nature ecology & evolution

ARTICLES

https://doi.org/10.1038/s41559-017-0355-8

Phylogenetic evidence for mid-Cenozoic turnover of a diverse continental biota

Paul M. Oliver[1*] and Andrew F. Hugall[2]

Rapid climatic change at the beginning of the Oligocene epoch is concordant with global biotic turnover in the fossil record. However, while Southern Hemisphere geological movement played a key role in shaping these global climatic shifts, given generally poor terrestrial fossil records, evidence for matching turnover in entire Austral biotas is lacking. Emerging comprehensive phylogenetic frameworks provide alternative avenues to explore for signals of mass turnover or restructuring. Here, we combine phylogenetic data with empirical and simulation-based approaches to understand the temporal dynamics of the origins of a diverse and highly endemic continental biota (Australian lizards and snakes). These analyses indicate that the temporal clustering of major radiation ages in Gondwanan endemic lineages and immigration into Australia is narrower than expected under time-continuous models assuming no overarching external perturbation. Independent phylogenetic dating analyses further indicate that the timing of both processes is concentrated in the period post-dating the Eocene–Oligocene transition (~34 million years ago). Epoch-defining processes around the start of the Oligocene appear to have also played a decisive role in reshaping a diverse Southern Hemisphere biota—by both re-setting Gondwanan endemic diversity and opening the way to successful immigration from the north.

Major climatic perturbations are linked to mass turnover of biodiversity throughout the Earth's history[1], most famously at the Cretaceous–Tertiary boundary[2]. The end-Eocene terminal event was another pronounced climatic shift (~34 million years ago (Ma))—in this case towards a cooler and drier climate[3]. This period is broadly congruent with biotic turnover in the fossil records of many marine and Northern Hemisphere terrestrial biotas, notably a marked turnover in European fossil mammals 'the Grande Coupere'[4–8]. To date, most evidence of biotic turnover at this timeframe has been derived from fossil data. In theory, these episodes of major biotic change through the Cenozoic could also be apparent in phylogenetic data, as has been investigated for the earlier Cretaceous–Tertiary boundary[9–11]. However, with few exceptions[12], the rapidly developing knowledge of the tree of life has only been sporadically exploited to test for evidence of mid-Cenozoic turnover and restructuring of entire biotas[13].

Southern Hemisphere plate tectonic movements were an integral driver of global climatic change through the mid-Cenozoic[3,14]. In particular, the northwards migration of the Australian continental plate is strongly linked to the formation of the Antarctic Circumpolar Current, rapid expansion of Antarctic ice sheets and a profound cooling and drying in the global biosphere around the end-Eocene terminal event 30–34 Ma[3,8,15,16]. Accordingly, it has been hypothesized that austral biotas were also acutely affected by mid-Cenozoic climatic perturbations[13]. However, the poor terrestrial fossil records of the southern continents in general, and Australia specifically, have generally precluded testing this hypothesis. New and emerging tree-of-life frameworks provide an alternative avenue for investigating mass turnover of regional biotas. In particular, while detecting extinction from phylogenies may be problematic[17,18], temporal clustering of biogeographic and radiation processes across distantly related clades is predicted by mass turnover and detectable with phylogenetic data.

Lizards and snakes (squamates) are the most diverse component of the Australian vertebrate fauna and probably include more than 1,000 species spanning numerous distantly related lineages[19]. This diversity includes both old endemic Gondwanan lineages with origins pre-dating the isolation of Australia and Antarctica around 45 Ma and more recent immigrant lineages that have subsequently colonized from the north[20]. Despite these disparate origins, recent phylogenetic work across all Australian squamates has shown that all major extant radiations post-date the mid-Cenozoic (Table 1). Mass turnover has also recently been inferred from analyses of diversification patterns within one major Australian lizard lineage[21]. Here, we use empirical and simulation-based methods to test whether the relative temporal clustering (a measure that controls for many potential biases in estimations of absolute ages) of two key processes—(1) immigration and (2) initial diversification of major extant (crown) radiations—is tighter in the observed data than expected under time-continuous models (that is, assuming no major external perturbation). Our results, coupled with estimates of the actual timing of key processes, indicate that extrinsic climatic perturbation and geological restructuring in the mid-Cenozoic, detectable in fossil records around the world, also fundamentally re-set the evolutionary dynamics of Australia's most diverse endemic vertebrate assemblage. We predict that further targeted analyses will reveal that signatures of mass restructuring and major evolutionary perturbations are more apparent in phylogenetic data than currently recognized.

Results

Summary of radiation ages and biogeographic origins.. We initially synthesized current information on the phylogenetic position, age and origins of the 13 most diverse (major) Australian groups (Fig. 1, Supplementary Fig. 1 and Table 1). As a further check on published dates, we also re-estimated absolute timeframes of

[1]Research School of Biology and Centre for Biodiversity Analysis, Australian National University, Canberra 0200 Australian Capital Territory, Australia. [2]Department of Sciences, Museums Victoria, Melbourne, Victoria 3001, Australia. *e-mail: paul.oliver@anu.edu.au

Table 1 | Summary of estimated origins, crown ages (this study and previously published) and species diversity for major Australian squamate groups

Major group	Origin	BEAST	PLRS	Published dates	Species diversity
Carphodactylidae	Endemic	30.7	31.9	33.4[22]	30
Core Diplodactylidae	Endemic	29.4	29.9	34.5[23]	96
Pygopodidae	Endemic	28.6	29.3	31.3[23]	48
Crenadactylus	Endemic	16.2	14.5	20.6[24]	12
All Egernia group	Equivocal	20.6	23.5	25.4[25]	51
Agamids	Immigrant	27.1	21.0	23.4[26]	90
Sphenomorphus group	Immigrant	24.2	20.1	24.9[25]	276
Eugongylus group	Immigrant	21.6	20.3	23.0[25]	124
Gehyra	Immigrant	16.2	21.4	30.0[27]	30
Varanus	Immigrant	14.7	15.2	26.8[28]	24
Blindsnakes	Immigrant	21.5	17.0	16.7[29]	50
Elapids	Immigrant	12.3	8.4	10.0[30]	97
Pythons	Immigrant	10.2	16.0	<24.0[31]	14

Endemic, Gondwanan radiation descended from ancestors present on Australia before the final break up of East Gondwana; immigrant, radiations with sister lineages distributed across islands and continents to the north of Australia; equivocal, post-Gondwanan divergences within Australiasia, but no close extralimital relatives. BEAST, fossil translated log-normal priors; PLRS, penalized likelihood rate smoothing; published dates from recent analyses in references. All dates in Ma. For all stem and crown ages, 95% confidence intervals are given in Supplementary Table 4.

diversification using a newly compiled nuclear gene alignment spanning the diversity of all major Australian lineages and extralimital taxa. This dataset was analysed using both fossil and secondary fossil calibration strategies, and both model- (BEAST) and non-model-based dating approaches (penalized likelihood rate smoothing). Across all published and new analyses, despite the length of the stem lineages and irrespective of origins (see below), estimated crown ages for Australian squamate radiations were young relative to the long history of squamates, with all of them post-dating the end of the Eocene (>33.9 Ma) and most concentrated in the Oligocene or early Miocene.

Of the 13 major Australian lineages, nine (comprising ~80% of standing species diversity) diverged from sister lineages currently distributed to the north of Australia (Asia or Melanesia) during the middle to late Cenozoic (Fig. 2). Eight of these have close relatives occurring in Asia (seven) or Africa (one) and are considered to be immigrant. One further lineage (*Egernia* group skinks) is allied to two engimatic lineages from islands to the north and east of New Guinea with no other near relatives—rendering inference of origins equivocal. The remaining four major lineages consist of distinct groups (Carphodactylidae, core Diplodacylidae, *Crenadactylus* and Pygopodidae; ~20% of Australian species diversity) within a larger and clearly Gondwanan lineage of geckos[23]. Given early Cenozoic divergences and long bare basal branches, we treated each of these four as a distinct major lineage (endemic) in all analyses.

Temporal clustering analyses. To test for temporal clustering, we developed three methods (Supplementary Note 1) that compared the observed distribution of ages of Australian squamates with null distributions based on (1) empirical squamate clade ages and (2) simulations using time-continuous models. Our prediction was that if the observed spread of ages was shaped by an extrinsic non-random Earth-history event, it would be significantly narrower than the spread of ages generated under the time-continuous models. We summarized temporal clustering as the normalized standard deviation of a set of ages (the s.d. divided by the mean) to control for variation in the s.d. linked to age (that is, sets of older nodes would have a larger s.d.). Using this measure, lower values indicate a tight spread of ages, while higher values indicate a wide spread. Importantly, these tests were designed to assess whether the relative spread of ages is tighter in the observed data than would be expected in the absence of a major environmental perturbation, rather than be restricted by any particular absolute age (for instance, 30 or 20 Ma), thereby making them more general and conservative tests of temporal clustering. Depending on the method of estimating divergence times (see above), the observed s.d./mean value ranges from 0.26 to 0.31 for the 8 immigrant groups and 0.26 to 0.35 for all 13.

To initially investigate temporal clustering of the Australian groups, we placed them in the context of a recent (~50% species complete, near-complete for genera and major lineages) squamate tree of life (SqToL)[32]. Empirically, the crown ages of the 13 major Australian groups are significantly clustered compared with subsets of 13 phylogenetically independent squamate clade ages restricted to a Cenozoic timeframe, defined by the age of the youngest and oldest endemic Australian radiations (for example, 10–70 Ma; $P=0.037$; 10,000 random draws; see Supplementary Fig. 2 for full results). Narrowing the test timeframe within Cenozoic reduced the significance of observed clustering, while widening it increased the significance (Supplementary Fig. 2).

This apparent temporal pattern involves two aspects—the age of immigration into Australia and the crown age of the Gondwanan endemic groups. These were investigated further with empirical and simulation analyses. First, immigration is a fixed event in time that is not intrinsically linked to subsequent patterns of endemic diversification within Australia, so we modelled it using a global phylogeny and inferred rates of immigration into and out of Australia. Conversely, expectations for the timing of endemic diversification within Australia (in particular crown age) are linked to lineage-specific variables (that is, the total length of time present in the continent, extant diversity and net speciation rates) and were modelled accordingly[33].

Maximum likelihood estimation of ancestral states[34] across the SqToL recovers the same pattern of an Australian fauna dominated by lineages with immigrant origins (plotted as the accumulation of immigration events in Fig. 3), including most of the major groups we focus on here (Table 1) plus a small number of other more recent minor taxa. We then used a time-dependent binary-state speciation and extinction (BiSSE) method[35] to chart the change in the 'rate of immigration' into Australia from the rest of the world (Fig. 3). Rates of immigration are negligible before the Eocene–Oligocene boundary, but undergo a sharp increase during the Oligocene and early Miocene. Finally, compared with distributions generated from

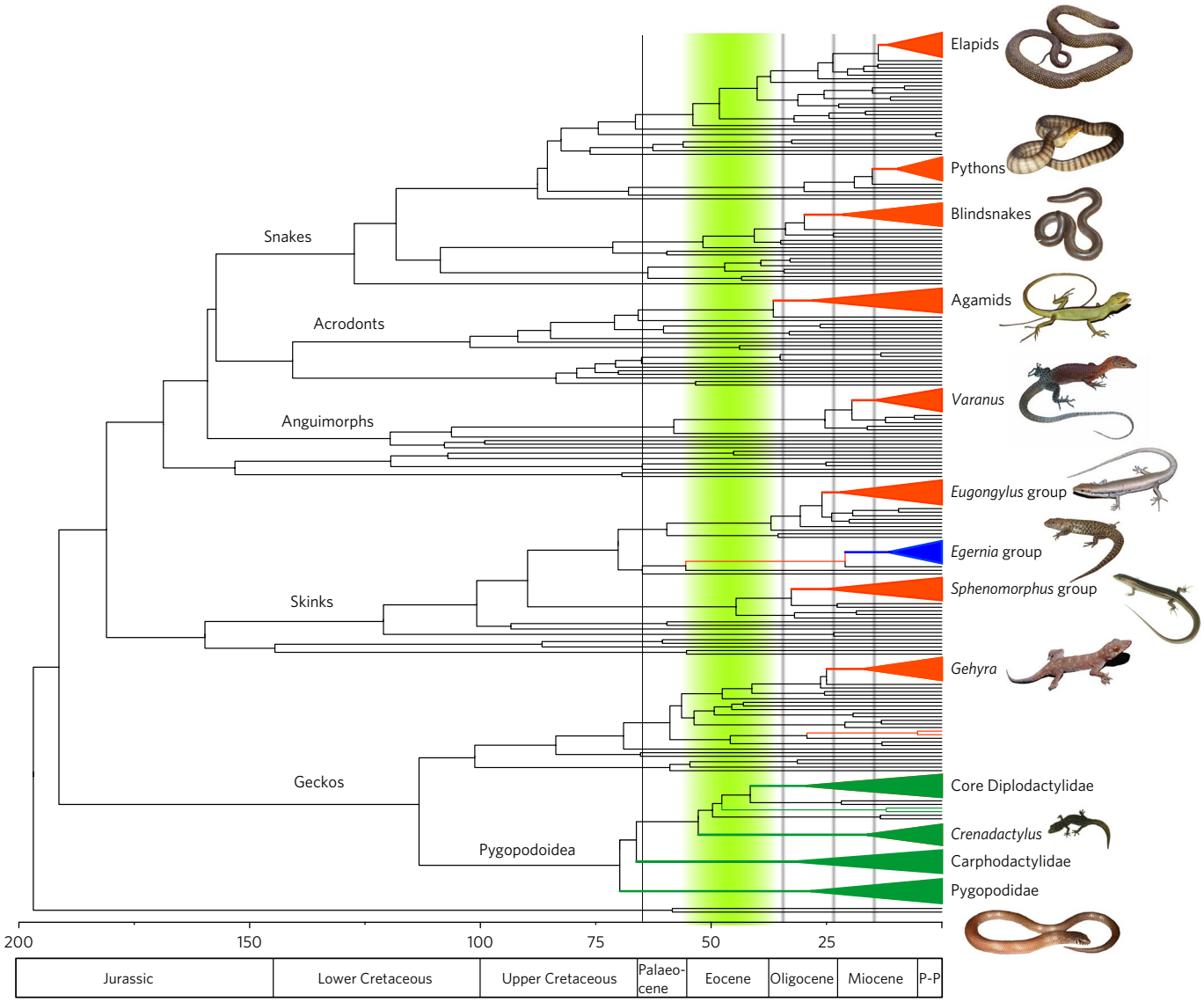

Fig. 1 | Phylogenetic position, age and origins of Australian squamates. Simplified version of the combined-data, fossil log-normal calibrated BEAST consensus tree. Extant Australian crown groups and stem lineages are highlighted: green represents Gondwanan origins, red immigrant origins and blue equivocal orgins. Key Earth-history events are also demarked, including the Eocene period of isolation of Gondwanan Australia from ca 55–35 Ma (lime shading) and the following major Cenozoic eras and events (from old to young): the Cretaceous–Tertiary boundary; the end-Eocene terminal event (corresponding to the Oi-1 glaciation and the opening of the Tasmanian Passage); the Mi-1 glaciation; and the mid-Miocene chill (see Fig. 2 for further details). P-P, Plio-Pleistocene. Credit images: Mark Hutchinson (elapids, pythons, blindsnake, *Eugongylus* group, *Sphenomorphus* group and *Ctenotus*); Brad Maryan (*Varanus*, *Crenadactylus* and *Pygopodidae*); Paul Oliver (*Gehyra* and Agamids); and Juniors Bildarchiv GmbH/Alamy Stock Photo (*Egernia*).

simulations of immigration (as a binary-state time-continuous stochastic model generated using rates of extinction, speciation and immigration estimated from the SqToL), the observed s.d./mean value for the arrival of the eight immigrant groups is significantly clustered ($P=0.049$; 10,000 simulations; again with null sampling restricted to Cenozoic timescales 10–70 Ma; Supplementary Fig. 2). If the equivocal *Egernia* group skink (see above) is also considered a potential immigrant (nine groups), the signal increases ($P=0.032$). Tightening the test timeframe again reduced significance, while widening it increased it (Supplementary Fig. 2).

Finally, to link the crown age of the Gondwanan endemic groups to the time of arrival of the immigrant groups, a complementary approach was taken, modelling the expected distribution of crown age s.d./mean conditioned on the the total period of time each major squamate lineage has been present in Australia (that is, stem ages). The eight immigrant lineages all arrived at a relatively similar time and diversified shortly afterwards, and the observed temporal clustering of crown radiation ages did not depart from expectations of time-constant diversification. In contrast, when the endemic lineages are included, the temporal clustering of crown ages is higher than expected, because the endemics have relatively long histories in Australia (long stem lineages) but similar clustering of crown ages (Fig. 4). These results indicate that the crown ages of the endemic Gondwanan lineages do not fit a time-constant diversification process but are consistent with an historically congruent shift effectively re-setting crown ages, broadly contemporaneous with the onset of immigration.

Discussion

While Southern Hemisphere continents played a critical role in shaping major climatic shifts in the mid-Cenozoic, evidence of corresponding mass restucturing in their biotas has remained sparse[13].

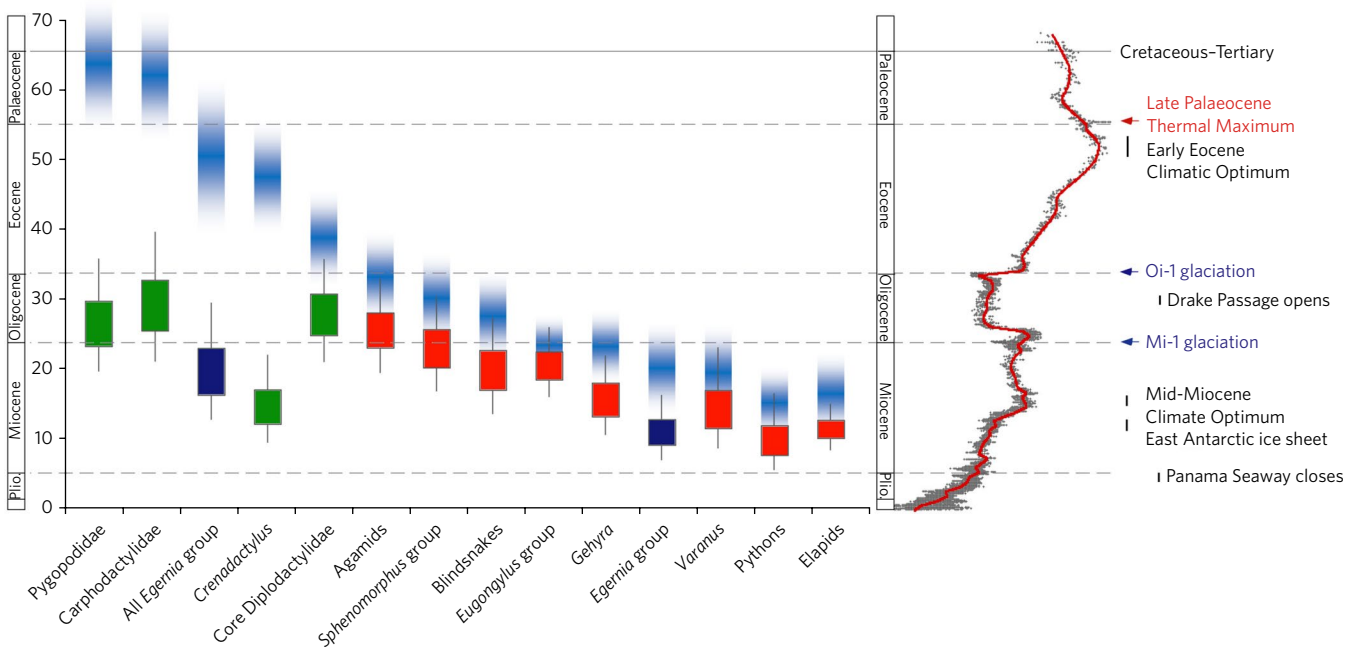

Fig. 2 | Crown and stem ages for extant Australian squamate groups. All age estimates (in Ma) were derived from BEAST analyses using fossil calibrations (log-normal distribution): 90% and quartile box and whisker plots show crown age estimates for lineages with endemic (green), immigrant (red) or equivocal (dark blue) origins. Light blue regions denote 90% confidence interval gradients for stem age. Two versions of crown definition for *Egernia* group skinks (equivocal origins) are included. Global deep-sea $\delta^{18}O$ records (modified from ref. [3]) and major climatic and tectonic events are indicated to the right.

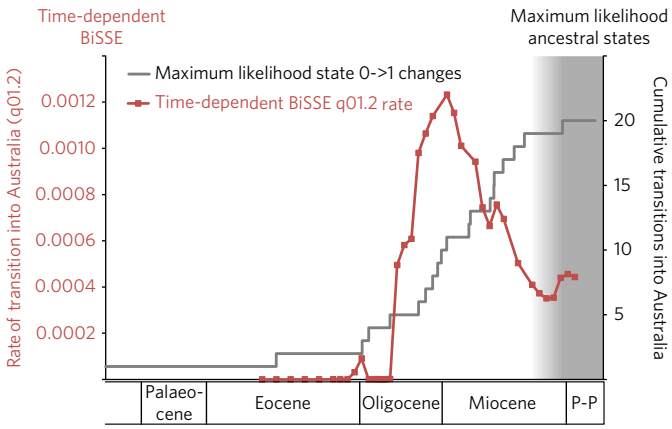

Fig. 3 | Maximum likelihood ancestral states and time-dependent BiSSE analyses of immigration into Australia. Based on a proxy SqToL. The grey line and right-hand *y* axis show the maximum likelihood ancestral state result for the cumulative number of inferred immigrations into Australia (changes in M-L state 0 to 1) against node age (*x* axis). The red line and left-hand *y* axis show the time-dependent BiSSE result for root to split-time inferred rate of immigration into Australia (q01.2) against split-time (*x* axis). The shaded 0–10 Ma zone on the right indicates the part of the tree substantially under-sampled (missing tip taxa). Note that the *x* axis scales should be regarded as only approximately equivalent.

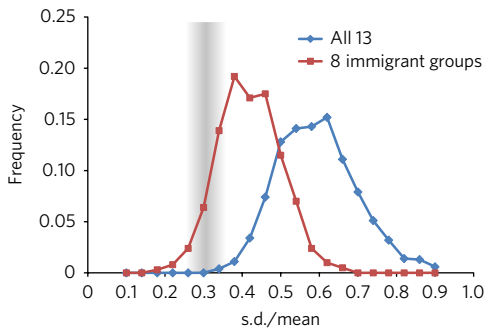

Fig. 4 | Observed versus expected temporal spread (s.d./mean) of crown ages for Australian lineages given the length of time they have been present in Australia. Distribution of the temporal clustering statistic (s.d./mean) from 1,000 simulations using a time-constant birth–death model based on observed stem ages and species diversities with 33% extinction. Red represents the 8 immigrant groups and blue all 13 Australian groups. The grey bar demarks the range of observed s.d./mean values for the 8 and 13 lineages using various dating estimates. Other analyses using extinction fractions of 0, 50, 67 and 75% also indicated significant clustering in the observed spread of crown ages across all 13 lineages.

Here, we are argue that two aspects of the phylogenetic evidence indicate there was a profound change in the basis of Australian squamate diversity around the mid-Cenozoic. First, the Australian squamate fauna is dominated by groups that have immigrated relatively recently from the increasingly proximate Asian region. Furthermore, this immigration is temporally constrained, with negligible (ultimately successful) colonization before the Oligocene (34 Ma) and a distinct period of change in influx peaking around the early Miocene. Second, for the minority Gondwanan endemic lineages, the juxtaposition of temporally clustered crown ages against long stem branches is suggestive of a congruent turnover (Table 1 and refs [21,23]). Taken together, the overall correlation of endemic crown ages with the onset of immigration implies that an overarching cause may link both immigration and the restructured age of endemic radiations (turnover).

We argue that this common link lies in the period of profound Earth biosphere change associated with the start of the Oligocene and formation of the Antarctic Circumpolar Current (30–34 Ma). This time period is marked by the initiation of the psychrosphere (the cold mass of ocean below the thermocline) and effectively demarcates the beginning of the modern ocean and global environment[8,16]. Analysis of paleoclimatic and geological records suggests that the onset of climatic change was rapid and unprecedented[3], most marked at higher latitudes[36] and in the Southern Hemisphere[16]. On this basis, the Australian terrestrial fauna should have been acutely affected. However, while a signature of biotic turnover through this period has been widely detected in marine fossil records from across the globe and from terrestrial fossil records from the Northern Hemisphere[4,5,7], matching signal in generally sparse Southern Hemisphere terrestrial fossil records is lacking. Likewise signatures of end-Eocene terminal event turnover from phylogenetic data have remained sporadic[12,13,21]. Thus, for regions in which phylogenetic data are the only source of information on lineages present historically (that is, those with sparse or no relevant fossil records, such as Australia), the detection of broadscale biological turnover has been challenging.

In light of this poor fossil record, we here focus on utilizing comprehensive phylogenies to look for temporal clustering of key events (immigration and endemic crown radiation ages) within a coherant biogeographic framework. Our focus is not on the rates of diversification and lineage accumulation through time, which have been examined elsewhere[21] and would often seem to have low power to detect extinction[17]. Our approach suggests that the assembly of an entire diverse regional biota in the Southern Hemisphere (in this case squamates in Australia) is inconsistent with long-term time-continuous processes throughout the Cenozoic, and instead effectively began in the early Oligocene. Broadly, our scenario is one of climate-driven mass turnover of the endemic lineages and onset of (successful) immigration driven by changes in ocean current patterns in conjunction with increasing proximity to Asia, resulting in the near-total restructuring of regional diversity. Subsequent immigration into Australia and biotic turnover has clearly also occured[37,38], but would only modify, not create, the correlation of Australian endemic and immigrant crown group ages laid down in the Oligocene era of distinct change.

The distinctive and highly endemic nature of the Australian biota has long been noted, and has variously been linked to Gondwanan inheritance and long isolation from other continents, combined with widespread intense aridification since the mid-Cenozoic[20,39,40]. It is therefore striking that the most diverse component of the Australian endemic terrestrial vertebrate biota (squamate) is dominated by taxa with non-Gondwanan origins, especially when compared with other terrestrial vertebrates (Supplementary Table 1) and plants[40]. This may reflect differing physiological limitations that are also captured by present-day distributional patterns[41]. In particular, a generally poor ability to deal with cool mesic environments among squamates may have both heightened extinction risk through climatic changes and/or reduced capacity to colonize higher-latitude Austral environments from tropical Asia before the Oligocene. However, even within other major Australian radiations, other studies have suggested post-Oligocene trends in crown radiation ages (in both Gondwanan and immigrant groups)[42,43], and in the inferred timing of immigration both into and out of Australia in plants[44], invertebrates[45,46], frogs[47] and birds[43,48]. Deep Gondwanan inheritance explains many distinctive elements of the Australia biota; however, the direct role of the Australian plate and surrounding currents in shaping global climatic change, coupled with the relatively small size and isolation of the island continent, may have shaped contemporary radiations across many groups, regardless of origins.

More broadly, global epochs are defined by distinct periods of change and turnover in the fossil record that should affect the phylogenetic record, but in ways that may not be obvious[12,13]. Increasing the utilzation of comprehensive phylogenetic datasets and emerging methods more attuned to key patterns, such as temporal clustering and shifts in birth–death processes, suggest that signals of global turnover may be more pervasive in phylogenetic data than widely recognized[12]. Recent analyses of global patterns of mammal diversification have also detected a (previously overlooked) but relatively pervasive upturn in the tempo of diversification beginning in the early Oligocene, which has again been linked to mid-Cenozoic climatic events[12]. Here, by considering immigrant and endemic processes in a specific biogeographic context, we have provided new evidence of a link between a period of profound global biosphere change around the Eocene–Oligocene transition and phylogenetic change in an entire regional fauna.

Methods

Definition of Australian squamate groups. We collated dating estimates (Table 1) from published phylogenetic studies focusing on Australian squamates (Supplementary Note 2). We focused on lineages with substantial Australian endemic diversification (>10 endemic species). Fifteen species-poor or recent immigrant Australian genera were not included (specifically colubroid snakes ($n=3$), gekkonine geckos ($n=4$), homolopsine snakes ($n=5$) and skink genera such as *Emoia*, *Cryptoblepharus* and *Eugongylus* ($n=3$)). Nine of these genera lack endemic Australian species. Where squamate lineages showed inter-digitation between Australia and New Guinea (Agamids, *Eugongylus* skinks, *Gehyra* and pythons), we used the age of the youngest clade including all Australian taxa. Otherwise, we focused on the Australian clade only.

Synthesis of the phylogenetic tree and dating. We unified the molecular phylogenetic data spanning extant endemic Australian groups and their closest known extra-limital relatives within a single overall tree representing current best estimates of squamate phylogeny (Supplementary Tables 2 and 3). We concatenated three slowly evolving nuclear genes appropriate for large-scale phylogenetics and most heavily sampled for Australian squamates (*BDNF*, *C-mos* and *RAG-1*) via the widely accepted supermatrix approach[32]. The final alignment of 4,551 sites included 215 squamates and four outgroups. We used exclusively nuclear genes to reduce the potential of inflating date estimates by combining heavily saturated mitochondrial data with fossil calibrations of 100 million years of age or more[49].

Phylogenetic and dating inferences were conducted with RAxML version 7.2.6 (ref. [50]), MrBayes version 3.2 (ref. [51]) and BEAST version 1.7.2 (ref. [52]) using optimized codon-partition models and linked branch lengths. Divergence ages were also estimated using penalized likelihood rate smoothing (r8s version 1.70)[53]. Maximum likelihood (RAxML) analyses used 1,000 fast bootstraps. Bayesian inference (MrBayes) used two independent five million step runs with burn-in of 25%, giving 15,000 trees for posterior estimation, with a parameter effective sample size of greater than 1,000. BEAST analyses used an uncorrelated log-normal rate variation model, a Yule speciation model, 50 million steps and 10% burn-in, leaving 9,000 samples for posterior estimation, with a parameter effective sample size of greater than 200. Penalized likelihood rate smoothing (an alternative method for generating chrongrams) was performed using the TN algorithm and ADD function on 1,000 post burn-in MrBayes trees (pruned of outgroups), each calibrated with ages drawn at random from the calibration probability distributions, together with a smoothing factor drawn at random from a uniform distribution spanning the likely range (5–50) determined by cross-validation[53]. To obtain dates as consistent as possible with most recent studies, we applied two calibration strategies—log-normal fossil calibrations and normal secondary priors—drawn from recent squamate phylogenetic studies (Supplementary Notes 2 and 3 and Supplementary Fig. 1). In both strategies, calibrations were relatively distant from Australian groups and concentrated towards the base of the entire SqToL; therefore, they were unlikely to bias the relative ages of groups. For each dating analysis, we recorded the distribution and median of stem and crown ages of the major Australian squamate groups. We also note that it is increasingly recognized that for relatively old radiations, date estimates derived from the combination of slowly evolving nuclear gene datasets with deep calibration points are relatively resistant to variation in the model choice and dating method, and even greatly reduced sampling of genes[54].

Additional published estimates for the age crown radiations were taken from phylogenetic studies of the relevant groups (Table 1, Supplementary Table 3 and Supplementary Notes 2 and 3). We preferentially focused on dates from studies that did not combine rapidly evolving mitochondrial data with deep old fossil calibrations (for the reasons outlined above).

Inferring origins and time of immigration. All of the tree and diversification analyses described below were performed in R version 3.0.2 using Comprehensive R Archive Network packages and summarized with the aid of custom R-scripts. As the Australian squamates comprise a limited number of mostly discrete groups of fairly clear-cut origins, negligible shared taxa and little back-migration, they are well suited to simple classification into Australian versus non-Australian taxa and immigrant versus endemic origins, and thus binary-state analysis methods. Defining the age of immigration as either the stem, mid-point or crown age has little effect because immigrant group stem lengths are relatively short (Fig. 1 and Supplementary Fig. 1).

For analytical investigations into the origins and temporal pattern of immigration into Australia we used a proxy SqToL[32], calibrated at 29 key nodes (Supplementary Table 4) via PATHd8 (ref. [55]) to make it consistent with most recent analyses of squamate ages (Supplementary Table 2). This tree of 4,161 tips includes all currently recognized squamate families and subfamilies, approximately 84% of genera and about 44% of recognized species[32]. It includes all Australian genera except *Cryptagama* and about 60% of Australian species. Taxa were categorized into two states, Australia (595 species; state 1) and the rest of the world (3,561 species; state 0) according to current taxonomy (http://www.reptile-database.org/ and ref. [19]).

To investigate patterns of immigration into Australia, maximum likelihood ancestral states[34] and a version of time-dependent BiSSE[35] were applied to the SqToL. Ancestral states were inferred by maximum likelihood using the R-package APE version 3.0-11 (ref. [34]), with results plotted as a net accumulation of transitions into Australia (Fig. 3), similar to the approach taken by McGuire et al.[56]. As immigration events into Australia are considered independent events, node state was defined by majority rule marginal likelihood and transitions were recorded as the age of a node with state 1 (Australia) with immediate ancestor with state 0 (rest of the world). Time-dependent BiSSE analysis splits a tree into two time sections, from the root to the split-time and from the split-time to the tip[35]. We used the root to split-time state 0 to state 1 transition rate (q01.2) as a measure of immigration into Australia. Maximum likelihood time-dependent BiSSE was conducted for split-times 5 to 50 Ma in steps of one million years using the R-package Diversitree version 0.9-6 (ref. [35]) and q01.2 plotted against split-time (Fig. 3). While the rate estimate is integrated across the root to split-time timespan, it is dominated by patterns close to the split-time due to the exponential increase in lineages. Shifting the split-time, can therefore effectively chart changes in the rate through the depth of the phylogeny. In these analyses, as per standard settings of BiSSE, we also estimated speciation (lambda) and extinction (mu) rates (Supplementary Table 5). BiSSE and maximum likelihood are appropriate for our purposes here because (1) all groups are essentialy either one state or the other, (2) empirically, BiSSE returns much the same pattern as maximum likelihood and (3) concerns about BiSSE inference (reference in Supplementary Note 2) are not relevant to our use for simulating immigration times.

Empirical comparisons of temporal clustering. While there are now numerous methods for the phylogenetic analysis of diversification dynamics[57], synchronicity of divergence events in phylogenetic data has rarely been statistically tested (although there are tests that are increasingly used at shallower phylogeographic scales[58,59]). Here, we investigated evidence of temporal clustering in the age of major Australian squamate radiations compared with expectations based on the SqToL. As a measure of temporal clustering, we used the normalized s.d. of the set of clade ages (s.d./mean). This normalized s.d. test statistic focuses on the relative spread of ages (that is, not absolute ages) across lineages of interest, making it a general test of temporal clustering rather than it being focused around any particular absolute age. This limits the confounding effects of uncertainty in estimates of absolute age and makes the test more conservative. Different dating methods and sampling strategies (stem, mid-point or crown) produced slight variation in the observed s.d./mean value; however, we conservatively focused comparisons on the method giving the widest range.

For an initial and preliminary empirical perspective, we compared the observed s.d./mean value with a distribution derived from 10,000 random draws of sets of 13 phylogenetically independent clade ages (that is, nodes not nested within one another) from the SqToL. This can be done across the whole age of squamates (from around 200 Ma) or limited to a taxonomic scale—and hence age range—more relevant to the questions at hand. Here, we focused node sampling on a broadly Cenozoic timespan comparable to potential crown ages for the major Australian groups, specifically 10–70 Ma: long enough to form a major group but not pre-dating the existence of anything that could be regarded as Gondwanan Australia. To explore the effect of these age range limits, we ran a series of analyses to describe the probability trend across age limits from 7–12 Ma to 55–90 Ma. Full details of the results are presented in Supplementary Fig. 2. Note that if the sampled empirical tree itself contains substantial temporal clustering, this merely weakens the significance of the test; that is, making it relatively conservative.

Stochastic null-model-based comparisons. The previous analysis empirically assesses temporal patterns among the Australian groups in the context of the observed global squamate patterns and tacitly treats all ages the same irrespective of lineage origins (that is, Gondwanan or immigrant). It could also be affected by sampling bias in the tree of life. In subsequent analyses, we seperated the age of immigration events, which are historical events fixed in time, and crown ages of endemic groups, which may be better considered as probabilistic expectations of a diversification process within a landmass and lineage. To account for these different expectations, we developed separate time-continuous stochastic null models for each process.

First, we modelled the expected frequency and temporal clustering of immigration events into Australia (conservatively defined as the crown ages of each immigrant radiation) using parameter estimates of speciation rate, extinction rate and transition rate (rest of the world to Australia) from the earlier BiSSE analysis using the SqToL (Supplementary Table 5 and Supplementary Fig. 3). We undertook 10,000 simulations to generate a null distribution for the test statistic (s.d./mean). Trees from these simulations resembled the SqToL in key aspects, allowing the age of transitions from state 0 to state 1 (q01) to provide a null distribution of immigration events into Australia modelled as a time-continuous two-state birth–death process. From each simulation, we sampled eight or nine immigration events to match the known number of immigrant lineages. The probability of the simulated s.d./mean of immigration being less than or equal to (that is, more temporally clustered) the observed value was then calculated, again, as with the empirical test, restricting the null sampling to the broadly Cenozoic timescale of between 10 and 70 Ma (full range 7–90 Ma; see Supplementary Fig. 2).

Finally, to link the crown ages of the endemic groups to the ages of the immigrant groups, we estimated the probability of the observed clustering of the crown ages, given expectations of a time-continuous diversification model based on the observed stem ages and species diversity of the Australian groups. This analysis is similar to the method used elsewhere[60] for modelling expected crown group stem length. A key aspect here is to show that the crown ages of the endemic groups do not fit stochastic birth–death expectations and hence represent historical events that are of a similar age to the immigration events. Birth and death rate parameters were determined for each group from stem age and species diversity (equation 7 in ref. [61]) using a range of background extinction levels from 0 to 90%. For each model, 1,000 birth–death trees were simulated and the appropriate s.d./mean test statistic was calculated from the crown ages using the GEIGER version 2.0 and TREESIM version 1.9.1 R-packages[33,62]. The results are shown for 33% extinction (Fig. 4). Analyses based on applying extinction fractions of 0, 50, 67, 75 and 90% all returned more significant P values (<0.006) for the observed temporal clustering of the 13 major lineages (Supplementary Table 6).

Data avaliabilty. The datasets generated and/or analysed during the current study are available in the Dryad repository; provisional DOI: 10.5061/dryad.674f7.

Received: 19 October 2016; Accepted: 25 September 2017;

References

1. Jablonski, D. Mass extinctions and macroevolution. *Paleobiology* **31**, 192–210 (2005).
2. Schulte, P. et al. The Chicxulub asteroid impact and mass extinction at the Cretaceous–Paleogene boundary. *Science* **327**, 1214–1218 (2010).
3. Zachos, J., Pagani, M., Sloan, L., Thomas, E. & Billups, K. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**, 686–693 (2001).
4. Raup, D. & Sepkoski, J. J. Periodic extinction of families and genera. *Science* **231**, 833–836 (1986).
5. Hooker, J. J., Collinson, M. E. & Sille, N. P. Eocene–Oligocene mammalian faunal turnover in the Hampshire Basin, UK: calibration to the global time scale and the major cooling event. *J. Geol. Soc. Lond.* **161**, 161–172 (2004).
6. Augé, M. & Smith, R. An assemblage of early Oligocene lizards (Squamata) from the locality of Boutersem (Belgium), with comments on the Eocene–Oligocene transition. *Zool. J. Linn. Soc.* **155**, 148–170 (2009).
7. Sun, J. et al. Synchronous turnover of flora, fauna, and climate at the Eocene–Oligocene boundary in Asia. *Sci. Rep.* **4**, 7463 (2014).
8. McGowran, B., Holdgate, G. R., Li, Q. & Gallagher, S. J. Cenozoic stratigraphic succession in southeastern Australia. *Aust. J. Earth Sci.* **51**, 459–496 (2004).
9. Jarvis, E. D. et al. Whole genome analyses resolve the early branches in the tree of life of modern birds. *Science* **346**, 1320–1331 (2014).
10. Meredith, R. W. et al. Impacts of the cretaceous terrestrial revolution and KPg extinction on mammal diversification. *Science* **334**, 521–524 (2011).
11. Feng, Y.-J. et al. Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. *Proc. Natl Acad. Sci. USA* **114**, E5864–E5870 (2017).
12. Stadler, T. Mammalian phylogeny reveals recent diversification rate shifts. *Proc. Natl Acad. Sci. USA* **108**, 6187–6192 (2011).
13. Crisp, M. D. & Cook, L. G. Explosive radiation or cryptic mass extinction? Interpreting signatures in molecular phylogenies. *Evolution* **63**, 2257–2265 (2009).

14. Hall, R. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer based reconstructions, model and animations. *J. Asian Earth Sci.* **20**, 353–431 (2002).
15. Lawver, L. A. & Gahagan, L. M. Evolution of Cenozoic seaways in the circum-Antarctic region. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **198**, 11–37 (2003).
16. Scher, H. D. et al. Onset of Antarctic circumpolar current 30 million years ago as Tasmanian Gateway aligned with westerlies. *Nature* **523**, 580–583 (2015).
17. Rabosky, D. L. Challenges in the estimation of extinction from molecular phylogenies: a response to Beaulieu and O'Meara. *Evolution* **70**, 218–228 (2016).
18. Beaulieu, J. M. & O'Meara, B. C. Extinction can be estimated from moderately sized molecular phylogenies. *Evolution* **69**, 1036–1043 (2015).
19. Wilson, S. & Swan, G. *A Complete Guide to Reptiles of Australia* 5th edn (New Holland, Sydney, 2017).
20. Keast, A. (ed.) in *Ecological Biogeography of Australia* 1999–2059 (Springer, Dordrecht, 1981).
21. Brennan, I. G. & Oliver, P. M. Mass turnover and recovery dynamics of a diverse Australian continental radiation. *Evolution* **71**, 1352–1365 (2017).
22. Oliver, P. M. & Bauer, A. M. Systematics and evolution of the Australian knob-tail geckos (*Nephrurus*, Carphodactylidae, Gekkota): plesiomorphic grades and biome shifts through the Miocene. *Mol. Phylogenet. Evol.* **59**, 664–674 (2011).
23. Oliver, P. M. & Sanders, K. L. Molecular evidence for Gondwanan origins of multiple lineages within a diverse Australasian gecko radiation. *J. Biogeogr.* **36**, 2044–2055 (2009).
24. Oliver, P. M., Adams, M. & Doughty, P. Molecular evidence for ten species and Oligo–Miocene vicariance within a nominal Australian gecko species (*Crenadactylus ocellatus*, Diplodactylidae). *BMC Evol. Biol.* **10**, 386 (2010).
25. Skinner, A., Hugall, A. F. & Hutchinson, M. N. Lygosomine phylogeny and the origins of Australian scincid lizards. *J. Biogeogr.* **38**, 1044–1058 (2011).
26. Chen, I., Stuart-fox, D., Hugall, A. F. & Symonds, M. R. E. Sexual selection and the evolution of complex color patterns in dragon lizards. *Evolution* **66**, 3605–3614 (2012).
27. Heinicke, M. P., Greenbaum, E., Jackman, T. R. & Bauer, A. M. Phylogeny of a trans-Wallacean radiation (Squamata, Gekkonidae, *Gehyra*) supports a single early colonization of Australia. *Zool. Scr.* **40**, 584–602 (2011).
28. Vidal, N. et al. Molecular evidence for an Asian origin of monitor lizards followed by tertiary dispersals to Africa and Australasia. *Biol. Lett.* **8**, 853–855 (2012).
29. Vidal, N. et al. Blindsnake evolutionary tree reveals long history on Gondwana. *Biol. Lett.* **6**, 558–561 (2010).
30. Sanders, K. L., Lee, M. S. Y., Leys, R., Foster, R. & Keogh, J. S. Molecular phylogeny and divergence dates for Australasian elapids and sea snakes (Hydrophiinae): evidence from seven genes for rapid evolutionary radiations. *J. Evol. Biol.* **21**, 682–695 (2008).
31. Scanlon, J. D. & Lee, M. S. Y. in *Reproductive Biology and Phylogeny of Snakes* (eds Sever, D. & Aldridge, R.) 55–95 (CRC, Boca Raton, 2010).
32. Pyron, R. A., Burbrink, F. T. & Wiens, J. J. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evol. Biol.* **13**, 93 (2013).
33. Hartmann, K., Wong, D. & Stadler, T. Sampling trees from evolutionary models. *Syst. Biol.* **59**, 465–476 (2010).
34. Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).
35. Fitzjohn, R. G. Diversitree: comparative phylogenetic analyses of diversification in R. *Methods Ecol. Evol.* **3**, 1084–1092 (2012).
36. Schumacher, S. & Lazarus, D. Regional differences in pelagic productivity in the late Eocene to early Oligocene—a comparison of southern high latitudes and lower latitudes. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **214**, 243–263 (2004).
37. Byrne, M. et al. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Mol. Ecol.* **17**, 4398–4417 (2008).
38. Byrne, M. et al. Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *J. Biogeogr.* **38**, 1635–1656 (2011).
39. Hooker, J. D. in *Botany of the Antarctic Voyage of H.M. Discovery Ships Erebus and Terror in the Years 1839–1843, III. Flora Tasmaniae* (ed. Hooker, J. D.) 1–36 (Reeve, London, 1860).
40. Crisp, M. D. & Cook, L. G. How was the Australian flora assembled over the last 65 million years? A molecular phylogenetic perspective. *Annu. Rev. Ecol. Evol. Syst.* **44**, 303–324 (2013).
41. Powney, G. D., Grenyer, R., Orme, C. D. L., Owens, I. P. F. & Meiri, S. Hot, dry and different: Australian lizard richness is unlike that of mammals, amphibians and birds. *Glob. Ecol. Biogeogr.* **19**, 386–396 (2010).
42. Mitchell, K. J. et al. Molecular phylogeny, biogeography, and habitat preference evolution of marsupials. *Mol. Biol. Evol.* **31**, 2322–2330 (2014).
43. Schweizer, M., Seehausen, O. & Hertwig, S. T. Macroevolutionary patterns in the diversification of parrots: effects of climate change, geological events and key innovations. *J. Biogeogr.* **38**, 2176–2194 (2011).
44. Sniderman, J. M. K. & Jordan, G. J. Extent and timing of floristic exchange between Australian and Asian rain forests. *J. Biogeogr.* **38**, 1445–1455 (2011).
45. Maekawa, K., Lo, N., Rose, H. A. & Matsumoto, T. The evolution of soil-burrowing cockroaches (Blattaria: Blaberidae) from wood-burrowing ancestors following an invasion of the latter from Asia into Australia. *Proc. R. Soc. Lond. B* **270**, 1301–1307 (2003).
46. Hugall, A. F. & Stanisic, J. Beyond the prolegomenon: a molecular phylogeny of the Australian camaenid land snail radiation. *Zool. J. Linn. Soc.* **161**, 531–572 (2011).
47. Kurabayashi, A. et al. From Antarctica or Asia? New colonization scenario for Australian–New Guinean narrow mouth toads suggested from the findings on a mysterious genus *Gastrophrynoides*. *BMC Evol. Biol.* **11**, 175 (2011).
48. Jønsson, K. A., Fabre, P., Ricklefs, R. E. & Fjeldså, J. Major global radiation of corvoid birds originated in the proto-Papuan archipelago. *Proc. Natl Acad. Sci. USA* **108**, 2328–2333 (2010).
49. Brandley, M. C. et al. Accommodating heterogenous rates of evolution in molecular divergence dating methods: an example using intercontinental dispersal of plestiodon (*Eumeces*) lizards. *Syst. Biol.* **60**, 3–15 (2011).
50. Stamatakis, A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690 (2006).
51. Ronquist, F. et al. Mrbayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012).
52. Drummond, A. J. & Rambaut, A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**, 214 (2007).
53. Sanderson, M. J. Evolution and divergence times in the absence of a molecular clock. *Evolution* **19**, 301–302 (2003).
54. Foster, C. S. P. et al. Evaluating the impact of genomic data and priors on Bayesian estimates of the angiosperm evolutionary timescale. *Syst. Biol.* **66**, 338–351 (2017).
55. Britton, T., Oxelman, B., Vinnersten, A. & Bremer, K. Phylogenetic dating with confidence intervals using mean path lengths. *Mol. Phylogenet. Evol.* **24**, 58–65 (2002).
56. McGuire, J. A. et al. Molecular phylogenetics and the diversification of hummingbirds. *Curr. Biol.* **24**, 910–916 (2014).
57. Rabosky, D. L. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE* **9**, e89543 (2014).
58. Crisp, M. D. & Cook, L. G. A congruent molecular signature of vicariance across multiple plant lineages. *Mol. Phylogenet. Evol.* **43**, 1106–1117 (2007).
59. Hickerson, M. J., Stahl, E. A. & Lessios, H. A. Test for simultaneous divergence using approximate Bayesian computation. *Evolution* **60**, 2435–2453 (2006).
60. Near, T. J. et al. Boom and bust: ancient and recent diversification in bichirs (Polypteridae: Actinopterygii), a relictual lineage of ray-finned fishes. *Evolution* **68**, 1014–1026 (2014).
61. Magallón, S. & Sanderson, M. J. Absolute diversification rates in angiosperm clades. *Evolution* **55**, 1762–1780 (2001).
62. Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**, 129–131 (2008).

Acknowledgements

P.M.O. was supported by a McKenzie Postdoctoral Fellowship from the University of Melbourne and an Australian Reseach Council Early Career Researcher Fellowship. We thank L. Bromham, M. Cardillo, M. Novosolov and C. Moritz for comments.

Author contributions

P.M.O. and A.F.H. conceived the project, undertook the analyses and wrote the paper.

Competing interests

The authors declare no competing financial interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41559-017-0355-8.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to P.M.O.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.