

Palaeoclimate change drove diversification among isolated mountain refugia in the Australian arid zone

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Abstract

Refugia featured prominently in shaping evolutionary trajectories during repeated cycles of glaciation in the Quaternary, particularly in the Northern Hemisphere. The Southern Hemisphere instead experienced cycles of severe aridification but little is known about the temporal presence and role of refugia for arid-adapted biota. Isolated mountain ranges located in the Australian arid zone likely provided refugia for many species following Mio/Pliocene (<15 Ma) aridification; however, the evolutionary consequences of the recent development of widespread sand deserts is largely unknown. To test alternative hypotheses of ancient vs. recent isolation, we generated a 10 gene data set to assess divergence history among saxicolous geckos in the genus *Heteronotia* that have distributions confined to major rocky ranges in the arid zone. Phylogenetic analyses show that each rocky range harbours a divergent lineage, and substantial intraspecific diversity is likely due to topographic complexity in these areas. Old divergences (~4 Ma) among lineages pre-date the formation of the geologically young sand deserts (<1 Ma), suggesting that Pliocene climate shifts fractured the distributions of biota long before the spread of the deserts.

Keywords: aridification, desert, diversification, geology, phylogenetics, refugia

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Introduction

Changes in climate and landscape can drive speciation. The effects of these forces on evolutionary history often can be visualized in molecular phylogenies of extant taxa, which in turn can facilitate tests of diversification hypotheses (Hewitt 2004; Venditti *et al.* 2009). However, molecular studies repeatedly confirm that interpretation of the patterns and processes of organismal diversification requires simultaneous examination of genetic, climate and landscape histories (Ricklefs & Schluter 1993; Birmingham & Moritz 1998; Avise 2000; Hewitt 2000; Carstens & Knowles 2007). For example, the extension of ice-sheets during Pleistocene glacial cycles is well known to have structured the genetic signatures of boreal taxa through repeated contraction to southern mountain refugia (Taberlet *et al.* 1998; Hewitt 2004). In

the un-glaciated tropics, fragmentation and expansion of forested habitats, also in response to climate cycling, are thought to have shaped congruent phylogenetic patterns across suites of taxa, both during the Pleistocene and in earlier time periods of the Miocene/Pliocene (Moritz *et al.* 2000; Thome *et al.* 2010). Studies of arid-zone biotic diversification lag behind those of other biomes (Beheregaray 2008; Byrne *et al.* 2008). However, there are a number of studies that show the development of increasing aridity and the recent development of major sand dune systems has shaped diversification patterns of arid-zone taxa (Scott *et al.* 2004; Sole *et al.* 2005; Murphy *et al.* 2006; Melville *et al.* 2009).

Tectonic uplift and mountain building also are known to influence species distribution and diversification independent of, or in conjunction with, climate shifts. In cases of the latter, it often can be difficult to distinguish the effects of geological activity from climatic fluctuations, particularly where active mountain building is ongoing, or has occurred in the very recent past,

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for example in the Andes (Brumfield & Capparella 1996; Elias *et al.* 2009), the Himalayas (Che *et al.* 2010) New Zealand (Trewick & Wallis 2001), and North American (Pook *et al.* 2000; Neiswenter & Riddle 2010). Therefore, while undeniably important, the role of recent uplift and topographic heterogeneity in the diversification of biota is complex, and testing competing hypotheses of geologic versus climate-induced diversification can be challenging.

The Australian landmass is old and weathered and as a result its 'mountainous' regions are more subdued than other continents. Recent tectonic events have been confined largely to the leading edge of the Australia-New Guinea Plate, and much of the continent has been geologically stable for hundreds of millions of years, enabling exclusive focus on climate driven evolutionary change. The palaeoclimatic history of Australia is characterized by erratic fluctuations, particularly from the mid-Neogene (~23–2.5 Ma) and throughout the Quaternary (2.5 Ma—Present) when a major shift from a stable climate characterized by warm and wet environments to volatile climate cycling led to aridification of much of the continent (Bowler 1976; Frakes *et al.* 1976; Mabbutt 1988; Martin 2006). In the intensively studied rainforests of northeastern Australia, the repeated incursions of these dry conditions were paramount in the diversification of temperate biota as fragmentation and contraction of rainforests pushed biota

into a number of mountain refugia (reviewed in Moritz *et al.* 2009).

But what of mountain ranges in xeric systems? Despite the immense size of the Australian arid zone, we still have much to learn about the effect of aridification on biotic diversification in this region (reviewed in Byrne *et al.* 2008), particularly regarding the presence and role of refugia, areas which promoted persistence of lineages throughout the Quaternary climate fluctuations. Fortunately, the physical origins of the arid zone and its landforms are well understood (Beard 1969; Wasson 1982; Mabbutt 1988; Fujioka *et al.* 2005, 2009), and, combined with regional palaeoecological inference, can provide the foundations to test evolutionary hypotheses concerning the spatial and temporal distribution and diversification of arid-adapted biota (Byrne *et al.* 2008).

Geological evolution of the arid zone

While much of arid Australia is flat, several mountain ranges, albeit limited in elevation (<1500 m), constitute prominent topographic features that rise above the surrounding lowlands (Fig. 1). The rocky ranges of the Pilbara (Pilbara block), Kimberley (Kimberley block) and central Australia (Arunta block) have been tectonically stable for more than 80 million years (van Kranendonk *et al.* 2002). They have a common geological

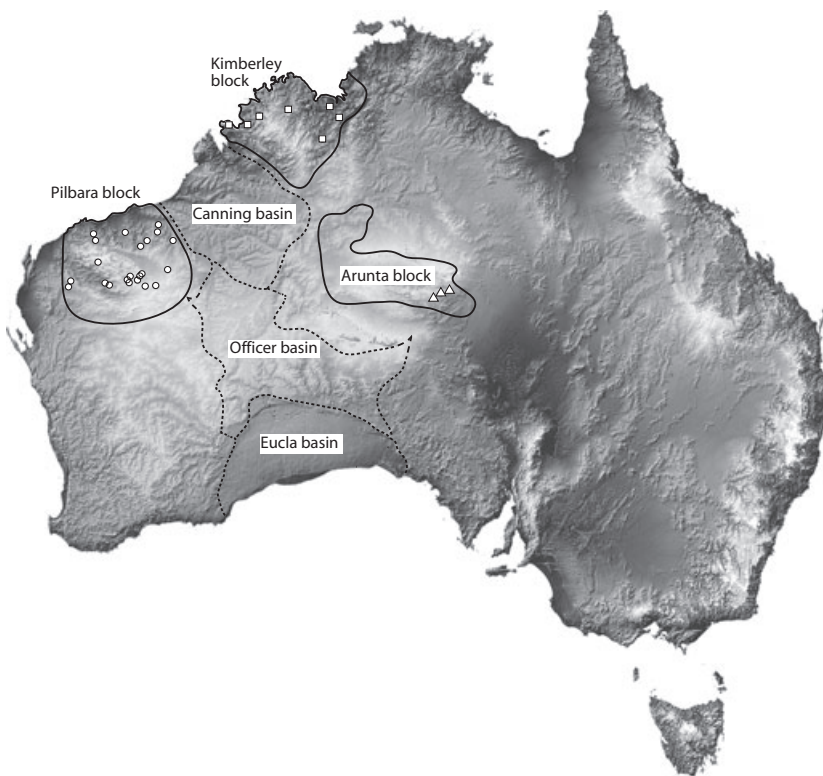


Fig. 1 Topographic map of the Australian continent, adapted from the digital elevation maps produced by NASA and the National Geospatial-Intelligence Agency. The thick lines define the boundaries of the uplifted pre-Cambrian craton that forms the mountain ranges of the Pilbara (Pilbara block), Kimberley (Kimberley block) and central Australian ranges (Arunta block). Dashed lines define the boundaries of the sedimentary basins that underlie the present sand deserts. Circles represent Pilbara *Heteronotia spelea*, triangles represent Central *H. spelea* and squares represent *H. planiceps* specimens included in our molecular data set.

history in that each is an uplifted remnant of the ancient pre-Cambrian craton (or shield) that underlies the westernmost two-thirds of the Australian continent. The topography and rugged characteristics of these regions are heavily influenced by the geological composition of the craton, as considerable tectonic and igneous activity in the past has produced a complex mosaic of rock types that are hard and resistant to erosion (Beard 1979; Wasson 1982). In contrast, between these uplifted regions the craton is buried under much younger sediments of a number of lowland basins, with the Canning, the Officer and the Eucla Basins forming a north–south chain through the arid interior (Fig. 1). The basins are comprised of younger, flat-lying sedimentary rocks laid down in a marine trough during the Palaeozoic when much of Western Australian was inundated by ocean (Wasson 1982). These sediments are strikingly different from those of the craton and have had a profound influence on the subdued and uniform nature of what is now low-lying arid zone.

The onset of aridity played a major role in landscape physiognomy. In particular, the termination of the warm and wet conditions of the early Neogene (~20 Ma) followed by heightened levels of erosion initiated an immense change to the basin regions. The recent establishment of sand plains and dune fields isolated the mountain uplands with vast tracts of sandy soils. Knowledge of the timing of sand dune initiation and development is impeded by a historical difficulty in determining the age of sand dune landforms by direct dating methods (Fujioka *et al.* 2009), as the constant reworking of sand grains over time limits the use of luminescence methods for dating individual dunes to only a few hundred thousand years (Hesse *et al.* 2004). However, novel cosmogenic methods for dating arid-zone dune fields recently have been applied in the Simpson Desert in central Australia, pushing back the age of dune field formation to a maximum of 1 Ma (Fujioka *et al.* 2009). The development of such an extensive and mobile landform system undoubtedly had a profound impact on the evolution and diversification of arid-zone biota.

Biotic response to aridification

The lack of information on the relative age of contemporary clades against the age of the habitats in which they now and historically have lived is a fundamental gap in our knowledge (Byrne *et al.* 2008). Numerous authors have alluded to topographically complex regions in the Australian arid zone acting as mesic refuges during periods of extreme aridity (e.g. Crocker & Wood 1947; Keast 1961; Schodde 1982, Heatwole 1987; Morton *et al.* 1995; Byrne *et al.* 2008), but we know little about the spatial and temporal scale of these putative refugia.

Based on geology, topography and the associated influence on moisture and microclimate, the primary refuge regions should be the Pilbara, central Australian ranges and the Kimberley (Fig. 1). These areas have been highlighted to varying degrees for their high species diversity and endemism (i.e. Cracraft 1991; Boden & Given 1995; Slatyer *et al.* 2007), but there have been surprisingly a few attempts to explicitly test the assumption that these mountainous regions constitute major historic refugia, largely because of inadequate molecular sampling (Byrne *et al.* 2008). In particular, analyses of species endemism and phylogeny cast doubt on whether the central Australian ranges can be considered a long-term (>2 Ma) refuge and isolate (Crisp *et al.* 2001; Laffan & Crisp 2003). Furthermore, while we know that the central deserts constitute barriers to dispersal for mesic-adapted birds (Keast 1959; Ford 1971; Schodde 2006, Joseph & Omland 2009), frogs (Littlejohn 1961; Main 1968; Read *et al.* 2001; Morgan *et al.* 2007) and reptiles (Pianka 1972; Chapple & Keogh 2004; Strasburg & Kearney 2005; Shoo *et al.* 2008), the evolutionary consequences of developing the vast inland sand deserts <1 Ma in Australia have received little attention.

Study system and hypotheses

Testing hypotheses concerning the interaction between ancient topographically complex mountain systems and young, low-lying desert regions ideally requires a group of organisms that currently span both landscapes. Geckos in the genus *Heteronotia* Wermuth, 1965 provide an ideal study system as their distributions span the Australian arid-zone, which includes the mountain regions of the Pilbara, Kimberley and central Australia that are separated by intervening uniform desert lowlands. The genus currently comprises three described species (*binoei*, *spelea* and *planiceps*) with numerous recognized chromosomal and genetically divergent lineages within *H. binoei* (Moritz 1993; Fujita *et al.* 2010). Of the three described taxa, the *H. binoei* complex has the largest distribution and broadest ecological range and is widespread throughout the Australian continent except the cooler southeast and southwest corners (Wilson & Swan 2003). In contrast, *H. spelea* and *H. planiceps* are rock-adapted specialists. *Heteronotia spelea* was described from the Pilbara region (Kluge 1963) with various populations from the Kimberley and central ranges also assigned to this taxon (Cogger 2000). *Heteronotia planiceps* is thought to occur in the Kimberley, with a purported disjunct population in the Pilbara (Storr 1989; Cogger 2000), while others extend its distribution into the Northern Territory (Wilson & Swan 2003).

The distribution of saxicolous *Heteronotia* across disjunct mountain ranges provides an ideal opportunity to

examine the role of refugia and long-term aridification versus more recent development of sand dune deserts in shaping the evolution and diversification of the arid Australian biota. If the mountain ranges served as ancient refugia throughout the Mio/Pliocene aridification, and if saxicolous populations were isolated at this time, there should be high divergence among refugia. Conversely, if each taxon was previously widespread (as implied by current taxonomy) but subsequently isolated by mid-late Pleistocene development of dune systems, divergence among current isolates will date to the late Pleistocene (<1 Ma). To the extent that lineages maintained high effective population sizes, either as large connected populations or as multiple subdivided populations, through recent climate oscillations, we expect to see high within-lineage genetic diversity.

Here, we use a multilocus, phylogenetic approach to carry out the first detailed genetic assessment of *H. spelea* and *H. planiceps* to distinguish between alternative hypotheses of ancient versus recent refugial isolation in the mountain ranges of the Pilbara, Kimberley and central Australia, and in doing so assess the role of mountain ranges and desert formation on their evolutionary histories. An analogous study, using the same suite of loci, on the widespread generalist *H. binoei* revealed mid-Pleistocene divergence and recent (<0.5 Ma) population expansion for the lineages spanning the deserts that presently separate Central and Western populations of *H. spelea* (Fujita *et al.* 2010). A further prediction is that isolation of the saxicolous lineages (*H. spelea* and *H. planiceps*) is contemporaneous with expansion of the generalist *H. binoei* throughout the western deserts.

Materials and methods

Taxonomic sampling

Tissue samples were obtained from the Western Australian Museum, the South Australian Museum and the Museum of Vertebrate Zoology at the University of California Berkeley for a total of 65 individuals (Table 1). This includes the best possible sampling given available tissues of the saxicolous members of the genus *Heteronotia* (*spelea* and *planiceps*) and representatives of the known major lineages of the *H. binoei* complex (Fujita *et al.* 2010). For all analyses, *Dixonius vietnamensis* (the sister taxon of *Heteronotia*) (Jackman *et al.* 2008) was used as the outgroup to root the phylogenetic trees.

Molecular data

Data used in this study were obtained from both mitochondrial DNA and multiple nuclear introns to provide

robust inference of relationships and divergence history. Initially, the entire mitochondrial NADH dehydrogenase subunit 2 (*nad2*) gene and the flanking transfer RNA (tRNA) genes tRNA^{Met} (partial), and tRNA^{Trp} (entire), tRNA^{Ala} (entire) and tRNA^{Asn} (partial) were sequenced. In addition, nine nuclear introns were amplified using primers developed by Fujita *et al.* (2010). These include *bzw1*, *lztfl1*, *dncl1*, *frih*, *erh*, *nmes*, *rpl14*, *rpl35* and *snrpd3*. Information on primers and marker length is presented in Table 2. Full details of marker development as well as PCR and sequencing amplification are presented elsewhere (Fujita *et al.* 2010).

Sequences were edited in Sequencher 4.0 (Genes Codes Corporation). Where occasional heterozygous indels were found, we concatenated the forward and reverse reads at the indel position, and subsequently coded the indel as missing data. Multiple alignments were performed using MUSCLE (Edgar 2004), which were then refined by eye. Protein coding regions of *nad2* were translated into amino acid sequences using the vertebrate mitochondrial code and were compared to *Gekko gecko* translations on GenBank to check for stop codons and frame shifts. We then used PHASE V2.1.1, running the algorithm 10 times, to phase the nuclear data into alleles (Stephens & Donnelly 2003), using the most probable reconstructed haplotypes for population genetic analyses. All sequences have been deposited in Genbank (Accession numbers HQ839864—HQ840286).

Phylogenetic analyses

Phylogenetic relationships were inferred using maximum-likelihood and Bayesian inference. Bayesian phylogenetic analyses were conducted in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003), for individual genes using genotype data for the nuclear markers and for the concatenated data set as a whole. For each marker, the best-fit models of molecular evolution were estimated under the Akaike Information Criterion (AIC) in MrModeltest v2.3 (Nylander 2004) (Table 2). For the concatenated data set, we calculated Bayes factors (Kass & Raftery 1995) to determine the best partitioning strategy (Brandley *et al.* 2005), comparing between (i) unpartitioned, (ii) two partitions (partitioned by nDNA and mtDNA), (iii) four partitions (partitioned by nDNA and by codon position for *nad2*), and (iv) twelve partitions (partitioned by gene and by codon position for *nad2*). The ratio of the harmonic mean likelihoods for competing models was computed using Tracer v1.4.1 (Rambaut & Drummond 2007). For the concatenated analyses, some samples were missing one of the nine genes, but this appeared to have no effect on the ultimate topology. All Bayesian analyses consisted of four

Table 1 Locality information for all individuals sampled in this study

Lab ID	Museum	Accession number	Genus	Species	Latitude (dec.)	Longitude (dec.)	Location (nearest)
31241	SAM	ABTC31241	<i>Heteronotia</i>	<i>binoei</i>	-11.96670	141.90000	Mapoon Mission, Qld
31253	SAM	ABTC31253	<i>Heteronotia</i>	<i>binoei</i>	-15.70000	126.36667	Drysdale, WA
32437	SAM	ABTC32437	<i>Heteronotia</i>	<i>binoei</i>	-27.00000	133.31700	Chandler, SA
MKF118	MVZ	MKF118	<i>Heteronotia</i>	<i>binoei</i>	-27.95000	134.31300	Copper Hills, SA
MKF302	MVZ	MKF302	<i>Heteronotia</i>	<i>binoei</i>	-26.13190	113.40334	Useless Loop, WA
Gko019	WAM	R96971	<i>Heteronotia</i>	<i>planiceps</i>	-15.71944	125.20694	Kings Cascade, WA
Gko020	WAM	R106220	<i>Heteronotia</i>	<i>planiceps</i>	-16.10000	124.63333	Red Cone Hill, WA
Gko021	WAM	R113996	<i>Heteronotia</i>	<i>planiceps</i>	-15.35000	126.61667	Carson Escarpment, WA
Gko022	WAM	R132761	<i>Heteronotia</i>	<i>planiceps</i>	-15.29111	128.66917	Carlton Hill Station, WA
Gko025	WAM	R158015	<i>Heteronotia</i>	<i>planiceps</i>	-16.11417	123.69417	Koolan Island, WA
Gko026	WAM	R158024	<i>Heteronotia</i>	<i>planiceps</i>	-16.14583	123.74917	Koolan Island, WA
Gko027	WAM	R158033	<i>Heteronotia</i>	<i>planiceps</i>	-16.14722	123.77056	Koolan Island, WA
Gko253	SAM	ABTC11740	<i>Heteronotia</i>	<i>planiceps</i>	-23.37300	120.14200	40km East of Mt. Newman, WA
Gko254	SAM	ABTC29848	<i>Heteronotia</i>	<i>planiceps</i>	-15.76806	129.09000	Jarrnarm Escarpment, NT
Gko255	SAM	ABTC32937	<i>Heteronotia</i>	<i>planiceps</i>	-23.37300	120.14200	40km East of Newman, WA
Gko256	SAM	ABTC31418	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.69611	134.03556	Undoolya Homestead, NT
Gko257	SAM	ABTC31432	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.59000	134.47972	Ross River, NT
Gko258	SAM	ABTC31433	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.69611	134.03556	Undoolya Homestead, NT
Gko259	SAM	ABTC31434	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.59000	134.47972	Ross River, NT
Gko260	SAM	ABTC31435	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.69611	134.03556	Undoolya Homestead, NT
Gko261	SAM	ABTC32871	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.69611	134.03556	Undoolya Homestead, NT
Gko262	SAM	ABTC32988	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.69611	134.03556	Undoolya Homestead, NT
Gko280	SAM	ABTC31199	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.69611	134.03556	Undoolya Homestead, NT
Gko281	SAM	ABTC31436	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.69611	134.03556	Undoolya Homestead, NT
Gko282	SAM	ABTC31437	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.69611	134.03556	Undoolya Homestead, NT
Gko279	SAM	ABTC24118	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.89000	133.71972	Mulga Dam MacDonnell Range, NT
Gko283	SAM	ABTC31636	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.59000	134.47972	Ross River, NT
Gko284	SAM	ABTC31659	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.59000	134.47972	Ross River, NT
Gko285	SAM	ABTC31726	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.59000	134.47972	Ross River, NT
Gko286	SAM	ABTC31731	<i>Heteronotia</i>	<i>spelea</i> (central)	-23.59000	134.47972	Ross River, NT
Gko004	WAM	R110056	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.03590	117.10658	30km South of Roebourne, WA
Gko005	WAM	R110075	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.03590	117.10658	30km South of Roebourne, WA
Gko006	WAM	R110076	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.03590	117.10658	30km South of Roebourne, WA
Gko028	WAM	R97258	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.36667	120.13333	Wheellarra Hill, WA
Gko029	WAM	R102227	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.41139	115.89389	Barlee Range, WA
Gko030	WAM	R102380	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.40000	115.88333	Barlee Range, WA
Gko031	WAM	R102436	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.13056	115.99444	Goodeman Pool, WA
Gko032	WAM	R113545	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-22.83333	119.46667	Capricorn Roadhouse, WA
Gko033	WAM	R114561	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.08333	119.28333	Newman, WA
Gko034	WAM	R115832	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.11889	118.78278	The Governor, WA
Gko035	WAM	R115833	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.11889	118.78278	The Governor, WA
Gko036	WAM	R121394	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-22.86667	119.43333	Weeli Wolli, WA
Gko037	WAM	R132488	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.20000	117.66667	Paraburdoo, WA
Gko038	WAM	R132681	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-20.60028	120.29167	Shay Gap, WA
Gko039	WAM	R135010	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.38611	119.62944	Mount Whaleback, WA
Gko040	WAM	R135388	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-22.31056	117.32861	Mount Brockman Station, WA
Gko041	WAM	R135446	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-22.31056	117.32194	Mount Brockman Station, WA
Gko042	WAM	R135456	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-22.31056	117.32194	Mount Brockman Station, WA
Gko043	WAM	R145600	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-20.92860	118.67780	Port Hedland, WA
Gko044	WAM	R157546	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.19417	118.81500	West Angeles, WA
Gko045	WAM	R157719	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-22.94083	118.90500	Newman, WA
Gko278	SAM	ABTC11763	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-20.92000	120.20972	Bamboo Creek Mine, WA
Gko287	SAM	ABTC32832	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-20.92000	120.20972	Bamboo Creek, WA
Gko288	SAM	ABTC32922	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-20.92000	120.20972	Bamboo Creek, WA
Gko435	WAM	R111667	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.0364	117.1060	TCMBE*, WA

Table 1 (Continued)

Lab ID	Museum	Accession number	Genus	Species	Latitude (dec.)	Longitude (dec.)	Location (nearest)
Gko436	WAM	R111927	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-22.6098	120.7290	BDRN09*, WA
Gko437	WAM	R111986	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-22.6098	120.7290	BDRN09*, WA
Gko438	WAM	R160085	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.3219	121.0020	NE09*, WA
Gko439	WAM	R160145	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.3219	121.0020	NE09*, WA
Gko440	WAM	R161285	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.3219	121.0020	NE09*, WA
Gko442	WAM	R165152	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.0538	119.1770	PW3*, WA
Gko443	WAM	R165222	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.3413	117.1890	DRC6*, WA
Gko444	WAM	R170295	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.0364	117.1060	TCMBC05*, WA
Gko445	WAM	R170828	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-23.3183	117.87	MBE06*, WA
Gko446	WAM	R170892	<i>Heteronotia</i>	<i>spelea</i> (Pilbara)	-21.5062	119.418	MBE1*, WA

separate runs, each with four chains (three heated, one cold, default heating parameters), for 20 000 000 generations sampled every 2000 generations, and with 3 000 000 generations burn-in. We assessed convergence of most of the Bayesian MCMC analyses in several ways. First, we examined the log-likelihood and parameter estimates over time using Tracer v1.4.1 (Rambaut & Drummond 2007). All runs had ESS values over 200 after discarding the burn-in, indicating we had at least 200 independent samples from the posterior for each analysis. Second, we examined the stability of posterior probabilities of nodes over time by plotting their values over the course of the analysis using Are We There Yet? (AWTY; Wilgenbusch *et al.* 2004). In all cases, the posterior probabilities had stabilized after we discarded the burn-in. Finally, we also examined the correlation between the posterior probabilities of the separate runs using AWTY, and in each case the runs were visually strongly correlated. Partitioned maximum-likelihood analyses of the combined nuclear data (partitioned by gene), and the mtDNA data (partitioned by codon position), were conducted using RAxML-VI-HPC v7.0.4 (Stamatakis 2006), using the GTR + G model of nucleotide substitution for each data partition. Support values were estimated from 1000 bootstrap replicates.

Recent arguments have been made that concatenation across loci may not be the most reliable approach to inferring the species phylogeny (Kolaczkowski & Thornton 2006; Edwards 2009) particularly when there is rate heterogeneity across loci (Carstens & Knowles 2007). Therefore, in addition to our concatenated approach, we used the hierarchical model implemented in *BEAST v. 1.6.0 (Heled & Drummond 2010), that co-estimates the species tree and all gene trees in one Bayesian MCMC analysis, to estimate the species tree phylogeny. This method requires a priori designation of species, so we divided our data based on the monophyletic groups that were consistent between mitochondrial and concatenated nuclear analyses resulting in four

'species'. Because species tree methods are more sensitive to missing data than supermatrix approaches (Edwards 2009), we only used individuals in our ingroup represented by complete data, resulting in a data set of 23 individuals for which each species was represented by at least five samples, with the exception of *H. planiceps* where we only had two. Our outgroup sample, BLS11160 *Dixonius vietnamensis*, contained missing data for one intron, *erh*. We partitioned our data by gene using models inferred in MrModeltest v2.3 (Nylander 2004) under the Akaike Information Criterion (Table 2). We conducted four separate runs, with samples drawn every 5000 steps over a total of 50 000 000 steps, with the first 10% discarded as burn-in. Acceptable convergence to the stationary distribution was checked by inspecting the posterior samples using the diagnostic software Tracer v1.5 (Rambaut & Drummond 2007). Effective sample sizes were >200 for all parameters. All runs produced the same topology with very similar posterior probabilities, so we combined runs to generate a single consensus tree.

To estimate genetic diversity within and between *H. binoei*, *H. planiceps* and *H. spelea* ('central' and 'Pilbara'), we calculated average sequence divergence (Dxy, Nei 1987) using the Tamura-Nei model for mtDNA and concatenated nuclear data separately, using individuals sequenced for all genes, as implemented in Arlequin v3.1.1 (Excoffier *et al.* 2005).

Divergence dating

Using molecular data to place a time frame on divergence events is an active area in phylogenetics, yet there is little agreement on how best to do this. Most of these methods require fossil calibration points to put bounds on internal divergence events (Ho & Phillips 2009), but the reality for most data sets is that the fossil record is limited or nonexistent or the fossils are poorly dated themselves (Heads 2005). The fossil record of

Table 2 Marker information used in this study

Gene	Abbreviation	Intron	Size (bp)*	T _A (°C)	Primers (5'-3') [†]	Model [‡]	Primer source
Small nuclear ribonucleoprotein D3	snrpd3	1	620	62	exon 1: ATATTGTGACTTGTGAGACCAATACG exon 2: CGAATGTACACTTGTCTAACTGTG	HKY	Fujita <i>et al.</i> (2010)
Enhancer of rudimentary homolog	erh	3	1750	62	exon 3: TTGATGATTTGGCTGATCTTAGCTG exon 4: CCAGTCTTTAATTGTAGGGCTGGTATG	GTR + G	Fujita <i>et al.</i> (2010)
Ribosomal protein L14	rpl14	1	900	62	exon 1: ACTGGTAGCAATGTGGATGTTATCG exon 2: GAACCTTGAGAAAGCAAGTCAGTCAGTTG	HKY + G	Fujita <i>et al.</i> (2010)
Ribosomal protein L35	rpl35	2	1500	72	exon 2: CAGAGTGTGACAGTCATTAACCCAGAC exon 3: GTCTTCAGACCCCTCTTCGTGCTTG	HKY + G	Fujita <i>et al.</i> (2010)
Basic leucine zipper and W2 domain-containing protein 1	bzw1	2	700	70	exon 2: CTTCTGGAGCAAAGCTTGATATCG exon 3: ATCGTTTCTAGGCTTCCTGIGCTG	HKY + I	Fujita <i>et al.</i> (2010)
Leucine zipper transcription factor-like 1	lzf1	1	450	65	exon 1: TGAAGTAATTAACATACATGCCGATTTCAC exon 2: TCCAGCATATCTGACACATTCATCTAATTG	GTR	Fujita <i>et al.</i> (2010)
Ferritin heavy chain	frlh	5	1250	70	exon 5: AAGAACAATCAACCAAGCTCTCTTGGAC exon 6: TCGTTGATAGCCTGCACITTGATCC	HKY + G	Fujita <i>et al.</i> (2010)
Dynein light chain 1	dnd1	1	1500	65	exon 1: TGATCAAAGAAATCGGGATATGCTGAG exon 2: TCTTCCCACAATACAGTGCACAAAGTAG	HKY + G	Fujita <i>et al.</i> (2010)
Normal mucosa of oesophagus-specific gene 1	nmes	3	1400	65	exon 3: ATAAACAAAACGTTGTAATCCGGAACC exon 4: CTCTCAATGCCGTTTCCACTCCTG	HKY + G	Fujita <i>et al.</i> (2010)
NADH dehydrogenase subunit 2	nd2		1200	65–42 [§]	L4437: AAGCTTTCGGGGCCCCATACC L4882: CAACATGACAAAAAATCGCCCC	GTR + I + G	Macey <i>et al.</i> (1998)
					hRNA ^{ASN} : CTAAAAATRTTRCGGGATCGAGGCC		Macey <i>et al.</i> (2000) (modified)
							Read <i>et al.</i> (2001)

*Intron sizes are approximate as measured in sample MKF99 (see Fujita *et al.* 2010).

[†]Primers anneal in the indicated exon.

[‡]Model of molecular evolution as inferred in MrModelTest v2.3 (Nylander 2004) using the AIC.

[§]Touchdown PCR.

geckos is particularly poor, and there are no fossils available of *Heteronotia* or any closely related genera. In addition, the problems associated with incorporating calibrations have received much attention (Graur & Martin 2004; Hedges & Kumar 2004; Benton & Donoghue 2007; Ho 2007; Ho & Phillips 2009), and it is becoming increasingly apparent that the type of calibrating information used in the dating analysis can significantly affect estimates of divergence times (Ho *et al.* 2008).

For a direct comparison with the analyses performed in Fujita *et al.* (2010) for divergences among lineages of *H. binoei*, we used a relaxed molecular clock in BEAST v1.5.2 (Drummond & Rambaut 2007), calibrated using a fixed calibration point from an inferred date of divergence within the *H. binoei* lineage (Strasburg & Kearney 2005), which itself is based on the *nd2* substitution rate in agamid lizards of Macey *et al.* (1998). We used our concatenated nDNA data set that included only those individuals that had complete data. We unlinked loci and substitution models, and assigned the most appropriate model of sequence evolution based on the results provided above. To allow for rate heterogeneity among lineages, we employed an uncorrelated lognormal relaxed clock (Drummond *et al.* 2006). We used a coalescent tree prior because of the recent relationships of the individuals. The constant population size model was chosen based on results from Bayesian Skyline Plots (not shown). For the prior age of the *H. binoei* node, we specified a normal distribution with a mean of 5.47 Ma and standard deviation of 0.7 (Fujita *et al.* 2010). We also specified a prior age for the root node using a gamma distribution (offset 19 Ma, shape = 2.0, scale = 0.5), corresponding to the estimated time when faunal exchange initiated between Southeast Asia and Australia (Heatwole 1987). Posterior estimates of parameters were obtained via Markov chain Monte Carlo (MCMC) sampling. We conducted three independent runs, with samples drawn every 20 000 steps over a total of 200 000 000 steps, with the first 10% discarded as burn-in. Acceptable convergence to the stationary distribution was checked by inspecting the posterior samples using the diagnostic software Tracer v1.5 (Rambaut & Drummond 2007).

There are a number of limitations and problems associated with this type of dating analysis, the most important of which are the use of a tertiary calibration point (Graur & Martin 2004) and the application of a date calibration based on a mtDNA substitution rate that we used on our combined nDNA data, which assumes the nuclear loci share the same time to most recent common ancestor (TMRCA) as the mtDNA locus. To explore the effect of incorporating different calibration methods, we also used two alternative approaches to

estimate the approximate timing of diversification events within *Heteronotia*, which we briefly outline below.

First, we used the Dxy mtDNA genetic distances between lineages and applied the mtDNA substitution rate of 1.3% pairwise divergence/million years as derived by Macey *et al.* (1998). This rate estimation has been applied widely in dating phylogenies for many groups of vertebrates, particularly reptiles (Schulte *et al.* 2000, Melville *et al.* 2004; Glor *et al.* 2005; Oliver *et al.* 2009). This rate also was used in several previous phylogeographic studies on *Heteronotia* based on the same region of mtDNA (Strasburg & Kearney 2005, Strasburg *et al.* 2007). In addition, we used a relaxed molecular clock in BEAST v1.5.2 calibrated using the mitochondrial substitution rate of 0.57% per lineage per million years ago as determined by Macey *et al.* (1999) based on *Teratiscincus* geckos, rather than using an inferred date. With no information on the evolutionary rates of the nuclear introns utilized in this study, we excluded the nDNA data and used the mtDNA data only. We assigned the GTR + I + G nucleotide substitution model with six rate categories for the discrete gamma distribution. Given the variation in topology from the nDNA data, we constrained the topology to that of the combined mtDNA and nDNA tree from MrBayes. We ran the same analysis without the topology constraints to assess any difference in divergence date estimates. To allow for rate heterogeneity among lineages, we employed an uncorrelated lognormal relaxed clock (Drummond *et al.* 2006). We used a constant-size coalescent prior for the tree. We specified a normally distributed prior (mean 6.5×10^{-9} subs/site/year, standard deviation 2.55×10^{-10} subs/site/year) for the *mean of branch rates parameter* (uclid.mean). Default priors were used for the remaining parameters. An important difference from the previous dating analysis is that we did not specify a prior age for the root node, which could result in discrepancies between the analysis methods. We conducted four independent runs, each for 50 000 000 steps.

Results

Following the removal of ambiguously aligned nucleotide sites, the final mtDNA data set consisted of 1041 base pairs (bp) and the nDNA data set consisted of 7872 bp. For the concatenated analyses, we compared partitioning strategies using Bayes factors. Comparing between (i) unpartitioned, (ii) two partitions (partitioned by nDNA and mtDNA), (iii) four partitions (partitioned by nDNA and by codon position for *nad2*) and (iv) twelve partitions (partitioned by gene and by codon position for *nad2*), support is strongest for the 12

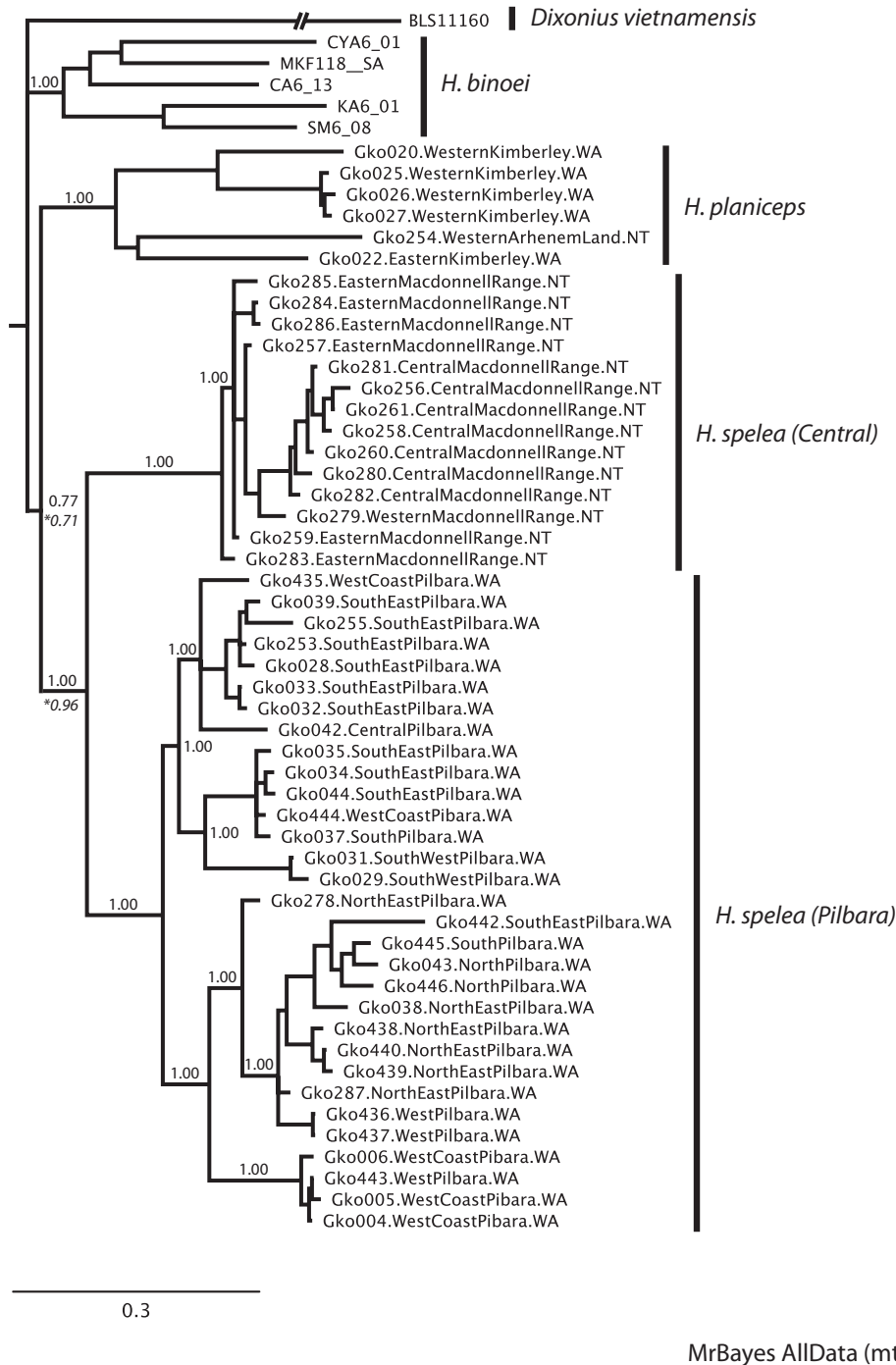


Fig. 2 Representative phylogram based on Bayesian analyses of the combined nuclear and mitochondrial data set. Values refer to Bayesian posterior probabilities. Values beside an “*” refer to Bayesian posterior probabilities from the *BEAST species tree analysis.

partition model (Table S1, Supporting Information). The partitioned Bayesian analyses of the concatenated nDNA (Fig. S1, Supporting Information) and the combined (mtDNA + nDNA) (Fig. 2) trees strongly support the monophyly of the three described species of *Heteronotia* (with 100% posterior probability support; pp

1.00). The mtDNA tree corroborated this result but with lower support for the monophyly of *H. binoei* and *H. planiceps* (pp 0.82 and 0.92, respectively) (Fig. S2, Supporting Information). All central Australian samples variously identified as *Heteronotia* sp. (Moritz *et al.* 1990), *H. spelea* (Cogger 2000) or *H. binoei* (Wilson &

Swan 2003) group together in our phylogenies as a highly divergent sister-clade to *H. spelea*. This relationship is strongly supported in both the concatenated nDNA tree as well as the combined tree (pp 1.00) but has poor support in the mtDNA tree (pp. 0.67). Samples of *H. planiceps* recorded from Newman in the Pilbara region are nested within the *H. spelea* clade in all analyses. The relationship between the lineages of *H. spelea*, *H. planiceps* and *H. binoei* were not resolved despite the large data set. The very short branch lengths in this part of the phylogeny suggests that the poor resolution is most likely due to rapid speciation of the three major clades over a short period of time (e.g. Willerslev *et al.* 2009). The partitioned maximum-likelihood analyses of both the concatenated nuclear data and the mtDNA data recovered the same topology as the partitioned Bayesian analyses (not shown), but the relationships between species are not strongly supported, with the exception of the sister relationship of the two *H. spelea* clades for which there is good support. The species tree estimated using *BEAST recovered a topology identical to the combined concatenated mtDNA and nDNA, again with support for the sister relationship of the two *H. spelea* clades (pp. 0.96) but with similarly weak support for the sister relationship of the *H. spelea* clades to *H. planiceps* (pp. 0.75) (Fig. 2).

Our sampling across the lineages of *Heteronotia* has revealed that rather than existing across multiple mountain ranges, *H. spelea* and *H. planiceps* have geographic distributions confined to separate mountain ranges (Fig. 1). *Heteronotia planiceps* is restricted to the Kimberley region, despite the morphologically similar individuals found in the Pilbara. The two divergent clades of

H. spelea have disjunct distributions, with one restricted to the Pilbara (herein referred to as Pilbara *H. spelea*) and the other found only in the central Australian ranges (herein referred to as Central *H. spelea*). Taxonomic revisions based on the results presented here are currently in preparation.

A substantial amount of variation was found within each of the three major lineages of saxicolous *Heteronotia*. Despite limited sampling, we found high genetic diversity across the Kimberley range of *H. planiceps*, with mtDNA diversity almost equal to that across the entire continental range of *H. binoei* (Table 3) (Fujita *et al.* 2010). Our sampling is greatest for Pilbara *H. spelea* and also highlights substantial genetic structure across the region. Six major clades were identified in the mtDNA tree, with an overall average nucleotide diversity of 7.77%. The geographically limited sampling of Central *H. spelea* revealed less genetic diversity than in the Pilbara population (dxy = 2.3%), and this result holds with recent extended mtDNA sampling across the species distribution (Fujita, Pepper & Moritz, unpublished data). Pairwise mitochondrial sequence divergences between the three major lineages ranged from 15.37% (Pilbara-Central *H. spelea*) to 18.87% (*H. binoei*-*H. planiceps*). Corresponding nuclear divergences ranged from 0.77% (Pilbara-Central *H. spelea*) to 1.51% (*H. planiceps*-Central *H. spelea*).

Divergence dating

Each of our dating approaches produced different age estimates, and this is expected given the different ways in which calibration points and rates of molecular evo-

Table 3 Measures of diversity and divergence

Taxon	mtDNA uncorrected "P" distance	mtDNA corrected Tamura-Nei diversity	Combined nDNA uncorrected "P" distance	Combined nDNA corrected Tamura-Nei diversity
<i>Heteronotia binoei</i> (n = 4)	0.1287 (0–0.1402)	0.1467	0.0167 (0–0.0207)	0.0162
<i>H. planiceps</i> (n = 6)	0.1146 (0–0.1623)	0.1323	0.0097 (0–0.0153)	0.0023
Central <i>H. spelea</i> (n = 14)	0.0218 (0–0.0365)	0.0226	0.0039 (0–0.0094)	0.0041
Pilbara <i>H. spelea</i> (n = 29)	0.0710 (0–0.1047)	0.0777	0.0082 (0–0.0164)	0.0078

Taxon	mtDNA uncorrected "P" distance	mtDNA corrected Tamura-Nei Dxy	Combined nDNA uncorrected "P" distance	Combined nDNA corrected Tamura-Nei Dxy
<i>H. binoei</i> and <i>H. planiceps</i>	0.1615 (0.1422–0.1787)	0.1887	0.0202 (0.0159–0.0233)	0.0205
<i>H. binoei</i> and Pilbara <i>H. spelea</i>	0.1382 (0.1201–0.1575)	0.1582	0.0211 (0.0156–0.0272)	0.0215
<i>H. binoei</i> and Central <i>H. spelea</i>	0.1437 (0.1239–0.1595)	0.1666	0.0199 (0.0151–0.0255)	0.0209
<i>H. planiceps</i> and Pilbara <i>H. spelea</i>	0.1575 (0.1431–0.1739)	0.1834	0.0197 (0.0144–0.0274)	0.0186
<i>H. planiceps</i> and Central <i>H. spelea</i>	0.1593 (0.1479–0.1691)	0.1862	0.0179 (0.0160–0.0228)	0.0182
Pilbara <i>H. spelea</i> and Central <i>H. spelea</i>	0.1340 (0.1076–0.1537)	0.1537	0.0129 (0.0087–0.0215)	0.0136

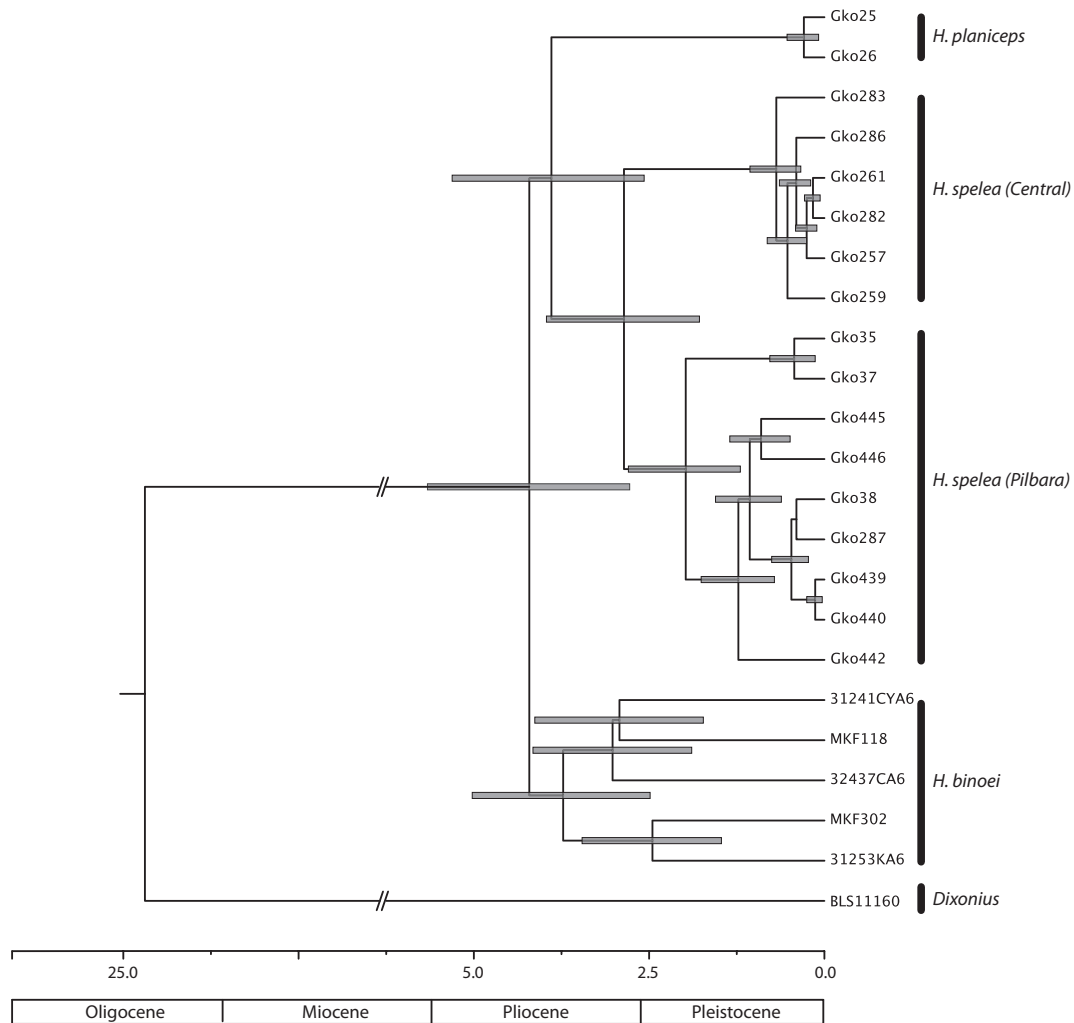


Fig. 3 Cladogram inferred using a relaxed molecular clock implemented in BEAST v. 1.5.3 using a subset of the nuclear data, with time axis indicated on the bottom. Node bars represent 95% confidence intervals.

lution were incorporated into the analyses. Dividing the genetic distance between lineages with the substitution rate of Macey *et al.* (1998) estimated major *Heteronotia* lineages diverged between 12 and 15 Ma. The relaxed lognormal clock analysis of the mtDNA data set using the substitution rate calibration produced the oldest date estimates, as expected because no age was specified on the root node. Divergence times from this method suggest that *Heteronotia* lineages split between 26 and 34 Ma. To facilitate comparison with divergence dynamics in *H. binoei*, we largely restrict our discussion to the dates derived via the relaxed lognormal clock analysis of the combined nDNA and a fixed calibration point (Fig. 3). Using this method, estimated divergence times between the major lineages (*H. planiceps*, *H. spelea* and *H. binoei*) occurred in the Pliocene between 3.9 and 4.2 Ma (2.5–5.7 Ma, 95% HPD estimate). The split between Pilbara and Central *H. spelea* occurred in the

late Pliocene/early Pleistocene, around 2.9 Ma (1.9–4.0 Ma, 95% HPD estimate) (Fig. 3). Our results exclude the hypothesis that these lineages separated in conjunction with the development of sand dune systems and at the same time as expansion of arid lineages *H. binoei* (<1 Ma, Fujita *et al.* 2010).

Discussion

We have produced a multilocus phylogeny using both mtDNA and nine independent nuclear genes allowing us to confidently identify the genealogical lineages within *Heteronotia*. In combination with what already is known of the geological and climate history of the arid zone, our molecular results give important insights into the mechanisms shaping biodiversity in the Australian arid zone. Our topologies show no recent connectivity between the saxicolous populations from the Pilbara,

Kimberley and central Australian ranges, as each region harbours its own divergent lineage of rock-dwelling *Heteronotia*. Furthermore, large amounts of genetic structure within *H. planiceps*, Pilbara *H. spelea* and, to a lesser extent, Central *H. spelea* indicate that each of these mountain ranges enabled population persistence as climates and landscapes changed across the arid zone. Finally, the timing of diversification both between and within lineages is too old to have arisen as a result of the development of the sand deserts >1 Ma and expansion of arid *H. binoei* lineages. Rather, the climate and habitat across the area presently occupied by the central sand deserts must have changed considerably following the onset and development of aridification through the late Miocene and Pliocene to fragment populations of *Heteronotia* ancestors early in the history of arid-zone development.

Biogeography and diversification of the rocky endemics

The rock-specialized habit of both *H. spelea* and *H. planiceps* highlights the relevance of an understanding of the geological setting around which these species have evolved. The regions where *H. spelea* and *H. planiceps* are found are geologically and topographically diverse compared to surrounding areas, primarily because of the unique geological structure of the pre-Cambrian Australian craton. The landforms of the Kimberley are rugged, characterized by intensely folded and faulted rocks, steeply scarped sandstone-capped hills and mountains and limestone gorges (Bridgewater 1987) that are bound to the south by the monotonous expanse of linear sand ridges of the inland deserts (Beard 1979). The south and central Pilbara is composed of two major mountain ranges, with deep gorges incised into the ironstone hills, while the northern Pilbara is dominated by gently rolling hills of deeply weathered granites. This region is bound on its north and eastern sides by the inland deserts. The central Australian ranges are comprised of rugged strike ridges and valleys of granites and limestones, with large Salinas and numerous dry-bed river systems (Mabbutt 1988; Boden & Given 1995). These inland ranges are entirely surrounded by desert lowlands, which has a strong influence on the extreme climates experienced in this region.

Contrary to previously reported distributions, *H. planiceps* and *H. spelea* (Pilbara *H. spelea* and Central *H. spelea*) have entirely disjunct distributions with each lineage confined to a separate mountain system within and peripheral to the arid zone. The sister relationship between lineages of *H. spelea* in the Pilbara and those in the central Australian ranges is concordant with the genetic results of a recent study on a dragon lizard

(*Tympanocryptis*; Shoo *et al.* 2008) and gecko ('CA6' *Heteronotia binoei*; Kearney *et al.* 2003; Fujita *et al.* 2010) where both show disjunct distributions across the central deserts. Similarly, Cracraft's (1991) assessment of the area relationships within a number of Australian vertebrate groups found the arid-zone regions of the Pilbara and 'Central Highlands' (included in his 'Western Desert') to be most closely associated, while the Kimberley region always is more distantly associated (Cracraft 1991). This genetic clustering agrees with what is known of the landscape history in the area. Despite the geological similarities of the Kimberley, central Australian ranges and the Pilbara, the climate of the Kimberley is substantially different to the southern mountain ranges, largely because of the dominating influence of the summer monsoon (Wyrwoll & Miller 2001). Accordingly, vegetation in the Kimberley also is very different compared to the Pilbara and central Australian ranges, with large areas dominated by tropical high-grass and woodland savannah (Beard 1979). In contrast, the shrubby *Acacia* habitat that dominates the Pilbara and central Australian ranges also exists in a very narrow belt through the sand deserts that separate these regions. Known as the 'Giles Corridor' (Pianka 1972), the extent of this habitat juncture is thought to have expanded and contracted in response to past changes in climate, periodically connecting the mountain ranges via a corridor of favourable habitat. Increased wind activity potentially could enable sandy top soils to be swept away, exposing harder subsoils underneath that allowed the spread of shrubby habitat. However, increased aridity could easily have tipped the balance in favour of *Triodia* grass habitat, which would isolate the Pilbara and central Australian ranges from one another with tracts of unsuitable habitat (Pianka 1972; Shoo *et al.* 2008). The large genetic distance between Pilbara and Central *H. spelea* lineages suggests that if the Giles Corridor promoted connectivity between the Pilbara and central Australian ranges for these species, it must have been insufficient to allow gene flow between these regions early in the speciation of the lineage that has persisted to the present time.

Persistence within mountain systems

The large amount of intraspecific diversity within the Pilbara lineages of *H. spelea* and Kimberley/Ord range of *H. planiceps* and their long-isolated distributions in different mountain ranges provides strong evidence that the Pilbara and Kimberley provided refugia during the unfavourably hyper-arid cycles during the Neogene and Quaternary. The ability of these regions to provide more thermally buffered climatic conditions is a direct result of topography, which in turn is intimately tied to

the history and geological composition of the Australian craton. While only represented by a small number of samples, *H. planiceps* displays extraordinarily high sequence diversity across its small geographic range of the Kimberley, on par with the diversity found across the entire continental range of *H. binoei* (Fujita *et al.* 2010). This may in part be explained by the particularly heterogeneous nature of the Kimberley landscape, with plateaus, gorges, rivers and numerous islands providing ample barriers to dispersal for nonviable organisms (Phillips *et al.* 2009). Our data further support that the Kimberley has been an important centre of diversification and endemism for terrestrial biota (Bowman *et al.* 2010) and highlights the need for further genetic studies in the area. Our sampling is greatest for Pilbara *H. spelea* and this too shows substantial genetic diversity across the region, consistent with what already has been found for other Pilbara reptiles (Pepper *et al.* 2006, 2008; Shoo *et al.* 2008). The central Australian uplands are a major topographic feature in Australia's arid region, further emphasized because they are completely isolated by surrounding deserts. While coastal mountain ranges such as the Pilbara or Kimberley would have been wetter and more thermally buffered (Byrne *et al.* 2008), the ranges in central Australia experienced more severe arid conditions, as well as temperatures that were much colder than at present (Miller *et al.* 1997). This hyper-arid climatic history is thought to have repeatedly extinguished narrowly endemic taxa in central Australia during the Pleistocene (Crisp *et al.* 2001). A similar pattern was identified by Linder (2000) of plant endemism in sub-Saharan Africa, where the expansion of deserts during arid cycles promoted a series of extinctions, even in apparently suitable mountain refugia. The low level of genetic diversity within Central *H. spelea* compared to lineages in other rocky ranges provides further support for a loss of genetic diversity in this region of Australia during periods of extreme aridity, but not for regional extinction in this case.

Divergence dating

The inland sand deserts of the Australian arid zone represent recently derived landforms, thought to only have existed for the last million years. Prior to this time, the landscape would most likely have consisted of hard, lateritic soils (Beard 1969; R. Wasson, personal communication). Contraction of the sclerophyll and wet forest that extended across inland Australia would have been concomitant with the initiation and spread of arid conditions from the mid-Neogene (<15 Ma) onwards (Martin 2006). Results from our dating analysis following the method of Fujita *et al.* (2010) suggest the divergence between *H. binoei*, *H. planiceps* and *H. spelea*

conservatively dates to the Pliocene/early Pleistocene, with short branch lengths and largely overlapping confidence intervals suggesting near simultaneous diversification. This timing is much older than the postulated maximum age of 1 Ma for the development of the sand deserts, implying the mobile desert landform was not responsible for isolating historical *Heteronotia* populations in the mountains. Similar ages were estimated for divergences between major lineages of the *H. binoei* complex (Fujita *et al.* 2010). These divergence times coincide with a time of extreme environmental change associated with deepening aridity and increased seasonality (reviewed in Byrne *et al.* 2008). In particular, the basin areas separating the uplands experienced a major termination of the warmer, wetter conditions experienced in the early Miocene, with the cessation of palaeodrainage channels in central and western Australia as well as unprecedented levels of erosion across the landscape (Martin 2006). While precise palaeoclimate reconstructions may never become available for the late Miocene because of a loss of a continuous geological record (Hill 1994; Byrne *et al.* 2008), it is likely that conditions in central and western Australia previously considered favourable by *Heteronotia* ancestors would have changed sufficiently in the lowlands to fragment populations into the moister refuges of the rocky uplands of the Pilbara, Kimberley and central Australia. Our dating exercise suggests the split between Pilbara and Central *H. spelea* occurred more recently around 2.9 Ma, and possibly much earlier, which is also older than the geological age of desert formation. In contrast to the saxicolous *Heteronotia*, divergences within the arid-zone lineages of *H. binoei*, a habitat generalist, were dated between 0.69 and 2.27 Ma (SM6) and 0.3 and 1.14 Ma (CA6) Ma, with strong signatures of recent population expansion <1 Ma (Fujita *et al.* 2010). This timing is consistent with the opening and spread of sand desert landforms in the arid interior, highlighting the recent connectivity between populations of this closely related species. Together, our studies on *Heteronotia* illuminate the contrasting roles of geological, landscape and climate evolution on the diversification of saxicolous and desert lineages, and make an important contribution to our understanding of wide-scale biogeography in the Australian arid zone.

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Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Representative phylogram based on Bayesian analyses of the concatenated nuclear data set. Values refer to Bayesian posterior probabilities.

Fig. S2 Representative phylogram based on Bayesian analyses of the mitochondrial set. Values refer to Bayesian posterior probabilities.

Table S1 Bayes factors comparisons of different data partitions for the concatenated data.

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