

## ORIGINAL ARTICLE

**Diversification dynamics of chameleons (Chamaeleonidae)**S. A. W. Giles & K. Arbuckle 

Department of Biosciences, Faculty of Science and Engineering, Swansea University, Swansea, UK

**Keywords**

chameleons; diversification rates; ecomorphology; habitat; dispersal; vicariance.

**Correspondence**Kevin Arbuckle, Department of Biosciences, Faculty of Science and Engineering, Swansea University, Swansea SA2 8PP, UK.  
Email: kevin.arbuckle@swansea.ac.uk

Editor: Anthony Herrel

Associate Editor: Anthony Herrel

Received 23 September 2021; revised 9 August 2022; accepted 30 August 2022

doi:10.1111/jzo.13019

**Abstract**

Chameleons are charismatic and common lizards across Madagascar, Africa, and some surrounding regions. Little is known about their diversification dynamics and how this relates to their ecology, so we estimated diversification rate variation and consider this in the context of three hypotheses previously proposed in the literature. First, that the transoceanic dispersal from Africa to Madagascar on two separate occasions has resulted in fast radiation of Malagasy chameleons. Second, that the substantial floral turnover in their distributions within South Africa has resulted in rapid radiations of the endemic dwarf chameleons (*Bradypodion*). Finally, that the evolution of distinct ecomorphs of chameleon has fuelled fast diversification via adaptive radiations. We use the most recent and complete phylogeny of chameleons to estimate the diversification dynamics of the group using three methods: BAMM (which estimates constant or gradually changing diversification regimes and tests for shifts in these), MEDUSA (which tests for rate shifts in particular clades), and ClaDS (which estimates branch-specific diversification rates). Our results from all analyses estimate a diversification rate increase in a clade containing most of the genus *Bradypodion*, a group containing the South African dwarf chameleons which occur in recognized biodiversity hotspots in diverse habitats. We find no evidence for shifts resulting from dispersal events to Madagascar or related to the strong ecomorphological divergence of short-tailed chameleon lineages (*Brookesia*, *Palleon*, *Rhampholeon*, and *Rieppeleon*). The single burst of diversification within chameleons was in a clade which was associated with geographic areas which have experienced rapid habitat turnover and vicariance over the last ~10 million years. This suggests that 'habitat vicariance' resulting from ecological changes in vegetation has contributed to the diversity of species in this area by increasing diversification rates.

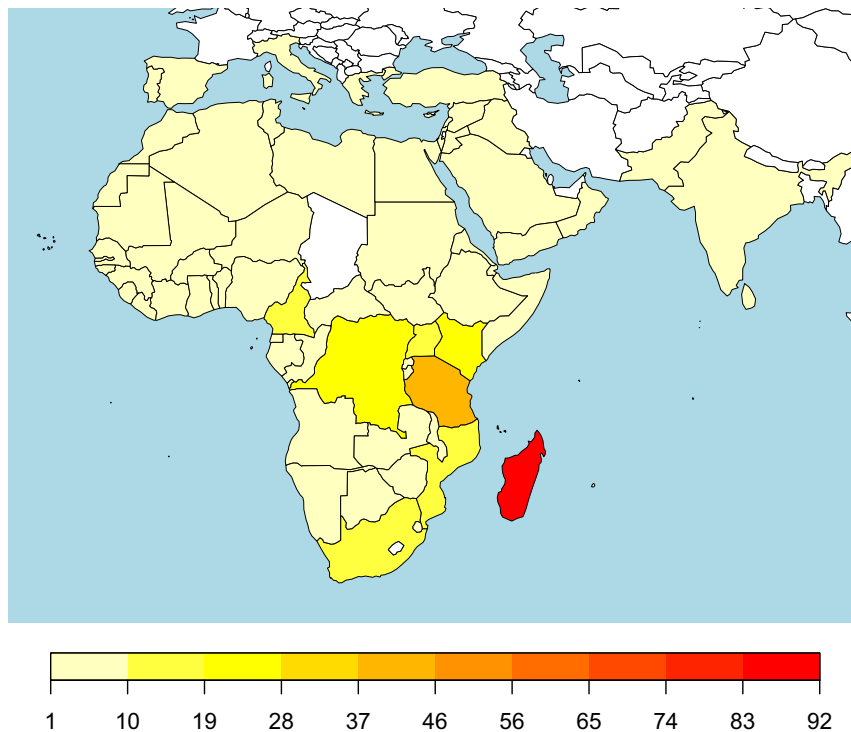
**Introduction**

Chameleons (Chamaeleonidae) are a moderately diverse family of lizards containing 222 currently recognized species (Pincheira-Donoso et al., 2013; Uetz et al., 2020), and their charismatic appearance and behaviour have led some to be used as flagship species (Gehring et al., 2010) and entrance into popular culture (e.g. Watterson, 2012). The family has a large geographic distribution extending throughout Africa (excluding the Sahara) and Madagascar into parts of Europe and Asia (Tolley & Menegon, 2014; Fig. 1). Previous phylogenetic and historical biogeographical analyses have shown that chameleons originated in Africa during the Late Cretaceous (~90 mya), after the break-up of Gondwanaland, and crown group lineages diversified at around the Cretaceous-Tertiary (KT) boundary (Tolley et al., 2013).

Although ~53% of chameleons are distributed across the African continent, two separate dispersal events have resulted

in Madagascar containing ~44% of species (Tolley et al., 2013), which is consequently a hotspot of chameleon diversity (Fig. 1). Smaller numbers of chameleon species are found in southern Europe, south Asia, the Seychelles, the Comoros, and Socotra, each of these five regions representing single dispersal events (Tolley et al., 2013). The extent of chameleon species richness in Madagascar has caused difficulties in explaining the geographic distribution of this diversity since large clades of both Madagascan and African species originate near the base of the phylogeny (Fig. 2). Earlier work suggested a Madagascan origin with multiple dispersals to Africa (Raxworthy et al., 2002), but more recent studies were better able to resolve the history of chameleons, leading to the biogeographic scenario described above, with an African origin and two dispersals to Madagascar (Tolley et al., 2013; Fig. 2).

Despite now having good information on phylogeny and biogeography, the diversification dynamics of chameleons remain poorly known across the family. Importantly,



**Figure 1** Country-level mapping of chameleon species richness, with heat map representing the number of species, based on the Reptile Database (Uetz et al., 2020) accessed on 19th December 2019. Each increment in the scale bar is 10% of the maximum value, shown rounded to the nearest whole number. As species richness is plotted at country-level, there is no assumption that species are found throughout the whole country (for instance, Northern Iberia and Northeastern India have no species). Note the markedly high species richness of Madagascar, the lower but still elevated diversity in non-arid regions of East and Southern Africa, and the relative paucity of species in most other parts of the distribution.

diversification *per se* has received great interest in chameleons, for instance, several speciation events have been tied to particular ecological drivers and hence how speciation occurs has been well studied (e.g. da Silva & Tolley, 2017; Tolley et al., 2006; Tolley et al., 2008). However, estimates of the rates of diversification and how these vary over the phylogeny are lacking. The time component of rates provides a much greater understanding of how the diversity of a clade accumulates over time, so estimating diversification rates gives deeper insight into the dynamics of the clade's biodiversity over evolutionary time.

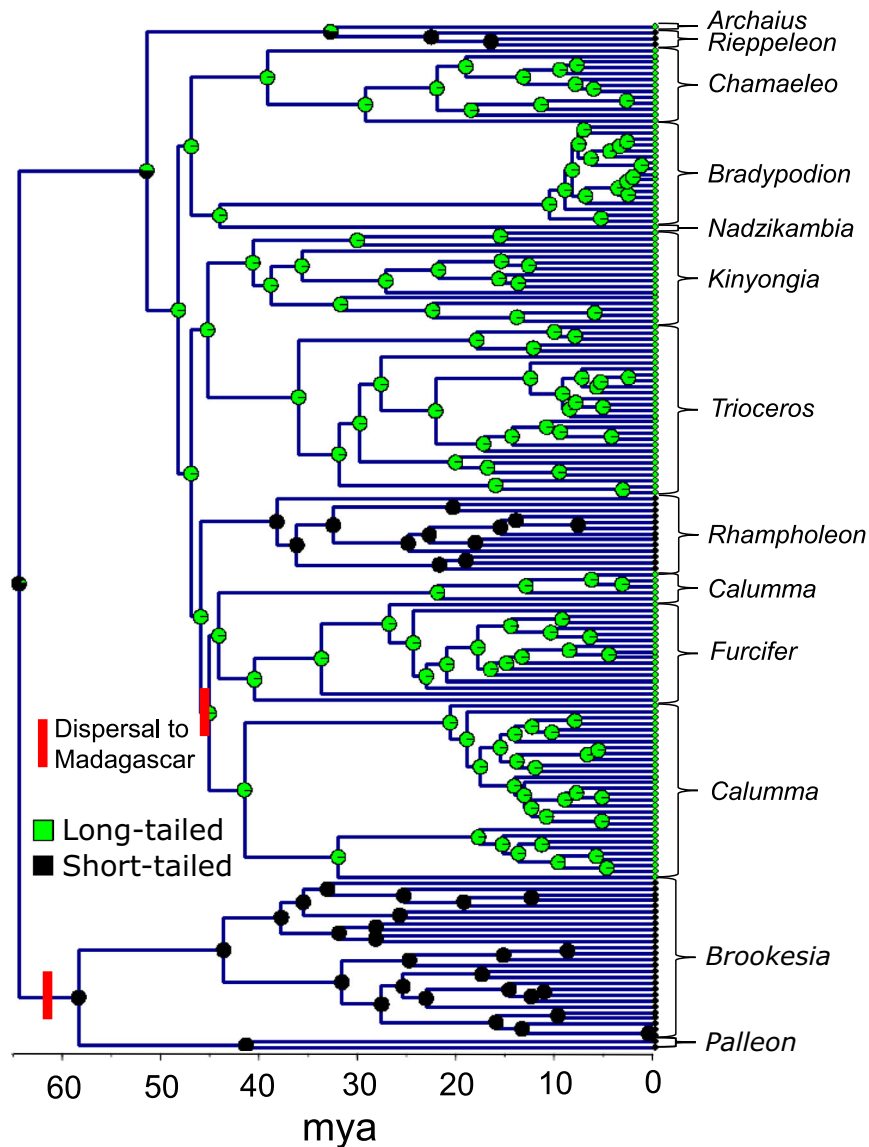
Here, we consider three biogeographic and ecological hypotheses that may have influenced diversification rates in chameleons and hence the structure of the present-day diversity: (1) transoceanic dispersal from Africa to Madagascar has resulted in fast radiation of Malagasy chameleons; (2) high rates of habitat turnover in South Africa has increased diversification rates of endemic dwarf chameleons (*Bradypodion*); (3) the evolutionary change between ecomorphs (distinct morphologies linked to ecology) at three different points in the phylogeny has fuelled diversification.

Note that, as discussed in more detail below, each of our hypotheses is tied to events that occurred only 1–3 times on the phylogeny (Fig. 2). Because of this, we cannot formally test for

statistical associations between these events and diversification rate shifts. Nevertheless, by estimating shifts in diversification rates independently of our hypothesised drivers, as above, we can look for evidence of rate shifts that are either concordant or inconsistent with each hypothesis. Hence, we can consider the locations of any detected upshifts in diversification rate as either supporting or arguing against each of our hypotheses. Specifically, we consider evidence supporting our hypotheses if we find evidence for an increase in diversification rates located on the branches (1) where dispersal to Madagascar occurred, (2) corresponding to the origin of *Bradypodion* or at least early in the history of the clade, and/or (3) associated with ecomorph transitions. Note that these are not mutually exclusive and our approach is capable of supporting any combination of these ranging from none to all three.

### *Hypothesis 1: Dispersal to Madagascar*

The two dispersal events which occurred early in the evolutionary history of chameleons (approximately 45 mya and 65 mya), and hence potentially have had time to generate substantial diversity, were both to Madagascar (Tolley et al., 2013; Fig. 2). Colonization of new biogeographical realms has frequently been implicated in faster rates of diversification in a



**Figure 2** Phylogeny of chameleons with schematic representations of the hypotheses considered herein mapped onto the tree. The phylogeny and locations of dispersal events to Madagascar are taken from Tolley et al. (2013). Ancestral states for long-tailed vs short-tailed ecomorphs were estimated using the re-rooting method (Yang et al., 1995) implemented in the R v 3.4.1 (R Core Team, 2017) package phytools (Revell, 2012) under an asymmetrical (Mk2) model. Species were coded for ecomorph following Tolley et al. (2013), Hughes and Blackburn (2020), and inspection of photographs.

wide range of taxa (Garcia-Porta & Ord, 2013; Herrera, 2017; Kennedy et al., 2016). Such dispersal events result in physical isolation of populations, which may contribute to speciation, but also introduces the colonizers to new environments (Schenk & Stepan, 2018). The new habitats and environmental conditions provide ecological opportunities that may promote rapid speciation as the lineage adapts to the variety of niches available (Price et al., 2014; Stroud & Losos, 2016; Wray & Stepan, 2017). For these reasons, colonization of new biogeographic areas is often associated with bursts of diversification (Burruss & Tan, 2017). Nevertheless, various processes (e.g. high competition with existing species, limited

resource partitioning, or lack of ecological opportunity) can prevent lineages from diversifying to fill empty niches, preventing an increase in speciation rates, and so faster radiation is not an inevitable consequence of dispersal to new areas (Burbrink et al., 2012; Burruss & Muñoz, 2022).

Specifically in the case of Madagascar, the high heterogeneity of climatically distinct biomes and the sharp boundaries between them likely drove evolutionary radiations (Pearson & Raxworthy, 2009; Vences et al., 2009; Wilmé et al., 2006). Indeed, recent work considering the evolutionary and environmental characteristics of biodiversity hotspots globally revealed that they tended to have such high spatial heterogeneity, and

tropical hotspots typically have higher speciation rates (Igea & Tanentzap, 2019). Consistent with this, higher rates of diversification have been found amongst endemic lemurs compared to continental relatives (Herrera, 2017). Moreover, the predicted pattern of adaptive radiation following colonization, whereby diversification rates start high and slow over time (due to niche filling) has been found in a wide range of Madagascan reptiles and amphibians, including one clade of chameleons (*Brookesia* + *Palleon*) (Scantlebury, 2013). Nevertheless, the latter study considered only endemic clades and only one of two Madagascan colonisations by chameleons, and hence it remains unknown whether the dispersal to Madagascar itself increased diversification rates beyond the African mainland clades. Consequently, we herein investigate whether dispersal to Madagascar (with its high spatial heterogeneity of biomes) has resulted in faster diversification rates, which have contributed to the island's role as a chameleon diversity hotspot (Fig. 1).

### *Hypothesis 2: Habitat instability in the genus Bradypodion*

The South African dwarf chameleons (*Bradypodion*) are a group which have several ecological and phylogenetic characteristics that suggest they may have diversified rapidly. Visual inspection of the chameleon phylogeny shows that the genus is a very young crown group compared to other clades with similar diversity (Tolley et al., 2013; Fig. 2). This in itself suggests the possibility that *Bradypodion* have experienced a rapid radiation, but the biogeographic history of their habitats provides *a priori* reason to suspect they might have experienced higher diversification rates (Ellis et al., 2014; Hoffmann et al., 2015; Tolley et al., 2008). The genus is mostly characterized by highly range-restricted species found in recognized biodiversity hotspots such as the Greater Cape Floristic and Maputo-Pondo-Albany regions (Tolley & Menegon, 2014). These areas have promoted diversification and high levels of endemism in the native biota due to their high spatial heterogeneity and frequent turnover and change in the habitats (Ellis et al., 2014; Tolley & Menegon, 2014). In particular, studies of the diversification of *Bradypodion* chameleons in these two regions have revealed an important role for habitat instability over recent evolutionary timescales (da Silva & Tolley, 2017; Tolley et al., 2006; Tolley et al., 2008).

Throughout the Miocene the climate in South Africa, and consequently the vegetation and habitats, changed substantially (Hoffmann et al., 2015; Hopley et al., 2007; Tolley et al., 2008), with forest reductions in many areas creating isolated forest patches across the landscape (Tolley et al., 2008). These shifts potentially led to population divergence via vicariance of habitats, especially likely in the case of *Bradypodion* since they comprise a mix of forest and shrub/grassland species (Tolley et al., 2004; Tolley et al., 2006; Tolley et al., 2008). The potential consequences of this scenario are evident in the taxonomic complexity of the genus, with many predicted cryptic species, and in the evidence that those geographic and ecological attributes have contributed to an example of ecological speciation (da Silva & Tolley, 2013; Tolley

et al., 2004; Tolley et al., 2008). Hence, we predict that habitat dynamics (the temporal contraction, expansion, and turnover of habitats) have led to a burst of diversification for the genus *Bradypodion*.

### *Hypothesis 3: Ecomorph transitions*

Although geographic isolation is important for speciation, and is the basis of the hypotheses outlined above (whether dispersal to Madagascar or vicariance of habitats), isolation in itself may not lead to increased rates of diversification, even if they result in speciation events (Schenk & Stepan, 2018; Stroud & Losos, 2016). Rapid radiations often result from adaptive ecological processes such as the combination of ecological opportunity and adaptive evolution that pushes species into a new 'adaptive zone' that can exploit those opportunities (Schluter, 2000; Stroud & Losos, 2016).

Chameleons exhibit two broad morphological types that are linked to ecology, or 'ecomorphs', which we refer to here as long-tailed and short-tailed. The chameleons we have called the 'short-tailed' ecomorph are also smaller, typically drab brown and more terrestrial (though they may also use small bushes and shrubs [Bickel & Losos, 2002; Luger et al., 2020]), and due to their shorter tails rely far less on caudal prehension for locomotion than the 'long-tailed' ecomorph. Previous researchers have often subdivided the long-tailed ecomorph into finer-scale ecomorphs, with diagnostic differences between them (e.g. Tolley et al., 2013); however, we restrict our coding to simple and very large scale variation which should reflect extreme scenarios that are most likely to lead to ecological radiations (or have greater effects that should improve our ability to find evidence for associations). The short-tailed ecomorph is ancestral, with long-tailed chameleons having evolved on one or two separate occasions early in the history of the family (Tolley et al., 2008; Tolley et al., 2013; Hughes & Blackburn, 2020; Fig. 2). Because these ecomorphs are associated with different habitats and a wide range of functional morphological adaptations to these habitats (Bickel & Losos, 2002; Herrel et al., 2013; Luger et al., 2020), they are highly likely to represent transitions to new adaptive zones. Consequently, we can hypothesise that evolutionary transitions between ecomorphs have provided both ecological opportunity and adaptive evolution to promote upshifts in diversification rate, though these may be temporary and decline to background levels after the new niches are filled.

## **Aims and hypotheses**

Herein, we estimate the diversification dynamics across the evolutionary history of chameleons for the first time and test for shifts in diversification rates. In doing so, we examine evidence for increased speciation rates corresponding to our previously stated hypotheses:-

- (1) Transoceanic dispersal from Africa to Madagascar has resulted in rapid radiations of chameleons that explain the particularly high species richness of Madagascan chameleons.

- (2) Vicariance of habitats due to forest fragmentation and climatic/vegetation turnover has caused adaptive radiation via isolation and ecological speciation in *Bradypodion*.
- (3) The evolution of ecomorphs has led to bursts of diversification as a result of ecological opportunity and evolution towards the new adaptive zone conferred by the morphological and ecological shifts.

## Materials and methods

The phylogenetic tree used as the basis for our analyses was obtained from Tolley et al. (2013), which represents the most comprehensive time-calibrated molecular phylogeny available for chameleons. We note that although this phylogeny remains the best available phylogeny for chameleons, it was not based on phylogenomics techniques and did not sample every species of chameleon, so we do not claim this is the last word in chameleon phylogeny. We recommend consulting Tolley et al. (2013) for full details of phylogenetic inference but, very briefly, this phylogeny was estimated using 6 genes (3 nuclear and 3 mitochondrial), with higher-level relationships estimated using a reduced taxon sampling approach with 13 genes (10 nuclear and 3 mitochondrial) and used as a topological constraint for inference of the more comprehensive 6-gene phylogeny. The resulting tree contained 174 of 222 currently recognized chameleon species (78%) and representing all genera of chameleons. Moreover, sampling of each genus was proportional to its species richness (Fig. S1) such that there was a strong correlation between total and sampled species richness of each genus (Pearson's correlation:  $r = 0.98$ ,  $t_9 = 14.75$ ,  $P = 1.3 \times 10^{-7}$ ) and no detectable relationship between the total species richness of a genus and the proportion of its species sampled (binomial GLM:  $\beta = -0.01$ ,  $z_{1,9} = -0.175$ ,  $P = 0.86$ ). Hence, when we control for sampling proportion in both of our analyses in a way which assumes random sampling, we are confident that the taxon sampling within our phylogeny meets this assumption and reflects actual diversity differences between clades.

We estimated diversification dynamics across the phylogeny using three approaches. The first was Bayesian Analysis of Macroevolutionary Mixtures version 2.5 (BAMM), with the R package BAMMtools used to analyse output (Rabosky, 2014; Rabosky et al., 2014). BAMM uses a Metropolis-coupled MCMC approach to estimate evolutionary rates (e.g. speciation rates) and shifts to different macroevolutionary rate regimes. Regimes, in this context, refers to distinct diversification processes which may be time-constant or time-varying. Priors for our analysis were calculated in BAMMtools (expectedNumberOfShifts = 1, lambdaInitPrior = 2.81041347, lambdaShiftPrior = 0.01773364, muInitPrior = 2.81041347), equal prior probability was given to time-constant vs time-varying models, and four Markov chains were run for 10 million generations, sampling every 1000 generations. Convergence was confirmed by examining the MCMC trace of log-likelihoods and effective sample sizes were calculated for the number of regime shifts and the log-likelihood (>900 in both cases). We provided the model with the global sampling proportion (80%) based on current known species richness of chameleons to

control for incomplete sampling. Although BAMM estimates both speciation and extinction rates, we here present only results based on speciation rates from this analysis since the latter are estimated far more reliably with this method, as is common practice (e.g. Rabosky, 2014; Rabosky, 2020; Shi & Rabosky, 2015; Title & Rabosky, 2019). In doing so, we ignore extinction rates for the purposes of interpreting these results, but instead treat their estimation as a background advantage in the method which allows extinction rate variation to be taken into account when estimating speciation rates. It should be noted that criticisms of BAMM have been reported in the literature, centring around implementation of priors, calculations of the likelihood function, and underestimation of the number of rate shifts (Meyer & Wiens, 2018; Moore et al., 2016), but these have been rebutted convincingly (Rabosky, 2018; Rabosky et al., 2017). Moreover, we also note that using three different methods to investigate diversification in our study reduces dependence on the inherent assumptions and limitations of any single method.

MEDUSA estimates shifts in net diversification rates by first fitting a single constant-rate birth-death model to the whole phylogeny, then systematically adding shifts to different rates at each branch of the tree in a step-wise manner (Alfaro et al., 2009). MEDUSA finds the best single shift (two-rate) model and assesses the support for it compared to a no shift model using AICc, with the threshold for support computed internally based on the total species richness of the clade. If a one-shift model is supported over a no-shift model, the procedure is continued with one more shift each time until no more rate shifts are supported. We ran MEDUSA in the R package geiger (Harmon et al., 2008), allowing rate shifts to occur either at nodes or on branches and permitting combinations of pure birth and birth-death models. As with our BAMM analysis, we controlled for incomplete sampling of the clade by providing MEDUSA with the sampling proportion of our dataset compared to the currently described species richness. We note that May and Moore (2016) explored the statistical behaviour of MEDUSA and described a tendency for the method to inflate rate estimates and numbers of rate shifts as a result of likelihood calculations which do not appropriately account for survival of the process and the existence of extinct lineages. Hence, we caution against interpreting the results from this approach individually, but use it here as part of a multi-pronged strategy to ensure results are consistent across diverse and contrasting limitations of the different methods and provide stronger conclusions via consilience, demonstrating robustness to issues with individual methods.

Finally, we used the ClaDS model (Maliot et al., 2019) to estimate branch-specific diversification rates across the phylogeny. This model is substantially different to both BAMM and MEDUSA as it assumes constant extinction rates across the phylogeny and speciation rates that change at each speciation event (resulting in frequent small changes in speciation rates throughout the tree). We fit the ClaDS model in the R package RPANDA (Morlon et al., 2016) incorporating sampling proportion to control for incomplete sampling of the clade as with our other analyses. We ran 3 Markov chains for 100 000 generations, sampling every 200 generations, and mapped speciation rate estimates for each branch onto the phylogeny.

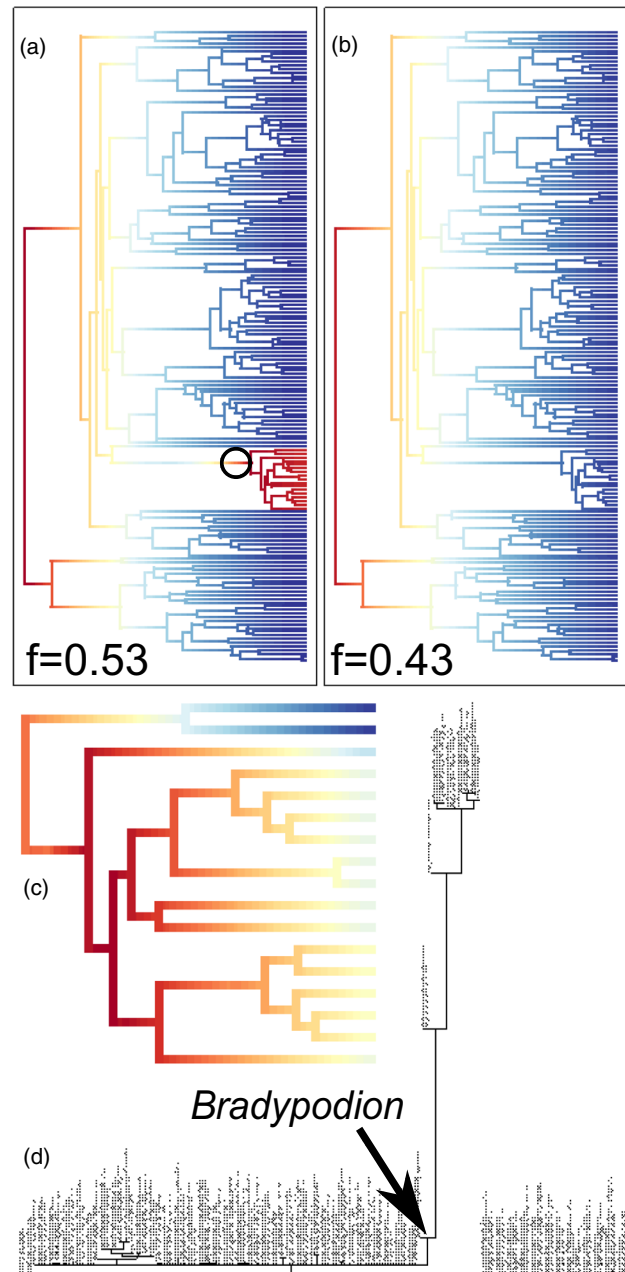


## Results

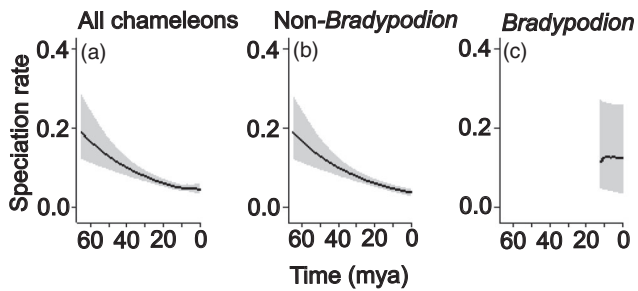
BAMM estimated a background regime of decreasing speciation rates over time, but with a single rate shift at the base of *Bradypodion* as the most likely pattern of diversification dynamics with a posterior probability of 0.53 (Fig. 3a). However, the next most likely scenario is a decreasing speciation rate with no rate shifts, which is estimated with a posterior probability of 0.43 (Fig. 3b). Nevertheless, the branch-specific marginal odds ratios for shifts strongly support a shift either at the base or very early in the history of *Bradypodion* (Fig. 3d), and comparison of prior and posterior distributions of the number of shifts also support a single shift (Fig. S2). Moreover, extracting the estimated speciation rates for *Bradypodion* and comparing them to other chameleons suggests an approximately two-fold increase in this genus (Fig. 4) with slightly lower rates in species from earlier diverging lineages compared to their congeners (Fig. 3c). The mean estimated speciation rate for *Bradypodion* was 0.115/my (95% CI 0.046/my–0.225/my) compared to that for other chameleons of 0.058/my (95% CI 0.050/my–0.068/my). Taken together, our results from BAMM suggest that the most likely scenario is decreasing speciation rates over time but with an increase to twice the background rate at or near the base of *Bradypodion*.

Despite being restricted to time-constant diversification (sub-)models, MEDUSA found concordant results to our BAMM analysis – a positive sign given that the limitations of MEDUSA are very different from those of BAMM (May & Moore, 2016). Specifically, the best-fitting model consisted of one rate shift ( $\Delta\text{AICc} = 8.91$  over a single rate model) estimated to occur very near the base of the genus *Bradypodion* (Fig. 5). This genus was completely sampled in the phylogeny and the shift excluded only 3 of the 17 species (*B. damaranum*, *B. pumilum*, and *B. caffer*), which were also estimated to have the slowest speciation rates in the genus by BAMM (the earliest diverging three species in Fig. 3c). The estimated net diversification rate following this shift was 0.163/my (95% CI 0.089/my–0.268/my), almost 4× higher than the background rate across the rest of the tree (0.048/my; 95% CI 0.041/my–0.056/my), further supporting a substantial burst of diversification early in the *Bradypodion* lineage. We also note that despite a documented tendency for MEDUSA to inflate rate estimates and numbers of rate shifts (May & Moore, 2016) our finding of similar estimates and shifts (concordant in number, location, and broadly in magnitude) between BAMM and MEDUSA lend additional confidence to our main findings.

Finally, and despite being limited to assuming constant extinction rates and that speciation rate changes at every speciation event (in sharp contrast to our other methods), our ClaDS model estimates also gave concordant results (Fig. 6). Specifically, there is a pattern of an ‘early burst’ in speciation rates where high rates occur earlier in the phylogeny followed by generally slower rates later in the tree, consistent with our results from BAMM. There is also evidence of markedly higher speciation rates within the genus *Bradypodion* starting at a location consistent with the rate shift from MEDUSA (and near that inferred by the most likely BAMM estimates).

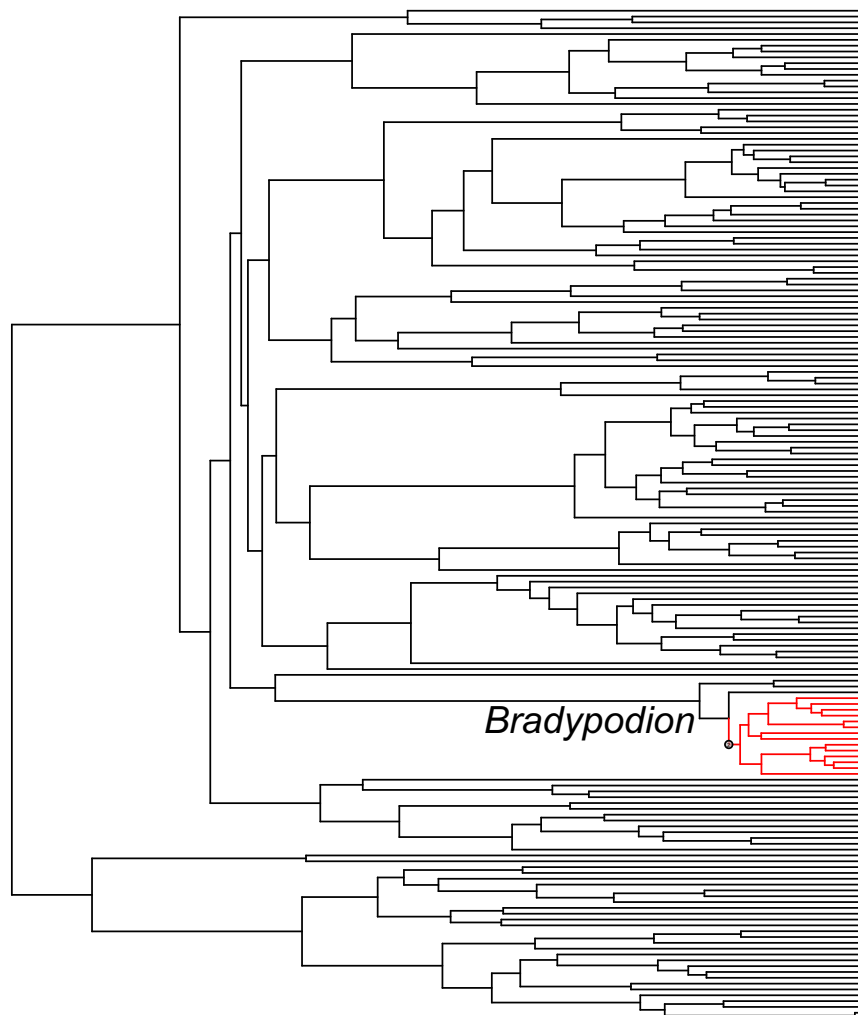


**Figure 3** BAMM estimates of speciation rates and regime shifts. The 95% credible shift set contains two scenarios, a single regime shift at the base of *Bradypodion* (a) and no regime shifts throughout the phylogeny (b);  $f$  is the frequency of each scenario in the posterior distribution, rates increase from blue to red, and the black circle in (a) shows the location of the estimated shift. Speciation rate estimates within the genus *Bradypodion* (c) show generally high rates but slightly lower in earlier-diverging species. Phylogeny with branch lengths representing the marginal odds ratio for a shift in that branch (d) shows that the strongest support for the location of the rate shift is at the branch leading to *Bradypodion* but with staggered support for other relatively basal branches within the genus.

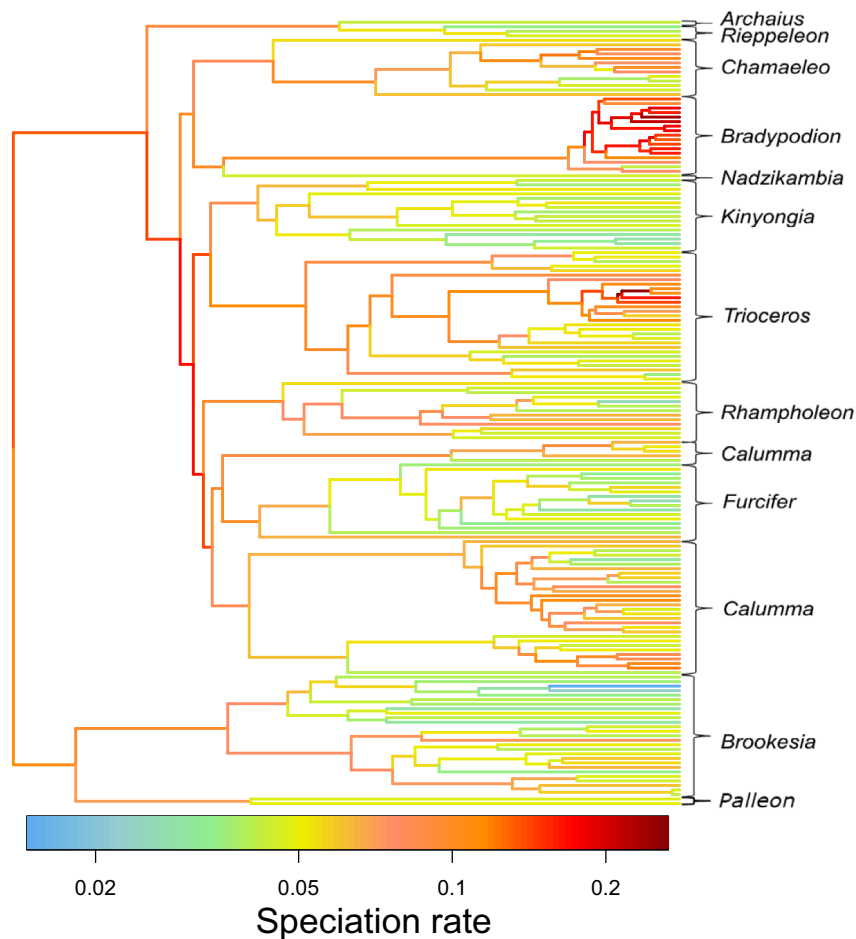


**Figure 4** BMM estimates of speciation rate over time for all chameleons (a), chameleons excluding *Bradypodion* (b), and the genus *Bradypodion* (c).

Despite a range of variation across the phylogeny, including a relatively high rate in three branches clustered around a single node within the genus *Trioceros*, there is little evidence of additional distinct shifts in speciation rates across the phylogeny beyond *Bradypodion*, particularly at locations consistent with our proposed hypotheses. Moreover, since the ClADS model enforces (typically small) changes in speciation rates on every branch, we are cautious about overinterpreting rare and isolated changes inferred from individual methods that may be linked to model assumptions. Hence, we focus our interpretation on the consistently inferred rate increase within *Bradypodion* since the consilience across all methods greatly reduces the chance of putting too much weight on potentially spurious model-specific findings.



**Figure 5** MEDUSA identified one net diversification rate shift across the evolutionary history of chameleons, shown as a small circle and descendant lineages in red. This shift is located near the base of *Bradypodion*, approximately where the speciation rates estimated by BMM peak within the genus (Fig. 3c), and represents a 3–4 fold upshift in *Bradypodion*.



**Figure 6** Branch-specific speciation rate estimates from the ClDS model showing a general pattern of decreasing rates over time and a clustering of high rates in the genus *Bradypodion* consistent in location with the inferred rate shift from MEDUSA. Note the general concordance between these results and those from both MEDUSA and BAMM in terms of magnitudes of rate estimate, locations of major shifts, and (for BAMM) an ‘early burst’ pattern across the whole tree.

## Discussion

Surprisingly, we find no support for increased diversification rates as an explanation of the remarkably high chameleon biodiversity of Madagascar (Fig. 1) nor do we find any evidence that evolutionary transitions between ecomorphs have resulted in adaptive radiations. In contrast, we find that most of the phylogenetic history of chameleons is characterized by relatively simple diversification dynamics, with a regime of decreasing speciation rates from  $\sim 0.2/\text{my}$  to  $\sim 0.05/\text{my}$  over the last  $\sim 60$  my. The one exception for which our results provide evidence is a burst of speciation coinciding with the origin or very early radiation of the genus *Bradypodion* in South Africa, corresponding to a 2–4 fold increase in speciation rate over the background rate. Interestingly, we find no evidence of substantial slowing of this radiation (Figs 3,4 and 6), though given the relatively short time period involved this may simply be an artefact of limited information to detect the exact shape of the temporal distribution of diversification dynamics in this genus.

One concern and possible reason for shifts in diversification rates in one particular clade, and which would likely impact all three of our methods, is potential variation in taxonomic opinion. Faurby et al. (2016) highlight that spurious variation in diversification rates can be found in clades if taxonomists working in that clade tend to be ‘splitters’ (or ‘lumpers’) compared to taxonomists working on other clades in the phylogeny. Faurby et al. (2016) suggest two warning signs of such problems. First, if shifts in diversification rates are found close enough to the tips of the tree that they may plausibly be in an area where intraspecific and interspecific variation diverge (and this ‘threshold’ could lead to differences in taxonomic opinion). Second, if an inferred shift occurs at a node representing a clade that is treated by different taxonomists than other clades on the phylogeny then it may simply represent differences in opinion of species delimitation. We note that the inferred shift at or near the origin of *Bradypodion* occurred on the order of  $\sim 10$  mya, but previous estimates of the duration of speciation have been far shorter than this ( $\ll 1$  mya to 5 mya in extreme cases) for



other taxa (Etienne et al., 2014; Etienne & Rosindell, 2012) so this seems unlikely to explain our results. Moreover, of the chameleon species described since 2000 (Uetz et al., 2020), the group responsible for all but one *Bradypodion* species description have been prominent in at least African chameleon taxonomy, including describing species in another 3 genera (*Kinyongia*, *Nadzikambia*, and *Rhampholeon*), 33% of all chameleon genera including *Bradypodion*, which again suggests this is unlikely to explain our results. Consequently, we believe our results are unlikely to be misled by differences in taxonomic opinion.

Since dispersal to (or presence on) Madagascar does not appear to have been associated with increased diversification rates in chameleons, the remarkable species richness must be predominantly a result of clade age (McPeck & Brown, 2007). This is consistent with the estimated timing of dispersal events in the history of the clade, with one occurring at the basal split of the crown group and the other occurring only ~25% of the time from root to tips (Tolley et al., 2013; Fig. 2). In other words, Madagascar is a biodiversity hotspot of chameleons not because it has resulted in rapid radiations, as in some other taxa (Herrera, 2017; Wood et al., 2015), but because it has had sufficient time to steadily accumulate large numbers of species. Previous work has revealed slowdowns in diversification rates of one clade of Madagascan chameleons (*Brookesia* + *Palleon*), amongst other endemic reptile and amphibian clades, and interpreted this as evidence for adaptive radiation following dispersal to the island (Scantlebury, 2013). Our results support the finding of diversification slowdowns in the *Brookesia* + *Palleon* clade, but as this is merely an extension of the diversification dynamics of chameleons in general it cannot be taken as revealing any special processes related to dispersal to Madagascar and the island's environment. This highlights that studies aiming to assess support for adaptive radiations following dispersal to islands by examining patterns of diversification can be unintentionally limited by excluding continental outgroups. In the case of chameleons, a decline in speciation rate was in progress before the dispersal events and was not altered after arriving in Madagascar, reducing support for adaptive radiation as an explanation for the high observed species richness.

Beyond Madagascar, other dispersal events lend additional support to our suggestion that dispersal has not been an important driver of diversification in chameleons. Tolley et al. (2013) document four distinct dispersals out of Africa to areas other than Madagascar, one each to Asia, Europe, the Seychelles, and Socotra Island. These dispersal events have resulted in very few extant species: one over ~10 my in Europe, three over ~8 my in Asia, one over ~35 my in the Seychelles, and one over ~20 my on Socotra (Tolley et al., 2013). The association of *both* fast and slow life histories with successful dispersal in chameleons (Weil et al., 2022) may help explain the lack of influence of dispersal on diversification. Fast life histories are expected to convey faster speciation rates (and also lower extinction rates) and have been associated with greater diversity in a wide range of taxa (Marzluff & Dial, 1991). If successful dispersers amongst chameleons included both species showing fast and slow life histories, then contrasting effects on diversification rates may cancel out any

general signal of dispersal with rate shifts. Consequently, dispersal is not always associated with burst of diversification (Hearn et al., 2018). Whatever the underlying mechanism, our results provide evidence that dispersal has not been an important driver of chameleon biodiversity in general.

The lack of evidence for ecomorph evolution as a driver of diversification dynamics is also surprising, particularly since finer scale ecomorphs (related to structure of vegetation rather than our broadscale short-tailed/long-tailed dichotomy) have shown good evidence of being related to early stages of ecological speciation in *Bradypodion* chameleons (da Silva & Tolley, 2013; da Silva & Tolley, 2017). Nevertheless, explicit studies of relationships between discrete ecomorphs and diversification rates are rare, and those considering quantitative ecomorphological measurements often find no or variable evidence for a relationship (Alhajeri & Steppan, 2018; Cantalapiedra et al., 2017; Seeholzer et al., 2017). Ecomorphs are highly likely to lead to ecological speciation since they typically meet the criteria for this process (Rundle & Nosil, 2005; Schluter, 2000), especially when they lead to occupancy of distinct habitats that therefore create spatial separation, as is the case in chameleons (Bickel & Losos, 2002; da Silva & Tolley, 2013; Luger et al., 2020). We suggest that, in the case of discrete ecomorphs, ecological speciation may happen as a result of the ecological shift involved in the evolution of the ecomorph, but once the ecomorph exists it does not prime the lineage for further diversification. In this scenario, no effect of ecomorph evolution would be found in speciation *rates*, even if particular speciation *events* are driven by this trait.

Our finding of substantially faster diversification in *Bradypodion* over the last ~10 my (peaking ~6 mya) is consistent with previous emphasis in the literature on ecological causes of past and incipient speciation in this genus (da Silva & Tolley, 2013; Tolley et al., 2004; Tolley et al., 2008). Moreover, we note that our diversification rate estimates for *Bradypodion* are likely to be underestimates given that the genus is predicted to harbour several cryptic, incipient and/or undescribed species that would further increase the diversity (Tilbury et al., 2006; Tolley et al., 2004; Tolley et al., 2022). The concentration of *Bradypodion* species in the Greater Cape Floristic and Maputo-Pondo-Albany biodiversity hotspots, combined with their (often) small ranges, suggests biogeographic or local ecological factors driving their exceptional diversification. These areas have been characterized by dynamic fluctuations in habitat types and distributions over the last ~10 my (Ellis et al., 2014; Tolley et al., 2006; Tolley et al., 2008; Tolley et al., 2014), and hence frequent separation of chameleon populations as their habitats shifted (combined with occasional shifts in habitat selection) may have been sufficient to drive rapid diversification in this genus (da Silva & Tolley, 2013; Tolley & Menegon, 2014). While changing habitats do not seem to have universally led to diversification in African reptiles (Barlow et al., 2019), phylogeographic and niche modelling analyses have demonstrated its importance in the genus *Bradypodion* (da Silva & Tolley, 2017; Tolley et al., 2006). Indeed, the clear linking of such patterns to speciation events in previous literature generated this hypothesis for our current study, and we add to previous findings by demonstrating that the *rate* of

diversification has also increased in this group. We therefore suggest that the association of *Bradypodion* with geographic areas which have experienced rapid habitat turnover and vicariance over the last ~10 million years has contributed to the unusually high speciation rates in the genus.

East Africa, particularly Tanzania, represents another hotspot of chameleon species richness (Fig. 1) and, although we did not explicitly address this in the current study, our results allow us to comment briefly on the diversity in this region. The substantial tectonic activity along the Rift Valley and Eastern Arc Mountains has led to many speciation events of chameleons via vicariance in this region (Matthee et al., 2004; Tolley et al., 2011); however, our results found no consistent evidence for increased rates of speciation in any chameleons except the Southern African *Bradypodion*. Similar to our explanation for the high species richness of Madagascar, we therefore suggest that the diversity of East African chameleons derives from a long period of time over which lineages gradually accumulated diversity. In agreement with this idea, Tolley et al. (2011) found that chameleons of the East African endemic genus *Kinyongia* consist of several clades which have diversified in situ within distinct areas of East Africa, and have remained in isolation in those areas since the mid-Miocene (with the whole genus remaining in East Africa since its origin in the Oligocene). Given the lack of diversification rate shifts across the chameleon phylogeny and the relative proximity of East Africa to Madagascar, combined with the early dispersal from Africa to Madagascar (Tolley et al., 2013), we speculate that chameleons originated in East Africa and that dispersals from that region have been infrequent. This would contribute further to explaining the distribution of chameleon diversity in Africa, and a historical biogeographic analysis with finer scale coding (including regions within Africa) across the whole chameleon phylogeny would enable this hypothesis to be tested in future studies.

## Acknowledgements

We are indebted to Krystal Tolley and her colleagues, whose sustained and excellent work has greatly enhanced our understanding of chameleons. More specifically, the current study was both inspired by, and only possible because of, their 2013 paper on the phylogeny and biogeography of chameleons.

## References

- Alfaro, M. E., Santini, F., Brock, C., Alamillo, H., Dornburg, A., Rabosky, D. L., Carnevale, G., & Harmon, L. J. (2009). Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 13410–13414.
- Alhajeri, B. H., & Steppan, S. J. (2018). Ecological and ecomorphological specialization are not associated with diversification rates in muroid rodents (Rodentia: Muroidea). *Evolutionary Biology*, **45**, 268–286.
- Barlow, A., Wüster, W., Kelly, C. M., Branch, W. R., Phelps, T., & Tolley, K. A. (2019). Ancient habitat shifts and organismal diversification are decoupled in the African viper genus *Bitis* (Serpentes: Viperidae). *Journal of Biogeography*, **46**, 1234–1248.
- Bickel, R., & Losos, J. B. (2002). Patterns of morphological variation and correlates of habitat use in chameleons. *Biological Journal of the Linnean Society*, **76**, 91–103.
- Burbrink, F. T., Ruane, S., & Pyron, R. A. (2012). When are adaptive radiations replicated in areas? Ecological opportunity and unexceptional diversification in West Indian dipsadine snakes (Colubridae: Alsophiini). *Journal of Biogeography*, **39**, 465–475.
- Burruss, E. D., & Muñoz, M. M. (2022). Ecological opportunity from innovation, not islands, drove the anole lizard adaptive radiation. *Systematic Biology*, **71**, 93–104.
- Burruss, E. D., & Tan, M. (2017). Ecological opportunity alters the timing and shape of adaptive radiation. *Evolution*, **71**, 2650–2660.
- Cantalapiedra, J. L., Prado, J. L., Fernández, M. H., & Alberdi, M. T. (2017). Decoupled ecomorphological evolution and diversification in Neogene-Quaternary horses. *Science*, **355**, 627–630.
- da Silva, J. M., & Tolley, K. A. (2013). Ecomorphological variation and sexual dimorphism in a recent radiation of dwarf chameleons (*Bradypodion*). *Biological Journal of the Linnean Society*, **109**, 113–130.
- da Silva, J. M., & Tolley, K. A. (2017). Diversification through ecological opportunity in dwarf chameleons. *Journal of Biogeography*, **44**, 834–847.
- Ellis, A. G., Verboom, G. A., van der Niet, T., Johnson, S. D., & Linder, H. P. (2014). Speciation and extinction in the greater cape floristic region. In N. Allsopp, J. F. Colville, & G. A. Verboom (Eds.), *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region* (pp. 119–141). Oxford University Press.
- Etienne, R. S., Morlon, H., & Lambert, A. (2014). Estimating the duration of speciation from phylogenies. *Evolution*, **68**, 2430–2440.
- Etienne, R. S., & Rosindell, J. (2012). Prolonging the past counteracts the pull of the present: Protracted speciation can explain observed slowdowns in diversification. *Systematic Biology*, **61**, 204–213.
- Faurby, S., Eiserhardt, W. L., & Svenning, J.-C. (2016). Strong effects of variation in taxonomic opinion on diversification analyses. *Methods in Ecology and Evolution*, **7**, 4–13.
- Garcia-Porta, J., & Ord, T. J. (2013). Key innovations and Island colonization as engines of evolutionary diversification: A comparative test with the Australasian diplodactyloid geckos. *Journal of Evolutionary Biology*, **26**, 2662–2680.
- Gehring, P.-S., Pabijan, M., Ratsoavina, F. M., Köhler, J., Vences, M., & Glaw, F. (2010). A Tarzan yell for conservation: A new chameleon, *Calumma tarzan* sp. n., proposed as a flagship species for the creation of new nature reserves in Madagascar. *Salamandra*, **46**, 167–179.

- Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E., & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics*, **24**, 129–131.
- Hearn, D. J., Evans, M., Wolf, B., McGinty, M., & Wen, J. (2018). Dispersal is associated with morphological innovation, but not increased diversification, in *Cyphostemma* (Vitaceae). *Journal of Systematics and Evolution*, **56**, 340–359.
- Herrel, A., Tolley, K. A., Measey, G. J., da Silva, J. M., Potgieter, D. F., Boller, E., Boistel, R., & Vanhooydonck, B. (2013). Slow but tenacious: An analysis of running and gripping performance in chameleons. *The Journal of Experimental Biology*, **216**, 1025–1030.
- Herrera, J. P. (2017). Testing the adaptive radiation hypothesis for the lemurs of Madagascar. *Royal Society Open Science*, **4**, 161014.
- Hoffmann, V., Verboom, G. A., & Cotterill, F. P. D. (2015). Dated plant phylogenies resolve Neogene climate and landscape evolution in the Cape Floristic Region. *PLoS One*, **10**, e0137847.
- Hopley, P. J., Marshall, J. D., Weedon, G. P., Latham, A. G., Herries, A. I., & Kuykendall, K. L. (2007). Orbital forcing and the spread of C4 grasses in the late Neogene: Stable isotope evidence from South African speleothems. *Journal of Human Evolution*, **53**, 620–634.
- Hughes, D. F., & Blackburn, D. G. (2020). Evolutionary origins of viviparity in Chamaeleonidae. *Journal of Zoological Systematics and Evolutionary Research*, **58**, 284–302.
- Igea, J., & Tanentzap, A. J. (2019). Multiple macroevolutionary routes to becoming a biodiversity hotspot. *Science Advances*, **5**, eaau8067.
- Kennedy, J. D., Borregaard, M. K., Jönsson, K. A., Holt, B., Fjeldså, J., & Rahbek, C. (2016). Does the colonization of new biogeographic regions influence the diversification and accumulation of clade richness among the Corvidae (Aves: Passeriformes)? *Evolution*, **71**, 38–50.
- Luger, A. M., Ollevier, A., De Kegel, B., Herrel, A., & Adriaens, D. (2020). Is variation in tail vertebral morphology linked to habitat use in chameleons? *Journal of Morphology*, **281**, 229–239.
- Maliet, O., Hartig, F., & Morlon, H. (2019). A model with many small shifts for estimating species-specific diversification rates. *Nature Ecology and Evolution*, **3**, 1086–1092.
- Marzluff, J. M., & Dial, K. P. (1991). Life history correlates of taxonomic diversity. *Ecology*, **72**, 428–439.
- Matthee, C. A., Tilbury, C. R., & Townsend, T. (2004). A phylogenetic review of the African leaf chameleons: Genus *Rhampholeon* (Chamaeleonidae): The role of vicariance and climate change in speciation. *Proceedings of the Royal Society B*, **271**, 1967–1975.
- May, M. R., & Moore, B. R. (2016). How well can we detect lineage-specific diversification-rate shifts? A simulation study of sequential AIC methods. *Systematic Biology*, **65**, 1076–1084.
- McPeck, M. A., & Brown, J. M. (2007). Clade age and not diversification rate explains species richness among animal taxa. *The American Naturalist*, **169**, E97–E106.
- Meyer, A. L., & Wiens, J. J. (2018). Estimating diversification rates for higher taxa: BAMM can give problematic estimates of rates and rate shifts. *Evolution*, **72**, 39–53.
- Moore, B. R., Höhna, S., May, M. R., Rannala, B., & Huelsenbeck, J. P. (2016). Critically evaluating the theory and performance of Bayesian analysis of macroevolutionary mixtures. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 9569–9574.
- Morlon, H., Lewitus, E., Condamine, F. L., Manceau, M., Clavel, J., & Drury, J. (2016). RPANDA: A R package for macroevolutionary analyses on phylogenetic trees. *Methods in Ecology and Evolution*, **7**, 589–597.
- Pearson, R. G., & Raxworthy, C. J. (2009). The evolution of local endemism in Madagascar: Watershed versus climatic gradient hypotheses evaluated by null biogeographic models. *Evolution*, **63**, 959–967.
- Pincheira-Donoso, D., Bauer, A. M., Meiri, S., & Uetz, P. (2013). Global taxonomic diversity of living reptiles. *PLoS One*, **8**, e59741.
- Price, S. L., Powell, S., Kronauer, D. J., Tran, L. A., Pierce, N. E., & Wayne, R. K. (2014). Renewed diversification is associated with new ecological opportunity in the Neotropical turtle ants. *Journal of Evolutionary Biology*, **27**, 242–258.
- R Core Team. (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Rabosky, D. L. (2014). Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS One*, **9**, e89543.
- Rabosky, D. L. (2018). BAMM at the court of false equivalency: A response to Meyer and Wiens. *Evolution*, **72**, 2246–2256.
- Rabosky, D. L. (2020). Speciation rate and the diversity of fishes in freshwaters and the oceans. *Journal of Biogeography*, **47**, 1207–1217.
- Rabosky, D. L., Grundler, M., Anderson, C., Title, P., Shi, J. J., Brown, J. W., Huang, H., & Larson, J. G. (2014). BAMMtools: An R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods in Ecology and Evolution*, **5**, 701–707.
- Rabosky, D. L., Mitchell, J. S., & Chang, J. (2017). Is BAMM flawed? Theoretical and practical concerns in the analysis of multi-rate diversification models. *Systematic Biology*, **66**, 477–498.
- Raxworthy, C. J., Forstner, M. R. J., & Nussbaum, R. A. (2002). Chameleon radiation by oceanic dispersal. *Nature*, **415**, 784–787.
- Revell, L. J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**, 217–223.

- Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, **8**, 336–352.
- Scantlebury, D. P. (2013). Diversification rates have declined in the Malagasy herpetofauna. *Proceedings of the Royal Society B*, **280**, 20131109.
- Schenk, J. J., & Steppan, S. J. (2018). The role of geography in adaptive radiation. *The American Naturalist*, **192**, 415–431.
- Schluter, D. (2000). *The Ecology of Adaptive Radiation*. Oxford University Press.
- Seeholzer, G. F., Claramunt, S., & Brumfield, R. T. (2017). Niche evolution and diversification in a Neotropical radiation of birds (Aves: Furnariidae). *Evolution*, **71**, 702–715.
- Shi, J. J., & Rabosky, D. L. (2015). Speciation dynamics during the global radiation of extant bats. *Evolution*, **69**, 1528–1545.
- Stroud, J. T., & Losos, J. B. (2016). Ecological opportunity and adaptive radiation. *Annual Review of Ecology, Evolution, and Systematics*, **47**, 507–532.
- Tilbury, C. R., Tolley, K. A., & Branch, W. R. (2006). A review of the systematics of the genus *Bradypodion* (Sauria: Chamaeleonidae), with the description of two new genera. *Zootaxa*, **1363**, 23–38.
- Title, P. O., & Rabosky, D. L. (2019). Tip rates, phylogenies and diversification: What are we estimating, and how good are the estimates? *Methods in Ecology and Evolution*, **10**, 821–834.
- Tolley, K. A., Bowie, R. C. K., Measey, G. J., Price, B. W., & Forest, F. (2014). The shifting landscape of genes since the Pliocene: Terrestrial phylogeography in the Greater Cape Floristic Region. In N. Allsopp, J. F. Colville, & G. A. Verboom (Eds.), *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region* (pp. 142–163). Oxford University Press.
- Tolley, K. A., Burger, M., Turner, A. A., & Matthee, C. A. (2006). Biogeographic patterns and phylogeography of dwarf chameleons (*Bradypodion*) in an African biodiversity hotspot. *Molecular Ecology*, **15**, 781–793.
- Tolley, K. A., Chase, B. M., & Forest, F. (2008). Speciation and radiations track climate transitions since the Miocene Climatic Optimum: A case study of southern African chameleons. *Journal of Biogeography*, **35**, 1402–1414.
- Tolley, K. A., & Menegon, M. (2014). Evolution and biogeography of chameleons. In K. A. Tolley & A. Herrel (Eds.), *The Biology of Chameleons* (pp. 131–150). University of California Press.
- Tolley, K. A., Tilbury, C. R., Branch, W. R., & Matthee, C. A. (2004). Phylogenetics of the southern African dwarf chameleons, *Bradypodion* (Squamata: Chamaeleonidae). *Molecular Phylogenetics and Evolution*, **30**, 354–365.
- Tolley, K. A., Tilbury, C. R., & Burger, M. (2022). Convergence and vicariance: speciation of chameleons in the Cape Fold Mountains, South Africa, and the description of three new species of *Bradypodion* Fitzinger, 1843. *African Journal of Herpetology*, **71**, 14–38.
- Tolley, K. A., Tilbury, C. R., Measey, G. J., Menegon, M., Branch, W. R., & Matthee, C. A. (2011). Ancient forest fragmentation or recent radiation? Testing refugial speciation models in chameleons within an African biodiversity hotspot. *Journal of Biogeography*, **38**, 1748–1760.
- Tolley, K. A., Townsend, T. M., & Vences, M. (2013). Large-scale phylogeny of chameleons suggests African origins and Eocene diversification. *Proceedings of the Royal Society B*, **280**, 20130184.
- Uetz, P., Freed, P., & Hošek, J. (2020). The reptile database. Retrieved from <http://www.reptile-database.org>
- Vences, M., Wollenberg, K. C., Vieites, D. R., & Lees, D. C. (2009). Madagascar as a model region of species diversification. *Trends in Ecology & Evolution*, **24**, 456–465.
- Watterson, B. (2012). *The Complete Calvin and Hobbes*. Andrews McMeel Publishing.
- Weil, S.-S., Gallien, L., Lavergne, S., Börger, L., Hassler, G. W., Nicolai, M. P. J., & Allen, W. L. (2022). Chameleon biogeographic dispersal is associated with extreme life history strategies. *Ecography (Early View)*, e06323.
- Wilmé, L., Goodman, S. M., & Ganzhorn, J. U. (2006). Biogeographic evolution of Madagascar's microendemic biota. *Science*, **312**, 1063–1065.
- Wood, H. M., Gillespie, R. G., Griswold, C. E., & Wainwright, P. C. (2015). Why is Madagascar special? The extraordinarily slow evolution of pelican spiders (Araneae, Archaidae). *Evolution*, **69**, 462–481.
- Wray, K. P., & Steppan, S. J. (2017). Ecological opportunity, historical biogeography and diversification in a major lineage of salamanders. *Journal of Biogeography*, **44**, 797–809.
- Yang, Z., Kumar, S., & Nei, M. (1995). A new method of inference of ancestral nucleotide and amino acid sequences. *Genetics*, **141**, 1641–1650.

## Supporting Information

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

**Figure S1** Plots of sampled and total species richness demonstrating proportional sampling in our study.

**Figure S2** Prior and posterior distribution for the number of rate shifts in our BAMM analysis.