic diversity in the monsoon tropics than previously thought, and a full morphological review and taxonomic revision of these genera is required. Relaxed molecular clock analyses indicate that species across both genera originated in the late Miocene and early Pliocene, with significant phylogeographic structure within species. We did not find any evidence that the monsoon tropics species were a monophyletic group that had diversified within the region; instead *Amphibolurus* and *Lophognathus* represent at least three independent evolutionary colonizations of the monsoon tropics. It is probable that the origins and phylogeographic patterns of the northern *Lophognathus* species have evolved under the climatic influence of the Australian monsoon, rather than being either an ancient Gondwanan lineage that pre-dates the monsoon or the result of a more recent dispersal event across Wallace's Line.

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1. Introduction

Tropical savannas constitute one of Earth's major biomes, covering 20–30% of the land surface, with high levels of biodiversity and endemism. Recently, the Cerrado in South America, which is dominated by tropical savannas, was listed as a global diversity hotspot (Myers et al., 2000). Even though tropical savannas have been recognized globally for their biodiversity and endemism, they have been relatively poorly studied. In addition, tropical savanna regions around the world are under increasing pressure from human population growth and development, as they occur in some of the most heavily populated areas, such as India and southeastern Asia. For these reasons the Australian tropical savannas are particularly important.

The Australian tropical savannas are one of the least developed and populated of these ecosystems, and they form approximately 25% globally of the remaining savannas that are in good ecological condition (Woinarski et al., 2007). Tropical savanna woodlands constitute a significant element of the Australian landmass. Vegetation of dense grass and scattered trees covers almost one quarter of the continent, extending the full east–west extent of the northern third of Australia (Bowman et al., 2010). These regions are dominated by a tropical monsoon climate (>85% of rainfall received between November and April; Bowman et al., 2010), which is stronger and more predicable near the coast. Further inland, the climate becomes generally less predictable and drier. Despite its importance very little is known about the biogeography and evolutionary history of the fauna in this Australian ecosystem.

The landscapes of northern tropical Australia have a long and complex history. There are geologically ancient formations (Precambrian basement rock), recent sediments and Proterozoic sandstones forming three escarpment blocks (Kimberley, Arnhem Land plateau and the ranges of Cape York Peninsula (Fig. 1)). These sandstone escarpments provide moister habitats than elsewhere in the monsoon tropics and are separated by topographic barriers formed from Cretaceous sea floors (Bowman et al., 2010): the Carpentarian

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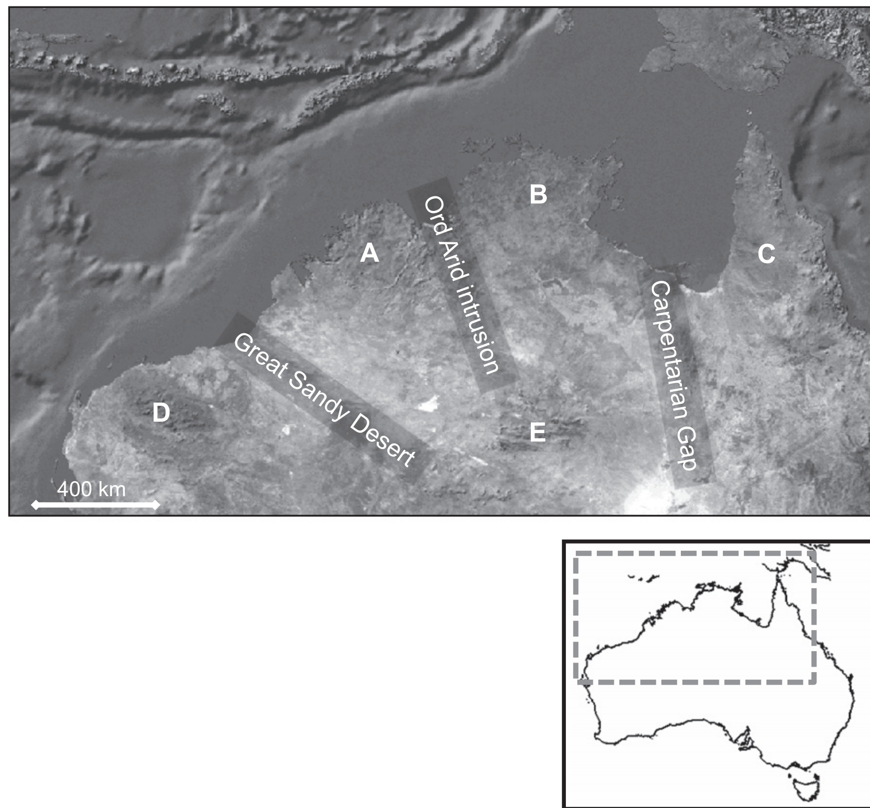


Fig. 1. Map of northern Australia with the major biogeographic barriers and topographic features: (A) the Kimberley; (B) Arnhem Land Plateau; (C) Cape York Peninsula; (D) the Pilbara; and (E) MacDonnell Ranges.

Gap, which separates Cape York and Arnhem Land, and the Ord Arid intrusion, which divides Arnhem Land and Kimberley (Fig. 1). The monsoon climate across this region also has a long history, with the uplift of the Tibetan plateau and increasing differentiation between equatorial and polar temperatures providing conditions conducive to the Australian Monsoon climate during the mid-late Miocene (15–8 Mya; Bowler, 1982; McGowran et al., 2004). The modern monsoon climate patterns had been established by the Pliocene (3–2.5 Mya; Haug and Tiedemann, 1998), although the intensity of the monsoon varied dramatically with the glacial-interglacial oscillations during the Pleistocene (Bowman et al., 2010).

Tropical savanna woodlands are home to a major component of Australian biodiversity, with some areas, particularly on the sandstone escarpments, possibly having similar biodiversity levels to Australian rainforests (Bowman et al., 2010). Yet, in tropical Australia most research efforts have focused on the tropical rainforests, which cover <1% of Australia's landmass. There have been extensive phylogeographic studies investigating the evolutionary histories of many rainforest vertebrate taxa, including frogs (e.g., James and Moritz, 2001; Schauble and Moritz, 2001), reptiles (e.g., Stuart-Fox et al., 2001; Phillips et al., 2004), and birds (e.g., Joseph and Moritz, 1994). These studies hypothesized long histories of vicariance and have identified numerous biogeographic barriers in northeastern Australia. More recently, there has been a rapid increase in phylogeographic research on Australia's arid centre, which, contrary to long-held assumptions, has found a long history of diversification and vicariance (Byrne et al., 2008 and references therein). However, very little is currently known about the diversification and biogeographic history of species in Australia's monsoon tropics, where there have been very few phylogenetic studies and virtually no phylogeographic studies undertaken.

There are many widespread species that occur in the tropical savannas, with distributions from Cape York, the Northern Territory, Kimberley and into the Pilbara. One such species, the common rock rat, has been found to have very little genetic divergence across its range (Baverstock et al., 1977). Alternatively, there are many vertebrate groups across the savannas that have high levels of endemicity, with species having restricted or disjunct distributions in the Kimberley, Arnhem Land and Cape York regions. For example, the black-throated finch complex experienced multiple vicariance events during the Pleistocene (300,000–600,000 years ago) across the Carpentarian Gap and the Ord Arid Intrusion (Fig. 1) (Jennings and Edwards, 2005; Schodde and Mason, 1999). Similarly, the red-backed fairy-wren experienced divergence across the Carpentarian Gap during the Pleistocene (~270,000 years ago; Lee and Edwards, 2008). Thus, these few molecular studies suggest that diversity in the monsoon tropics originated in the Pleistocene. However, more research is needed to determine whether other taxa share a similar evolutionary history.

Although there is evidence for a long history of the monsoon climate across northern Australia, dating back to the Late Eocene (Bowman et al., 2010), it may be that the development of the modern monsoon climate during the Pliocene and the subsequent Pleistocene oscillations has been the major influence on the diversification and distribution patterns of the current vertebrate faunas. If this is the case, we would expect vertebrates in the tropical savannas to have more recent origins and patterns of diversification than those in arid and semi-arid regions, which have been found to date back to the Miocene (Byrne et al., 2008). We sought to test this supposition using two genera of agamid lizards that have broad distributions in the monsoon tropics and arid regions.

The lizard genera *Amphibolurus* and *Lophognathus* are medium-large bodied agamid lizards endemic to the Australasian region.

The distributions of these genera span much of continental Australia, including arid, semi-arid, temperate and tropical monsoon regions (Fig 2). They are generally semi-arboreal and associated with savanna woodlands, woodlands and riverine vegetation (Greer, 1989).

Amphibolurus and *Lophognathus* have long been considered closely related, but the composition of these two genera has varied over time and among authors. For example, Greer (1989) and Wilson and Swan (2008) assign all species to the genus *Amphibolurus*, while Cogger (2000) places three species in *Amphibolurus* and three in *Lophognathus*. In our study, we follow the current catalogue (ABRS, 2010; Table 1), which lists four species in *Lophognathus* (*L. burnsi*, *L. gilberti*, *L. longirostris* and *L. temporalis*) and three species in *Amphibolurus* (*A. muricatus*, *A. norrisi* and *A. nobbi*). Our study incorporated all current species of *Amphibolurus* and *Lophognathus*, except *A. nobbi*. Recent molecular work and morphological analyses clearly indicate that *A. nobbi* is a member of the genus *Diporiphora* and unrelated to either *Amphibolurus* or *Lophognathus*. For this reason *A. nobbi* has not been included in our study (Schulte et al., 2003; Edwards and Melville, submitted for publication).

Recent molecular work indicates that further taxonomic problems may exist in *Lophognathus* and *Amphibolurus*. Schulte et al. (2003) provided the first molecular phylogeny of Australian agamids and identified a clade containing four species in three genera that form a clade with the *Amphibolurus* type species (*A. muricatus* [the type], *A. norrisi*, *L. gilberti*, and *Chlamydosaurus kingii*). This molecular work also suggests that *L. temporalis* and *L. lophognathus* are sister species but are an independent evolutionary lineage to *L. gilberti* (Schulte et al., 2003). *L. burnsi* has not been included in any molecular study. In addition, molecular work (Schulte et al., 2003) and field observations (Melville unpublished data) lead us to hypothesize that a number of populations that have been relegated

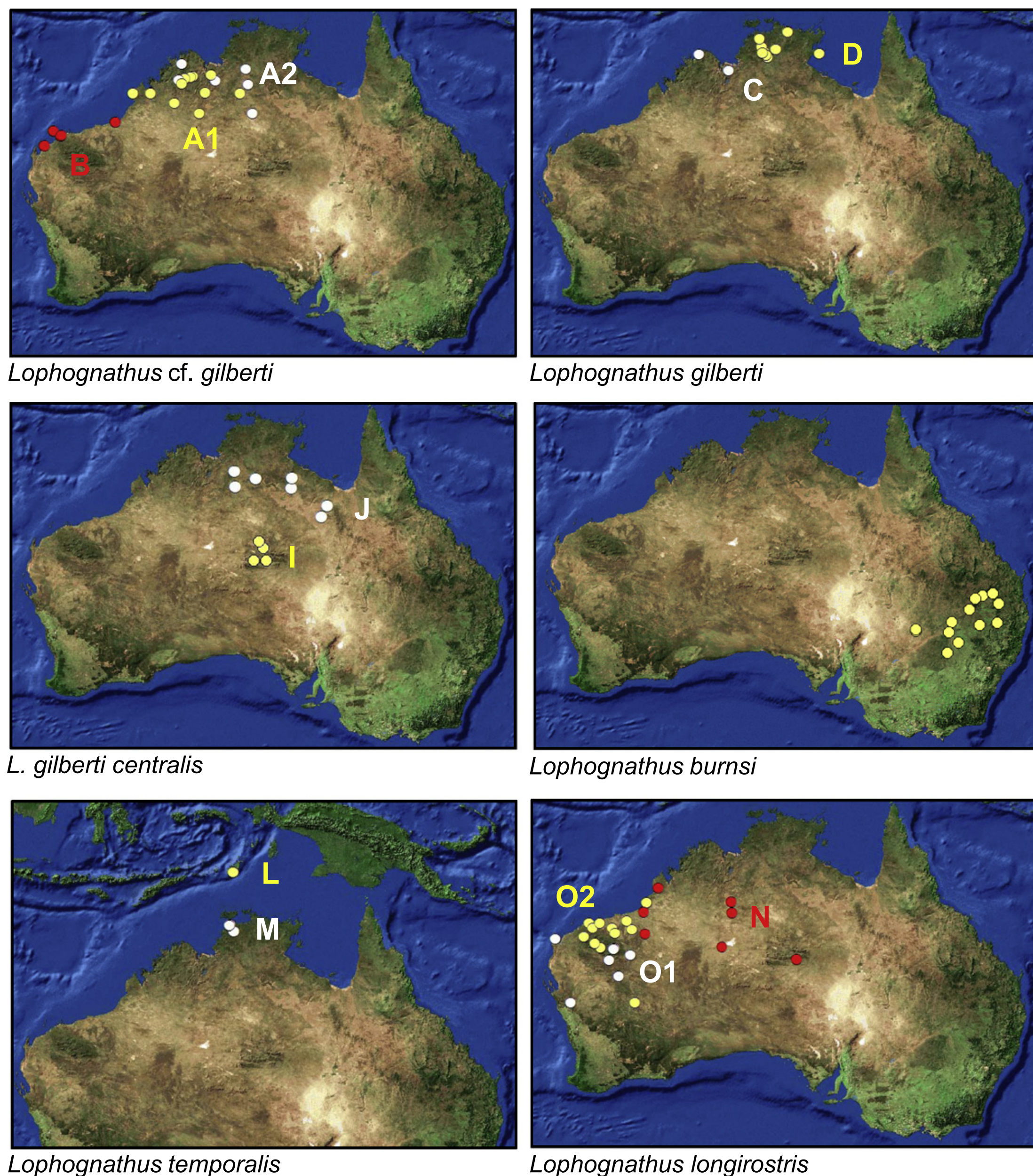
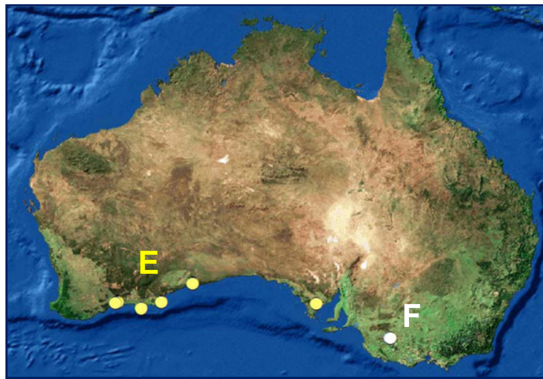


Fig. 2. Distribution maps of the samples sequenced in the current study for eight *Lophognathus* and *Amphibolurus* species. *Lophognathus gilberti* has been separated into *L. gilberti*, *L.* cf. *gilberti* and *L. gilberti centralis*, as shown in phylogenetic results (Figs. 3–5). Samples are colored to designated different mtDNA clades and clade letters refer to those in Fig. 3.



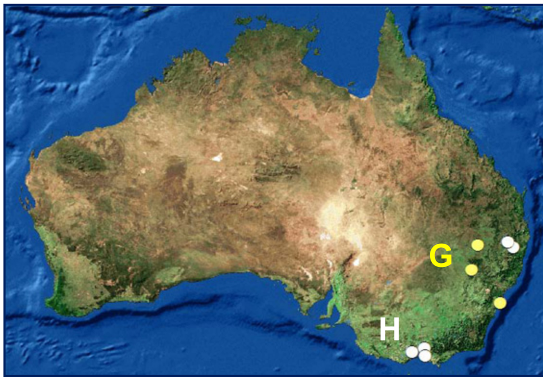


Fig. 2 (*continued*)

Table 1
Agamidae study species, detailing current taxonomic designations from ABRS (2010). Primary types of species with original genus designations are highlighted in bold.

Species, including primary types and junior synonyms	Described	No. sequenced
1. *Amphibolurus muricatus*	White (1790)	10
Lacerta muricata	**White (1790)**	
Agama jaksoniensis	Cloquet (1816)	
2. ***Amphibolurus norrisi***	**Witten and Coventry (1984)**	16
3. ***Chlamydosaurus kingii***	**Gray (1825)**	
4. ***Lophognathus burnsi***	Wells and Wellington (1985)	15
5. ***Lophognathus gilberti*** *Redtenbacheria fasciata* *Physignathus incognitus* *Physignathus gilberti centralis*	**Gray (1842)** Steindachner (1867) Ahl (1926) Loveridge (1933)	63
6. ***Lophognathus longirostris*** *Physignathus eraduensis* *P. longirostris quattuorfasciatus*	**Boulenger (1883)** Werner (1909) Sternfeld (1924)	31
7. ***Lophognathus temporalis*** *Lophognathus lateralis* *Lophognathus labialis*	**Günther (1867)** Macleay (1877) Boulenger (1883)	14

to *L. gilberti*'s synonomy (e.g., *Physignathus gilberti centralis*) may represent valid species. In the present study, we attempt to resolve problems raised by previous studies of *Amphibolurus* and *Lophognathus* by conducting more extensive sampling within and among species.

We present a comprehensive phylogeographic study of all the *Amphibolurus* and *Lophognathus* species to examine the origins and diversification of tropical savanna species. Specifically, we compare phylogeographic patterns in the tropical savanna species (*L. temporalis*, *L. gilberti*) to the remaining species, which occur in arid, semi-arid and temperate Australia. We sequenced two gene regions, incorporating mtDNA (*ND2*) and nuclear (*RAG1*) genes, and undertook extensive phylogenetic analyses. We also used a relaxed molecular clock to estimate divergence events in these species. Our study not only contributes valuable information in resolving longstanding taxonomic issues in two problematic genera but also provides important insight into the origins and diversification of species in a globally significant biome, Australia's tropical savannahs.

2. Materials and methods

2.1. Field collection of specimens and tissue samples

We collected specimens for morphological analysis and associated tissues for sequencing with two main objectives. First, we endeavored to maximize geographic spread and, second, we sought to fill geographical gaps in tissue samples already held in Australian museums. We collected a total of 88 specimens and associated tissue samples across seven species (see Appendix A for details), including *C. kingii*, *L. gilberti*, *L. longirostris*, *L. temporalis*, *L. burnsi*, *A. muricatus*, and *A. norrisi*. We also ensured that we collected samples of *P. gilberti centralis*, a junior synonym of *L. gilberti*, which we call *Lophognathus gilberti centralis* in phylogenetic analyses (see Table 1 for current taxonomy).

2.2. DNA sequencing and alignment

Genomic DNA was isolated using Proteinase K digestion and chloroform-isoamyl alcohol extraction. For all specimens, a fragment (~1200 bp) of the mtDNA genome was targeted that includes the entire protein-coding gene *ND2* (NADH dehydrogenase subunit two) and flanking genes encoding $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$. For a subset of specimens, we sequenced a ~1200 bp of recombination activating gene-1 exon in the N-terminal domain (see Melville and Hale, 2009). Oligonucleotide primer pairs used in PCR amplification and sequencing of mitochondrial and nuclear genes are listed in Table 2.

Mitochondrial and nuclear PCR amplification protocols used in the current study have been outlined in Shoo et al. (2008). Negative controls were run for all amplifications. PCR products were purified using SureClean Plus (BIOLINE). Purified product was sent to a commercial company (Macrogen, Korea) for sequencing. Sequence chromatograms were edited using SeqMan version 5.07 (DNASTAR Inc., USA) to produce a single continuous sequence for each specimen. Mitochondrial DNA sequences were aligned using tRNA secondary structure models (Macey et al., 1997), and protein-coding regions were translated to amino acids to check alignment and for stop codons.

Table 2
Oligonucleotide primer sequences.

Gene region	Sequence (5′-3′)	Source
mtDNA		
$tRNA^{Met}$ (forward)	AAGCAGTTGGGCCCATRCC	Macey et al. (2000)
COI (reverse)	AGRGTTCCRATRTCTTTRTGRTT	Modified Macey et al. (1997)
Nuclear		
RAG1(forward)	CAAAGTGAGACSACTTGGAAAGCC	Shoo et al. (2008)
RAG1(reverse)	CATTTTTCAAGGGTGGTTTCCACTC	Shoo et al. (2008)

2.3. Phylogenetic analyses

Additional tissue samples for DNA sequencing were obtained from museum collections (see Appendix A for details). These museum samples combined with the tissues we collected in the field provided comprehensive sampling for the study species (Table 1). Published sequences were also incorporated into phylogenetic analyses to allow placement of the agamid species sampled in the present study in a wider phylogenetic context, providing a representative species from all other Australian agamid genera where available. For the mtDNA and *RAG1* nuclear regions, 29 and 7 previously published samples respectively were included (see Appendix A).

Phylogenetic analyses for each of the individual gene regions were undertaken using maximum likelihood in RAxML (Stamatakis et al., 2008) and Bayesian analyses in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). Phylogenetic trees were estimated using a priori partitioned mixed model maximum likelihood (ML) with RAxML in the CIPRES web portal with separate analyses of the mitochondrial and nuclear regions. Analyses were performed using a general time-reversible model of sequence evolution with rate variation among sites estimated using a gamma distribution (GTR+Γ+I; Tavaré, 1986). This model was chosen based on preliminary analyses using Modeltest v3.7 (Posada and Crandall, 1998) that chose the TVM+Γ+I as the optimal model with the Akaike Information Criterion for the *RAG1* data but the GTR + Γ + I model for mtDNA. However, current implementation of RAxML does not permit specification of the TVM substitution model. Proportion of invariant sites also was estimated, and all model parameter values were estimated from the data. For each of the separate genes, default parameters were used with 25 rate categories and an initial rearrangement limit of 10. We assumed three (codon positions 1–3) partitions for the protein-coding mtDNA and *RAG1* genes and a fourth partition for the mtDNA tRNAs regions included. Bootstrap resampling (Felsenstein, 1985) was applied to assess support for individual nodes in each above-mentioned analysis using 100 bootstrap pseudoreplicates in RAxML with the same settings as above. We considered branches with a bootstrap value of $\geqslant$95% as strongly supported (Felsenstein and Kishino, 1993), <95 to $\geqslant$70% as moderately supported, and <70% as weakly supported.

Bayesian analyses were performed using the evolutionary model selected by ModelTest with parameters estimated from data during the analysis. Four Markov chains were used in each of two simultaneous runs starting from different random trees. Analyses were run for 5 million generations for each dataset. Standard deviation of split frequencies was used as a convergence diagnostic to confirm suitability of run length. For all analyses, it was confirmed that potential scale reduction factor (PSRF) values were close to 1.0, indicating that an adequate sample of the posterior probability distribution had been achieved (Ronquist and Huelsenbeck, 2003). In addition, the output was examined using Tracer v1.3 (Drummond and Rambaut, 2003) to check that stationarity had been reached.

A phylogenetic analysis of the combined mitochondrial and nuclear data was conducted using a partitioned Bayesian approach. For all tissue samples collected for this study, the mitochondrial and nuclear sequences in the partitioned analysis were derived from the same specimen. However, for the previously published sequences, concatenated data were for the same species but not from the same samples. The evolutionary models selected by ModelTest were employed for individual gene regions, parameters were estimated during analysis and the same run length, and sample frequency was used. Partitions corresponding to individual gene regions were unlinked, allowing each partition to have its own set of parameters. The rate parameter was set to 'variable' to allow all partitions to evolve under different rates. Default settings were utilized for all other parameters.

2.4. Partitioned Bremer support

We used a partitioned Bremer support (PBS) analysis to determine whether there was congruence between haplotype clades in the mtDNA and *RAG1* phylogenies. The data were analyzed using the maximum parsimony criterion in PAUP with heuristic searching and TBR swapping, using the following two-step strategy to reduce time spent swapping non-optimal trees. For each replicate only the five best trees were saved. We used 10,000 replicates (hsearch start = stepwise addseq = random nreps = 10,000 nchuck = 5 chuckscore = 1). This generated a subset of "better" trees which was used as the starting point for a second MP analysis in which only this subset was swapped to completion (hsearch start = current chuckscore = no). (Edgecombe et al., 2000; Larkin et al., 2006).

To determine partitioned Bremer support indices the program TreeRot.v3 was used (Sorenson and Franzosa, 2007). We time-limited the searches by modifying the TreeRot constraints file search commands to read as follows: hsearch enforce start = stepwise timelimit = 30 limitperrep = yes converse constraints = [node name] addseq = random nreps = 20 (e.g. Buffington et al., 2007). To confirm that this was an appropriate search strategy, we confirmed that the original most parsimonious tree was also found when the search was limited in this way. We also examined the TreeRot logfile to see that within each set of twenty search replicates, trees of similar or the same length were hit by each replicate.

2.5. Estimates of divergence time

A relaxed molecular clock method within the program BEAST v1.4.5 (Drummond and Rambaut, 2003) was used to estimate divergence times for each species of *Lophognathus* and *Amphibolurus*. Separate analyses were conducted on the mtDNA and nuclear phylogenies. Additional published sequences were used in the analysis to allow placement of calibration points (see Appendix A). A subset of in-group taxa were used in these analyses to ensure that assumptions of the Yule speciation prior were not violated (see Appendix A). Six calibration points were used, including five fossil calibrations and one biogeographic calibration point (Table 3). As fossils are a minimum estimate of age, we used lognormally distributed calibrations for fossils and a normal distribution for the biogeographic calibration (Table 3). The node of all iguanians (Agamidae + Chameleonidae + Iguanidae) sampled was constrained to be monophyletic compared with the outgroups. A user defined starting tree was implemented for each analysis, in which the iguanians were monophyletic. A GTR+I+Γ model of evolution was employed on all datasets, using an uncorrelated lognormal relaxed molecular clock. A Speciation: Yule Process tree prior was used with a user specified starting tree, incorporating the monophyly of Iguania. Each analysis was run for 10 million generations. The output was examined using Tracer v1.3 (Drummond and Rambaut, 2003) to check that stationarity had been reached and to assess the autocorrelation of rates from ancestral to descendant lineages, as detailed in the results (Drummond et al., 2006).

3. Results

3.1. Mitochondrial DNA

To investigate phylogenetic relationships of lineages within *Amphibolurus* and *Lophognathus* 148 new sequences and 29 previously published sequences were analyzed for the *ND2*

Table 3
Fossil and biogeographic calibrations for estimation of divergence times, including the settings implemented in the BEAST relaxed clock analyses.

Fossil age	Description	Calibration	BEAST settings (lognormal distribution)	
			Zero off-set	Std. dev.
Middle Jurassic (154–180 Mya)	Primitive acrodont iguanian, from the Kota Formation in peninsular India (Evans et al., 2002)	Common ancestor of all iguanians (Iguanidae + Agamidae + Chameleonidae)	154	1.25
Early Miocene (22.8 Mya)	Fossils assigned to "sceloporine" genera, from Wyoming and Nebraska (Robinson and Van Devender 1973)	Minimum age estimate for the clade including *Sceloporus*, *Uma*, *Holbrookia*, *Phrynosoma*, *Callisaurus*	22.8	1.0
Early Miocene (18 Mya)	Fossil *Chameleo*, but with morphological similarities to *Rhampholeon,* from Rusinga Island, Lake Victoria, Kenya (Rieppel et al., 1992)	Minimum age estimate for the clade including *Chameleo* and *Rhampholeon*	18	1.0
Pliocene (5 Mya)	Oldest known *Phrynocephalus* fossil, from eastern Turkey (Zerova and Chkhikvadze, 1984)	Minimum age estimate for *Phrynocephalus*	5	1.25
Age of Biogeographic Event	Description	Calibration	BEAST settings (normal distribution)	
			Mean	Std. dev.
Late Miocene (10 Mya)	The rise of the Pamir-Tien Shan (Abdrakhmatov et al., 1996)	Divergence between Pamir *Laudakia* (*L. lehmanni*, *L. himalayana*, *L. stoliczkana*) and those from the Iranian Plateau (*L. caucasia*, *L. microlepis*, *L. erythrogastra*)	10	1.0

protein-coding gene. The alignment comprised 1434 characters: 804 characters were variable and 701 characters were parsimony informative. A GTR+I+Γ model was selected as the best fitting model for likelihood analysis using AIC criteria. Model parameters were: gamma = 0.6648; proportion of invariable sites = 0.2141; substitution rates $A \leftrightarrow C$ 0.2480, $A \leftrightarrow G$ 5.9776, $A \leftrightarrow T$ 0.2815, $C \leftrightarrow G$ 0.3194, $C \leftrightarrow T$ 3.2307, $G \leftrightarrow T$ 1.0000; and, nucleotide frequencies A = 0.4085, C = 0.3500, G = 0.0622 and T = 0.1794.

The mtDNA Bayesian (mean ln-likelihood −19777.01) and ML trees (Fig. 3) did not recover three monophyletic genera consisting of *Lophognathus*, *Amphibolurus* and *Chlamydosaurus*. Instead, the analysis recovered a well supported monophyletic group (99% bootstrap; 100% posterior probability) consisting of taxa from three genera. Within this clade there are three well supported subclades of haplotypes sampled from: (1) *C. kingii* (100% bootstrap; 100% posterior probability); (2) *A. muricatus*, *A. norrisi*, *L. burnsi*, *L. gilberti centralis* (100% bootstrap; 100% posterior probability); and (3) *L. gilberti* (91% bootstrap; 100% posterior probability).

Two species of *Lophognathus* (*L. longirostris* and *L. temporalis*) do not belong to this large clade and are more closely related to outgroups in the genera *Rankinia*, *Tympanocryptis* and *Pogona* than they are to *L. gilberti*. However, *L. longirostris* and *L. temporalis* are not supported as sister taxa.

There is deep phylogeographic structure in a number of species. In particular, *L. gilberti* consists of two distinct, well supported haplotype clades (100% bootstrap; 100% posterior probability). These two groups correspond to a northern and more southern haplotype clade (Fig. 2), which come into close contact south of Katherine in the Northern Territory and north of Kununurra in Western Australia. For clarity the southern *L. gilberti* haplotype clade will be called *L.* cf. *gilberti* hence forth, as the type locality for *L. gilberti* is at Port Essington and falls within the northern clade. In addition, four species contain well supported haplotype clades that are geographically circumscribed (96–100% bootstrap; 98–100% posterior probability): *L. gilberti centralis, L. longirostris*, *L. temporalis* and *A. norrisi* (see Section 3.4 below for further details).

3.2. Nuclear DNA

One hundred and seven new sequences and eight previously published sequences were analyzed for the recombination activating gene-1 exon (*RAG1*). The alignment comprised 1385 characters of which 294 were variable and 167 were parsimony informative. The TVM+I+Γ model was selected as the best fitting model for likelihood analysis using AIC criteria. Model parameters were: gamma = 1.0186; proportion of invariable sites = 0.3459; substitution rates $A \leftrightarrow C$ 1.4086, $A \leftrightarrow G$ 5.1376, $A \leftrightarrow T$ 0.4306, $C \leftrightarrow G$ 0.9440, $C \leftrightarrow T$ 5.1376, $G \leftrightarrow T$ 1.0000; and, nucleotide frequencies A = 0.3168, C = 0.2222, G = 0.2204 and T = 0.2405. The Bayesian tree (Fig. 4) was largely consistent with that obtained from mitochondrial data (mean ln-likelihood −5248.81). The clade containing *Lophognathus* (*L. gilberti* and *L.* cf. *gilberti*), *Amphibolurus* and *Chlamydosaurus*, as with the mtDNA, is well supported (100% posterior probability). The phylogenetic relationships within this clade lack the resolution recovered using mtDNA. *C. kingii* is well supported as a monophyletic group that is the sister taxon to a clade comprising the other taxa (100% bootstrap; 100% posterior probability). However, the phylogenetic relationships between *L. gilberti centralis*, *A. muricatus*, *A. norrisi*, *L. burnsi*, and *L. gilberti* are not well resolved and form a large polytomy. Only *A. norrisi* and *A. muricatus* are supported as sister taxa (70% bootstrap; 86% posterior probability). *L. gilberti* and *L.* cf. *gilberti*, which formed a well supported clade in the mtDNA, are not supported as sister taxa with *RAG1*.

As with the mtDNA, *L. temporalis* and *L. longirostris* are more closely related to the outgroup genera *Rankinia*, *Tympanocryptis* and *Pogona* (96% bootstrap; 100% posterior probability) in the nuclear gene phylogeny (Fig. 4). Similarly, they are not supported as sister taxa. Instead *L. longirostris* forms a clade with the outgroup genera (91% bootstrap; 96% posterior probability), and *L. temporalis* is the sister lineage to *L. longirostris* and the three outgroup genera.

3.3. Combined analysis

The alignment of concatenated data (*ND2* + *RAG1*) comprised 3225 characters of which 1333 were variable and 1054 were parsimony informative. The Bayesian topology (mean ln-likelihood –20527.38; Fig. 5) was largely congruent with that returned from the analysis of mtDNA. The analysis recovered a well supported monophyletic group (100% posterior probability) consisting of taxa from three genera. Within this clade there are three well supported subclades of haplotypes: (1) *C. kingii* (100% posterior probability); (2) *A. muricatus*, *A. norrisi*, *L. burnsi*, *L. gilberti centralis* (100% posterior probability); and (3) *L. gilberti* (100% posterior probability). The second and third of these haplotype clades are supported as sister groups (97% posterior probability). As with both mtDNA and nuclear trees, *L. gilberti* and *L.* cf. *gilberti* form two deeply divergent

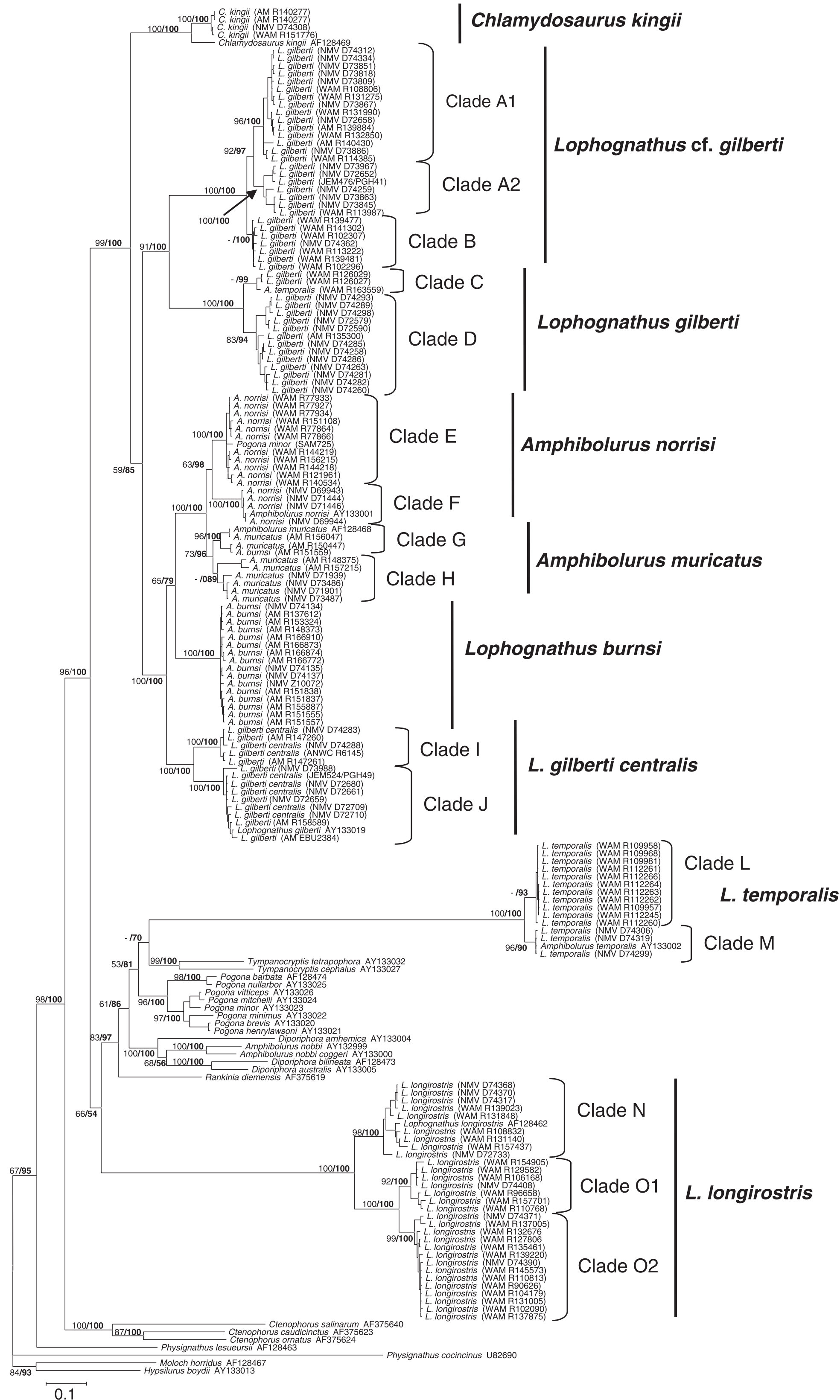


Fig. 3. Bayesian 50% majority-rules phylogenetic tree for the genera *Lophognathus*, *Amphibolurus* and *Chlamydosaurus* based on ∼1400 bp mitochondrial DNA (*ND2*). Samples sequenced in the current study and previously published sequences are designated by tissue or museum registration numbers and GENBANK numbers (see Appendix A for details). Bayesian posterior probabilities and ML bootstraps are provided on branches. Letters designate clades mapped in Fig. 2.

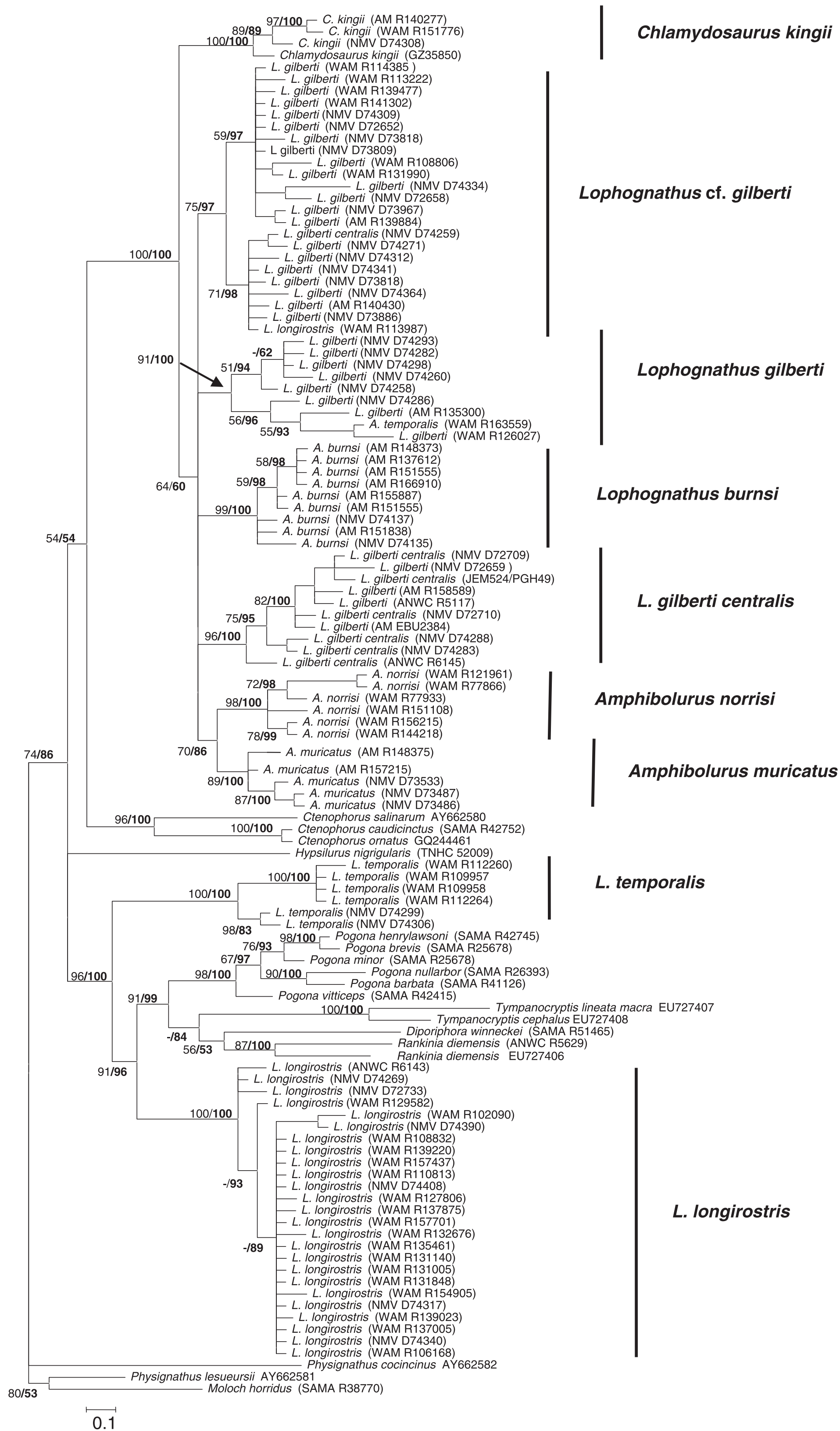


Fig. 4. Bayesian 50% majority-rule phylogenetic tree for the genera *Lophognathus*, *Amphibolurus* and *Chlamydosaurus* based on ~1400 bp for the recombination activating gene-1 exon (*RAG1*). Samples sequenced in the current study and previously published sequences are designated by tissue or museum registration numbers and GENBANK numbers (see Appendix A for details). Bayesian posterior probabilities and ML bootstraps are provided on branches.

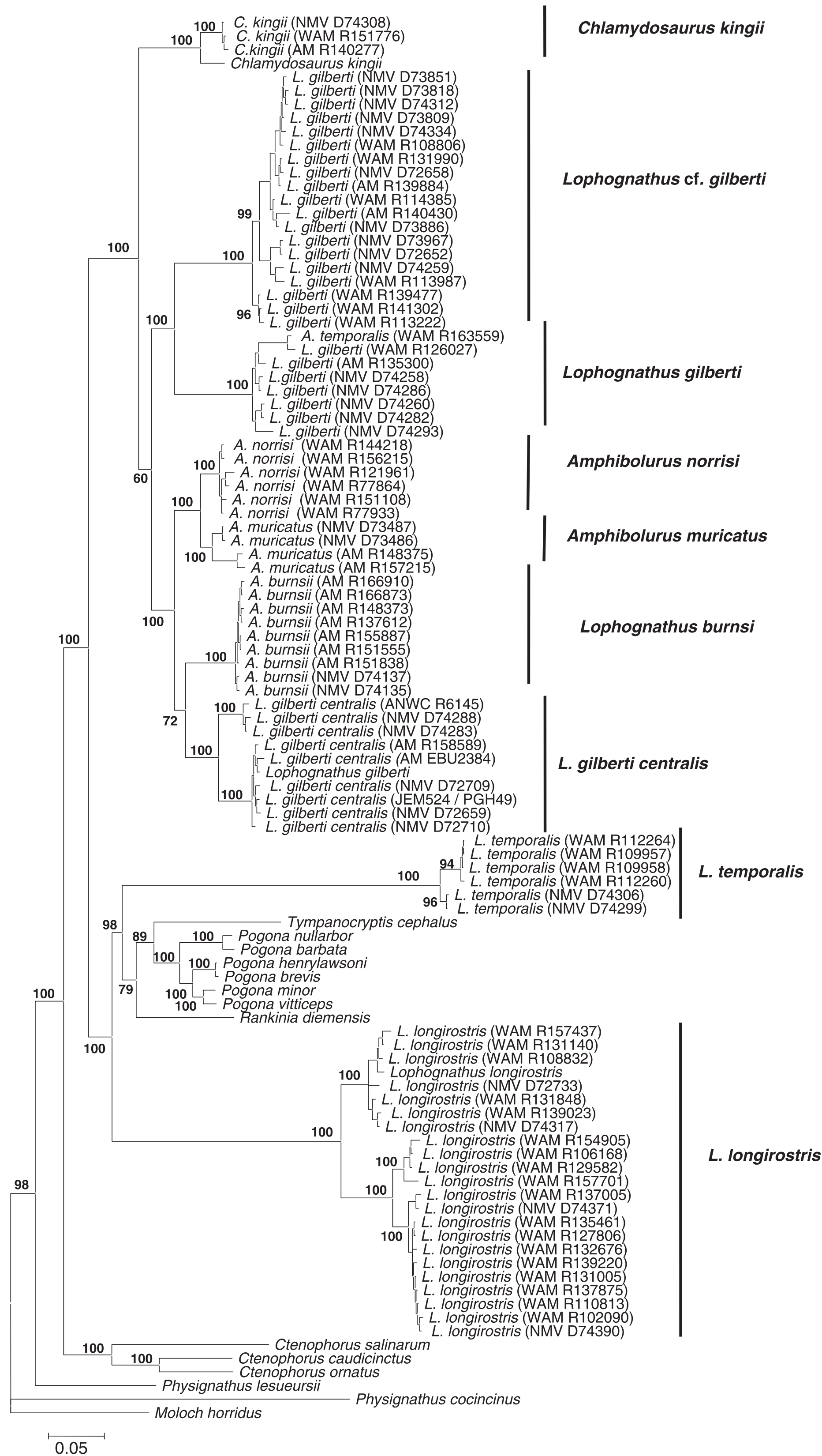


Fig. 5. Bayesian 50% majority-rule phylogenetic tree of partitioned analysis of *ND2* and *RAG1* data. Samples sequenced in the current study and previously published sequences are designated by tissue or museum registration numbers and GENBANK numbers (see Appendix A for details). Bayesian posterior probabilities and ML bootstraps are provided on branches.

lineages (100% posterior probability). *L. longirostris* and *L. temporalis* in the combined analysis, as with both mtDNA and *RAG1* based trees, are more closely related to the outgroups in the genera *Rankinia*, *Tympanocryptis* and *Pogona* than they are to *L. gilberti* (100% posterior probability). However, *L. longirostris* and *L. temporalis* are not supported as sister taxa.

3.4. Partitioned Bremer support

Maximum-parsimony analysis found 21 equally parsimonious trees (length = 3443). MP and ML analysis recovered similar topologies, and the topology most similar to the ML tree was used for the PBS analysis. The 16 haptlotype clades (A–O) and eight in-group species, which were defined on the mtDNA ML tree (Fig. 3) were also recovered in the MP analysis of the combined dataset (Table 4), except for clade F which was missing because no *RAG1* data was collected for this clade. PBS analysis showed conflict at five of the 16 haplotype clades A-O, specifically, *RAG1*did not provide support for haplotype clades: A2 (within *L.* cf. *gilberti*), D (within *L. gilberti*), I (within *L. gilberti centralis*), N or O2 (*within L. longirostris*). For the eight in-group species, conflict was found for two haplotype clades: *A. muricatus* (the mtDNA partition did not support this node) and *L. burnsii* (not supported by *RAG1*). This result for *L. burnsi* is in contrast to this species receiving high ML bootstrap and Bayesian support in both the mtDNA and *RAG1* phylogeny. Overall the PBS analysis found conflict at 55 of 94 nodes present in the MP tree used for the MP analysis. However, most of these conflicts where at the tips of the tree: 35 of the 55 conflicts were for clades with four or fewer individuals.

3.5. Estimates of divergence times

The relaxed lognormal clock analysis of the mtDNA and *RAG1* datasets produced the same in-group topology as the Bayesian and ML phylogenetic analyses (Fig. 3). The evolutionary rates for each of the gene regions were similar to those reported in previously for squamates: mtDNA 0.0062 (Schulte et al., 2003) and *RAG1* 0.0008 (Sanders et al., 2010 – concatenated nuclear data incorporating *RAG1*).

Examination of the log file in Tracer v1.3 indicated a slight tendency toward a positive correlation in the rate of ancestral to descendant branches (covariance: mtDNA = 0.101; *RAG1* = 0.012), but zero was included in the 95% HPD (mtDNA: −0.042–0.284; *RAG1*: −0.136–0.162); thus, this autocorrelation was not considered significant (Drummond et al., 2006). The coefficient of rate variation was estimated to be 0.543 (95% HPD: 0.424–0.755) for the mtDNA and 0.496 (95% HPD: 0.369–0.637) for *RAG1*, indicating that the dataset is not strictly clock-like and that a lognormal relaxed clock is appropriate.

There are discrepancies between the age estimates of the *ND2* and *RAG1* gene regions. However, there is significant overlap in the 95% credibility intervals of both analyses, indicating age estimates of Miocene to Pliocene for most species. The age of the large lineage containing the genera *Chlamydosaurus* (*C. kingii*), *Amphibolurus* (*A. muricatus* and *A. norrisi*) and *Lophognathus* (*L. burnsi*, *L. gilberti*, *L. gilberti centralis*) was estimated to date back to the mid-Miocene: mtDNA – 19.6 Mya (95% CI: 14.4–22.1) and *RAG1* – 14.5 Mya (95% CI: 12.2–17.5). The age of the clade containing the *Lophognathus* type species (*L. gilberti*) contained *L. gilberti* and *L.* cf. *gilberti* in the mtDNA analysis and was estimated to date to the mid to late Miocene, with an estimate of 11.2 Mya (95% CI: 8.9 – 14.3), while the age of this group could not be estimated using *RAG1* because it was not monophyletic. The clade containing the type species of *Amphibolurus* (*A. muricatus*) also contained *A. norrisi*, *L. gilberti centralis*, *L. burnsi* and was also estimated to date to the mid to late Miocene: mtDNA – 10.4 Mya (95% CI: 7.6–13.0) and *RAG1* – 8.4 Mya (95% CI: 7.3–12.4).

Table 4
Results from partitioned Bremer support analysis. Haplotype clades refer to those designated in Fig. 3. Positive values indicate support for haplotype clades, while negative values indicate a lack of support.

Haplotype clade	Genus	Species	mtDNA	*RAG1*
A + B + C + D	***Lophognathus***	**cf. *gilberti* + *gilberti***	6.27	−1.27
A + B	***Lophognathus***	**cf. *gilberti***	45.48	1.52
A1	”	”	7.08	0.92
A2	”	”	9.06	−0.06
B	”	”	8.67	2.33
C + D	***Lophognathus***	***gilberti***	43.67	1.33
C	”	”	10.29	4.71
D	”	”	7.46	−1.46
E + G + H	***Amphibolurus***	***norrisi* + *muriactus***	14.34	1.66
E + F	***Amphibolurus***	***norrisi***	N/A	N/A
E	”	”	13.93	4.07
F	”	”	[not in combined dataset]	
G + H	*Amphibolurus*	*muricatus*	−0.19	1.19
G	”	”	18.75	1.25
H	”	”	13.00	1.00
	Lophognathus burnsi + *Amphibolurus*		7.27	0.73
	Lophognathus	*burnsi*	0.47	−0.49
I + J	*Lophognathus gilberti centralis*		15.78	3.22
I	”	”	27.81	−0.81
J	”	”	31.49	1.51
L + M	*Lophognathus temporalis*		145.5	12.51
L	”	”	12.52	5.48
M	”	”	11.69	1.31
N + O	*Lophognathus longirostris*		83.05	7.95
N	”	”	23.91	−2.91
O1+O2	”	”	22.93	−0.93
O1	”	”	9.1	0.9
O2	”	”	16.1	−0.1

3.6. Intraspecific variation within Lophognathus and Amphibolurus lineages

Arid-zone species (*L. longirostris*, *L. gilberti centralis*, *L. burnsi*) – Relaxed molecular clock analyses suggest that *L. longirostris* and *L. gilberti centralis* originated in the late Miocene, while *L. burnsi* is younger, with an age estimate of the Plio-Pleistocene (Table 5). MtDNA and *RAG1* analyses showed that there is deep genetic structure within *L. longirostris* and *L. gilberti centralis*.

L. longirostris consists of two well supported clades in the mtDNA analysis (Fig 3: clades N & O), and within clade O there are two monophyletic groups. Geographically, clade N extends from central Australia, northwest through the Great Sandy Desert to coastal regions, while clade O is concentrated in the Pilbara region of northwestern Australia (Fig. 2). The two subclades within clade O exhibit extensive geographic overlap. All three clades converge on the southwestern boundary of the Great Sandy Desert. Within clade N the haplotype from the most easterly individual (NMV D72733), which was collected in the East MacDonnell Ranges of the Northern Territory, separates from all others at the basal phylogenetic split. The next phylogenetic split separates haplotypes from the northwestern edge of the Great Sandy Desert from those collected to the east of the Great Sand Desert. The *RAG1* data do not show the same geographic pattern as the mtDNA in *L. longitrositris* (Fig. 4). However, the basal phylogenetic splits within *L. longirostris* in the *RAG1* phylogeny separate haplotypes of central Australia in the Northern Territory (ANWC R 6143, NMV D74269, NMV D72733) from those sampled elsewhere.

L. gilberti centralis contains two well supported mtDNA clades (Fig. 3), a northern clade and a central Australian clade (Fig. 2). Further sampling is required to determine where or whether these two clades make geographic contact in the Northern Territory. The *RAG1* analysis does not resolve two well supported clades (Fig. 4) within *L. gilberti centralis*; however, northern haplotypes cluster to the exclusion of those from the southern extent of the sampling (ANWC R6145, NMV D74283, NMV D74288).

L. burnsi shows very little structure in mtDNA (Fig. 3), even though sampling extends across a large area of northern New South Wales and eastern Queensland. In contrast, *RAG1* for *L. burnsi* shows a higher level of structuring, with the most northerly sampled haplotypes (NMV D74135, NMV D74137, AM R151838) forming a group to the exclusion of others.

Semi-arid and temperate-zone species (*A. norrisi*, *A. muricatus*) – These two species have the most southerly distributions of the *Amphibolurus* and *Lophognathus* species, with *A. norrisi* spanning southern semi-arid regions and *A. muricatus* occurring in semi-arid and temperate regions of southeastern Australia (Fig. 2). Relaxed molecular clock analyses indicate that both species originated in the Plio-Pleistocene and show structure in both the mtDNA and *RAG1* phylogenies (Figs. 3 and 4).

In *A. norrisi* there are two well supported mtDNA clades (Fig. 3), one of which occurs in western Victoria and eastern South Australia (clade F) and a second clade that extends from central South Australia, west of the Spencer Gulf, across the Nullabor Plain into Western Australia's southern coastal region (clade E).

The *A. muricatus* samples sequenced in this study form two haplotype clades in the mtDNA phylogeny, of which only one is well supported (Fig. 3). Clade G, which is a well supported haplotype clade, occurs in central and northern New South Wales, while the sister clade F has a disjunct population in Victoria and southeastern Queensland. Sampling included in our study for both *A. muricatus* and *A. norrisi* does not span the full range of these species, and further work could shed light on phylogeographic patterns.

Tropical savanna species (*L. temporalis*, *L. gilberti*, *L.* cf. *gilberti*) - relaxed molecular clock analyses suggest that all three tropical savanna species originated in the late Miocene (Table 5). Additionally, mtDNA and *RAG1* analyses showed that there is deep genetic structure within each species.

L. temporalis samples included in our study come from the Darwin area of the far northern coast of the Northern Territory and from the Laut Islands in Indonesia (Fig. 2). Haplotypes from each of these localities are supported as clades in both the mtDNA analysis and *RAG1* (Figs. 3 and 4). However, there is little phylogenetic structure within either of these clades, which is probably because individuals were collected in close proximity in each region.

L. gilberti contains two haplotype clades in both the mtDNA and *RAG1* analyses (Figs. 3 and 4). However, the haplotype clades in each of these analyses contain different groupings of individuals. In the mtDNA analysis, clade C occurs in the Kimberley region of Western Australia, while clade D is distributed in the far north of the Northern Territory, including the Arnhem Land escarpments (Fig. 2). These two haplotype clades are separated by the Ord Arid intrusion, and relaxed molecular clock analysis indicates that the common ancestor of each of these mtDNA clades are Plio-Pleistocene in age (clade C: 3.4 Mya (95% CI 1.3–4.2); clade D: 2.3 Mya (95% CI 0.7–3.5)). The *RAG1* analysis shows no clear geographic pattern, with both clades overlapping extensively.

Lophognathus cf. *gilberti* has a more southerly distribution than *L. gilberti*, occurring from central-northern Northern Territory, across the Kimberly region of Western Australia and down the coast to the Pilbara. MtDNA analysis revealed two clades, only one of which, clade A, is well supported (Fig. 3). Clade A occurs in the Northern Territory and northern Western Australia but does not extend south along the coastal region of the Great Sandy Desert. Clade A consists of a well supported clade of more northern and eastern haplotypes and another well supported clade of more southern and western haplotypes. These clades overlap in the central Kimberley region (Fig. 2). Clade B occurs in the coastal Pilbara and was not found north along the coastal area of the Great Sandy Desert. Relaxed molecular clock analysis indicates that the common ancestor of each of these mtDNA clades is Plio-Pleistocene in age (clade A1: 1.9 Mya (95% CI 1.3–3.5); clade A2: 2.1 Mya (95% CI 1.7–4.6); clade B: 1.3 Mya (95% CI 0.5–1.8)). The *RAG1* anal-

Table 5
Estimated age of the common ancestor of *Lophognathus* and *Amphibolurus* species and clades in millions of years, using relaxed molecular clock analyses. The mean estimate and in brackets the 95% credibility intervals are presented for two independent gene regions.

	mtDNA	*RAG1*
Amphibolurus norrisi	2.3 Mya (0.6–2.6)	2.7 Mya (1.2–5.5)
Amphibolurus muricatus	3.5 Mya (1.8–4.9)	1.6 Mya (0.3–4.6)
Lophognathus burnsi	1.9 Mya (1.3–2.5)	1.1 Mya (0.8–4.8)
Lophognathus gilberti	7.6 Mya (3.4–10.3)	4.8 Mya (3.6–8.3)
Lophognathus cf. gilberti	6.7 Mya (4.3–8.6)	4.5 Mya (3.1–9.4)
Lophognathus gilberti centralis	8.8 Mya (5.1–14.5)	4.1 Mya (1.7–7.1)
Lophognathus longirostris	6.4 Mya (4.5–11.2)	4.6 Mya (2.9–8.7)
Lophognathus temporalis	5.4 Mya (3.2–8.3)	6.4 Mya (2.5–9.6)
Amphibolurus + *Lophognathus* (excluding *L. longirostris* and *L. temporalis*)	11.17 Mya (8.9–16.31)	6.7 Mya (6.4–12.9)

ysis on the other hand shows no clear geographic pattern, with both clades overlapping extensively.

4. Discussion

4.1. Taxonomic relationships

Our molecular work, incorporating both mitochondrial and nuclear gene regions, shows that there is far greater species diversity in *Amphibolurus* and *Lophognathus* than is presently recognized. These molecular results provide compelling support for a taxonomic revision of the genera *Amphibolurus* and *Lophognathus*. Our results clearly indicate that the species in these genera span three ancient independent evolutionary branches: 1. *C. kingii*, *L. gilberti*, *L. gilberti centralis*, *L. burnsi*, *A. norrisi*, *A. muricatus*; 2. *L. temporalis*; and 3. *L. longirostris*. The most taxon-rich of these branches (1) incorporates the type species for three genera: *Chlamydosaurus*, *Lophognathus*, *Amphibolurus*. Thus, the remaining two branches require designation as new monotypic genera. Currently, the generic name *Gowidon* is available for *L. longirostris* (*Gowidon longirostris*; Wells and Wellington, 1985), and *L. temporalis* awaits taxonomic revision.

Within the genera *Lophognathus* and *Amphibolurus*, taxonomic revision is also required. Our results show that the junior synonym *L. gilberti centralis* is unrelated to *L. gilberti* and is instead nested within *Amphibolurus*. In addition, *L. gilberti* comprises two subgroups that were sister clades in the mtDNA phylogeny but not in the nuclear phylogeny. Thus, it is probable that *L.* cf. *gilberti* is a new and as yet undescribed species. Finally, our results provide evidence that *L. burnsi* is a valid species and is sister to *L. centralis*. Although partitioned Bremer support analysis found conflict between the gene regions for *A. burnsi*, this species was highly supported by both ML bootstrap and Bayesian posterior probabilities across both gene regions. This conflict may be because the parsimony analysis used in the PBS does not incorporate a model of evolution.

Based on the mtDNA phylogeny, the genus *Amphibolurus* should contain the following species: *A. norrisi*, *A. muricatus*, *A. burnsi*, and *A. centralis*; while *Lophognathus* comprises *L. gilberti* and *L.* cf. *gilberti*. However, these genera are not recovered in the *RAG1* phylogeny. Although, the PBS analysis confirmed congruence across gene regions for *Amphibolurus*, it found conflict between the mtDNA and *RAG1* gene regions for the monophyly of *Lophognathus*. Thus, further work, incorporating morphological data, is required to resolve these taxonomic problems. Our results suggest that the morphological characters that have previously been used to define *Lophognathus* and *Amphibolurus*, such as dorsal scalation, femoral pores, body proportions and ornamentation, are not diagnostic (i.e., they are not unique to the genera for which they are considered characteristic). In light of our molecular findings, we recommend that a complete review of the morphological characters for each species and each genus undertaken. A comprehensive morphological review of these agamid lizards would provide an important contribution to our understanding of Australia's lizard diversity.

4.2. Phylogeography of tropical savanna agamids

A recent review of biogeographic patterns in the Australian monsoon tropics (Bowman et al., 2010) posed questions about the evolutionary origins of its constituent taxa. Are they species that have arrived in the recent geologic past across Wallace's line or are they ancient relicts from Gondwana that have persisted in ancient refugia? Our results provide information of particular relevance to these questions. The most recent molecular dating work in the Australian agamid subfamily Amphibolurinae, which is a monophyletic lineage, suggests that this radiation dates back to the late Oligocene – early Miocene (Hugall et al., 2008), with a burst of diversification and speciation in the Miocene (e.g., Shoo et al., 2008; Edwards and Melville, submitted for publication). Thus, the Australian agamids diversified within Australasia but are not a Gondwanan element.

The evolutionary origins of the Australian monsoon tropical agamids do not fit into either of the scenarios posed by Bowman et al. (2010) and reviewed in the preceding paragraph. Our molecular results reveal that two of the monsoon tropics species (*L. gilberti* and *L.* cf. *gilberti*) form a monophyletic group which is the sister taxon to a clade containing arid, semi-arid and temperate-zone species (*A. muricatus*, *A. norrisi*, *L. burnsi*, *L. centralis*). These taxa collectively form are the sister clade to the frill-necked lizard (*C. kingii*), which is also a monsoon tropics species. The remaining monsoon tropics species, *L. temporalis*, does not form a clade with the other monsoon tropics species but is instead most closely related to *Pogona*, *Tympanocryptis*, *Diporiphora* and *Rankinia*, which cover virtually all climatic areas of Australia. Thus, *Lophognathus* and *Amphibolurus* have a complex history of diversification across most of continental Australia during the late Miocene, which may have been associated with increasing aridification (Byrne et al., 2008). Australia has become progressively desiccated over the last 20 million years, culminating in extreme aridity during glacial cycles (Williams, 2000), which has provided the background for the evolution of an arid-adapted biota (Byrne et al., 2008). Numerous vertebrate groups diversified during this period of increasing aridification, including birds, squamates and marsupials (see Table 1 in Byrne et al., 2008). Thus, it is probable that the evolutionary origins of the genera *Amphibolurus* and *Lophognathus* are also associated with these large-scale continental climatic changes.

Our study revealed that within the monsoon tropics, *L. gilberti*, *L.* cf. *gilberti* and *L. temporalis* show latitudinally abutting distributions. The most northerly species, *L temporalis*, has previously been thought to have a distribution across much of northern Australia, from eastern Western Australia, through the Northern Territory, across northern Queensland and into Cape York (Wilson and Swan, 2008). This species also occurs on islands north of Australia and coastal regions of New Guinea. We could not obtain samples from Queensland; however, we sampled extensively in the Northern Territory and Western Australia. We found no molecular evidence of this species anywhere except coastal regions of the Northern Territory. Thus, it is probable that this species has a far more restricted distribution than previously thought, and some specimens identified as *L. temporalis* are instead *L. gilberti* (e.g., Western Australian specimen WAM R163559). Thus, *L. temporalis* appears to be a far northern coastal and island species, which has phylogeographic structure associated with populations separated by water bodies. However, land-based dispersal between islands in the Arafura and Timor Seas would have been possible during the glacial maxima of the Pleistocene oscillations, where low sea levels (−150 m) exposed much of these areas (Byrne et al., 2008; Bowman et al., 2010). Thus, there would probably have been a repeating pattern of alternating isolation and contact in *L. temporalis.*

L. gilberti has previously been considered a widely distributed species, covering most of the northern third of Australia (Wilson and Swan, 2008). Our molecular work provides strong evidence across mtDNA and nuclear genes that this species is composed of two species, a more northerly species (*L. gilberti*) and a more southerly one (*L.* cf. *gilberti*). Thus, *L. gilberti* has a much more restricted distribution that previously thought, occurring in the Arnhem Land region of the Northern Territory and the eastern Kimberley region of Western Australia. We did not obtain samples from Cape York, so it is yet to be confirmed whether this species occurs there. *L. gilberti* appears to be restricted to the moister sandstone escarpment regions of the monsoon tropics, and our phylogenetic

analysis indicates that there has been a vicariance event across the Ord Arid intrusion during the Plio-Pleistocene. Similar distributions occur in numerous vertebrate species, including the Kimberley and Kakadu rock rats (Bowman et al., 2010). Such a distribution pattern is also occurs in endemic birds (e.g., Ford, 1982; Johnstone, 1990) and vicariance across the Carpentarian Gap is also common (e.g., Jennings and Edwards, 2005). At the species level it has been found that divergence across these biogeographic barriers in black-throated finches and the red-backed fairly wren are Pleistocene in origin (Jennings and Edwards, 2005; Lee and Edwards, 2008). Thus, these biogeographic barriers may play an important role across vertebrates in the monsoon tropics.

The most southerly of the monsoon tropics species that we studied was *L.* cf. *gilberti*. This species has a broad distribution from the Northern Territory, through northern Western Australia and along the coastal regions of the Pilbara. This agamid lizard occurs in the more southerly and drier savanna woodlands, skirting the northern extent of the Australian arid-zone (see Byrne et al. (2008) for the extent of the arid-zone). There is deep phylogeographic structure in *L.* cf. *gilberti*, with a disjunct distribution across the Great Sandy Desert, which probably dates to the Pliocene. This desert extends right up to the ocean in its northwestern extent and was composed of active sand dunes during the glacial maxima of the Pleistocene oscillations (Byrne et al., 2008). Sandy desert landforms in Australia are relatively young, with their origin in the early Pliocene and mid-Pleistocene (Fujioka et al., 2005, 2009). Thus, we are unable to determine whether the coastal Pilbara *L.* cf. *gilberti* pre-dates the expansion of the Great Sandy Desert or whether this population results from a long-distance dispersal event across or around the sand desert. The short branch length with low support in the mtDNA and the lack of divergence of the Pilbara clade in the nuclear gene *RAG1* may provide evidence for a long-distance dispersal event rather than a long history of isolation.

In the northern extent of *L.* cf. *gilberti*'s distribution there are two divergent and well supported mtDNA clades that overlap geographically. This indicates that there is probably a past history of vicariance with subsequent dispersal, with the clades overlapping in the southern and western Kimberley region. It is unclear what biogeographic barrier might have led to this past divergence, but future work may provide data on whether other species in the Kimberley region display a similar complex phylogeographic pattern.

Interestingly, the species *L. gilberti centralis* has a distribution that encompasses the southern portions of the monsoon tropics. This species covers broad climatic regimes from arid central Australia to the Carpentarian Gulf and central Northern Territory (Fig. 2). Our current genetic sampling shows a distributional disjunction for this species between the northern and central Australian populations. However, this cannot be considered a true distributional disjunction as museum voucher specimens, which lacked associated tissue samples, have been collected in this region. Further genetic sampling across this region will help to clarify the phylogeographic structure of this broadly distributed species.

Our results show that some of the monsoon tropics *Lophognathus* species span a broad range of climatic regions (*L.* cf. *gilberti*) from monsoonal to the arid-zone, while others are more tightly distributed within a climatic region (*L. gilberti* and *L. temporalis*). With origins dating back to the Miocene or early Pliocene, these species were found to be of a similar age to related species in the arid-zone (*L. gilberti centralis* and *L. longirostris*) and older than species that occur in semi-arid and temperate areas (*L. burnsi*, *A. muricatus* and *A. norrisi*). Thus, our results provide evidence that the origin of agamid species in the monsoon tropics coincides with climatic conditions conducive to a monsoon climate 15–8 Mya (Bowman et al., 2010), and it is probable that the origins and phylogeographic patterns of these northern *Lophognathus* species have evolved under the influence of these climatic conditions. Additionally, the broad temporal patterns of speciation and diversification in the tropical savanna agamids are similar to those summarized in a review of the Australian arid-zone, where a burst of speciation occurred during the Miocene and diversification in the Plio-Pleisctocene was at an intraspecific level (Byrne et al., 2008).

4.3. Conclusions

We provide the most comprehensive phylogenetic and historical biogeographic study of vertebrates in Australia's monsoon tropics and the most complete molecular systematic study of *Lophognathus* and *Amphibolurus*. Our results do not support an ancient Gondwanan origin of agamids in the monsoon tropics but also do not support a recent dispersal event across Wallace's Line into tropical Australia. Instead our results support Miocene autochthonous diversification in *Lophognathus* and *Amphibolurus* species across the monsoon tropics and the arid-zone. Within the monsoon tropics *Lophognathus* species, our results support the Ord Arid intrusion and the Great Sandy Desert as biogeographic barriers to savanna woodland species. We also report considerable phylogeographic structuring over relatively small distances in the Kimberleys and Arnhem Land Plateau. These results highlight a need for detailed phylogeographic research on more species in these regions to identify refugia and species dispersal routes. Future studies of other ground-dwelling vertebrate groups, such as skinks, geckos, frogs and small mammals, would provide an ideal comparison to the phylogeographic patterns we have identified in *Lophognathus*. Thus, there remains great scope for furthering our understanding of the complex biogeographic history of the Australian monsoon tropics.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.11.025.

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