



Insights into Himalayan biogeography from geckos: A molecular phylogeny of *Cyrtodactylus* (Squamata: Gekkonidae)



Ishan Agarwal^{a,*}, Aaron M. Bauer^b, Todd R. Jackman^b, K. Praveen Karanth^a

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

^b Department of Biology, Villanova University, 800 Lancaster Avenue, Villanova, PA 19085, USA

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ABSTRACT

The India–Asia collision profoundly influenced the climate, topography and biodiversity of Asia, causing the formation of the biodiverse Himalayas. The species-rich gekkonid genus *Cyrtodactylus* is an ideal clade for exploring the biological impacts of the India–Asia collision, as previous phylogenetic hypotheses suggest basal divergences occurred within the Himalayas and Indo-Burma during the Eocene. To this end, we sampled for *Cyrtodactylus* across Indian areas of the Himalayas and Indo-Burma Hotspots and used three genes to reconstruct relationships and estimate divergence times. Basal divergences in *Cyrtodactylus*, *Hemidactylus* and the Palearctic naked-toed geckos were simultaneous with or just preceded the start of the India–Asia collision. Diversification within *Cyrtodactylus* tracks the India–Asia collision and subsequent geological events. A number of geographically concordant clades are resolved within Indo-Burmese *Cyrtodactylus*. Our study reveals 17 divergent lineages that may represent undescribed species, underscoring the previously undocumented diversity of the region. The importance of rocky habitats for *Cyrtodactylus* indicates the Indo-Gangetic flood plains and the Garo-Rajmahal Gap are likely to have been important historical barriers for this group.

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1. Introduction

The most visible evidence of the India–Asia collision are the Himalayas, bounding the Indian subcontinent with the highest mountains in the world (Fig. 1). This colossal geological event profoundly affected Asian biodiversity by bringing together the relatively insular biota of the Indian Plate and mainland Asia (Conti et al., 2002; Gower et al., 2002; Datta-Roy and Karanth, 2009), with subsequent Himalayan uplift causing regional climate change (Patnaik et al., 2012) and creating mountain barriers to dispersal. Though the exact configuration and timing of the India–Asia collision is an active area of research, evidence indicates India and Asia began colliding about 50 million years ago (mya), preceded by the collision of Tethyan island arcs with India and/or Asia, and final India–Asia suturing was between 45 and 35 mya (Rowley, 1996; Ali and Aitchison, 2008; Hall, 2012; Bouilhol et al., 2013; Metcalfe, 2013; but see Van Hinsbergen et al., 2012 for an opposing model), culminating in the uplift of the Himalayas by ~23 mya (Clift et al., 2008). While the geology of the India–Asia collision is relatively well studied, its biological impacts are still being revealed (Che et al., 2010; Li et al., 2013). Large-scale faunal

exchange between the drifting Indian plate and Southeast Asia initiated in the Eocene (Klaus et al., 2010; Li et al., 2013), and the appearance of Holarctic mammal fossils in northwest India suggests some connectivity to mainland Asia as early as 54 mya (Clementz et al., 2011). Molecular evidence supports Eocene–Oligocene biotic links between Peninsular India and mainland Asia, with dispersal both out of India (Gower et al., 2002; Datta-Roy and Karanth, 2009) and into India (Van Bocxlaer et al., 2009; Bansal and Karanth, 2013; Li et al., 2013). Dispersal of at least some groups ceased in the Middle to Late Miocene, coincident with the final uplift of the Himalayas and increasing seasonality (Li et al., 2013). However, vagile taxa such as mammals and birds have moved into the Himalayas in the last 10 million years (my), with the influence of recent glaciation and climate change implicated in diversification (Reddy, 2008; den Tex and Leonard, 2013; Srinivasan et al., 2013; Yuan et al., 2013).

The contemporary Himalayan region is climatically and topographically heterogeneous, the rugged mountains finely dissected by river valleys, with local environmental variation linked to altitude, slope, and aspect; and broad east–west gradients in rainfall, temperature and seasonality. Highly biodiverse, this area includes the Himalaya Hotspot and part of the Indo-Burma Hotspot (Mittermeier et al., 2005). A combination of location and geological history make the Himalayas biogeographically unique, the fauna a

* Corresponding author.

E-mail address: ishan.agarwal@gmail.com (I. Agarwal).

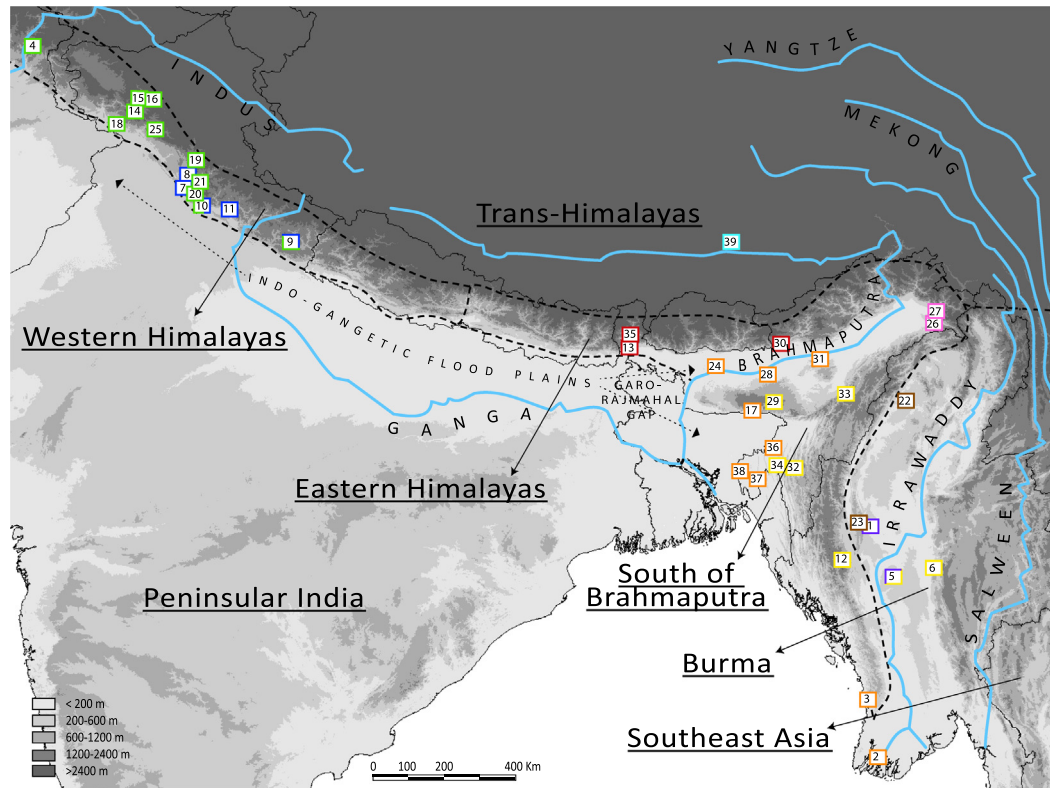


Fig. 1. Map of the circum-Himalayan region showing sampling locations of Himalayan and Indo-Burmese *Cyrtodactylus*. Numbered localities are referenced in Table 1; multiple samples of a species are numbered serially; colors correspond to clades marked in Fig. 2: Blue = Clade F, brown = Clade E, cyan = Clade A, green = Clade B, orange = Clade N, pink = Clade L, purple = Clade G, red = Clade H, yellow = Clade M. Major rivers are marked by a bold line and capitalized text, areas used in ancestral area reconstruction delineated by dotted lines and river courses, labelled with underlined text (refer to methods for definitions). The Indo-Gangetic Flood Plains (dotted arrows indicate the extent) lie south of the Himalayas and the Garo-Rajmahal Gap separates the Shillong Plateau and Rajmahal Hills. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mix of peninsular Indian, Oriental and Palaeartic elements (Mani, 1974; Das, 1996). The recent discoveries of numerous morphologically distinct vertebrates from the Himalayas and northeast India (Bauer, 2002, 2003; Sinha et al., 2005; Athreya, 2006; Das et al., 2010; Sondhi and Ohler, 2011; Kamei et al., 2012; Datta-Roy et al., 2013) are indicative of gross underestimation of biodiversity. Well sampled phylogenies are needed to reveal patterns of diversity and assembly in the Himalayas, and to further understanding of the processes that shaped this biodiversity, with the potential utility to predict impacts of future change (Richardson and Whittaker, 2010; Sinervo et al., 2010).

Cyrtodactylus is the most speciose gekkonid genus globally, with over 175 described species, distributed from the Western Himalayas through Southeast Asia to the western Pacific (Wood et al., 2012; Uetz, 2014). With a probable Laurasian origin in the Palaeocene, global phylogenies of *Cyrtodactylus* reveal a west to east pattern of diversification, and basal divergences in the Eocene separate clades in the Trans-Himalayas, Western Himalayas, Indo-Burma and Southeast Asia (Wood et al., 2012; Bauer et al., 2013). The onset of the India–Asia collision is coincident with early diversification within this group (Wood et al., 2012; Bauer et al., 2013), though it is unclear how this geological event has impacted diversification within circum-Himalayan *Cyrtodactylus* from the middle Eocene to present, as few Himalayan species have been sampled. *Cyrtodactylus* is the most speciose lizard genus in the Himalayan and Indo-Burma region, with 17 species known from relatively well surveyed Burma, three from Nepal, and two from Pakistan (Bauer, 2002, 2003; Schleich and Kästle, 2002; Zug et al., 2003; Khan, 2006; Venugopal, 2010; Wood et al., 2012; Bauer et al., 2013; Masroor pers. comm.). A probable artefact of

inadequate sampling, just five species are known from India – the catchall species *C. khasiensis* is reported from across northeast India, *C. gubernatoris* and *C. himalayanus* are known only from their type descriptions from the Eastern and Western Himalayas respectively, and *C. fasciolatus* and *C. lawderanus* are known from the Western Himalayas (Sharma, 2002). The distribution of *Cyrtodactylus* species and the timing of diversification, with basal divergences seemingly correlated with the India–Asia collision, make the genus an ideal candidate to explore the faunal impacts of the collision. To this end, we sampled for *Cyrtodactylus* across Indian regions of the Indo-Burma and Himalayan hotspots (Fig. 1) and used molecular data to build phylogenies and estimate divergence times.

2. Materials and methods

2.1. Taxon sampling, DNA data, and sequence alignment

We generated sequence data for 41 individuals of *Cyrtodactylus* from 32 localities across the Himalayas, northeast India and the Andaman and Nicobar Islands (Table 1 and Fig. 1). We targeted type localities and other likely habitats and spotted geckos at night by eyeshine on fieldtrips from 2009 to 2011. Tissue samples were collected in the field from subsequently vouchered specimens and preserved in 95–100% ethanol, and a few tissue samples were contributed by other researchers (see acknowledgements). Collections from protected areas were made with forest department permits. Genomic DNA was extracted using Qiagen DNeasy™ tissue kits.

The final sequence alignment contained 2477 aligned base pairs (bp) of data including one mitochondrial gene, NADH

Table 1

Cyrtodactylus samples from the Himalayas, Indo-Burma, and the Andaman and Nicobar Islands used in this study with tissue sample number, locality, and GenBank accession numbers. Locality numbers reference Fig. 1, multiple samples of a species are numbered serially. Collection abbreviations: CAS = California Academy of Sciences, CES = Centre for Ecological Sciences, Bangalore, MVZ = Museum of Vertebrate Zoology, Berkeley, PMNH = Pakistan Museum of Natural History, Islamabad, USNM = United States National Museum, Washington.

Species	Musuem No.	Locality	GenBank Accession Numbers			Locality number
			ND2	RAG1	PDC	
<i>Cyrtodactylus annandalei</i>	CAS 215722	Myanmar, Sagaing Divisioin, Alaungdaw Kathapa National Park	JX440524	JX440683	JX440633	1
<i>Cyrtodactylus ayeyarwadyensis</i> 1	CAS 216459	Myanmar, Rakhine State, Than Dawe District	JX440526	JX440634	JX440685	2
<i>Cyrtodactylus ayeyarwadyensis</i> 2	CAS 222812	Myanmar, Ayeyarwady Division, Mwe Hauk Village	GU550715			3
<i>Cyrtodactylus battalensis</i>	PMNH 2301	Pakistan, NWFP, Battagram City	KC151983	KC152035	KC152007	4
<i>Cyrtodactylus brevidactylus</i> 1	CAS 214104	Myanmar, Mandalay Division, Popa Mountain Park	JX440527	JX440636	JX440687	5
<i>Cyrtodactylus brevidactylus</i> 2	CAS 214105	Myanmar, Mandalay Division, Popa Mountain Park	GU550714			5
<i>Cyrtodactylus chrysopylos</i>	CAS 226141	Myanmar, Shan State, Ywa Ngan Township	JX440531	JX440639	JX440690	6
<i>Cyrtodactylus fasciolatus</i> 1	CES11/1337	India, Himachal Pradesh, Shimla District, Nr. Subathu	KM255184	KM255120	KM255143	7
<i>Cyrtodactylus fasciolatus</i> 2	CES11/1269	India, Himachal Pradesh, Shimla District, Nr. Tattapani	KM255171	KM255121	KM255144	8
<i>Cyrtodactylus cf fasciolatus</i> 1	CES11/1345	India, Uttarakhand, Almora District, Almora	KM255169		KM255145	9
<i>Cyrtodactylus cf fasciolatus</i> 2	CES11/1257	India, Himachal Pradesh, Sirmaur District, Nr. Nahan	KM255185	KM255122	KM255146	10
<i>Cyrtodactylus cf fasciolatus</i> 3	CES09/1196	India, Uttarakhand, Dehradun District, Mussoorie-Kempton Road	KM255172	HM622351	HM622366	11
<i>Cyrtodactylus feae</i>	USNM 559805	Myanmar, Mandalay Division, Popa Mountain Park	JX440536	JX440645	JX440696	5
<i>Cyrtodactylus gansi</i>	CAS 222412	Myanmar, Chin State, Min Dat Township	JX440537	JX440646	JX440697	12
<i>Cyrtodactylus gubernatoris</i>	CES10/1235	India, West Bengal, Kalimpong District, Nr. Lower Mongpong	KM255204	KM255123	KM255147	13
<i>Cyrtodactylus cf. gubernatoris</i>	CES09/1101	Northeast India			KM255148	-
<i>Cyrtodactylus himalayanus</i> 1	CES11/1297	India, Jammu and Kashmir, Doda District, Bhaderwah	KM255173	KM255124	KM255149	14
<i>Cyrtodactylus himalayanus</i> 2	CES11/1307	India, Jammu and Kashmir, Kishtwar District, Kishtwar-Atholi Rd.	KM255186	KM255125	KM255150	15
<i>Cyrtodactylus himalayanus</i> 3	CES11/1317	India, Jammu and Kashmir, Kishtwar District, Nr. Kishtwar	KM255187	KM255126	KM255151	16
<i>Cyrtodactylus khasiensis</i>	CES10/1229	India, Meghalaya, East Khasi Hills District, Cherrapunjee Resort	KM255188	KM255127	KM255152	17
<i>Cyrtodactylus lawderanus</i>	CES11/1343	India, Uttarakhand, Almora District, Almora	KM255189	KM255128	KM255153	9
<i>Cyrtodactylus cf. lawderanus</i> 1	CES11/1330	India, Jammu and Kashmir, Samba District, Nr. Mansar	KM255174	KM255129	KM255154	18
<i>Cyrtodactylus cf. lawderanus</i> 2	CES11/1275	India, Himachal Pradesh, Mandi District, Aut	KM255175	KM255130	KM255155	19
<i>Cyrtodactylus cf. lawderanus</i> 3	CES11/1276	India, Himachal Pradesh, Mandi District, Aut	KM255190			19
<i>Cyrtodactylus cf. lawderanus</i> 4	CES11/1262	India, Himachal Pradesh, Solan District, Sadhupul	KM255176	KM255131	KM255156	20
<i>Cyrtodactylus cf. lawderanus</i> 5	CES11/1253	India, Himachal Pradesh, Sirmaur District, Nr. Nahan	KM255177	KM255132	KM255157	10
<i>Cyrtodactylus cf. lawderanus</i> 6	CES11/1264	India, Himachal Pradesh, Shimla District, Nr. Jutogh	KM255178			21
<i>Cyrtodactylus russelli</i>	CAS 226137	Myanmar, Sagaing Division, Htamanthi Wildlife Sanctuary	JX440555			22
<i>Cyrtodactylus slowinskii</i>	CAS 210205	Myanmar, Sagaing Division, Alaungdaw Kathapa National Park	JX440559		JX440719	23
<i>Cyrtodactylus</i> sp. Abhayapuri	CES09/1137	India, Assam, Bongaigaon District, Nr. Abhayapuri		KM255133	KM255158	24
<i>Cyrtodactylus</i> sp. Chamba	CES11/1291	India, Himachal Pradesh, Chamba District, Nr. Chamba	KM255191	KM255134	KM255159	25
<i>Cyrtodactylus</i> sp. CHNG 1	CES11/1349	India, Arunachal Pradesh, Changlang District, Miao	KM255179			26
<i>Cyrtodactylus</i> sp. CHNG 2	CES13/1459	India, Arunachal Pradesh, Changlang District, Miao	KM255192			26
<i>Cyrtodactylus</i> sp. Glow	CES13/1465	India, Arunachal Pradesh, Changlang District, Glow Lake	KM255193			27
<i>Cyrtodactylus</i> sp. Guwahati	CES09/1127	India, Assam, Guwahati District, Guwahati	KM255194	KM255135	KM255160	28
<i>Cyrtodactylus</i> sp. Jowai	CES10/1228	India, Meghalaya, Jaintia Hills District, Nr. Jowai	KM255195			29
<i>Cyrtodactylus</i> sp. Khellong	CES13/1464	India, Arunachal Pradesh, West Kameng District, Khellong	KM255196			30
<i>Cyrtodactylus</i> sp. Kohora	CES09/1128	India, Assam, Golaghat District, Kohora	KM255170	KM255136	KM255161	31
<i>Cyrtodactylus</i> sp. Mizoram 1	CES13/1455	India, Mizoram, Nr. Aizawl	KM255197			32
<i>Cyrtodactylus</i> sp. Mizoram 2i	CES13/1456	India, Mizoram, Nr. Aizawl	KM255198			32
<i>Cyrtodactylus</i> sp. Nagaland	CES10/1233	India, Nagaland, Kohima District, Khonoma	KM255199	KM255137	KM255162	33
<i>Cyrtodactylus</i> sp. Phuldungsei 1	CES10/1211	India, Tripura, North District, Phuldungsei	KM255200	KM255138	KM255163	34
<i>Cyrtodactylus</i> sp. Phuldungsei 2	CES10/1214	India, Tripura, North District, Phuldungsei	KM255180		KM255164	34
<i>Cyrtodactylus</i> sp. Sikkim	CES09/1197	India, Sikkim, East District, Singtam	KM255181	KM255139	KM255165	35
<i>Cyrtodactylus</i> sp. Tripura 1	CES10/1210	India, Tripura, North District, Rowa Wildlife Sanctuary	KM255201	KM255140		36
<i>Cyrtodactylus</i> sp. Tripura 2	CES10/1225	India, Tripura, North District, Gumti	KM255202			37
<i>Cyrtodactylus</i> sp. Tripura 3	CES10/1218	India, Tripura, West District, Sepahijhala Wildlife Sanctuary	KM255182	KM255141	KM255166	38
<i>Cyrtodactylus</i> sp. Tripura 4	CES10/1216	India, Tripura, West District, Sepahijhala Wildlife Sanctuary	KM255183	KM255142	KM255167	38
<i>Cyrtodactylus tibetanus</i>	MVZ 233251	Tibet, Lhasa, 3 km WNW of Potala Palace	JX440561	JX440722		39
<i>Cyrtodactylus adleri</i>	CES09/1126	India, Nicobar Islands, Nancowry Island			KM255168	-
<i>Cyrtodactylus rubidus</i>	CES13/1445	India, Andaman Islands, South Andaman, Wandoor	KM255203			-

dehydrogenase 2 (ND2, 1041 bp) and partial sequences of two nuclear genes, recombination activating gene (RAG1, 1041 bp) and phosphatidylcholine (PDC, 395 bp). Primers and PCR conditions follow those given in [Bauer et al. \(2013\)](#). We also included published sequences representing the broad *Cyrtodactylus* lineages recovered by [Wood et al. \(2012\)](#), using species of its sister genus *Hemidactylus* as outgroups ([Table S1](#)). Divergence dating analyses used an expanded dataset, in order to broadly represent phylogenetic diversity within the Gekkota and Gekkonidae, and for compatibility with fossil calibrations. We removed multiple closely related sequences and included representative gekkonids and other gekkotans in the divergence dating dataset ([Table S1](#)).

Purification and sequencing of PCR products was carried out at Amnion Biotech Pvt. Ltd. (Bangalore, India). Complementary strands were sequenced in most cases to ensure sequence accuracy. Sequences were aligned with the ClustalW algorithm ([Thompson et al., 1994](#)) implemented in MEGA 5.2 ([Tamura et al., 2011](#)) and finally edited by eye. As these are protein coding genes, translated amino acid alignments were examined to check for premature stop codons to rule out the possibility of sequencing pseudogenes.

2.2. Phylogenetic analyses

Phylogenetic relationships were reconstructed using maximum likelihood (ML) and Bayesian inference (BI). As strongly supported relationships were congruent across single gene trees and the concatenated data, subsequent analyses were performed using the concatenated data (ND2 + RAG1 + PDC). We selected both the optimum partitioning scheme and best-fit models of sequence evolution using Bayesian information criteria and the greedy algorithm with linked branch lengths in PartitionFinder 1.1.0 ([Lanfear et al., 2012](#)). The selected models and partitioning schemes for all analyses are listed in [Table 2](#). Partitioned ML analyses were conducted using RAxML 7.4.2 ([Stamatakis, 2006](#)) implemented in raxmlGUI 1.3 ([Silvestro and Michalak, 2012](#)). As RAxML can apply only a single model across partitions, analyses used GTR + I + G, the most complex selected model ([Table 2](#)), with support assessed using 1000 rapid bootstraps. Partitioned Bayesian analyses were conducted in MrBayes 3.2.2 ([Ronquist et al., 2012](#)) applying the best fit models of sequence evolution to each partition ([Table 2](#)). We used default priors and melting temperature, with branch lengths linked across partitions; and statefreq, revmat, shape, and pinvar unlinked, and ratepr set to variable. Analyses had two parallel runs

with four chains each (three hot and one cold) and were carried out for 5 million generations, sampling every 5000 generations. The two independent runs were determined to have converged to a stationary distribution as the standard deviation of split frequencies was <0.01 long before analyses were terminated. We plotted log likelihood scores and other parameter values from each run against generation time in Tracer 1.6 ([Rambaut et al., 2013](#)) to determine adequate effective sample size (>200) and conservatively discarded the first 25% as burn-in.

2.3. Divergence dating

Divergence dates were estimated in BEAST 1.8.0 ([Rambaut and Drummond, 2007](#); [Drummond et al., 2013](#)) from the concatenated dataset, with input xml files created in BEAUti 1.7.5 ([Drummond et al., 2012](#)). We constrained the analysis to reflect the topology recovered by ML and BI, and applied Yule tree priors ([Drummond et al., 2007](#)) as this is a species-level phylogeny. We used the six partition scheme and substitution models selected in PartitionFinder ([Table 2](#)), with a relaxed uncorrelated lognormal clock model and an uninformative uniform prior for the mean rate of each partition (ucln.mean, $1-10^{-7}$). The lower bound of GTR rate parameters in the substitution model element ([Drummond et al., 2007](#)) were set at 10^{-6} (rare mutations caused issues with convergence) with default values for all other priors. The analysis was run for 130 million generations, sampling every 10,000 generations. We assessed stationarity in Tracer 1.6 ([Rambaut et al., 2013](#)) based on plots of all parameters vs. generation time and effective sample sizes (>200). The first 25% trees were conservatively discarded as burn-in and the maximum clade credibility tree with median heights was summarized using TreeAnnotator 1.7.5 ([Drummond et al., 2012](#)). Unless otherwise specified, divergence estimates are written in the format 'mean' ('95% HPD') mya or ('mean', '95% HPD' mya).

We used four fossil calibrations that have previously been used to date gekkotan phylogenies ([Heinicke et al., 2011](#); [Agarwal et al., 2014](#); [Table 3](#)). We used published values for all calibration priors, except for the most recent common ancestor (mrca) of the Gekkota ([Table 3](#)). The oldest gecko-like lizard is *Eichstaettisaurus schroederi* ([Bauer, 2013](#)) from the Jurassic, and though previously assigned to the Gekkota ([Evans, 1993, 1994](#); [Gauthier et al., 2012](#); [Bauer, 2013](#)) was not considered a gecko in a recent comprehensive review of gekkotan fossils ([Daza et al., 2014](#)). *Cretaceogekko* is the oldest known gekkotan fossil, dated to 97–110 mya ([Arnold and Poinar,](#)

Table 2

Best-fit models of sequence evolution and partitioning schemes selected in PartitionFinder for reconstructions of phylogeny using maximum likelihood (RAxML) and Bayesian inference (MrBayes), and divergence dating using BEAST. Codon position is denoted by cp1–cp3.

	Partition no.	Best model	Partition
RAxML	1	GTR + I + G	ND2 cp1
	2	GTR + I + G	ND2 cp2
	3	GTR + G	ND2 cp3
	4	GTR + G	PDC, RAG1, cp1 + cp2
	5	GTR + G	PDC, RAG1 cp3
MrBayes	1	GTR + I + G	ND2 cp1
	2	GTR + I + G	ND2 cp2
	3	GTR + G	ND2 cp3
	4	HKY + G	PDC, RAG1, cp1 + cp2
	5	HKY + G	PDC, RAG1 cp3
BEAST	1	GTR + I + G	ND2 cp1
	2	GTR + I + G	ND2 cp2
	3	GTR + I + G	ND2 cp3
	4	HKY + I + G	PDC, RAG1, cp1 + cp2
	5	HKY + G	PDC cp3
	6	TrNef + G	RAG1 cp3

Table 3

Calibration priors used in divergence dating analyses.

Node	Prior distribution	Reference for prior distribution
<i>Sphaerodactylus torrei</i> vs. <i>S. roosevelti</i>	Exponential, mean = 3, offset = 15	Heinicke et al. (2011)
<i>Pygopus nigriceps</i> vs. <i>Delma butleri</i>	Exponential, mean = 10, offset = 20	Heinicke et al. (2011)
<i>Woodworthia maculata</i> vs. <i>Oedura marmorata</i>	Exponential, mean = 17, offset = 16	Heinicke et al. (2011)
mrca Gekkota	Exponential, mean = 20, offset = 110	This study

2008), though its placement within the Gekkota is unstable (Daza et al., 2014). While this fossil has been previously used to calibrate the Gekkonidae–Phyllodactylidae split (Heinicke et al., 2011), we interpret it as representing a hard minimum for the Gekkota (Pepper et al., 2011; Agarwal et al., 2014; Daza et al., 2014). The mrca of the Gekkota was calibrated using an exponential prior with mean = 20, offset = 110 (Table 3); the lower bound of which represents the upper age estimate for the oldest known gecko (*Cretaceogecko*, Arnold and Poinar, 2008) and the 95% upper bound overlaps with the oldest gecko-like lizard (*Eichstaettisaurus*, Evans et al., 2004).

2.4. Ancestral area reconstructions

We reconstructed ancestral distributions using the Bayesian binary MCMC method (BBM) implemented in RASP 2.1 (Yu et al., 2012), and ML in Mesquite 2.75 (Maddison and Waddison, 2011). BBM analyses used a 1000 post burn-in trees from MrBayes outputs, with outgroups and multiple samples of single species pruned. We carried out a single run of 1 million generations with 10 chains, sampled every 1000 generations, with the first 25% discarded as burn-in, fixed state frequencies and G among-site variation, maximum number of areas set to four and outgroup distribution considered null. ML ancestral area reconstructions applied model MK1 on the final tree from BEAST analyses, with distribution treated as a categorical multistate variable. While we did not explicitly test for dispersal, dispersals were inferred if any descendant lineage was distributed in an area not strongly predicted (<0.2) as the ancestral area of its mrca.

There are few published biogeographic analyses of the study area (Fig. 1), and we modified the biogeographic provinces of Rodgers and Panwar (1988). We used geological features (mountains, lowlands, rivers) across which there is turnover of *Cyrtodactylus* lineages to designate seven broad areas for reconstruction (Fig. 1). These were defined as, (1) the “Trans-Himalayas” (TH), or areas of the Tibetan Plateau; (2) the “Western Himalayas” (WH), defined as the Himalayas west of western Nepal; (3) the “Eastern Himalayas” (EH), or areas north and west of the Brahmaputra including the Himalayas from eastern Nepal east to northeastern Arunachal Pradesh; (4) “South of Brahmaputra” (SB), the mountains and lowlands of Indo-Burma south and east of the Brahmaputra up to and including the eastern syntaxis (the Patkai-Naga-Arakan Yoma ranges) of the Himalayas; (5) “Burma” (BR), or interior Burma east of the eastern syntaxis and west of the Salween; (6) “Southeast Asia” (SE), areas east of the Salween as far as the Western Pacific, including the Andaman and Nicobar Islands; and (7) “Peninsular India” (PI), defined as areas south of the Himalayas and west of the Garo-Rajmahal Gap including Sri Lanka.

3. Results

3.1. *Cyrtodactylus* phylogeny

ML and Bayesian analyses recovered similar topologies, a few nodes well supported by BI alone, and overall high support for the genus *Cyrtodactylus* and well sampled clades (Fig. 2).

Cyrtodactylus tibetanus (Clade A, Fig. 2) is sister to the main *Cyrtodactylus* radiation, which includes three well supported clades corresponding to those recovered by Bauer et al. (2013): Western Himalayas (Clade B, Fig. 2), Indo-Burma (Clade C, Fig. 2) and Southeast Asia east of the Salween (including the subgenus *Geckoella*; Clade D, Fig. 2). Our samples of *Cyrtodactylus* show high genetic diversity, with 18 distinct lineages at a conservative cutoff of >10% mtDNA genetic distance, and an additional four lineages when the cutoff is lowered to 8%. Clade B includes small-medium sized, morphologically similar species (SVL < 75 mm) distributed in the Western Himalayas corresponding to the subgenus *Siwaligekko* (Khan, 2003). Clade C is the most well sampled in our phylogeny, adding at least 14 putative species to the 10 species sampled by Wood et al. (2012). A number of monophyletic groups with distributions closely related to geological features are recovered as part of Clade C. Very short internodes at the base of Clade C separate a clade with species distributed in interior Burma (Clade E), and the Western Himalayan *C. fasciolatus* Clade (Clade F). A second clade including species distributed in interior Burma (Clade G) is recovered as sister to species from the Eastern Himalayas (Clade H, Fig. 2). The remaining species are members of a large clade distributed largely south of the Brahmaputra (Clade I, Fig. 2), including topotypical material of *C. khasiensis*. With very short internodes that receive moderate support, geckos from eastern Arunachal Pradesh (Clade L) branch just outside a final well supported clade; within which a deep divergence separates the Mountain and Lowland clades (Clade M, N; Fig. 2). The remaining sampled *Cyrtodactylus* are recovered as part of a large clade distributed from Indochina east of the Salween to Papua New Guinea and Australia (Clade D, Fig. 2). Broad relationships within this clade are similar to the more detailed sampling of Wood et al. (2012). *Cyrtodactylus* (*Geckoella*) cf. *collegalensis* is sister to *C. deccanensis*, both Indian species in turn sister to *C. triedra* from Sri Lanka, and the monophyly of the subgenus *Geckoella* is supported by Bayesian analyses only (Clade K, Fig. 2). *C. adleri* from the Nicobar Islands and *C. rubidus* from the Andaman Islands are nested within a largely Sundaic Clade (Clade J, Fig. 2; Clade J, Wood et al., 2012) and are not sister taxa.

3.2. Divergence dating

The BEAST timetree recovered a well supported primarily old world radiation, the (*Cyrtodactylus* + *Hemidactylus*) + Palaeartic naked-toed geckos (PAL) clade (entire timetree with other geckos and outgroups in Fig. S1), in agreement with recently published gekkonid phylogenies (Heinicke et al., 2011, 2012; Gamble et al., 2012; Bauer et al., 2013). The mrca of PAL and *Hemidactylus* + *Cyrtodactylus* was inferred to have begun diversifying 69 (77–60) mya, and *Hemidactylus* and *Cyrtodactylus* diverged from each other 64 (73–56) mya. The divergence between *C. tibetanus* and other *Cyrtodactylus* occurred at 55 (64–47) mya, followed by the Western Himalayan Clade which split off from main *Cyrtodactylus* at 51 (59–45) mya, its mrca dated to 18 (22–15) mya (Fig. 3). The final broad split within *Cyrtodactylus* separating the Indo-Burma Clade from the Southeast Asian Clade occurred 45 (51–39) mya. Among species of the Indo-Burma Clade, almost simultaneous consecutive basal divergences separate Clade E (35, 40–30 mya) and Clade F

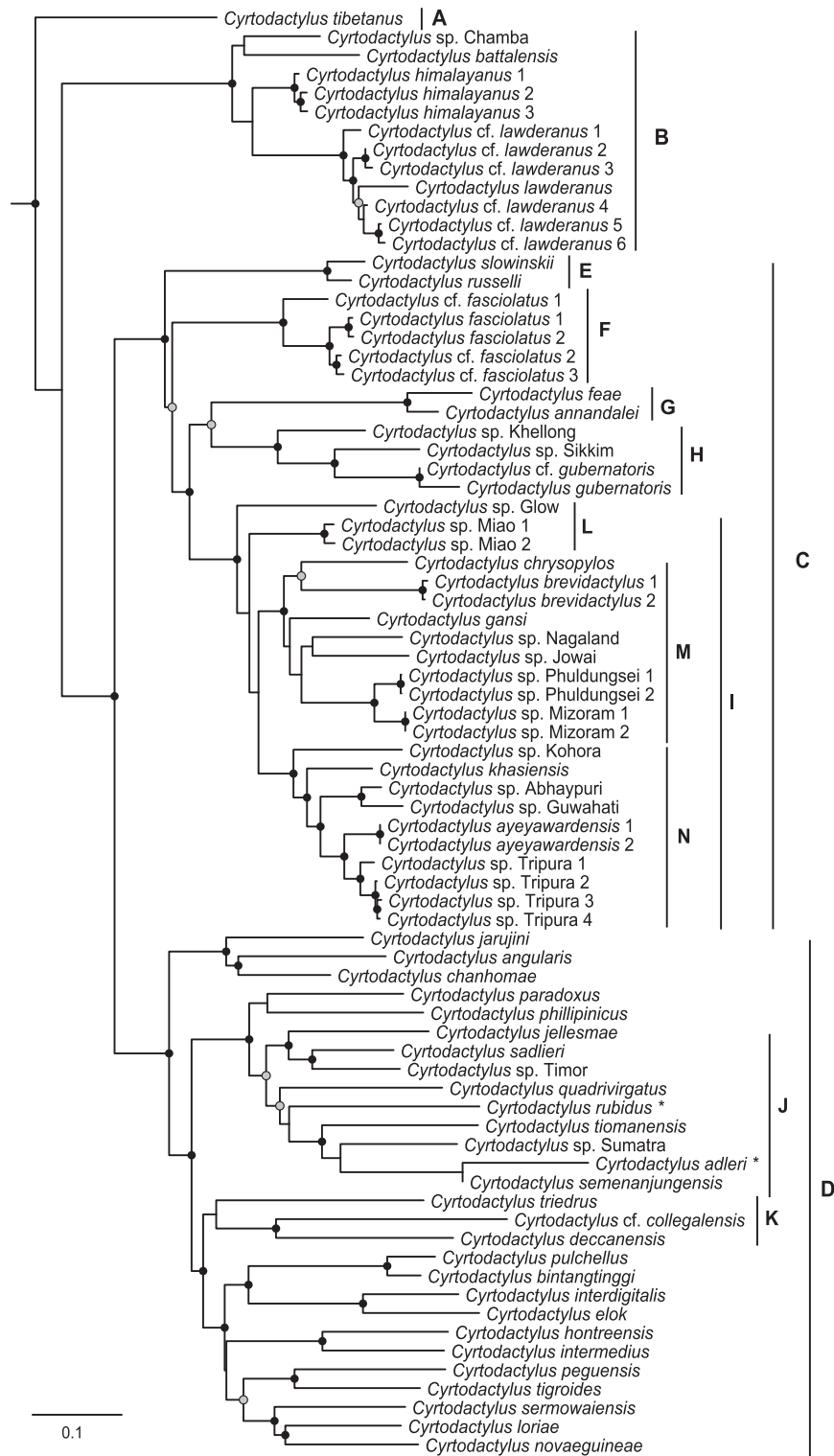


Fig. 2. Maximum-likelihood phylogeny of *Cyrtodactylus* (concatenated data: ND2, RAG1, PDC). Nodes with high support indicated by black-filled circles (ML bootstrap ≥ 70 , Bayesian PP ≥ 0.95), nodes with support only from Bayesian analyses by gray-filled circles (Bayesian PP ≥ 0.95 , ML bootstrap < 70), and nodes with support only from ML analyses by white-filled circles (ML bootstrap ≥ 70 , Bayesian PP < 0.95). Multiple samples of a species are numbered serially (Table 1, S1). Major clades are marked by bold letters: A = Trans-Himalayas, B = Western Himalayas, C = Indo-Burma, D = Southeast Asia, E = Burma 1, F = *C. fasciolatus* Clade, G = Burma 2, H = Eastern Himalayas I = South of Brahmaputra, J = Sunda, K = *Geckoella*. *Hemidactylus* spp. used as outgroups not shown.

(33, 39–29 mya). The South of the Brahmaputra Clade diverged from Clade G + Clade H 31 (36–27) mya, while clades G and H diverged from one other about 28 (33–24) mya. Within the South of the Brahmaputra Clade, species from eastern Arunachal Pradesh diverged about 23–21 (27–18) mya, and the Mountain and

Lowland clades diverged from each other 20 (23–17) mya. The mrca of Southeast Asian *Cyrtodactylus* was dated to 38 (43–33) mya, the mrca of the Sunda clade to 25 (29–22) mya and the Andaman species *C. rubidus* diverged from its closest sisters 22 (26–19) mya while *C. adleri* from the Nicobars diverged

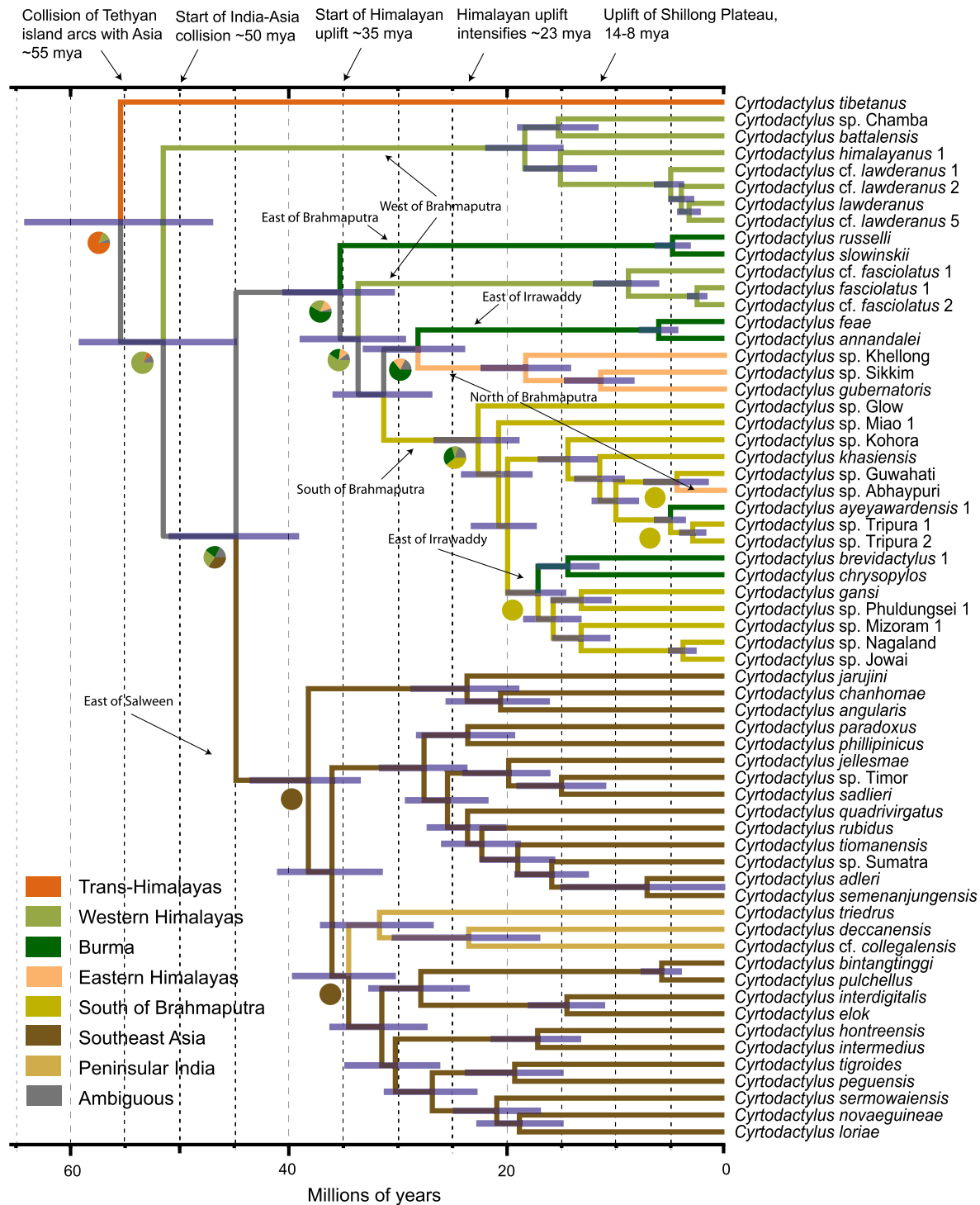


Fig. 3. Bayesian timetree of *Cyrtodactylus* showing ML ancestral range reconstructions with probabilities from BBM analysis shown diagonally below and to the left of key nodes (pie charts). Blue bars at nodes represent 95% HPD, branches are colored by distributional area (areas shown in Fig. 1, details in methods). Timing of major geological events indicated in top row, distributions of *Cyrtodactylus* clades relative to major rivers indicated on branches. Complete timetree with other geckos and outgroups shown in Fig. S1.

from *C. semenanjungensis* 7 (15–0) mya. The mrca of *Geckoella* began diversifying 32 (37–27) mya and the two sampled Indian species split from each other 23 (30–17) mya.

Our divergence estimates for the mrca of Gekkota are similar to a number of studies using overlapping calibrations (Heinicke et al., 2011; Wood et al., 2012; Bauer et al., 2013; Agarwal et al., 2014) though they do not overlap with one recent study, not unexpected given the (inappropriate) use of the gekkonid *Yantarogekko balticus*

(Bauer et al., 2005) as representing the eublepharid/sphaerodactylid split (Jones et al., 2013).

3.3. Ancestral area reconstructions

BBM and ML analyses recovered similar ancestral areas within *Cyrtodactylus* (Fig. 3). A number of geographically cohesive clades were inferred to occupy unambiguous ancestral areas (Fig. 3). We

report the results of BBM analyses for key nodes, discussing only inferred ancestral areas with ≥ 0.2 probability. We recovered the broad pattern of diversification known in *Cyrtodactylus*, indicative of dispersal from west to east (Wood et al., 2012; Bauer et al., 2013). From an ancestral root distribution in the Trans Himalayan region, successive ancestral distributions are in the Western Himalayas, Indo-Burma and Southeast Asia. A number of deeper nodes had ambiguous reconstructed ancestral areas and could not be resolved. The mrca of the Indo-Burma Clade + Southeast Asia Clade was inferred to be distributed in Southeast Asia, the Western Himalayas or Burma (SE 0.4, WH 0.3, BR 0.2); the mrca of the Indo-Burma Clade in Burma or the Western Himalayas (BR 0.6, WH 0.2). Within the Indo-Burma Clade, the distribution of the mrca of clades F–I is inferred as Western Himalayas or Burma (WH 0.6, BR 0.2), of clades G–I as south of the Brahmaputra or Burma (SB 0.4, BR 0.3), and of Clade G + Clade H as Burma or the Eastern Himalayas (BR 0.6, EH 0.2). The mrca of the South of Brahmaputra Clade and all nested clades, except (*C. breviodactylus* + *C. chrysopylos*), was reconstructed to have been distributed south of the Brahmaputra (SB > 0.9). The Andaman and Nicobar species are nested within the Sundaic Clade while *Geckoella* is nested within Clade D, the ancestral distributions of these groups unambiguously reconstructed as Southeast Asia east of the Salween (SE > 0.9). There appears to be a single origin each for lineages in the Trans-Himalayas, south of the Brahmaputra, in Southeast Asia and Peninsular India (Fig. 3). There are two independent lineages each in the Western Himalayas, Eastern Himalayas; and four lineages that are distributed in Burma.

4. Discussion

4.1. Phylogeny, species diversity and endemism

Our reconstructions of *Cyrtodactylus* relationships are similar to those of Wood et al. (2012) and Bauer et al. (2013) and this work has provided greater resolution within the Himalayan and Indo-Burma clades, allowing the recognition of geographically coherent groups. We sampled all described Indian *Cyrtodactylus* from the Himalayas, northeast India as well as the Andaman and Nicobar Islands. Our phylogeny recovers 22 genetically divergent *Cyrtodactylus* lineages from the Himalayas and Northeast India that are likely to represent distinct species. The five valid species of *Cyrtodactylus* from the region are represented in our phylogeny by topotypical material, and our conservative estimate of 17 undescribed species potentially increases the currently recognized diversity of this group from these regions by more than threefold. The outcome of the first extensive geographic sampling across this region, our results highlight the biodiversity of *Cyrtodactylus* in the Himalayas and Indo-Burma, as well as how little is known of the biodiversity of the region. These geckos are common across their range and many of the populations sampled by us have been observed by field biologists during the last 20 years (e.g. Pawar and Birand, 2001; Chettri and Bhupathy, 2007; Das et al., 2007; Mahony et al., 2009; Agarwal et al., 2010; Purkayastha et al., 2011), but lack of obvious morphological variation and no systematic taxonomic effort has resulted in their placement in the catchall species *C. khasiensis*. Wood et al. (2012) observed that the Indo-Burma clade of *Cyrtodactylus* encompasses the morphological diversity of the entire genus. Interestingly, the morphologically distinct Burmese species (Bauer, 2002, 2003) are nested within larger clades that include the sampled Indian *Cyrtodactylus*. This may simply be reflective of poor taxonomic practice, with ongoing work to identify diagnostic morphological characters and describe the genetically divergent unnamed lineages as new species (Agarwal and Giri, unpublished data).

The Eastern Himalayas and Northeast India are emerging as important regions of endemism, with recent molecular studies of amphibians and lizards uncovering a deep history and/or endemic radiations in the region (Che et al., 2010; Kamei et al., 2012; Wood et al., 2012; Datta-Roy et al., 2013). *C. sp.* Sikkim and *C. gubernatoris* were collected just 33 km straight-line distance apart, from the same river valley at altitudes between 200 and 450 m, yet are estimated to have diverged 11 (15–8) mya with 17% ND2 sequence divergence. Species pairs from the mountain and lowland clades South of the Brahmaputra diverged 20 (23–17) mya with an average of 21% ND2 sequence divergence between the clades, but at least two pairs were found within 60 km of each other, indicative of similar long-term restrictions on gene flow. Species turnover within *Cyrtodactylus* is at a much finer scale than our geographic sampling, with only a few samples from unique localities likely to represent conspecific lineages (ND2 sequence divergence < 6%), in the Western Himalayas and lowlands of Northeast India and adjacent Burma. It is clear that *Cyrtodactylus* diversity in the region remains vastly underestimated, as most areas of this complex mountainous landscape remain completely unexplored by field biologists, and we expect additional sampling to reveal many more undescribed species. Potential distributions of amphibians and reptiles in northeast India demonstrate poor congruence with the existing protected area network (Pawar et al., 2007), and baseline information on diversity and distribution across taxonomic groups from the Himalayas and northeast India is essential if we are to make informed decisions to most effectively conserve biodiversity. Broad geographic sampling and robust phylogenies are key to characterizing basic alpha diversity and unraveling patterns of historical diversification across these biodiversity hotspots.

4.2. The story of the India–Asia collision as told by geckos

With over 400 described species, the clade containing the groups (*Cyrtodactylus* + *Hemidactylus*) + PAL includes over 40% of described gekkonid species (Uetz, 2014). With a likely Palaeartic/Laurasian origin for the entire clade (Carranza and Arnold, 2006; Bauer et al., 2010, 2013; Wood et al., 2012), divergences of these groups from one another overlap with the K/T boundary (69–64 mya; 95% HPD 77–56 mya). Basal divergences within each group are in the time interval 62–52 mya (95% HPD 70–45 mya), coincident with the timing of initial India–Asia contact and/or collision of micro-continental blocks with India/Asia (Rehman et al., 2011; Meng et al., 2012; Metcalfe, 2013) and the start of faunal exchange (Rust et al., 2010; Clementz et al., 2011). Endemic to mid and high elevations of the Western Ghats, *Davidogecko anamallensis* diverged from ancestral *Hemidactylus* in the Eocene (Fig. S1), suggesting potential transmarine dispersal from the Palaeartic preceding India–Asia collision (Bansal and Karanth, 2013). Simultaneous basal divergences within PAL and *Cyrtodactylus* separate a single lineage each on the Tibetan Plateau, sister to the remaining members within that group, *Altiphylax stoliczkae* on the south-western margin and *C. tibetanus* on the south-eastern margin, respectively. The timing of divergence of these lineages currently restricted to high elevations of the Tibetan Plateau suggests that Tibetan Plateau uplift may have driven their isolation, also concordant with evidence that suggests coincident India–Asia collision to the east and west (Zhang et al., 2012). Whereas *Altiphylax* is distributed further west in mountainous regions (Bauer et al., 2013), the lineage leading to *C. tibetanus* does not seem to have moved out of the eastern Tibetan Plateau (see next section for a discussion on Tibetan Plateau *Cyrtodactylus*). During the same time period, species in the Western Himalayan clade separated from remaining *Cyrtodactylus* distributed eastward.

Temporally overlapping basal divergences within three independent groups whose ancestors were in proto-Tethyan regions

or mainland Asia north of the Ceno-Tethys by the early Eocene, indicates the India–Asia collision has profoundly influenced diversification, possibly by creating barriers to east–west gene flow or through fragmentation of existing ranges, with post-collision orogeny contributing to isolation of Tethyan lineages (Wood et al., 2012). Refined geological models and dated phylogenies of multiple taxa will uncover generalities in the biological impacts of the India–Asia collision.

4.3. *Cyrtodactylus* biogeography

Ancestral *Cyrtodactylus* were in the proto-Himalayan region by the early Eocene, and basal divergences in a broadly west–east pattern (Wood et al., 2012) within just 10 million years in the middle Eocene separated geographically coherent clades – Trans-Himalayas, Western Himalayas, Indo-Burma, and Southeast Asia east of the Salween. *Cyrtodactylus* appears to have first dispersed east of the present-day course of the Brahmaputra in the middle Eocene, and one lineage then crossed the Irrawaddy and Salween in the late Eocene to early Oligocene giving rise to the Southeast Asian Clade. Subsequently, a single lineage appears to have dispersed south of the Brahmaputra in the early Miocene, with a single back-dispersal in the Pliocene, and sampled members of the Indo-Burma Clade appear to crossed the Irrawaddy twice in the middle to late Miocene. Our results confirm the Salween River as a historical barrier for *Cyrtodactylus*, with only a single dispersal across it, separating the Indo-Burma and Southeast Asian clades (Wood et al., 2012). Our broad sampling across Indian regions of *Cyrtodactylus* distribution, including the most geographically proximate regions of *Cyrtodactylus* distribution to the range of the endemic Indian clade *Geckoella*, allows us to rule out an over-land back dispersal of *Geckoella* into India, and trans-oceanic dispersal across the Bay of Bengal is the most likely explanation for the origin of *Geckoella* (Wood et al., 2012). The Andaman and Nicobar Islands were also formed by the India–Asia collision and represent the submerged southern extensions of the eastern syntaxis (Valdiya, 2010). Broad biogeographic affinities of reptiles in the Andamans are with Burma and Indochina, while those of the Nicobars with Sunda (Smith, 1943; Das, 1996, 1999). *Cyrtodactylus* on the islands deviate from this pattern, having moved in from proximate Sundaic regions with an independent colonization each in the Andamans and Nicobars.

Poor support for relationships at the base of the *Cyrtodactylus* phylogeny may be the result of a lack of taxon sampling in this part of the tree; or may reflect relatively rapid diversification early in the history of the genus, although the time calibrated tree (Fig. 3) suggests less rapid speciation than the very short internal branches of the likelihood tree (Fig. 2). The position of *C. tibetanus* as the sister to other *Cyrtodactylus* may be an artefact of missing data, as this taxon has only 865 bp (492 ND2, 373 RAG1) of sequence data, and some unconstrained BEAST runs recovered a poorly supported *C. tibetanus* + Western Himalayan clade. These bent-toed geckos are morphologically similar, and further gene and taxon sampling may reveal a monophyletic Western Himalayan + Trans Himalayan clade. *C. zhaomii* and *C. medogensis* are also distributed in the Tibetan Plateau and resemble both *Altiphylax* and *C. tibetanus* in aspects of superficial external morphology. Additional molecular data is essential to ascertain relationships, although based on color photographs (Daming, 2002; Shi and Zhao, 2010), both species are likely to be allied with *Altiphylax*.

Strikingly, while the Himalayan and Indo-Burma clades have similar stem ages, the Western Himalayan clade is relatively species-poor, and crown-group diversification begins in the middle Miocene, compared to Oligocene diversification in the Indo-Burma clade. This may be an artefact of the much larger geographic area across which the Indo-Burma clade has diversified and/or unequal

sampling effort in these regions, and we have certainly missed lineages in the vast Himalayas and Trans-Himalayas; though our geographic sampling spans the known east–west range of most clades. While recent Pleistocene glaciation is thought to have affected the Western Himalayas more severely (Srinivasan et al., 2013), this data is indicative of relatively long-term stability in northeast India from the Oligocene onward and/or increased extinctions in the Western Himalayas from the Eocene to Middle Miocene.

Ancestral area reconstructions for the mrca of the Indo-Burma clade were ambiguous, though we interpret the distribution of its sub-clades to indicate a possible ancestral distribution in the mountains north of the present day Eastern Himalayas and/or Burma, as basal divergences separate species in the Western Himalayas, Eastern Himalayas north of the Brahmaputra, and Burma west of the Salween; with only a single dispersal south of the Brahmaputra. Species south of the Brahmaputra are separated into a Mountain Clade (average elevation 740 m, range 340–1300 m) and a Lowland Clade (170 m, 6–900 m). While at least one lineage from the Mountain Clade has dispersed into Burma and is found at lower elevations, members of this clade have not crossed the Brahmaputra, perhaps because appropriate mountain habitats do not occur close to the river. In contrast, the lowland clade appears to show a single dispersal north of the Brahmaputra, though this clade has not moved into Burma except along the coastal lowlands, indicating mountains may be a barrier for its members (Fig. 1). This clade also has the only known widely distributed species in Indo-Burma, with *C. sp. Tripura* and *C. ayeyawardensis* known from multiple localities across the lowlands of Tripura and of Burma and Bangladesh respectively (Bauer, 2003; Mahony et al., 2009). A single lineage each from the mountain and lowland clades were found on the Shillong Plateau, each having diverged from its sister lineage 13–11 (16–9) mya, concordant with uplift-linked diversification as orogeny in the Shillong Plateau was complete between 14 and 8 mya (Clark and Bilham, 2008). Further sampling from eastern Arunachal Pradesh and adjacent Burma may reveal that species from this region form a single clade; while additional sampling from the Eastern Himalayas may reveal that the deep divergence between *C. sp. Sikkim* + *C. gubernatoris* and *C. sp. Khellong* represents a case similar to the Mountain and Lowland clades in the South of Brahmaputra Clade, with one lineage restricted to the foothills and the other extending deeper into the mountains.

More than 20 *Cyrtodactylus* species have been described from cave and karst habitats across Southeast Asia in the last twenty years, affirming the affinities of this group with rocky habitats (Ellis and Pauwels, 2012; Wood et al., 2012). We observed *Cyrtodactylus* only on rocks, apart from *C. lawderanus*, which is also found on the ground, and *C. sp. Tripura* which was also observed on muddy substrates and a tree. Our sampling was biased toward rocky areas, however, based on earlier field surveys that revealed *Cyrtodactylus* in the Western Himalayas and northeast India are almost always found among rocks (Agarwal unpubl. data), although we did also search for geckos on trees, foliage and on the ground. *Cyrtodactylus* have a long history in the Himalayas and northeast India, but have not moved south or west out of these regions into Peninsular India. The dominant geckos in Peninsular India, also mainly scansorial and overlapping in size with *Cyrtodactylus*, are the brookii group of *Hemidactylus* (Bauer et al., 2010). *Cyrtodactylus* and *Hemidactylus* have largely complementary global distributions, and the brookii group came into India in the Oligocene, after *Cyrtodactylus* had begun diversifying in the Himalayas and Indo-Burma (Fig. S1). *Cyrtodactylus* are good dispersers, distributed throughout mainland areas they occur in and on many islands in Southeast Asia, some of which have never had terrestrial connections, and the Brahmaputra and Irrawaddy have been crossed multiple times. *Cyrtodactylus* have also made historical climatic shifts into arid regions, with species in interior Burma, the

Tibetan Plateau and members of the subgenus *Geckoella* in Peninsular India. The fact that these geckos have a deep history in the region, have been able to cross aquatic and mountain barriers multiple times and have made historical climatic shifts, combined with their close association with rocks, leads us to believe that alluvial river flood plains and lack of suitable rocky habitat are important barriers to gene flow in *Cyrtodactylus*, and dispersal into Peninsular India may have been restricted from northeast India by the Garo-Rajmahal Gap (Hora, 1949) and from the Himalayas by the Indo-Gangetic flood plains.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2014.07.018>.

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