



Concordance in phylogeography and ecological niche modelling identify dispersal corridors for reptiles in arid Australia

Jane Melville^{1*}, Margaret L. Haines¹, Joshua Hale¹, Stephanie Chapple¹ and Euan G. Ritchie²

¹Department of Sciences, Museum Victoria, Melbourne, Victoria 3001, Australia, ²Deakin University, Geelong, Australia, School of Life and Environmental Sciences, Centre for Integrative Ecology (Burwood Campus), 221 Burwood Highway, Burwood, Victoria 3125, Australia

ABSTRACT

Aim Using the rock-specialist agamid *Ctenophorus caudicinctus* as a model, we test hypothesized biogeographical dispersal corridors for lizards in the Australian arid zone (across the western sand deserts), and assess how these dispersal routes have shaped phylogeographical structuring.

Location Arid and semi-arid Australia.

Methods We sequenced a *c.* 1400 bp fragment of mtDNA (*ND2*) for 134 individuals of *C. caudicinctus* as well as a subset of each of the mtDNA clades for five nuclear loci (*BDNF*, *BACH1*, *GAPD*, *NTF3*, and *PRLR*). We used phylogenetic methods to assess biogeographical patterns within *C. caudicinctus*, including relaxed molecular clock analyses to estimate divergence times. Ecological niche modelling (*MAXENT*) was employed to estimate the current distribution of suitable climatic envelopes for each lineage.

Results Phylogenetic analyses identified two deeply divergent mtDNA clades within *C. caudicinctus* – an eastern and western clade – separated by the Western Australian sand deserts. However, divergences pre-date the Pleistocene sand deserts. Phylogenetic analyses of the nuclear DNA data sets generally support major mtDNA clades, suggesting past connections between the western *C. c. caudicinctus* populations in far eastern Pilbara (EP) and the lineages to the east of the sand deserts. Ecological niche modelling supports the continued suitability of climatic conditions between the Central Ranges and the far EP for *C. c. graafi*.

Main conclusions Estimates of lineage ages provide evidence of divergence between eastern and western clades during the Miocene with subsequent secondary contact during the Pliocene. Our results suggest that this secondary contact occurred via dispersal between the Central Ranges and the far EP, rather than the more southerly Giles Corridor. These events precede the origins of the western sand deserts and divergence patterns instead appear associated with Miocene and Pliocene climate change.

Keywords

Agamidae, Australia, *Ctenophorus caudicinctus*, desert lizards, dispersal corridors, phylogeography

*Correspondence: Jane Melville, Department of Sciences, Museum Victoria, GPO Box 666, Melbourne, Vic. 3001, Australia.
E-mail: jmelv@museum.vic.gov.au

INTRODUCTION

The distribution of related lineages across a landscape and evidence of historical gene flow between them provide valuable insight into the process of divergence and ultimately speciation (Milá *et al.*, 2013). Phylogeographical studies at a

large spatial scale and which encompass major geographical barriers to gene flow, as well as regions of secondary contact, are of particular interest because they can shed light on historical factors driving divergence. The Australian arid zone provides an ideal system to study the role of geographical barriers in intraspecific diversification at a large spatial scale,

over long time periods. Unlike regions in the Northern Hemisphere that have complex glaciation histories and significant topographic barriers to dispersal, Australia has had a relatively stable climate, largely without glaciation during the Pleistocene, and 'mountainous' regions are more subdued (Byrne *et al.*, 2008). The Australian arid zone is immense, consisting of c. 5.25 million km² covering almost 70% of the landmass (Byrne *et al.*, 2008). Although the Australian continent is relatively flat compared with other continents, the arid zone has a number of desert ranges (elevation < 1500 m) in central, western and northern regions (Fig. 1), constituting prominent topographic features that rise above the surrounding lowlands. These lowland regions in the western deserts, in particular, are dominated by sand plains and dune fields that have isolated the desert ranges with vast tracts of sandy soils. Thus, the mosaic of rocky and sandy habitats in Australia's arid zone has the potential to play an important role in diversification patterns and ultimately speciation in desert fauna.

Pianka (1972) developed a simple model to explain extensive speciation of lizards within the Australian deserts based on the spatial and temporal fluctuation of habitats, hypothesizing that the arid zone was comprised of a complex array of spatially differentiated habitats. As part of his model, Pianka (1972) identified Pleistocene dispersal routes across the western deserts, with the Giles Corridor extending across the central portion of the western sand deserts (Fig. 1). Although the late Miocene saw an increase in the aridity in this region but the spread of sand deserts in Western Australia occurred during the Pleistocene (Hill, 1994; Byrne *et al.*, 2008). Pianka (1972) suggested that the Giles Corridor functioned as an intermittent Pleistocene dispersal route between eastern and western arid-zone fauna and remains the only proposed dispersal route for reptiles in the region. It is a continuous band of *Acacia* shrublands linking the east Murchison goldfields in Western Australia to the Central Ranges by extending through the Lake Carnegie region in the Great Victoria Desert and the southern part of the Gibson Desert (Van Oosterzee, 1991). A number of recent studies have tested Pianka's (1972) hypotheses using phylogenetic and phylogeographical approaches, with the prediction that arid-zone lizards should exhibit phylogeographical structuring concordant with the distribution of the major vegetation communities in central Australia (e.g. Chapple *et al.*, 2004; Shoo *et al.*, 2008; Pepper *et al.*, 2011a,b). Results from these studies are mixed, where a study on *Egernia* skinks failed to provide strong support for ecological and habitat factors being responsible for the diversification (Chapple *et al.*, 2004), while Shoo *et al.* (2008) found strong genetic divergence between habitat patches in pebble-mimic dragon lizards. Pepper *et al.* (2011a) found that geological, landscape and climate evolution have played an important role in the diversification of saxicolous and desert lineages of *Heteronotia* geckos. However, these studies have found no genetic evidence supporting historical dispersal routes

across the western deserts, instead both studies of rock specialists found deeply divergent lineages on either side of the sand deserts, with no evidence of secondary contact (Shoo *et al.*, 2008; Pepper *et al.*, 2011a,b).

Ctenophorus caudicinctus (Günther 1875) provides an ideal opportunity to further investigate the relationship between habitat distributions, dispersal routes and diversification in Australia's western deserts. *Ctenophorus caudicinctus* is a rock-dwelling species that occurs in the western half of Australia (Fig. 1). It consists of six subspecies, distinguished on male morphology and colour patterns (Storr, 1967): *C. c. caudicinctus*, *C. c. mensarum*, *C. c. infans*, *C. c. macropus*, *C. c. slateri* and *C. c. graafi*. Despite the morphological distinctiveness of the subspecies, Storr (1967) was unable to assign all specimens examined to particular subspecies, particularly in contact zones between *C. c. caudicinctus*, *C. c. mensarum* and *C. c. infans*. Additionally, populations from the northern extent of the range were left unidentified, with the exception of the description of *C. c. macropus* from western Arnhem Land. In a later publication the distribution of *C. c. macropus* was extended to incorporate these formerly unidentified northern populations from the Kimberley in Western Australia across to north-western Queensland (Storr *et al.*, 1983). As these morphological studies there has been very little research conducted on *Ctenophorus caudicinctus*, although more recent molecular work has indicated that it is closely related to the rock-dwelling species *C. ornatus* from south-western Western Australia (Melville *et al.*, 2001; Schulte *et al.*, 2003).

We investigate dispersal routes and diversification in Australia's western deserts in a comprehensive phylogeographical study of *Ctenophorus caudicinctus*, incorporating six genes and ecological niche modelling (ENM), to examine the relative importance of habitat distributions. We focus, in particular, on the Great Sandy Desert, the Gibson Desert and Great Victoria Deserts, testing the hypothesis that the Giles Corridor provides a dispersal route across the western sand deserts. Based on the distributions of subspecies, we would expect evidence of gene exchange between *C. c. mensarum* and *C. c. graafi* if the Giles Corridor has provided a dispersal route through the sand deserts (Fig. 1). We predicted that the Giles Corridor – a band of *Acacia* shrublands – would not make an obvious dispersal corridor for a rock-specialist species, such as *C. caudicinctus*. We employ relaxed molecular clock analyses to estimate divergence times within *C. caudicinctus* in order to determine whether phylogeographical patterns are consistent with the Giles Corridor being a Pleistocene dispersal route in this species. We also use ENM to determine if suitable climatic conditions currently exist for dispersal of *C. caudicinctus* across the western deserts. ENM has been successfully incorporated into phylogeographical studies to address such concepts. GIS-based models predicting the geographical distribution of sister species have provided a valuable method of investigating the role of ecological factors in driving diversification (Kozak *et al.*, 2008).

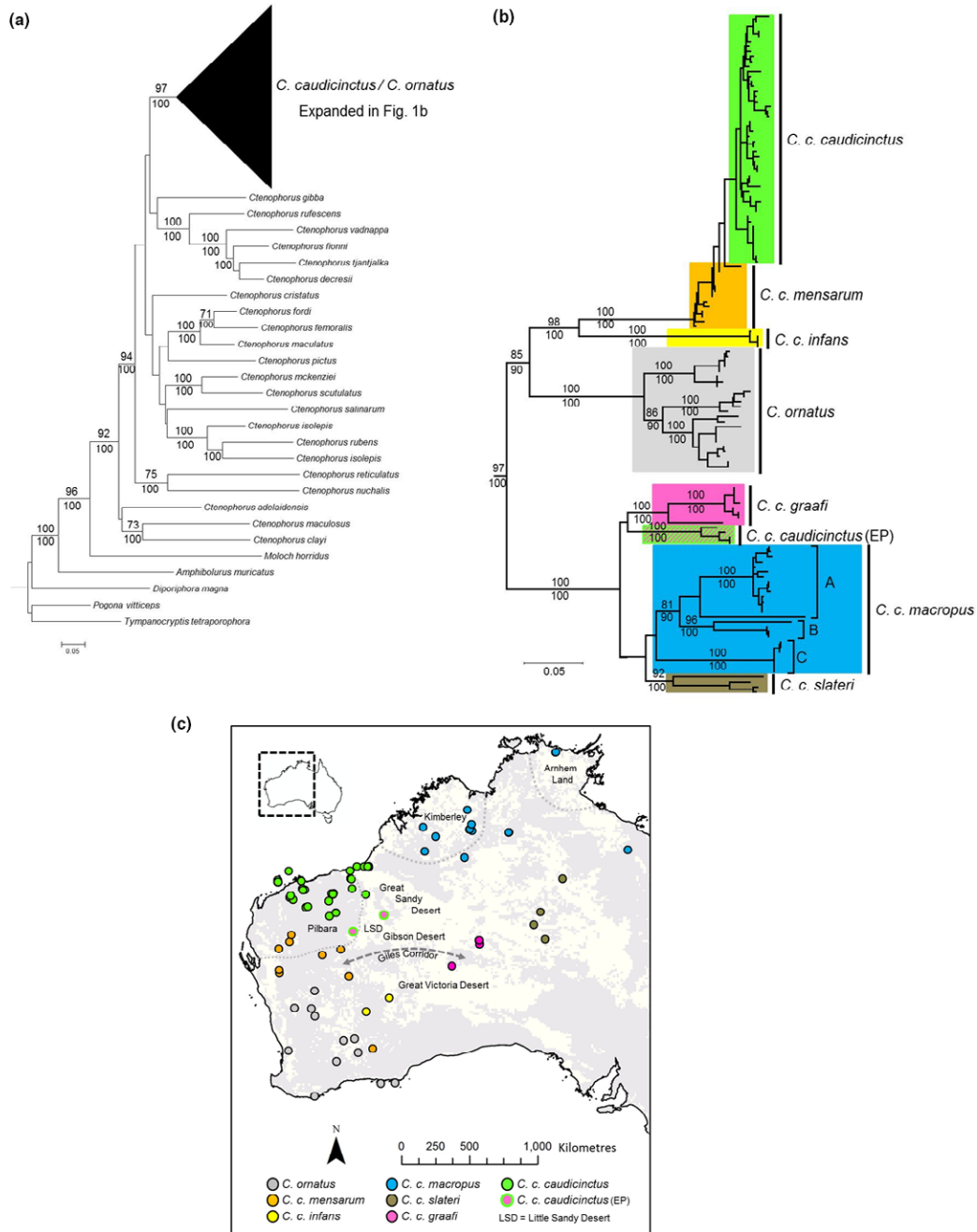


Figure 1 Maximum likelihood phylogenetic tree for *Ctenophorus ornatus* and the six subspecies of *C. caudicinctus* based on *c.* 1400 bp mitochondrial DNA (*ND2*). Samples sequenced in this study and previously published sequences are designated by tissue or museum registration numbers and GenBank numbers (see Appendix S1 for details). ML bootstraps > 70% (above) and Bayesian posterior probabilities > 90% (below) are provided on branches. Colours designate clades, which are mapped – pale yellow shading on the locality map indicates the distribution of sand deserts and major desert systems, the Giles Corridor and other biogeographical features have been labelled.

MATERIALS AND METHODS

Tissue samples

We collected specimens of *Ctenophorus caudicinctus* and associated tissues for sequencing with two main objectives. First, we endeavoured to maximize geographical spread and

second we sought to fill in geographical gaps in tissue samples already held in Australian museums. Thirty-eight field-collected tissue samples of *C. caudicinctus* were included in the study (see Appendix S1 in Supporting Information). An additional 96 tissue samples of *C. caudicinctus* and *C. ornatus* were obtained from museum collections (see Appendix S1). These museum samples combined with the field-collected tis-

sues provided comprehensive sampling for the study species. We also included previously published sequences as outgroups for our analyses and sequenced additional outgroup species, where required.

Laboratory protocols and alignment of DNA sequences

Genomic DNA was extracted from tail tips or liver samples using a DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD, USA) as per manufacturer's instructions or using a using Proteinase K digestion and chloroform-isoamyl alcohol extraction. For all specimens, a fragment (*c.* 1400 bp) of the mtDNA genome was amplified that includes *ND2* and flanking tRNAs (see Melville *et al.*, 2011 for primer sequences and protocols). For a subset of specimens, we sequenced five nuclear loci (*BDNF*, *BACH1*, *GAPD*, *NTF3*, and *PRLR*). In previous phylogeographical studies of Australian agamids it has been common to use the nuclear exon *RAG1*, however, previous work on *C. caudicinctus* and *C. ornatus* found that there is significant intraspecific length variation in the N-terminal domain of this gene in these species (Melville & Hale, 2009), thus, this regions was not included in our study. Oligonucleotide primer pairs for the mitochondrial and five nuclear genes are listed in Appendix S2.

Amplifications for *ND2* and *BDNF* were performed in 25 μ L volumes in the presence of 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μ M of forward and reverse primer, 1x Qiagen polymerase chain reaction (PCR) buffer and 1 U of HotStarTaq DNA polymerase (Qiagen). For *BACH1*, *GAPD*, *NTF3* and *PRLR*, amplifications were performed in 20 μ L volumes in the presence of 0.25 μ M of forward and reverse primer and 50 U of GoTaq Hot Start (Promega, Madison, WI, USA). PCR protocols for mitochondrial and nuclear genes are listed in Appendix S2. PCR amplifications were visualized on a 1.2% agarose mini-gel and amplified products were purified using either GFX spin columns, using SureClean Plus (BIOLINE, London, UK), or ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). Purified product was sent to Macrogen (Korea) for sequencing. Sequence chromatograms were edited using GENEIOUS 6.1.8 (Biomatters Ltd, Auckland, New Zealand) 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.

Phylogenetic analyses

Phylogenetic analysis of all samples for the mtDNA gene region was undertaken using maximum likelihood and Bayesian analyses. Published *ND2* sequences of 22 other *Ctenophorus* species, plus five species from other Australian agamid genera were included in analyses as outgroups. To investigate phylogenetic relationships between the *C. caudicinctus* subspecies, 134 new sequences and 28 previously published sequences were analysed for the *ND2* protein-coding gene. The alignment comprised 1425 characters: 865 characters were variable and 721 characters were parsimony informative. Maximum likelihood phylogenetic trees were estimated using PHYLML 2.1.0 (Guindon & Gascuel, 2003) implemented in GENEIOUS 6.1.2 (Biomatters Ltd), using a BEST topology search. Analyses were performed using a GTR+I+ Γ model, estimated using MRMODELTEST 2.3 with the Akaike information criterion: $\gamma = 0.7936$; proportion of invariable sites = 0.2805; substitution rates A \leftrightarrow C 0.5529, A \leftrightarrow G 7.3567, A \leftrightarrow T 0.6780, C \leftrightarrow G 0.2001, C \leftrightarrow T 4.5134, G \leftrightarrow T 1.0000; and, nucleotide frequencies A = 0.4054, C = 0.3199, G = 0.0747 and T = 0.2000. Bootstrap resampling (Felsenstein, 1985) was applied to assess support for individual nodes in each above-mentioned analysis using 100 bootstrap replicates in PHYLML with the same settings as above.

Bayesian analyses were performed in MRBAYES 3.2 (Ronquist *et al.*, 2012) using the evolutionary model selected by MRMODELTEST 2.3 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 10 million generations for each data set. 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 values were close to 1.0, indicating that an adequate sample of the posterior probability distribution had been achieved (Ronquist *et al.*, 2012). In addition, the output was examined using TRACER 1.5 (Rambaut & Drummond, 2003) to check that stationarity had been reached. Bayesian analyses for *BDNF*, *BACH1*, *GAPD*, *NTF3*, and *PRLR* were repeated for the subset samples, consisting of 68 ingroup and four outgroup samples. Identical run conditions to the *ND2* analyses were used for these analyses, except for the models of evolution implemented (Table 1). Models of sequence evolution were selected using MRMODELTEST 2.3.

Table 1 Details of sequence length, number of parsimony informative characters models of evolution, Mr Bayes settings and mean log likelihood of the Bayesian tree for each of the nuclear gene regions.

Gene region	Length (bp)	Number of sequences	Number of parsimony informative characters	Model of evolution	Log likelihood ($-\ln$)
<i>BACH1</i>	1030	68	63	GTR+I+ Γ	-2745.76
<i>BDNF</i>	841	68	26	GTR+I	-1783.73
<i>PRLR</i>	512	66	55	GTR+I+ Γ	-1939.35
<i>NTF3</i>	610	68	20	HKY+I	-1243.57
<i>GAPD</i>	253	65	23	HKY+ Γ	-821.98

We used a Bayesian framework for subspecies tree estimation, incorporating all gene regions (*ND2*, *BDNF*, *BACH1*, *GAPD*, *NTF3* and *PRLR*), to determine phylogenetic relationships between subspecies across the six gene regions. We used a reduced *ND2* data set, matching sequence data for individuals in the nuclear DNA data set, resulting in six data sets of 72 individuals. Populations of *C. c. caudicinctus* from the eastern Pilbara (EP), which aligned with *C. graafi* in the mtDNA data set, was coded as a stand-alone group for the species tree analysis, while all other samples were coded according to their subspecies designation. We used *BEAST, enabled in BEAST 1.7.5, to co-estimate the six gene trees embedded in a shared species tree (see Heled & Drummond, 2010). Unlinked substitutions models were employed across the loci, based on preliminary analyses using MRMODELTEST 2.3 (Table 1). A Yule process species tree prior was specified and the gene tree priors were automatically specified by the multispecies coalescent. The analysis was run for 50 million generations. The output was examined using TRACER 1.5 (Rambaut & Drummond, 2003) to check that stationarity had been reached.

Divergence times estimates

A relaxed molecular clock method within the program BEAST 1.7.5 was used to estimate divergence times for each of the *C. caudicinctus* subspecies, based on the mtDNA data set. Additional published sequences were used in the analysis to allow placement of calibration points, as detailed in Melville *et al.* (2011). We used lognormally distributed fossil calibrations, including four *Iguania* fossils detailed in previous studies (see Melville *et al.*, 2011): a middle Jurassic acrodont iguanian fossil (154–180 Ma), an early Miocene sceloporine (22.8 Ma), a *Chamaeleo/Rhampholeon* fossil (18 Ma) and a Pliocene *Phrynocephalus* fossil (5 Ma). Specific BEAST settings for these calibrations are as per table 3 in Melville *et al.* (2011). In addition, we added a minimum age estimate for the ingroup of Australian amphibolurine species, with a fossil of the *Physignathus lesueurii* lineage, of 20 Ma (Covacevich *et al.*, 1990) with BEAST settings detailed in Edwards & Melville (2011). The analysis was run for 20 million generations using a GTR+I+Γ model of evolution with a Speciation: Yule Process tree prior and a random starting tree. The output was examined using TRACER 1.5 (Rambaut & Drummond, 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).

Ecological niche modelling

We used the environmental niche modelling algorithm MAXENT 3.3.3 (Elith *et al.*, 2011), with default settings, to create predicted current distributions (climate envelopes) for each of the *Ctenophorus* subspecies, except for *C. c. infans*, which was omitted due to insufficient records. Locality records for all subspecies were collected from across their distributions by E.G.R. and J.M. and supplemented with further records from

additional museum databases (see Acknowledgements). Reliability of all records was assessed with reference to current known distributions and according to our own and expert knowledge of each subspecies; dubious records were excluded from the final data set. To reduce problems of over-fitting and co-linearity of variables in our models, we used eight climatic variables (annual mean temperature, temperature seasonality, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, precipitation seasonality, precipitation of the wettest quarter and precipitation of the driest quarter, as per McMahon *et al.* (1995) and a soil layer (categorized as sand or other substratum, modified from Fordham *et al.*, 2012). We chose these variables as they are known to be important in influencing the distributions of vertebrates, including reptiles, across our study region (Ritchie *et al.*, 2008; Melville *et al.*, 2011).

RESULTS

Phylogenetic relationships

Mitochondrial DNA

The mtDNA Bayesian and ML trees (Fig. 1) recovered two monophyletic lineages within the *C. caudicinctus* and *C. ornatus* group. These lineages represent a western and eastern clade, with the western clade comprising *C. ornatus*, *C. c. infans*, *C. c. mensarum* and *C. c. caudicinctus*. The monophyly of this western lineage only received moderate support (85% bootstrap; 90% posterior probability), with *C. ornatus* being the basal group; however, the monophyly of all the western *C. caudicinctus* subspecies was highly supported (98% bootstrap; 100% posterior probability). Within the western *C. caudicinctus* subspecies, the *C. c. infans* clade received high support (100% bootstrap; 100% posterior probability), while *C. c. caudicinctus* and *C. c. mensarum* were not supported as being independent evolutionary lineages, although all the *C. c. mensarum* samples formed the basal lineages in a clade containing *C. c. caudicinctus* and *C. c. mensarum*. These two subspecies were highly supported as a single monophyletic lineage (100% bootstrap; 100% posterior probability).

The eastern clade received strong support (100% bootstrap; 100% posterior probability), with the monophyly of both *C. c. graafi* (100% bootstrap; 100% posterior probability) and *C. c. slateri* being well supported (92% bootstrap; 100% posterior probability). *C. c. macropus* was not supported as monophyletic but instead contained three well supported lineages: A. Western Australia and western Northern Territory; B. Arnhem Land; and C. western Queensland. One sample of *C. c. macropus* from the south-western Kimberley was highly diverged from the remainder of the Kimberley samples. Additionally, there were four samples of *C. c. caudicinctus* from the far eastern Pilbara area (designated as 'EP' hence forth) that fell within the eastern phylogenetic clade, as the sister lineage to *C. c. graafi*, although this relationship was not well supported.

Examination of mean uncorrected *P* distance of mtDNA between subspecies (Table 2) indicates deep divergences between all subspecies (8.97–14.88%), a level often seen between species, indicating a long history of vicariance. The exception to these deep divergences is the low level of mean uncorrected *p* distance of mtDNA between *C. c. caudicinctus* and *C. c. mensarum* (1.97%). In addition, the far EP *C. c. caudicinctus* populations that fall within the eastern clade phylogenetically are also deeply diverged from all other *C. caudicinctus* subspecies (8.08–15.04%).

Nuclear DNA

A subset of *C. caudicinctus* and *C. ornatus* samples were sequenced for *BDNF*, *BACH1*, *GAPD*, *NTF3*, and *PRLR*, ensuring all mtDNA lineages were represented. Sequence length, number of phylogenetically informative sites and models of evolution implemented in Bayesian analyses for each gene region are provided in Table 1. All nuclear data sets were complete, except for *GAPD*, which was missing three sequences (NMVD73949, SAMAR91440, WAMR131013), and *PRLR*, which was missing two ingroup sequences (WAMR139051, NMVD74380) and one outgroup (*C. adelaidensis*). Although there was less resolution of phylogenetic relationships in the nuclear regions, compared with the mtDNA tree, a number of consistent patterns were present across the gene regions. The Bayesian trees (see Appendix S3) for each of the five nuclear genes recovered mtDNA monophyletic lineages of *C. c. infans* and *C. ornatus*. In three genes (*BDNF*, *GAPD*, *NTF3*) *C. c. graafi* and *C. c. caudicinctus* (EP) were recovered as a monophyletic lineage. In the *BACH1* Bayesian tree, both *C. c. graafi* and *C. c. caudicinctus* (EP) were resolved as being in the eastern lineage including, *C. c. slateri* and *C. c. macropus*. Similarly, in *PRLR*, *C. c. graafi* and one of the *C. c. caudicinctus* (EP) samples (WAMR102635) were resolved as being in a clade containing the eastern lineages (*C. c. slateri* and *C. c. macropus*), while the two remaining *C. c. caudicinctus* (EP) samples (WAMR102611, WAMR102084) fell outside this clade in a

Table 2 Mean uncorrected mtDNA sequence divergence between subspecies of *Ctenophorus caudicinctus* and *C. ornatus*. Values given are percentages.

	1	2	3	4	5	6	7
1. <i>C. c. macropus</i>							
2. <i>C. c. slateri</i>	8.97						
3. <i>C. c. infans</i>	14.74	14.86					
4. <i>C. c. graafi</i>	9.09	9.36	14.88				
5. <i>C. c. caudicinctus</i>	14.28	14.48	11.33	13.95			
6. <i>C. c. caudicinctus</i> (EP)	8.90	8.67	15.04	8.08	13.68		
7. <i>C. c. mensarum</i>	13.88	14.14	11.15	13.71	1.97	13.33	
8. <i>C. c. ornatus</i>	14.25	13.95	13.38	14.07	13.04	13.26	12.96

number of samples for which phylogenetic relationships were unresolved.

Subspecies tree estimation

A reduced *ND2* data set, matching sequence data for individuals in the nuclear data sets, was used, resulting in two data sets of 72 individuals. A new MRMODELTEST analysis was conducted on this reduced mtDNA data set to estimate the optimal model of evolution, with a GTR+I+ Γ model selected and implemented in the species tree analysis. The posterior parameter value estimates from the *BEAST species tree analysis were characterized by high (> 200) effective sample sizes and convergence of the individual runs was confirmed from assessments using TRACER. The maximum clade credibility trees from the posterior sets of species trees differed between each gene (Fig. 2). The topology of the mtDNA and nuclear trees were similar to that in the Bayesian analyses, although the nuclear trees show a higher level of resolution. The monophyly of *C. ornatus* and *C. c. infans* was highly supported across all gene regions. In all genes, except *PRLR*, *C. c. graafi* and *C. c. caudicinctus* (EP) are resolved as being sister lineages. In the *PRLR* tree, as in the Bayesian tree, *C. c. graafi* and one of the *C. c. caudicinctus* (EP) samples (WAMR102635) were resolved as being in a clade containing the eastern lineages (*C. c. slateri* and *C. c. macropus*); however, in this analysis the two remaining *C. c. caudicinctus* (EP) samples (WAMR102611, WAMR102084) were resolved as being part of the lineage containing *C. c. caudicinctus* and *C. c. mensarum*. In the *ND2* and *BACH1* trees *C. c. graafi* and the *C. c. caudicinctus* (EP) samples, were part of the eastern lineage with *C. c. slateri* and *C. c. macropus*, while in the *BDNF* and *GAPD* trees *C. c. graafi* and the *C. c. caudicinctus* (EP) samples, were part of the western lineage with *C. c. caudicinctus* and *C. c. mensarum*. In the overall species tree, *C. ornatus* and *C. c. infans* were highly supported as basal lineages in the complex. There was strong support for a western clade containing *C. c. caudicinctus* and *C. c. mensarum* in the overall species tree, although the *C. c. caudicinctus* (EP) samples were strongly supported as belonging to the eastern clade. The highly supported eastern clade contained *C. c. slateri*, *C. c. macropus*, *C. c. graafi* and the *C. c. caudicinctus* (EP) samples. Within this eastern clade *C. c. graafi* and the *C. c. caudicinctus* (EP) samples are highly supported as sister lineages, as are *C. c. slateri* and *C. c. macropus*.

Divergence times

The relaxed lognormal clock analysis of the mtDNA data set produced the same ingroup topology as the Bayesian and ML phylogenetic analyses (Fig. 1). Examination of the log file in TRACER 1.3 indicated a slight tendency towards a negative correlation in the rate of ancestral to descendant branches but zero was included in the 95% HPD (covariance: -0.0522; 95% credibility interval -0.3301 to 0.2478); thus, this autocorrelation was not considered significant (Drummond *et al.*, 2006). The coefficient of rate variation

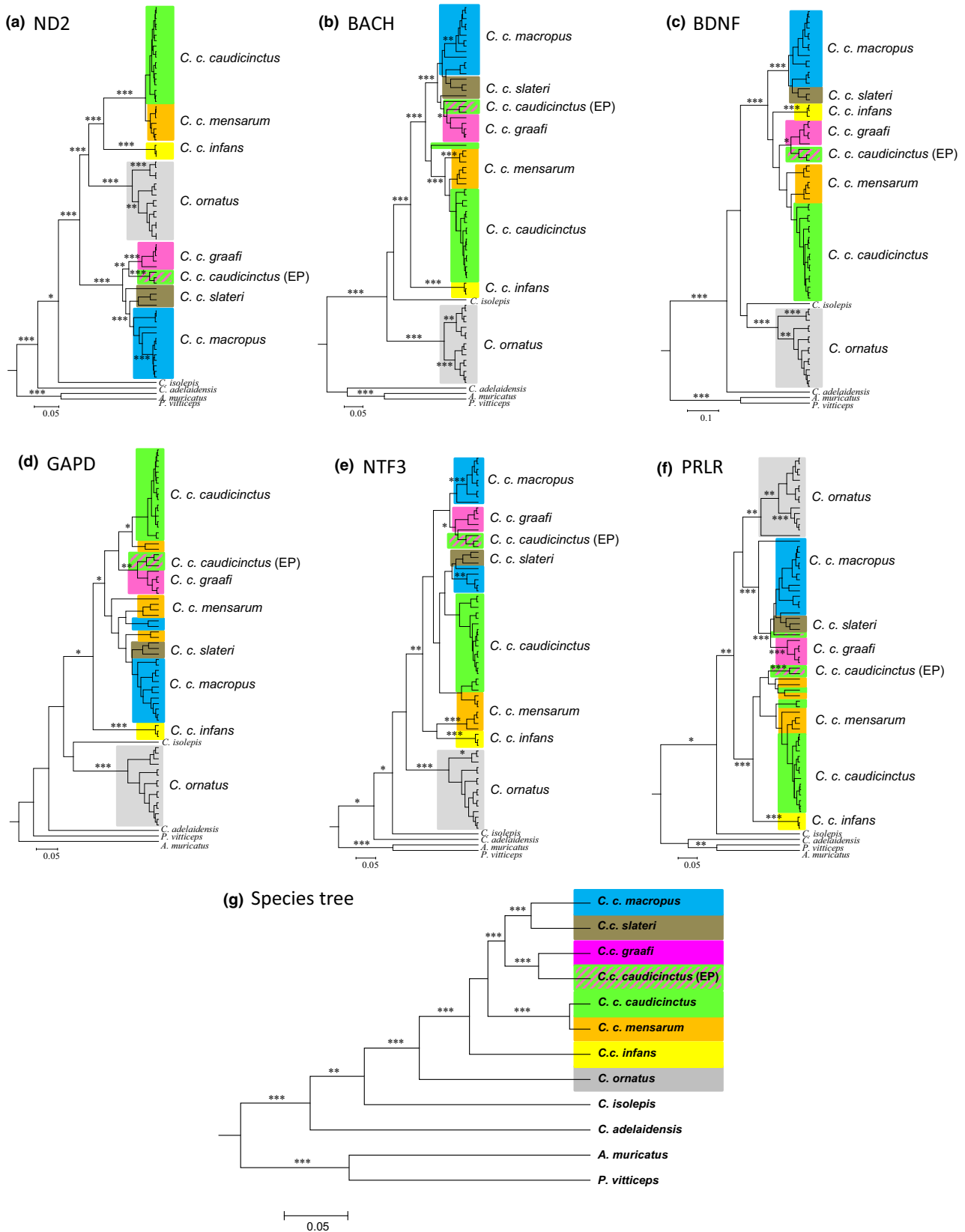


Figure 2 Gene and species tree phylogenies based on data sets inferred using *BEAST for the mtDNA and five nuclear genes (*BDNF*, *BACH1*, *GAPD*, *NTF3* and *PRLR*). Clade posterior probabilities are indicated on branches: ***> 98%; **90–97%; *80–89%. Colours designate clades mapped in Fig. 1.

was estimated to be 0.3374 (95% credibility interval 0.2096–0.4847), indicating that the dataset is not strictly clock-like and that a lognormal relaxed clock is appropriate. Age estimates (Table 3) indicate a mid-Miocene origin of the common ancestor of *C. caudicinctus* and *C. ornatus*. The *C. caudicinctus* lineages, both eastern and western, were estimated to be of late Miocene origins, as were each of the subspecies clades. An exception to this was the age of the common ancestor of *C. c. mensarum* and *C. c. caudicinctus*, which was much younger and probably Pleistocene in origin. In contrast, *C. c. graafi* and the *C. c. caudicinctus* populations from the far EP, which were grouped phylogenetically within the eastern *C. caudicinctus* mtDNA clade (Fig. 1), were estimated to have diverged in the Pliocene.

Ecological niche modelling

Overall our distribution models had very high predictive power, with an average area under curve of 0.98 (range 0.97–0.99). *Ctenophorus c. caudicinctus*'s climate envelope was restricted to the Pilbara region and *C. c. mensarum*'s climate envelope to the Southern Pilbara, Gascoyne and MidWest regions (Fig. 3). *Ctenophorus c. graafi*'s climate envelope, predominantly Central Australian, also spanned an area between *C. c. slateri* (Central Australia) and *C. c. caudicinctus*. *Ctenophorus c. macropus*'s distribution differed from all other subspecies (arid distributions) in being restricted to the monsoonal region of Northern Australia.

DISCUSSION

Dispersal routes through the western sand deserts

Previous phylogeographical studies have examined how the distributions of sand deserts in arid Australia have shaped

Table 3 Estimated age of the common ancestor of *Ctenophorus caudicinctus* and *C. ornatus* clades in millions of years, using relaxed molecular clock analyses, and mean estimate and 95% credibility intervals are provided based on mtDNA (ND2).

	Age of common ancestor (Ma)	95% credibility interval
<i>C. ornatus</i> and <i>C. caudicinctus</i>	9.4	7.1–12.1
Western <i>C. caudicinctus</i> : <i>C. c. infans</i> , <i>C. c. mensarum</i> , <i>C. c. caudicinctus</i>	5.5	3.4–7.6
Eastern <i>C. caudicinctus</i> : <i>C. c. macropus</i> , <i>C. c. slateri</i> , <i>C. c. graafi</i> , <i>C. c. caudicinctus</i> (EP)	5.3	3.7–7.2
<i>C. c. mensarum</i> and <i>C. c. caudicinctus</i>	0.9	0.4–1.7
<i>C. c. macropus</i> and <i>C. c. slateri</i>	5.0	3.5–6.7
<i>C. c. graafi</i> and <i>C. c. caudicinctus</i> (EP)	4.2	2.0–4.9

the evolutionary history and diversity of lizard species. Each of the studies investigating rock-dwelling lizards have found that the divergence of clades pre-dates the sand deserts and that geographically isolated species form deeply divergent and highly supported monophyletic lineages (e.g. Shoo *et al.*, 2008; Pepper *et al.*, 2011a). Shoo *et al.* (2008) and Chapple *et al.* (2004) had sought to test the role of dispersal routes through the sand deserts in shaping the biogeographical history of lizard species, but again found no genetic evidence of these dispersal routes. Our results provide information of particular relevance to these questions.

The Giles Corridor, proposed by Pianka (1972), has been central to these studies investigating dispersal routes through the western sand desert. This corridor of *Acacia* shrublands links the east Murchison goldfields region in Western Australia to the Central Ranges by extending through the Lake Carnegie region in the Great Victoria Desert and the southern part of the Gibson Desert (Van Oosterzee, 1991). Our data, however, find no evidence of a dispersal route via the Giles Corridor during the Pleistocene, with relaxed molecular clock analyses providing support for divergences and secondary contact that pre-date the Pleistocene sand deserts. In addition, we found no evidence of historic hybridization, gene flow or introgression between *C. c. graafi* and *C. c. mensarum*, as would be expected if the Giles Corridor had been the dispersal route. This result seems logical as *C. caudicinctus* is a rock-specialist for which *Acacia* shrublands would not provide an ideal habitat-type for dispersal. Instead, our results, provide evidence of a more northerly historical dispersal route from the Central Ranges to the far EP, at the western and northern edges of the Little Sandy Desert (Fig. 1), with historic phylogenetic connections between the Central Ranges and the far EP. We also find, using ENM, that there are currently suitable climatic conditions and habitat distributions between the Central Ranges and the far EP for potential dispersal routes for the rock-specialists.

A number of rock-dwelling gecko species have been found to have sister lineages in the Central Ranges and the Pilbara (e.g. Oliver *et al.*, 2010; Pepper *et al.*, 2011a,b). In fact, in the saxicolous gecko *Heteronotia spelea* the sister lineages in the Central Ranges and Pilbara (now *H. fasciolatus* – Pepper *et al.*, 2013) were found to have diverged in the Pliocene or early Pleistocene (Pepper *et al.*, 2011a). The age of this divergence is similar to the estimates we provide for the age of the common mtDNA ancestor of *C. c. graafi* in the Central Ranges and the *C. c. caudicinctus* populations from the far EP (Table 3). The formation of the Australian sand deserts are younger than these Pliocene divergences, with luminescence dating estimating dune activity in Australia to at least 300 ka and cosmogenic isotope dating revealing that dune-fields in the western part of the Simpson Deserts began to form 1 Ma (Fujioka & Chappell, 2010). Consequently, the phylogeographical patterns observed in both the dragon lizards and geckos pre-dates the formation of sand deserts. Results from our study suggest that the major lineages (eastern and western) of the *C. caudicinctus* species complex

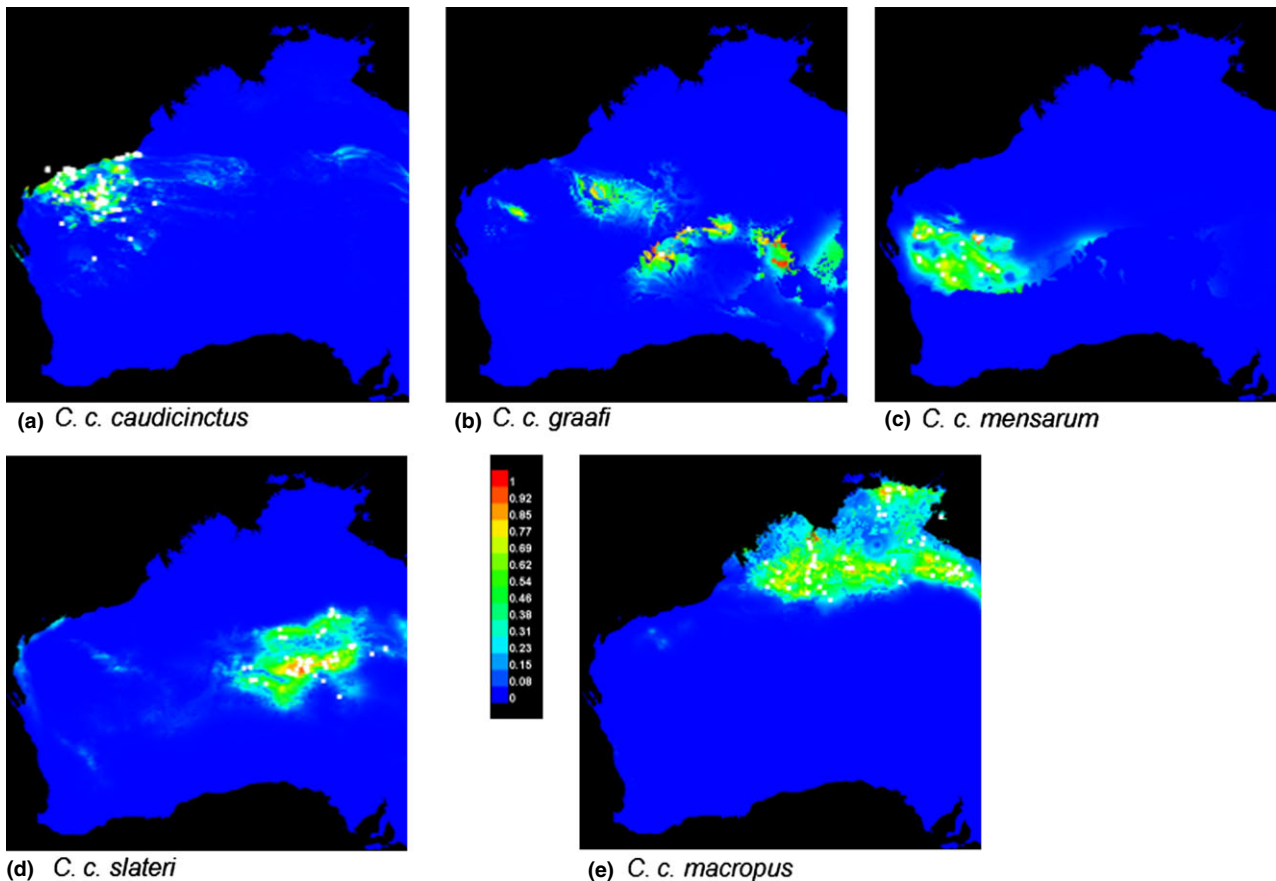


Figure 3 Predicted distributions of *Ctenophorus* subspecies as modelled by MAXENT (Phillips *et al.*, 2006). Warmer colours represent greater predicted environmental suitability with dark blue representing regions not suitable for the species. White dots are locality records.

diverged in the late Miocene, with later secondary contact during the Pliocene, through the Gibson Desert and Little Sandy Desert region.

The Miocene was a time of dramatic climatic shifts, with increasing aridity and seasonality (Byrne *et al.*, 2008) and the Western Australian lowland basins experienced an end to the warmer and wetter conditions of the early Miocene (Martin, 2006). Although a continuous geological record for the period is not available, the late Miocene would have continued to change with increasing aridity through the region (Hill, 1994; Byrne *et al.*, 2008). It is probable that in the late Miocene conditions were unfavourable in the lowlands for species that had occurred there earlier, fragmenting populations as they took refuge in the rocky uplands of the Pilbara and central Australia (Pepper *et al.*, 2011a). In the Pliocene there was a 'mesic pulse', with a temporary return to wet and warm conditions (Byrne *et al.*, 2008). Thus, it is possible that these climatic changes were the major drivers of the phylogeographical patterns observed in the *C. caudicinctus* species complex rather than the expansion of the sand deserts. Increasing Miocene aridity may underlie the original divergence of *C. caudicinctus* into an eastern and western lineage, with a temporary return to mesic conditions in the Pliocene

allowing dispersal and secondary contact with gene exchange between the Central Ranges and Pilbara.

The spread of the sand deserts during the Pleistocene has probably limited subsequent dispersal and gene exchange within the *C. caudicinctus* species complex. However, our ENM, which included a sand layer, does suggest that there are potentially suitable habitats for *C. c. graafi* across the Little Sandy Desert and Gibson Desert into the EP. In fact, the distribution of suitable habitats based on ENM is in concordance with our genetic results. The genetic markers we used in our study provide a snapshot of historic gene exchange, while the ENM suggest that there may be suitable habitats through the western deserts currently. Thus, it would be of particular interest to use genetic markers to explore whether there is infrequent dispersal and gene exchange occurring currently. Further sampling in the rocky habitats through the Gibson Desert area would provide additional important data, if *C. c. caudicinctus* or *C. c. graafi* occur in these areas.

Phylogenetic relationships

Our molecular work, incorporating both mitochondrial and nuclear gene regions, reveal a complex evolutionary and bio-

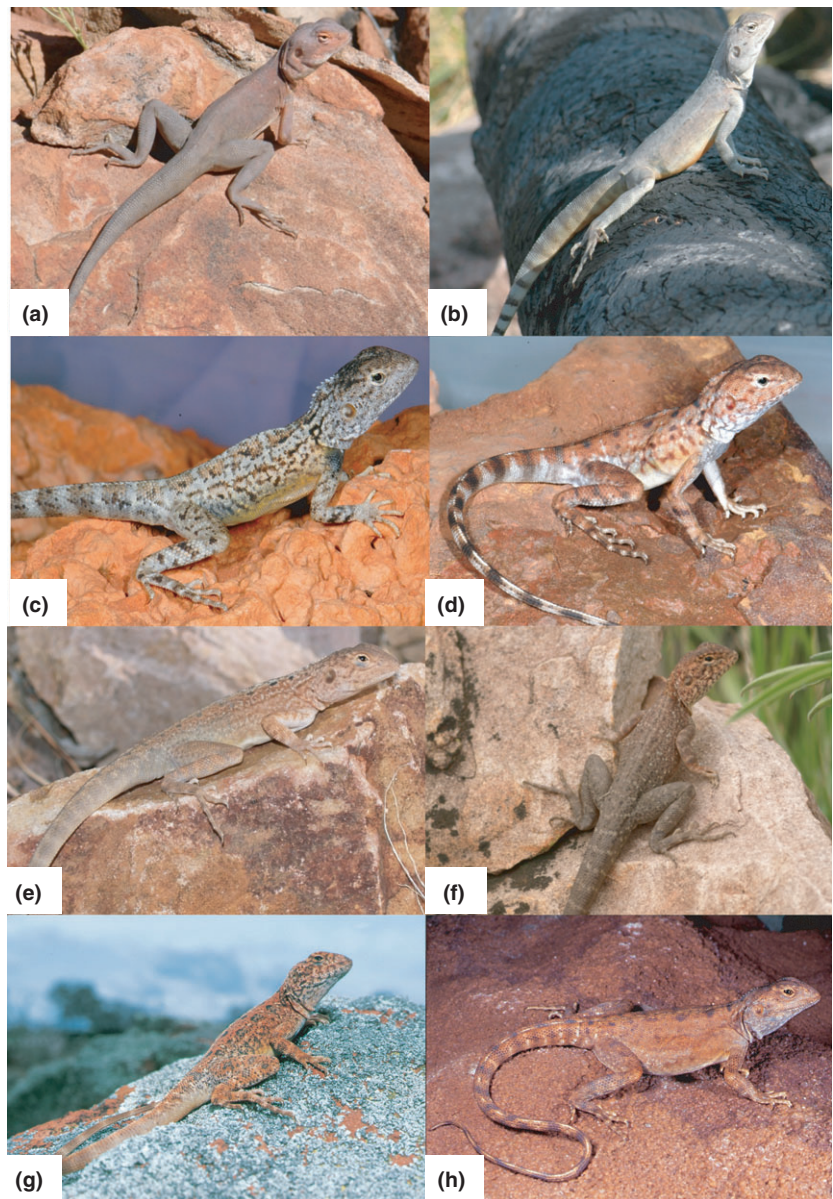


Figure 4 Photographs of each of the *Ctenophorus caudicinctus* subspecies, including two images each of *C. c. caudicinctus* and *C. c. macropus* to demonstrate variation within lineages. Locality information: (a) *C. c. macropus*, Wolfe Creek Crater, Western Australia (image: R. Glor); (b) *C. c. macropus*, western Arnhem Land, Northern Territory (image: R. Glor); (c) *C. c. caudicinctus*, 80 mile beach, Western Australia (image: J. Melville); (d) *C. c. caudicinctus*, Port Headland, Western Australia (image: J. Melville); (e) *C. c. graafi*, Pungkulpirri, Western Australia (image: M. Hutchinson); (f) *C. c. slateri*, Waterhouse Range, Northern Territory (image: M. Hutchinson); (g) *C. c. infans*, Laverton, Western Australia (image: G. Harold); (h) *C. c. mensarum*, Meekatharra, Western Australia (image: G. Gaikhorst).

geographical history in *Ctenophorus caudicinctus*. Using a Bayesian framework for subspecies tree estimation, which is the most appropriate approach when there is conflict between gene trees (see Heled & Drummond, 2010), we provide evidence of phylogenetic congruence between data sets and the unexpectedly deep genetic divergences between subspecies, which suggests that a revision of *C. caudicinctus* subspecies is warranted.

Our genetic data support a close relationship between *C. c. caudicinctus* and *C. c. mensarum*, with no evidence that these subspecies are independent evolutionary lineages. However, we provide strong support that these two subspecies together form an independent evolutionary lineage (with the exception of the two far eastern populations of *C. c. caudicinctus*, which we address further down), which diverged from *C. c. infans* in the late Miocene. *Ctenophorus c. infans* is morphologically distinct, comprising the smallest of the sub-

species with adult male coloration differing little from females and juveniles (Storr, 1967; Fig. 4). Thus, based on both morphological and molecular evidence, we recommend that *C. c. infans* and *C. c. caudicinctus* be raised to full species level and that *C. c. mensarum* should be synonymized into *C. caudicinctus*.

The monophyly of the eastern lineages of *C. caudicinctus* are strongly supported in the mtDNA and species tree analyses, with the inclusion of the far EP populations (discussed below). Morphologically, there is significant variation between *C. c. macropus* and *C. c. slateri* (Storr, 1967; Fig. 4); however, there is also within-lineage diversity, both in terms of genetic diversity (Fig. 1) and morphological variation (Storr, 1967; Fig. 4). There is clearly a need for further research into the diversity within and between these two lineages. For the moment, we recommend raising this lineage as a whole (*C. c. macropus*

and *C. c. slateri*) to species level, with precedence requiring it to become *C. slateri* (Storr, 1967), while *C. c. macropus* is synonymized into *C. slateri*, but with the obvious need of reviewing the diversity within this group in the immediate future.

Finally, we found evidence of historic gene exchange between the eastern and western lineages of *C. caudicinctus*. In particular, two populations in the far EP region, identified as *C. c. caudicinctus*, were supported as belonging to the eastern clade across two gene regions (*ND2* and *BACH1*; Fig. 2). Our results are unable to shed light on whether *C. c. graafi* is the result of gene exchange between *C. c. slateri* and *C. c. caudicinctus* (i.e. a hybrid origin) or whether *C. c. graafi* diverged independently with subsequent gene exchange with *C. c. caudicinctus*. Storr (1967) describes *C. c. graafi* as having morphological characters that differ from either *C. c. caudicinctus* or *C. c. slateri*, thus, further work incorporating more loci (e.g. next generation sequencing) should shed light on the origins of *C. c. graafi*. Until then we recommend the *C. c. graafi* be raised to species level, reflecting its morphological difference, isolated distribution and genetic distinctiveness.

With our recommendations the *C. caudicinctus* species group, which currently incorporates six subspecies, would become four species: *C. caudicinctus*, *C. infans*, *C. slateri* and *C. graafi*. However, we acknowledge that further work is required, particularly for *C. slateri* and *C. graafi*.

CONCLUSIONS

The *Ctenophorus caudicinctus* species complex represents an important test case for investigations of phylogeographical structure of species spanning western sand deserts, as it provides the first genetic evidence of biogeographical dispersal routes, which are also supported by ENM. We were able to show that there has been a historic dispersal route between the Central Ranges and Pilbara, probably during the Pliocene, but we found no evidence that the previously hypothesized Giles Corridor has been used as a dispersal route for *C. caudicinctus*. Our results further highlight the biogeographical connection between the Central Ranges and Pilbara and provide additional evidence that divergences in specialist rock-dwelling lizards in this region pre-date the Pleistocene sand deserts.

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Melbourne Animal Ethics Committee and animals were collected under permits from the WA Department of Parks and Wildlife and the NT Parks and Wildlife Commission.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Details of *Ctenophorus caudicinctus* and *C. ornatus* samples.

Appendix S2 Supplementary materials and methods, including primers and PCR protocols.

Appendix S3 Supplementary figures, including Bayesian trees for each of the five nuclear genes.

BIOSKETCH

Jane Melville studies the evolution, systematics and eco-morphology of Australian agamids. Her research focuses on understanding adaptive responses of agamids to environmental change and the evolutionary mechanisms underlying these changes.

Author contributions: J.M. and J.H. initiated the research; J.M. and E.R. conceived the ideas; J.M., M.H. J.H. and S.C. acquired the data; J.M. and E.R. analysed the data; J.M. led the writing; M.H. J.H., S.C. and E.R. contributed to the writing; all authors discussed the ideas and commented on the manuscript.

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