



Eocene–Oligocene cooling and the diversification of *Hemidactylus* geckos in Peninsular India

Aparna Lajmi^{a,b,*}, Praveen K. Karanth^a

^a Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, Karnataka, India

^b Institute of Evolution, Department of Evolutionary and Environmental Biology, University of Haifa, Haifa 3498838, Israel

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ABSTRACT

The Eocene–Oligocene cooling marks a global shift towards a cooler and drier climate, concurrent with significant turnover in biota globally. In Peninsular India, palynological investigations suggest a shift from wet rainforest vegetation to dry and seasonal species during this period. However, the grassland and open habitats that dominate this region at present expanded relatively recently due to Late Miocene intensification of monsoon seasonality. We test the possible role of these climatic shifts in generating the dry habitat diversity of an endemic radiation of *Hemidactylus* geckos distributed in both wet and dry habitats, by investigating whether (a) the beginning of the diversification of dry habitat lineages overlap with the Eocene–Oligocene cooling or late Miocene aridification and (b) accompanied by an increase in diversification. Molecular data (two nuclear and two mitochondrial gene fragments) were generated for samples collected across Peninsular India, and along with previously published data, a time-calibrated phylogeny was reconstructed. Ancestral state reconstruction of dry and wet habitat states was carried out on the time-calibrated phylogeny and γ -statistics along with a test for diversity-dependent diversification utilized to examine the trend in lineage accumulation. Results show the ancestral node of this radiation to have a dry habitat state, which began diversifying between 39 and 32 million years ago, concurrent with the Eocene–Oligocene cooling. Furthermore, lineage diversification fit a diversity-dependent model of diversification and the γ -statistics revealed an early increase in diversification followed by a slowdown later. The deep divergences of dry habitat lineages concurrent with the Eocene–Oligocene cooling suggest that this could have served as an ecological opportunity facilitating an early increase in lineage diversification of *Hemidactylus* in this region. These results suggest an ancient origin and long persistence of arid ecosystems in Peninsular India.

1. Introduction

Changing climate is a major driver of macroevolutionary dynamics (Erwin, 2009; Ezard et al., 2011). The Cenozoic era is marked by a global trend towards a cooler and drier climate, particularly two abrupt shifts in temperature—the Eocene–Oligocene cooling and Late Miocene aridification (Zachos et al., 2001). These changes in climate have influenced the diversification of biota across the globe (Mayhew et al., 2008; Morley, 2000; Prothero and Berggren, 2014; Zhang et al., 2012). Peninsular India and Sri Lanka support an extensive dry zone that formed during this period. However, the relative roles of these climatic shifts in shaping the diversity of dry habitats in this region are poorly understood. Phylogenetic reconstruction of evolutionary histories of organisms can aid in this endeavor. For example, with the help of time-calibrated phylogenies, plant groups specific to certain biomes have

been studied to estimate the time of origin of these biomes (Becerra, 2005; Byrne et al., 2011; Davis et al., 2005; reviewed in Pennington et al., 2006; Richardson et al., 2001). In groups that have representative species in multiple biomes, such studies have given insights into what the ancestral biome was and the timing of biome shifts (reviewed in Donoghue and Edwards, 2014). However, few studies on animal taxa have used this framework (but see Marin et al., 2013; Agarwal and Ramakrishnan, 2017; Brennan and Oliver, 2017). Thus, one could test specific hypothesis regarding the ancestral state/biome of the organism or biome shifts, given the paleoclimate and the biome preferences of the organism.

Eocene–Oligocene cooling (EOC) is the most prominent climatic shift after the Cretaceous (Prothero, 1994). Following the warm and humid early Eocene Climatic Optimum 52–50 million years ago (Mya), the temperature began to decline and the trend towards cooler

* Corresponding author at: Institute of Evolution, Department of Evolutionary and Environmental Biology, University of Haifa, Haifa 3498838, Israel.
E-mail address: aparna@evo.haifa.ac.il (A. Lajmi).

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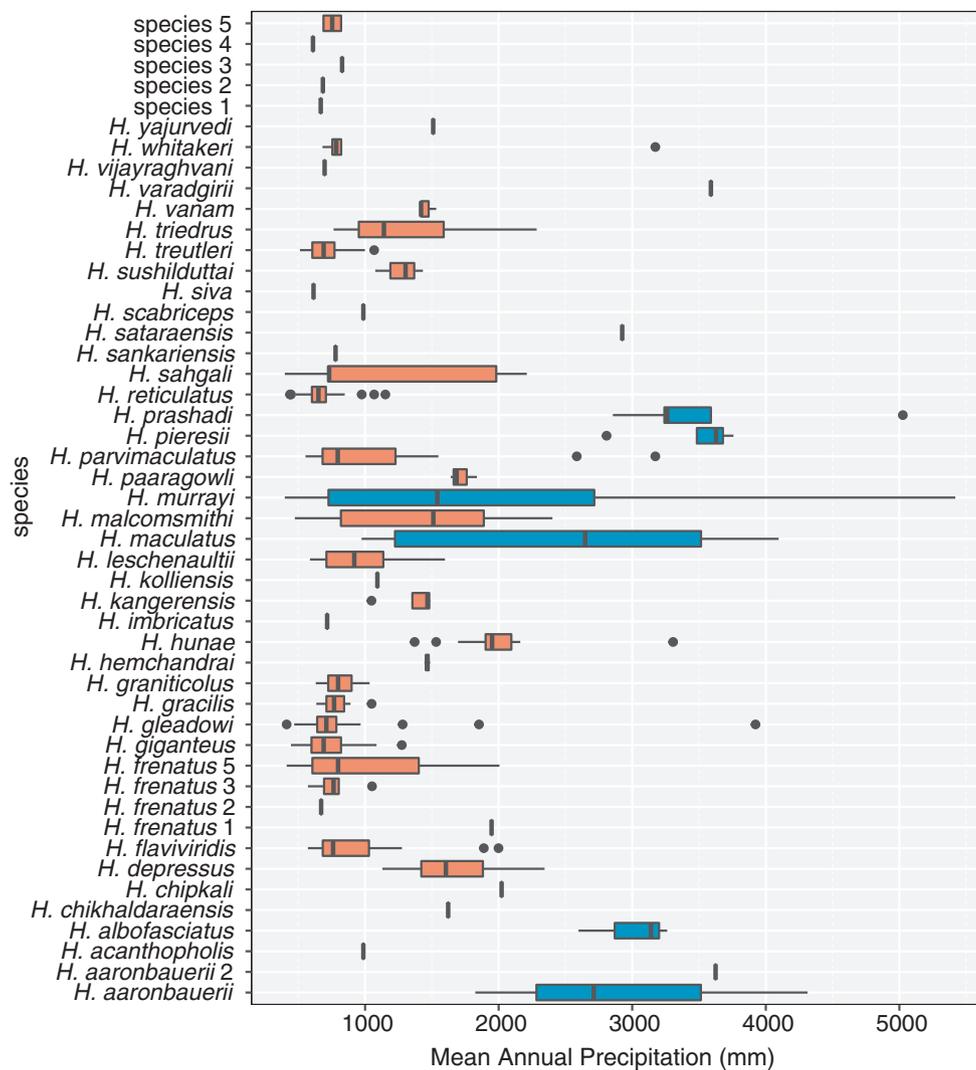


Fig. 1. Range of annual precipitation experienced by each divergent lineage. Lineages restricted below 2500 mm rainfall are depicted in orange and those experiencing more than 2500 mm or having a wide range spanning low and high rainfall are in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

conditions lasted for 17 million years with a rapid drop in temperature at the Eocene–Oligocene boundary ~ 33.7 Mya (Zachos et al., 2001). This period of a rapid decrease in temperature is called the Eocene–Oligocene transition. The cooling starting from late middle-Eocene up until early Oligocene resulted in increased aridification (Abels et al., 2010; Dupont-Nivet et al., 2007; Hoorn et al., 2012; Kargaranbafghi and Neubauer, 2017; Passchier et al., 2013) and temperature seasonality (Hren et al., 2013; Ivany et al., 2000; Wade et al., 2012). This led to a mass biotic turnover of terrestrial as well as marine flora and fauna from warm and humid adapted to more cold- and dry adapted taxa, across this time period (Brennan and Oliver, 2017; Meng and McKenna, 1998; Prothero, 1994; Champion and Seth, 1968; Stadler, 2011; Sun et al., 2014; Zhang et al., 2012).

The cold period was interrupted by a relatively warm temperature from the late Oligocene up until the middle Miocene 26–15 Mya (Zachos et al., 2001). The cooling and aridification after the mid-Miocene had a profound influence on the diversification of biota in Australia, Eurasia, as well as Africa (Cerling et al., 1997; Richardson et al., 2001; Tang and Ding, 2013). Although C4 grasses evolved ~ 35 Mya, their range expanded considerably in the Late Miocene 8–6 Mya, which facilitated the opening up of forested habitats (Edwards et al., 2010; Edwards and Smith, 2009; Pound et al., 2012). This led to a global turn over from forest-dwelling to open habitat organisms that occupied this

novel habitat (Fortelius et al., 2006).

The Indian subcontinent also experienced these climatic shifts and associated turnover of flora. Palynological studies reveal that while the early Eocene harbored the richest and most diverse palynomorph assemblages, there was a marked reduction in the diversity of palynomorphs during the EOC. After this period, regional differences in their assemblages become more apparent (Morley, 2000; Prasad et al., 2009). The Late Eocene also saw the immigration of several plant taxa that indicate the development of drier or more seasonal climate (Morley, 2000). By early Miocene (~ 23 Mya) the monsoonal seasonal climate became more pronounced in Peninsular India (Clift et al., 2008). The last phase of intense aridification occurred between 11 and 7 Mya, concurrent with the global Miocene aridification, leading to the establishment of open habitats and grasslands especially in the North-western Indian subcontinent (Molnar and Rajagopalan, 2012). Today, much of Peninsular India can be characterized as a dry zone that receives less than 2500 mm average rainfall annually (Karanth, 2003). The wet evergreen forests, that covered most of the Indian plate during Paleocene, are today largely restricted to the Western Ghats and very few pockets in Sri Lanka (Prasad et al., 2009). These shifts in climate and the concomitant changes in vegetation are bound to have influenced the diversification of many taxa. Thus, Peninsular India and Sri Lanka provide an ideal setting to examine the role of past climate in the

diversification of biota.

Little is known about how these early climatic shifts might have shaped the current diversity and distribution of Indian fauna. A phylogenetic study on wet zone restricted *Gegeniophis* caecilian revealed that the eastern and western lineage diverged around 37.5 (45.1–30.2) Mya, and the establishment of dry climate in the intervening areas was hypothesized to have caused this separation (Gower et al., 2016). During the late Miocene, dry and open grassland restricted lizards such as *Sitana* and *Ophisops* show increased diversification (Agarwal and Ramakrishnan, 2017; Deepak and Karanth, 2018), suggesting prevalence and expansion of open habitats during the late Miocene. Fossorial snakes that have a preference for mesic habitats showed a decrease in diversification and turnover rate from late Miocene to present (Cyriac and Kodandaramaiah, 2017). Apart from these handful of studies, our understanding of the evolution of dry habitat biota is limited.

Peninsular India and Sri Lanka harbor a radiation of *Hemidactylus* geckos, referred here as the Indian radiation, that is largely restricted to this region (Bansal and Karanth, 2010; Bauer et al., 2010b). Most species of the Indian radiation are distributed in the dry zone of Peninsular India, with a few distributed in areas with higher rainfall of > 2500 mm mean annual precipitation, particularly in the Western Ghats (Fig. 1). Additionally, the Indian radiation began diversifying during the Late Eocene–Early Oligocene (41–28 Mya; Lajmi et al., 2019). This overlaps with the time when Peninsular India was covered with tropical forest to the appearance of drier flora. Thus, the Indian radiation is an ideal model system to investigate whether the EOC or the late Miocene aridification generated the current dry habitat diversity. In this study, the EOC hypothesis predicts (a) the ancestor/ancestors of dry habitat lineages to overlap with the EOC and (b) a constant diversification rate or an early increase in diversification rate coinciding with the EOC. Under this hypothesis, the wet habitat lineages would have evolved more recently from dry habitat ancestors. On the other hand, the Late Miocene aridification hypothesis predicts (a) the ancestor/ancestors of dry habitat lineages to overlap with the late Miocene and (b) increase in diversification rates to coincide with late Miocene aridification. Therefore, under this hypothesis, the basal nodes of the Indian radiation would be wet habitat and the dry habitat lineages would have evolved during the late Miocene. Both these hypotheses are rejected if the evolution of dry habitat ancestor (along with the credible interval) does not overlap with either of these climatic shifts. We test these hypotheses (Fig. 2) using ancestral state reconstruction on a time-calibrated phylogeny along with diversification analyses.

The Indian radiation includes 39 described species. However, a

large part of the diversity in this radiation has not been described, as the dry zone of Peninsular India is largely unexplored. Therefore, through intensive taxon sampling, we first identify putative species using species delimitation methods and then test the hypotheses stated above using a hypothesis testing framework.

2. Material and methods

2.1. Taxon sampling and molecular work

Opportunistic sampling was carried out across the distribution range of this radiation in Peninsular India (Table S2 in Appendix S1). Tissue samples were preserved in 100% alcohol and DNA was extracted using Qiagen DNeasy blood and tissue kit. Four fragments of DNA were PCR amplified—two partial mitochondrial genes cytochrome *b* (cyt *b*; 307 bp) and NADH dehydrogenase 2 (ND2; 981 bp) and two partial nuclear genes, Recombination Activation Gene1 (RAG1; 1044 bp) and Phosducin (PDC; 395 bp) amounting to 2727 bp. PCR protocol was as per Bansal and Karanth (2010) and Bauer et al. (2010). These four markers have been used extensively in phylogenetic studies of the genus *Hemidactylus* and are known to resolve the relationships within the Indian radiation (Bansal and Karanth, 2010; Bauer et al., 2010b; Lajmi et al., 2019). Our sampling included all the described species from the Indian radiation except *H. peiresii* from Sri Lanka which is believed to be sister to *H. depressus*.

2.2. Phylogenetic analysis

Sequence data from all four markers mentioned above was used for phylogenetic reconstruction. Previously published sequence data of Indian and Sri Lankan species were included from Bauer et al. (2010), Bansal and Karanth (2010), Lajmi et al. (2019), Agarwal et al. (2019), Chaitanya et al. (2018), Mirza et al. (2018), Mirza and Raju (2017), Srikanthan et al. (2018) (Table S2 in Appendix S1). Representatives of other major *Hemidactylus* clades were also incorporated in the analyses, along with three species of *Cyrtodactylus*, which were used as outgroup (based on Gamble et al., 2012; Pyron et al., 2013). There were 1617 variable sites and 1356 parsimony informative sites in the dataset. Individual gene sequences were aligned in MEGA 5.2.2 (Tamura et al., 2011) using clustalW (Thompson et al., 1994) and then concatenated. PartitionFinder v1.1.1 (Lanfear et al., 2012) was used to find the best partitioning scheme and appropriate models of sequence evolution (Table S1 in Appendix S1). Phylogenetic trees were constructed using

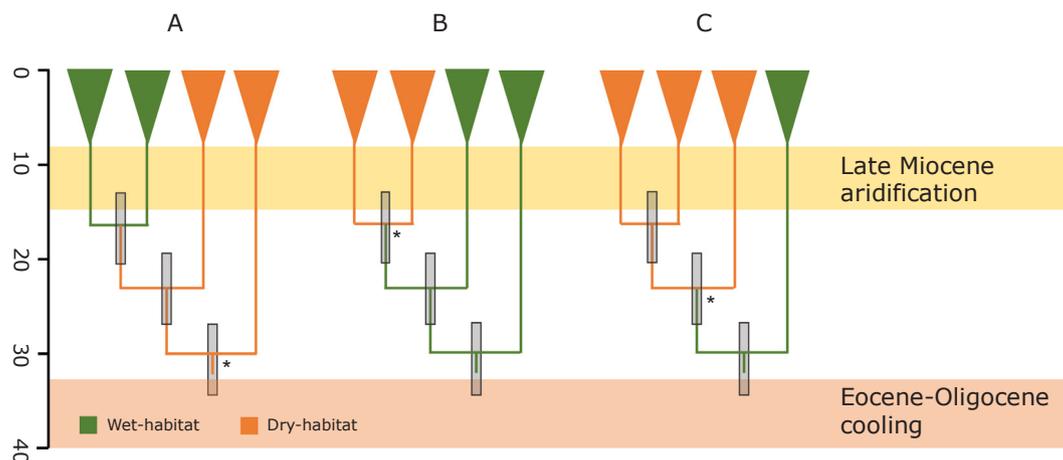


Fig. 2. Three scenarios depicting different timings of evolution of dry habitat ancestor that allows us to test the climatic hypotheses. (a) The EOC hypothesis cannot be rejected if the nodes along with their credible intervals overlapping with the EOC have a dry habitat ancestor (depicted by *). We expect a constant rate or early increase in diversification rates concurrent with EOC. (b) The late Miocene hypothesis cannot be rejected if evolution of the dry habitat ancestors is concurrent with the late Miocene aridification. Here, increase in diversification rates will coincide with late Miocene aridification. (c.) Both these hypotheses are rejected under this scenario as the evolution of dry habitat ancestor along with the credible interval does not overlap with either of these climatic events.

maximum likelihood (ML) approach implemented in RAxML HPC 7.4.2 (Stamatakis, 2006) using the graphical interface raxmlGUI v1.3.1 (Silvestro and Michalak, 2012) and Bayesian inference in MrBayes 3.2.1 (Ronquist et al., 2012). In RAxML, a thorough ML search with 1000 bootstraps for 10 runs was carried out. The analysis in MrBayes was carried out for 5 million generations sampling every 500 generations. The likelihood values of the two runs were visualized in Tracer v1.6 (Rambaut et al., 2014) to check for stationarity. To evaluate convergence across runs the standard deviation of split-frequency of < 0.01 was used. The initial 25% of sampled trees were discarded as 'burn-in' before constructing the consensus tree.

2.3. Species delimitation

DNA sequence data from nuclear marker RAG1 and PDC, and mitochondrial marker *cyt b* was used for species delimitation. Two methods were used to identify divergent lineages within the Indian radiation—the single locus multi-rate Poisson Tree Processes (mPTP; (Kapli et al., 2017) using the *cyt b* data and multilocus Bayesian species delimitation and species tree estimation using the program BPP v3.3 (Yang and Rannala, 2014) on the *cyt b*, RAG1, and PDC datasets. ND2 was not be used for species delimitation as many samples lacked this data. mPTP is an improved method to the previously published PTP and bPTP methods as it tries to accommodate different levels of intraspecific genetic diversity and differential sampling of each species. The partitioned mitochondrial *cyt b* dataset was used for building the ML phylogenetic tree in raxmlGUI v1.3.1 (Silvestro and Michalak, 2012). This analysis was run for 100 million generations in 4 independent runs, and 25% of the initial samples were discarded as burn-in. Multilocus Bayesian method BPP (Yang, 2015) requires a priori species hypothesis and the mPTP output along with the currently known described species was used as the input for the Bayesian analysis. The BPP analysis was done on each individual clade separately as the program cannot handle a large number of "species". A combination of different gamma prior (Γ) was used to account for different demographic scenarios of large and small population size (θ s) and deep and shallow divergence times (τ_0)— $\theta = G(1, 10)$ and $\tau = G(1, 10)$, $\theta = G(1, 10)$ and $\tau = G(2, 2000)$, $\theta = G(2, 2000)$ and $\tau = G(1, 10)$, and $\theta = G(2, 2000)$ and $\tau = G(2, 2000)$ as employed by (Leache and Fujita, 2010; Huang and Knowles, 2016). Each analysis was run twice for 1 million generations with parameters sampled every 2 generations and a burnin of 80,000 generations.

2.4. Divergence date estimation

Divergence dates were estimated in a Bayesian framework with the program BEAST v1.8.3 (Drummond et al., 2012) using a combined dataset consisting of the two nuclear genes, RAG1 and PDC, and the mitochondrial gene ND2. We used one representative of each of the divergent lineage identified to build the dated phylogeny. This ensured that the data across all the markers were near complete for these lineages. Additional outgroup taxa were added as mentioned in Agarwal et al. (2014b), which were used to calibrate the phylogeny. The dataset was partitioned and models of sequence evolution were applied as proposed by PartitionFinder v1.1.1 (Lanfear et al., 2012). The analysis was constrained to obtain the same topology as the ML tree. A relaxed uncorrelated lognormal distribution and Yule process tree prior were used. Five calibration points were used as in previously published studies (Gamble et al., 2008; Heinicke et al., 2011; Nielsen et al., 2011; Agarwal et al., 2014a; Agarwal and Karanth, 2015) (Table S4 in Appendix S1). The Markov Chain Monte Carlo (MCMC) was run for 100 million generations, sampling every 10,000 generations and estimated sample sizes (> 200) calculated in Tracer v1.6 to ensure appropriate chain length. The initial 25% of the trees were discarded as burnin. Two independent analyses were run and the trees of the two runs were combined using the program Logcombiner v1.8.3 included in

the BEAST package. A maximum clade credibility (MCC) tree with median heights was generated in TreeAnnotator v1.8.3. To test the sensitivity of the age estimates to the calibration priors, we ran five independent analyses by dropping each of the five calibration points at a time.

2.5. Ancestral state reconstruction

To understand the rainfall regimes in which each lineage was distributed, mean annual precipitation (MAP; BIO12) data were extracted from WorldClim Version 2 (Fick and Hijmans, 2017) for a total 550 locations (Fig. S1). To this end, location data from fieldwork and published literature (Agarwal et al., 2019, 2011; Bauer et al., 2010b; 2010a; Chaitanya et al., 2018; Dandge and Tiple, 2015; Gaikwad et al., 2009; Giri and Bauer, 2006; Giri, 2008; Giri and Bauer, 2008; Giri et al., 2017; Lajmi et al., 2019; 2016; Mirza and Sanap, 2014; Mirza, 2018; Mirza et al., 2018, 2017; Mirza and Raju, 2017; Murthy et al., 2014; Srikanthan et al., 2018; Srinivasulu et al., 2018, 2014) was collected for 48 divergent lineages in the Indian radiation. No information on species from the Indian radiation was available on Global Biodiversity Information Facility (GBIF; <http://www.gbif.org/>) except in the case of human commensal species from outside Peninsular India and Sri Lanka, which are likely to have been moved around recently. The range of MAP experienced by each divergent lineage was visualized with a box plot to determine whether they are restricted to dry or wet zone (Fig. 1). Similarly, all the other 18 bioclimatic variables were also examined post-hoc.

Tropical evergreen, semi-evergreen to moist deciduous forests in Peninsular India experience MAP of > 2500 mm (Karanth, 2003; Prasad et al., 2009; Champion and Seth, 1968). Whereas areas that experience < 2500 mm rainfall are largely covered with dry deciduous to scrub forests as well as open savanna habitats. Therefore, all lineages that experienced > 2500 mm of MAP were considered as wet habitat lineages. Fig. 2 also shows a clear trend where most lineages found in the dry zone experience < 2500 mm MAP. Therefore, lineages restricted to this rainfall conditions were categorized into dry habitat lineages. However, no such trend was observed with respect to other bioclimatic variables that we examined. Moreover, a priori data to categorize these variables is not known. MAP Ancestral state reconstruction was employed to determine whether the ancestral state of the Indian radiation was wet habitat or dry habitat lineage. MAP was used as a continuous variable as well as discretized by categorizing these lineages into dry and wet habitat.

The time-calibrated phylogeny of the Indian radiation was used for ancestral state reconstruction. For discrete character mapping of dry and wet habitat states, we first tested between four different rate transition models to find the best fit model using the function fitMk 1. equal rates (ER) model 2. all rates different (ARD) 3. and 4. the two possible irreversible models. The best fitting model was chosen based on AIC scores. For the reconstruction of the discrete traits, 1000 stochastic character map simulations were generated using the function make.simmap in the phytools package v0.6-00; (Revell, 2012) for R team (2014) using the rate transition matrix. For continuous character mapping, we used mean values of MAP raw as well as log-transformed values as the trait value. We compared the model fit between a Brownian motion (BM) and Ornstein-Uhlenbeck (OU) models using Akaike Information Criterion (AIC) scores in the R package Geiger (Harmon et al., 2008). The ancestral state was estimated using the function fastAnc in the R package phytools, assuming the best fitting model. The reconstructions were then visualized using the functions contMap and phenogram.

2.6. Diversification analyses

Gamma statistics (γ -test; Pybus and Harvey, 2000) was used to understand the trend in lineage accumulation through time on 1000

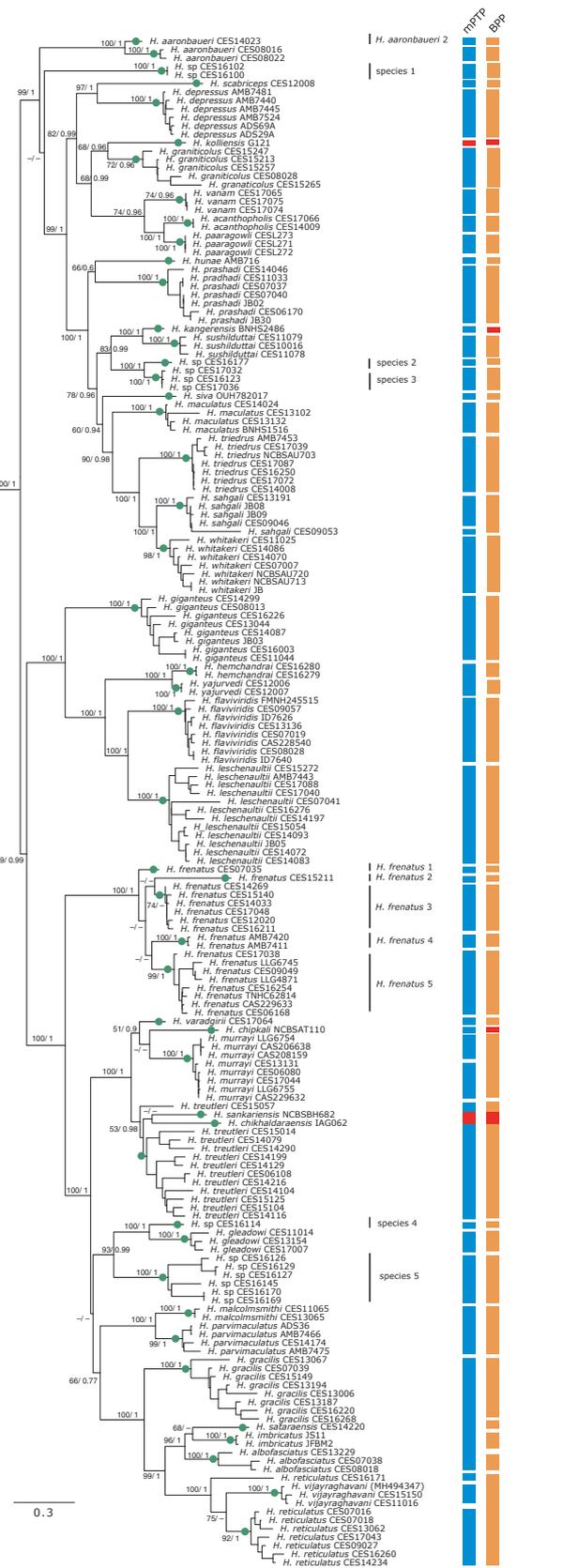
trees sampled from the posterior distribution of the BEAST run. When lineages follow a pure-birth model of diversification, $\gamma < 0$ implies that lineages have accumulated early in the history of the clade while $\gamma > 0$ means accumulation of lineages closer to the tips. The γ distribution of 1000 trees from this data was compared with that of 10,000 trees simulated under a pure birth model. To make sure that the slowdown in diversification rates towards the tips is not due to lack of time for speciation (protracted speciation; Etienne and Haegeman, 2012) or failure to identify cryptic lineages, we excluded the last 2 million years of branches from the tree after which there are no speciation events, and recalculated γ statistics. This analysis was also done using the package Phytools v0.6-00 (Revell, 2012) in R team (2014). Given the caveats of γ -statistics (Fordyce, 2010), we also tested for diversity-dependent diversification using the R package DDD (Etienne and Haegeman, 2012). DDD is a maximum likelihood approach which allows us to fit a diversity-dependent model and contrast it with a diversity-independent constant rate model. We used a likelihood ratio test to compare these two models. We refrained from using other statistical programs to estimate rate shifts since most of these rely on large phylogenetic trees for a robust estimate of diversification rate and rate shifts.

3. Results

Molecular data from a total of 224 samples, including published data, were analyzed. Maximum likelihood and Bayesian phylogenies of the concatenated dataset revealed similar topologies. The Indian radiation was retrieved with high ML and BI support (Fig. 3), although its relationship with other *Hemidactylus* could not be ascertained due to low support. The Indian radiation has three major well-supported clades — clade 1, 2 and 3—similar to Lajmi et al., 2019, Bansal and Karanth (2010) and Bauer et al. (2010). The phylogeny unveiled high genetic diversity ($> 12\%$ in *cyt b*) within a few species, which are likely to be cryptic species complexes—*H. reticulatus* and *H. frenatus*. Information on sampling location, species, museum ID and Genbank numbers are reported in Table S2 in Appendix S1.

The mPTP analysis estimated 43 divergent lineages within the Indian radiation (Fig. 3) based on the *cyt b* phylogeny. While most of the described species were correctly identified to be distinct (24 species), some clades with high genetic diversity were split into multiple divergent lineages—*H. reticulatus*, *H. frenatus*, and *H. aaronbaueri*. In four cases two or more distinct species were grouped together—*H. malcolmsmithi* + *H. parvimaculatus*, *H. gracilis* + *H. sataraensis* + *H. imbricatus* + *H. albofasciatus*, *H. hemchandrai* + *H. yajurvedi*, and *H. treutleri* + *H. varadgirii*. However, all these species are morphologically distinct (Bauer et al., 2008; Dandge and Tiple, 2015; Gaikwad et al., 2009; Giri and Bauer, 2008; Lajmi et al., 2016; Murthy et al., 2014). The 43 divergent lineages identified by mPTP and the additional described species were taken as input for the Bayesian analysis. This approach supported 41 species. It recognized all the described species except in case of *H. parvimaculatus* and *H. malcolmsmithi*, and *H. reticulatus* and *H. vijayraghavani*, where two species were grouped into one. These were considered to be separate lineages for further analysis. *H. parvimaculatus* and *H. malcolmsmithi* differ considerably in morphology and appear to have disjunct geographical distributions (Lajmi et al., 2016). However, these two cases need to be investigated more thoroughly. Furthermore, three recently described species, *H. kollensis*, *H. sankartiensis*, and *H. chikhaldaraensis* (Agarwal et al., 2019) could not be included in the species delimitation as only ND2 data was available for these species. They were also considered distinct lineages for further analysis. Thus, 48 divergent lineages were identified in the Indian radiation (Fig. 3) and the posterior probabilities associated with each of these potentially distinct species are given in Table S3 in Appendix S1.

The time-calibrated phylogeny revealed that the diversification in this radiation began 39–32 Mya (Fig. 4a). For further analysis, this tree was used (complete dated phylogeny in Appendix S2). The overall



(caption on next page)

results do not change when the calibration priors are excluded one at a time from the analysis. We also estimated the divergence dates after dropping the mitochondrial 3rd position. The 95% HPD of the most

Fig. 3. Maximum likelihood phylogeny of the Indian radiation of *Hemidactylus* geckos with concatenated data (2727 bp). Values at the nodes denote maximum likelihood bootstrap support and posterior probability. Divergent lineages identified by mPTP are depicted in blue and BPP results are indicated in orange, with missing taxa for the analysis in red. Green dots on each of the branches represent candidate lineages chosen for further analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recent ancestor of the Indian radiation overlapped with the EOC in all the analyses. The results of these analyses are mentioned in Table S5 in Appendix S1. The mean annual precipitation data for each divergent

lineage showed that 38 out of the 48 species experienced < 2500 mm of MAP, while nine species were distributed in the wet zone (Fig. 1). Three lineages from the wet zone showed comparatively large variation in the amount of annual precipitation they experience. For discrete character mapping, the ER model was chosen based on AIC scores which fit marginally better than the irreversible model (dry to wet habitat only; $\Delta AIC = 0.08$). The dry habitat phenotype was retrieved as the ancestral state in the discrete character mapping analysis with a probability of 0.99 (Fig. 5a). BM model fit the dataset better than OU model for the MAP raw as well as log-transformed data, and the BM model was used for the ancestral state reconstruction. The continuous trait mapping of raw MAP gave an ancestral state value of 1381 mm for the Indian

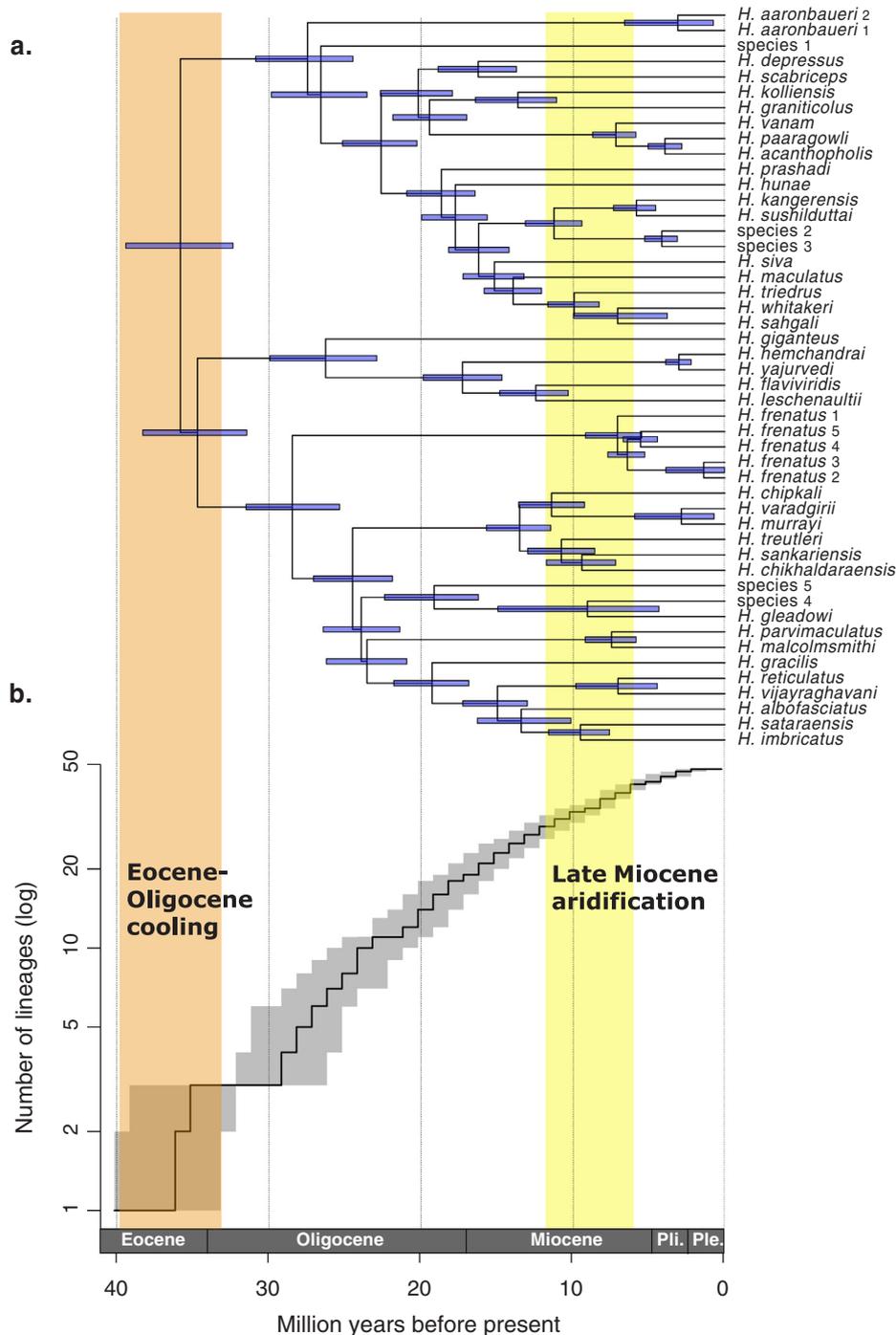


Fig. 4. a. Time-calibrated phylogeny of the Indian radiation along with credible intervals associated with each node. b. Lineage through-time plot with the black line showing the median age of lineages and the grey area denoting credible interval drawn from the posterior distribution.

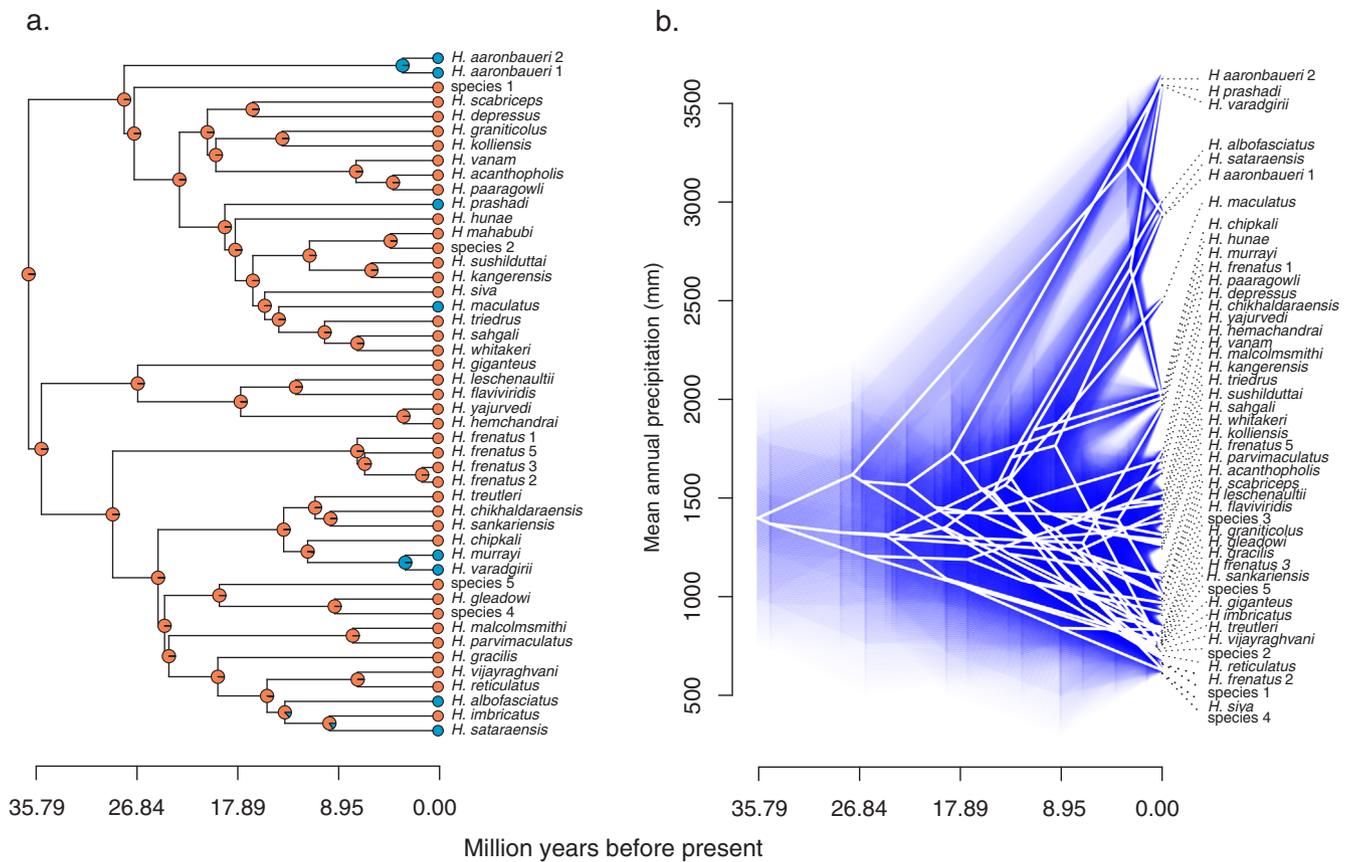


Fig. 5. a. Ancestral state reconstructed for discrete data on the time-calibrated phylogeny. Reconstructed states are depicted at each node where orange depicts dry habitat and green denotes wet habitat states. The colors at the tip show the assigned state for each divergent lineage. b. ancestral state reconstruction of continuous rainfall data using means annual precipitation on the y-axis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

radiation with a credible interval ranging from 428 to 2333 mm. The ancestral state with log-transformed value was found to be 1176 mm with a credible interval of 617–2244 mm. Although large, the credible interval lies below 2500 mm (Fig. 5b). Lineages have shifted from low to high rainfall regimes more recently and independently at least six times (Fig. 5a).

The gamma value of 1000 trees from the posterior distribution ranged from -4.49 to -2.85 (median -3.45 ; p -value < 0.001 ; Fig. 4b). All trees were significantly different from the null distribution of simulated trees. Removing the last two million years increased the γ values (-2.47 , p -value 0.014), but it was still significantly lower than null suggesting that slowing down of diversification is unlikely to be due to lack of time for recent speciation. The MCC tree of Indian radiation fit diversity-dependent diversification model better, further confirming slow-down of diversification rates later. The loglikelihood of the diversity-dependent model (-139.74) was greater than that of a constant rate model (-145.78).

4. Discussion

We examined whether the EOC or late Miocene aridification led to an increase in the dry habitat diversity of *Hemidactylus* geckos in Peninsular India and Sri Lanka. We tested these hypotheses by reconstructing a time-calibrated phylogeny with near complete taxon sampling and used ancestral state reconstruction of dry and wet habitat states to investigate whether the dry habitat ancestor evolved during the EOC or late Miocene aridification. The trend in lineage accumulation was also examined to test if the increase in diversification rate was concurrent with the evolution of dry habitat ancestor. We found that

the reconstructed ancestral state of the Indian radiation, 39–32 Mya, was a dry habitat state and the credible interval of this node overlaps with the EOC. Furthermore, the trend in lineage diversification showed an early increase in diversification rate and fit diversity-dependent diversification. Thus, the hypothesis proposing the role of EOC in facilitating the dry habitat diversity in Peninsular India cannot be rejected. On the other hand, late Miocene aridification hypothesis can be rejected as the evolution of dry habitat ancestor does not coincide with this event. Moreover, there is a slowdown in diversification rates during the Miocene. These results suggest that the EOC played a role in generating the dry habitat diversity of *Hemidactylus* in Peninsular India.

We first performed lineage delimitation, as a robust and complete phylogeny is prerequisite to test these hypotheses in a phylogenetic framework. Given the underestimation of alpha diversity in the tropics, we first addressed this issue through broad sampling followed by using two coalescent-based methods for species delimitation. More thorough sampling focusing on each of the species complexes, along with species delimitation using multiple lines of evidence is required to establish the species status of the lineages delimited in this study. If these are indeed valid species, this would be a substantial increase in the species diversity of *Hemidactylus* geckos from the Indian radiation (from 39 species).

4.1. Dry and wet habitat lineages

The mean annual precipitation (MAP) data extracted for each divergent lineage revealed that most of them were distributed in low rainfall conditions of < 2500 mm MAP. Additionally, many lineages were restricted to < 1000 mm MAP. While confined geographical

distribution could result in a narrow range of precipitation in some lineages, many species like *H. giganteus*, *H. gleadowi*, *H. parvimaculatus*, *H. flaviviridis*, *H. treutleri*, *H. gracilis*, and *H. reticulatus* are widely distributed. In fact, *H. parvimaculatus* and *H. flaviviridis* are human commensal species. These species often disperse widely through human-mediated dispersal (Carranza and Arnold, 2006) and therefore, would be expected to span different rainfall conditions. Yet, all these species are restricted to the dry zone, suggesting the sensitivity of dry habitat species to higher precipitation. Another explanation for low *Hemidactylus* diversity in the wet zone could be that the pre-established fauna of the wet zone competitively excluded *Hemidactylus*. A total of six lineages were restricted to areas with > 2500 mm MAP. These species are present only in the wet zone and experience little variation in precipitation. This could be a result of one or more factors—inability to cope with the dry climate, geographic barrier restricting species to a smaller area in the wet zone (in case of *H. sataraisensis* and *H. albofasciatus*), and/or paucity of sampling (in case of *H. varadgirii* and *H. aaronbaueri* 2).

Other than species restricted to wet or dry habitats, three species were distributed in a wide range of rainfall conditions, namely *H. murrayi*, *H. maculatus*, and *H. aaronbaueri*. These species were categorized as wet habitat species in the ancestral state reconstruction. While this could have implications on the ancestral state, coding these species as wet and dry would result in higher support for an ancestral state of dry habitat species and will not change the overall pattern. This would further strengthen the argument that the ancestral lineage of this radiation was a dry habitat species. To avoid biased outcomes resulting from the inaccurate categorization of species to wet and dry habitat, we also used MAP as a continuous trait. Nevertheless, it must be noted that when lineages are assigned to wet or dry habitat, there is an implicit assumption that these lineages are adapted to these conditions. However, these “traits” have not been measured on the organism.

4.2. Diversification shaped by drying climate

The ancestral state reconstruction of discrete as well as continuous data revealed the dry habitat state to be the reconstructed ancestral state at the basal node of the Indian radiation. Furthermore, the wet habitat lineages have evolved independently multiple times relatively recently. The dry habitat ancestral lineage began diversifying 39–32 Mya, soon after the Indian plate collided with Asia. The docking of the Indian plate with Asia is proposed to have facilitated the dispersal of *Hemidactylus* into India (Bansal and Karanth, 2013). The change in climate during the EOC from humid to a more arid environment in Peninsular India could have provided a conducive environment for these geckos. Moreover, this region was yet to be occupied by other dry habitat lizard taxa which could have been potential competition. These factors could have served as an ecological opportunity for a dry habitat preferring ancestor, giving rise to the observed lineage accumulation pattern. Most of this early diversification in the Indian radiation seems to have occurred in Peninsular India, with dispersals into Sri Lanka only after early to mid-Miocene. Moreover, most of the dry habitat *Hemidactylus* diversity of Sri Lanka, which is a subset of the Indian *Hemidactylus*, is a result of relatively recent range expansion from Peninsular India (Lajmi et al., 2019). Although the deep divergences of dry habitat lineages overlapping the EOC point towards the persistence of dry climate in Peninsular India, the occurrence of dry habitats in Sri Lanka during this period remains unclear.

Given that the subcontinent has experienced a reduction of forest cover through the Cenozoic (Morley, 2000), there could have been extinctions of wet habitat lineages of Indian radiation. This could influence the ancestral state reconstruction, as the phylogenetic reconstruction here accounts for only extant lineages. However, most extant species of the genus *Hemidactylus*, which has a global distribution across the tropics and sub-tropics, are distributed in dry forest or open habitats. Therefore, it is likely that the ancestral lineage of the

Indian radiation that arrived on the Indian plate was a dry habitat species. Understanding the niche preferences of the sister clade could also shed light on this aspect. However, the sister clade of the Indian radiation is not known as the higher-level phylogeny of this genus is not yet resolved. Climate could have directly influenced diversification by providing conducive environment and/or could have indirectly influenced by facilitating diversification of certain kind of vegetation (giving rise to a new niche), diversification of prey species, and/or extinction of competition. Moreover, multiple factors other than climate could have facilitated the diversification of the Indian radiation, such as topographic and/or microhabitat heterogeneity (Deepak and Karanth, 2018; Vijayakumar et al., 2016), which needs detailed investigation.

The disparity in the number of dry and wet habitat species in the Indian radiation is not surprising as lizards often exhibit higher richness in arid regions, unlike most other vertebrates (Powney et al., 2010; Roll et al., 2017; Wiens et al., 2013). An investigation of diversification pattern across major squamate lineages in Australia dated the crown ages of these lineages to be after the Eocene (Oliver and Hugall, 2017). Particularly in pygopodoid lizards, the arid environment is thought to have facilitated the diversification of these lizards (Brennan and Oliver, 2017). Although squamates have a much deeper history, most of the diversification in the Australian arid zone is associated with the Miocene aridification.

The diversification pattern of the Indian radiation fits a diversity-dependent model. Such pattern of diversification has been reported in adaptive radiations (Burbrink et al., 2012; Wagner et al., 2014) as well as several continental radiations (Phillimore and Price, 2008; Rabosky, 2013; Rabosky and Lovette, 2008a; Wagner et al., 2014). The slowdown in diversification rate is often proposed to be a result of ecological limits on resources that could lead to an equilibrium between speciation and extinction processes (Rabosky and Hurlbert, 2015). This decrease in diversification rates could be a result of decreasing speciation rate or increasing extinction rate. Rabosky and Lovette, 2008b demonstrate that phylogenies with a later slowdown in diversification rates result from decreasing speciation rate rather than an increasing extinction rate. In Peninsular India, diversification in other lizards such as *Sitana* and *Ophisops* inhabiting the dry zone increased relatively recently during the Late Miocene (Agarwal and Ramkrishnan, 2017; Deepak and Karanth, 2018). The intensification of monsoon seasonality and aridification during this period is proposed to have led to the establishment of savanna habitats, which facilitated increased diversification in these open habitat lizards. Many species of the Indian radiation are specific to these open habitats and grasslands. The clade of terrestrial geckos consisting of *H. reticulatus*, *H. sataraisensis*, *H. imbricatus*, *H. gracilis*, *H. vijayraghavanii*, and *H. albofasciatus*, began diversifying during this period. The overall trend of decrease in lineage accumulation during the Miocene in the Indian radiation is intriguing and contrary to our expectation. Further examination of the evolution of these microhabitat specific species could shed light on this apparent paradox.

5. Conclusion

We tested whether the EOC or late Miocene aridification could have facilitated the diversification of dry habitat diversity of *Hemidactylus* geckos using a hypothesis testing framework. The two climatic hypotheses predict that the shift in climate should overlap with the evolution of a dry habitat ancestor and an increase in diversification rate. Results from both ancestral state reconstruction and diversification analyses allow us to reject the late Miocene aridification hypothesis. Furthermore, the role of EOC in generating the dry habitat diversity of *Hemidactylus* geckos in Peninsular India cannot be rejected. The Indian radiation of *Hemidactylus* geckos, being the oldest known radiation among the dry zone reptiles of Peninsular India, suggests a much older date for the establishment of the dry climatic regime in Peninsular India. This study, consistent with the palynological work, strongly points towards presence of dry habitats in Peninsular India during the

late Eocene–early Oligocene.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympbev.2019.106637>.

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