Phylogenetic Analysis and Molecular Dating Suggest That *Hemidactylus anamallensis* Is Not a Member of the *Hemidactylus* Radiation and Has an Ancient Late Cretaceous Origin

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**Abstract**

**Background of the Work:** The phylogenetic position and evolution of *Hemidactylus anamallensis* (family Gekkonidae) has been much debated in recent times. In the past it has been variously assigned to genus *Hoplodactylus* (Diplodactylidae) as well as a monotypic genus 'Dravidogecko' (Gekkonidae). Since 1995, this species has been assigned to *Hemidactylus*, but there is much disagreement between authors regarding its phylogenetic position within this genus. In a recent molecular study *H. anamallensis* was sister to *Hemidactylus* but appeared distinct from it in both mitochondrial and nuclear markers. However, this study did not include genera closely allied to *Hemidactylus*, thus a robust evaluation of this hypothesis was not undertaken.

**Methods:** The objective of this study was to investigate the phylogenetic position of *H. anamallensis* within the gekkonid radiation. To this end, several nuclear and mitochondrial markers were sequenced from *H. anamallensis*, selected members of the *Hemidactylus* radiation and genera closely allied to *Hemidactylus*. These sequences in conjunction with published sequences were subjected to multiple phylogenetic analyses. Furthermore the nuclear dataset was also subjected to molecular dating analysis to ascertain the divergence between *H. anamallensis* and related genera.

**Results and Conclusion:** Results showed that *H. anamallensis* lineage was indeed sister to *Hemidactylus* group but was separated from the rest of the *Hemidactylus* by a long branch. The divergence estimates supported a scenario wherein *H. anamallensis* dispersed across a marine barrier to the drifting peninsular Indian plate in the late Cretaceous whereas *Hemidactylus* arrived on the peninsular India after the Indian plate collided with the Eurasian plate. Based on these molecular evidence and biogeographical scenario we suggest that the genus *Dravidogecko* should be resurrected.

**Introduction**

*Hemidactylus anamallensis*, a gekkonid endemic to the Western Ghats of South India has undergone many taxonomic revisions, yet its phylogenetic position and taxonomic status remains unresolved. This species was originally described as a member of *Hoplodactylus* [1,2], a genus in the family Diplodactylidae that is confined to New Zealand. Smith [3] assigned it to a new monotypic genus 'Dravidogecko' on the basis of its phalangeal taxonomy [3,6,10,11]. Russell [6] synonymised *Hemidactylus* with *Hoplodactylus* and genera closely allied to *Hemidactylus*. Underwood [4] and Kluge [5] also demonstrated that *Dravidogecko* was a gekkonid gecko and not a member of the family Gekkonidae. Bauer et al. [13] sequenced from 30 species sampled from around the world. Their phylogenetic analysis resulted in five well supported clades. Three subsequent studies that included additional species (around 14) also retrieved similar clades. They also suggested that *H. anamallensis* could be a primitive *Hemidactylus*. *Hemidactylus* is a species rich genus with 122 recognised species [9] distributed worldwide and has been identified predominantly on the basis of its phalangeal taxonomy [3,6,10,11]. Russell [6] suggested that the genera *Briba*, *Cosymbotus*, *Dravidogecko* and *Teratolepis* also belong to *Hemidactylus*. Carranza and Arnold [12] undertook one of the most comprehensive phylogenetic studies of *Hemidactylus* based on mitochondrial 12S rRNA and cytochrome b sequences from 30 species sampled from around the world. Their phylogenetic analysis resulted in five well supported clades. Three subsequent studies that included additional species (around 14) also retrieved similar clades [13–15]. In Carranza and Arnold [12] phylogenetic relationships of *Hemidactylus* (distributed in Southeast Asia) and *Briba* (monotypic genus from Brazil) were deeply nested within the *Hemidactylus* group, hence they synonymised these genera with *Hemidactylus*. Bauer et al. [13], using molecular data from five
genes, showed that *Tarentola* was deeply embedded within the tropical Asian clade of *Hemidactylus* along with the ground dwelling geckos endemic to Indian subcontinent. Therefore, they synonymised it with *Hemidactylus*, renaming it as *Hemidactylus imbricatus*. These studies did not include *H. anamallensis*. Thus, its affinity to *Hemidactylus* based on morphological data needs to be evaluated using molecular data.

Within the *Hemidactylus* radiation, *H. anamallensis* has been assigned to the *H. bowringii* complex in the tropical Asian clade by Zug et al. [16]. Whereas Bauer et al. [13] suspected that *H. anamallensis* is part of a highly derived lineage, consisting of *H. albofasciatus-imbricatus-reticulatus* within the *H. bowringii* complex in the tropical Asian clade. Thus, both the above scenarios would predict *H. anamallensis* to be deeply nested within the *Hemidactylus* radiation, but differ with respect to its exact phylogenetic position. These scenarios are in sharp contrast to Bauer and Russell's [8] hypothesis, wherein they considered *H. anamallensis* to be a primitive *Hemidactylus*, thereby suggesting that phylogenetically it could be sister to all the *Hemidactylus* species. These putative phylogenetic positions of *H. anamallensis* generate very different biogeographical scenarios for the origin and spread of both *H. anamallensis* and other *Hemidactylus* species of the Indian subcontinent. Interestingly, in a recent molecular work by Bansal and Karanth [15], *H. anamallensis* was indeed sister to all the *Hemidactylus* thus supporting Bauer and Russell [8] hypothesis. Nevertheless their results also suggested that “*H. anamallensis*” was genetically distinct from other *Hemidactylus*. However, in their study genera closely allied to *Hemidactylus* were not included, thus a robust evaluation of the phylogenetic position of *H. anamallensis* with respect to the genus *Hemidactylus* could not be undertaken. Therefore, the authors called for a re-examination of its allocation to the genus *Hemidactylus* with additional molecular data from related genera.

The objective of this study was to investigate the phylogenetic position of *H. anamallensis* within the gekkonid radiation. To this end, several nuclear and mitochondrial markers were sequenced from multiple *H. anamallensis* samples and these sequences were combined with published sequences of gekkonids. These alignments were then subjected to multiple phylogenetic analyses. Results from these analyses in conjunction with molecular dating were used to understand the origin and biogeography of *H. anamallensis*.

**Results**

**Phylogenetic position of *H. anamallensis* within Gekkonidae (C-mos and 12S rRNA dataset)**

All tree building methods retrieved a strongly supported clade consisting of the genera *Agamura, Crosohama*, *Cytodactylus, Cynotopus, Geckoella, Hemidactylus, Sterodactylus* and *Tropidolecotes*. Members of this clade, henceforth referred to as deletion clade, also shared a 21 bp deletion in the *C-mos* gene (Bayesian tree shown in figure 1a and b). The relationships between members of the deletion clade were also identical across tree-building methods. Within the deletion clade, *Hemidactylus* (excluding *H. anamallensis*) formed a clade with high support. Additionally it was observed that the members of this *Hemidactylus* clade shared a unique 9 bp insertion in the *C-mos* gene (figure 1b). However, this insertion was not seen in *H. anamallensis*. In all the trees *H. anamallensis* emerged as sister to the rest of the *Hemidactylus* radiation. For a list of sequences used and their accession numbers see table 1.

Clarifying the position of *H. anamallensis* within the clade consisting of *Hemidactylus* and other closely related genera (*RAG-1* and *PDC* dataset)

In all the methods of phylogenetic inference, *H. anamallensis* emerged as sister to *Hemidactylus* and was separated from *Hemidactylus* by a long branch (Bayesian tree shown in figure 2). Genera *Cytodactylus* and *Geckoella* were sister to *Hemidactylus-H. anamallensis* clade. The overall topology of the Bayesian, ML, and MP trees were similar with respect to the relationships among *Cytodactylus, Geckoella, Hemidactylus* and *H. anamallensis*. For a list of sequences used and their accession numbers see table 1.

**Divergence dates estimates**

Bayesian estimation of divergence dates suggests that the ancestral lineage leading to *H. anamallensis* and the remaining *Hemidactylus* (node C) diverged from each other around 68.9 million years ago (mya) (95% HPD 45.15–92.65 mya) (figure 2, table 2). Additionally the lineage leading to the remaining *Hemidactylus* underwent radiation much later around 49.62 mya (Node D, 95% HPD 32.12–67.12 mya) (figure 2, table 2). The divergence dates estimated at the other nodes in this analysis were concordant with the divergence dates from previous studies [17–19].

**Discussion**

The molecular data presented in the current study provided interesting insights into the phylogenetic position of *H. anamallensis* within Gekkonidae. The *C-mos* and 12S rRNA dataset suggested that *H. anamallensis* was part of a large clade consisting of genera such as *Agamura, Cytodactylus, Cynotopus, Geckoella, Hemidactylus, Sterodactylus, and Tropidolecotes* (figure 1). This clade received high posterior probability and bootstrap support and, more importantly the members of this clade shared a 21 bp deletion that was not seen in any other gekkonid. Within the deletion clade *H. anamallensis* was sister to *Hemidactylus. H. anamallensis* and *Hemidactylus* were also retrieved as sister to each other by RAG-1 and PDC dataset. Thus the nuclear markers support Bauer and Russell’s [8] hypothesis that *H. anamallensis* might be a primitive Hemidactylus.

Interestingly in the *C-mos* gene, a 9 bp insertion was observed among *Hemidactylus* (figure 1b). This insertion was unique to the *Hemidactylus* lineage and was not shared with any other Gekkonid including *H. anamallensis*. Furthermore in the RAG-1 + PDC tree *H. anamallensis* was separated form the rest of the *Hemidactylus* by a long branch. Thus among nuclear markers *H. anamallensis* appeared distinct from the remaining *Hemidactylus*.

Our divergence date estimates based on both fossils as well as biogeographical events suggested that the divergence between the lineage leading to *H. anamallensis* and the rest of the *Hemidactylus* lineage occurred around 68.9 mya (95% HPD 48.15–89.65) (figure 2, table 2) in the late Cretaceous. However, the remaining members of the *Hemidactylus* lineage radiated much later around 49.62 mya (95% HPD 36.12–63.12) (figure 2, table 2) in the Eocene. During the late Cretaceous period peninsular Indian landmass was isolated from all other landmasses having separated from Madagascar around 80 mya. Nevertheless fossil evidence suggested that peninsular India, during its northward journey, remained close to Africa and Eurasia until it collided with the Asian plate around 55 mya [20,21]. Thus faunal links between peninsular India and these landmasses were maintained by vulture animals, which were able to surmount minor marine barriers [20]. Interestingly members of the deletion clade (figure 1a), which consisted of genera closely related to *H. anamallensis*, are distributed...
predominantly in Northern Africa and Asia. This distribution pattern suggested that basal radiation within this clade might have occurred on these landmasses. Furthermore during the early stages of this radiation one of the lineages might have dispersed on to the drifting peninsular Indian plate where it eventually evolved into \textit{H. anamallensis}. Much later, around 49.62 mya, the genus \textit{Hemidactylus} underwent radiation (figure 2, table 2) probably on the Asian plate \cite{12} and dispersed to other parts of the world including peninsular India. Recent molecular studies on \textit{Hemidactylus} revealed that India harboured an endemic radiation \cite{14,15}. According to our dating estimate, this Indian radiation occurred around 36.47 mya (Node E) (95% HPD 19.89–53.05 mya) (figure 2, table 2). Taken together these dates suggested that \textit{Hemidactylus} arrived on the Indian plate after peninsular India collided with Asia. During this time \textit{H. anamallensis} was already present in India, having dispersed on to drifting peninsular India before collision. In a recent molecular study a similar late Cretaceous dispersal of frogs on to drifting peninsular India has been reported \cite{22}.

Thus, the dating estimates suggests that \textit{H. anamallensis} has a unique biogeographical history that appears to be very different from that of the remaining \textit{Hemidactylus}. Additionally \textit{H. anamallensis} also appears to be genetically distinct from the remaining \textit{Hemidactylus}. Taken together, these results support the reassignment of \textit{H. anamallensis} to a separate genus by resurrection of \textit{Dravidogecko}, the genus to which \textit{H. anamallensis} was previously assigned. In the past, authors have sunk \textit{Dravidogecko} into \textit{Hemidactylus}, as there were no morphological features that were unique to \textit{Dravidogecko} \cite{7,8,23}. According to Bauer et al. \cite{8} the characteristic undivided lamellae seen in \textit{H. anamallensis} is not unique to this species as it is shared with a highly derived lineage of ground dwelling \textit{Hemidactylus} spp. of South Asia. They suggested that \textit{H. anamallensis} was part of this highly derived lineage within the \textit{H. brookii} complex. However the present study does not support this relationship as in both the phylogenies \textit{H. anamallensis} is not sister to \textit{H. brookii} within the \textit{Hemidactylus} radiation. Thus this character (undivided lamellae) appears to have been secondarily derived in one of the lineages of \textit{Hemidactylus}. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{(a): Bayesian tree based on combined dataset of C-mos and 12S rRNA genes showing the relationships among the members of the family Gekkoninae. The numbers at the nodes represent the maximum likelihood bootstrap/posterior probability. */* Indicates the bootstrap support ≥90%/Bayesian posterior probability of 1, -/- indicates bootstrap support ≤50% and Bayesian posterior probability of ≤0.5. Black arrow represents the node that constitutes the members of the deletion clade and the white arrow represents the node, which separates the taxa with insertion (\textit{Hemidactylus}). (b) C-mos DNA sequence alignment-showing indels among some members of the family Gekkoninae. doi:10.1371/journal.pone.0060615.g001}
\end{figure}
| Sample name                     | Voucher Number | Locality                     | C-mos | 12S rRNA       | RAG-1       | PDC   |
|--------------------------------|----------------|-------------------------------|-------|----------------|-------------|-------|
| *Aristelliger lar*             | JB 01          | Dominican Republic            | -     | -              | EF534805    | EF534847 |
| *Bavayia cyclopa*              | AMB 7683       | New Caledonia                 | -     | -              | HQ 426264   | HQ 426176 |
| *Blasodactylus antongilensis*  | ZCMV 2187      | Nosy Mangabe, Madagascar      | -     | -              | EU054229    | EU054205 |
| *Blasodactylus sakalava*       | WRBM 18        | Wilf's Track, Tolara District, Madagascar | - | -              | EU054227    | EU054203 |
| *Carphodactylus laevis*        | AMS 143258     | Queensland, Australia         | -     | -              | EF534781    | EF534821 |
| *Cnemaspis limi*               | LLG 6267       | Pulau Tioman, Malaysia        | -     | -              | EF534809    | EF534851 |
| *Coleonyx variegatus*          | CAS 205334     | California, USA               | -     | -              | EF 534777   | EF 534817 |
| *Cyrtodactylus aeyawadyensis*  | CAS 216446     | vic. Kanthaya Beach, Rakhine State, Myanmar | - | -              | EU268287    | EU268317 |
| *Cyrtodactylus consobrinus*    | LLG 4062       | Niah Cave, Sarawak, Malaysia  | -     | -              | EU268288    | EU268318 |
| *Cyrtodactylus fasciolatus*    | CES 091196     | Kempty road, Mussoorie, Uttarakhand, India | KC735108 | KC735096 | HM622351 | HM622366 |
| *Cyrtodactylus gubernatoris*   | CES 1197       | Singhtum, Sikkim             | -     | -              | KC735086    | KC735091 |
| *Cyrtodactylus khasiensis*     | CES 1101       | Northeast India              | -     | -              | KC735109    | KC735097 |
| *Cyrtodactylus laroe*          | FK 7709        | N slope of Mt. Simpson, Bunisi, Milne Bay Province, Papua New Guinea | - | -              | EU268289    | EU268319 |
| *Cytopodiscus scabrum*         | CES1104        | Sam, Rajasthan               | KC73510 | KC735098 | - | - |
| *Cytopodiscus kachensis*       | CES1146        | Kutch, Gujarat               | KC73511 | KC735099 | - | - |
| *Cytopodiscus species*         | CES1107        | Kuno, Madhya Pradesh         | KC73512 | KC735100 | - | - |
| *Delma Tincta*                 | AMS 151607     | Sturt Natl.PK., NSW, Australia | - | -              | HQ 426277   | HQ 426188 |
| *Diploactylus conspicillum*     | AMS 158426     | Sturt Natl. Park, NSW, Australia | - | -              | HQ 426278   | HQ 426189 |
| *Elgaria kingii*               | TG 00065       | Navajo County, Arizona, USA   | -     | -              | AY662603    | HQ426252 |
| *Eublepharis macularius*       | JS 2           | Pakistan                     | -     | -              | EF 534776   | EF 534816 |
| *Euleptes europaea*            | -              | Liguria, Italy               | -     | -              | EF534806    | EF534848 |
| *Geckoella collargensis*       | CES 1136       | Mumbai, Maharashatra         | -     | -              | KC735087    | KC735092 |
| *Gekko gecko*                  | No ID          | unknown                      | -     | -              | EF534813    | EF534854 |
| *Goniurosaurus araneus*        | JFBM 15830     | Vietnam                      | -     | -              | HQ 426286   | HQ 426197 |
| *Gymnactylus amarali*          | CHUNB 38646    | Cocalzinho, Goiás, Brazil    | -     | -              | HQ 426288   | HQ 426199 |
| *Heloderma suspectum*          | TG 00068       | Arizona, USA                 | -     | -              | AY662606    | HQ426254 |
| *Hemidactylus anamallensis 1*  | CES 08029      | Vadiyoor, Eravikulam, Tamil Nadu, India | KC735113 | HM595680 | HM622353 | HM622368 |
| *Hemidactylus anamallensis 2*  | CES 08030      | Vadiyoor, Eravikulam, Tamil Nadu, India | KC73514 | KC735101 | KC735088 | KC735093 |
| *Hemidactylus anamallensis 3*  | CES 10002      | Wayanad, Tamil Nadu, India    | KC73515 | KC735102 | KC735089 | KC735094 |
| *Hemidactylus anamallensis 4*  | CES 10003      | Wayanad, Tamil Nadu, India    | KC73516 | KC735103 | - | - |
| *Hemidactylus anamallensis 5*  | CES 10004      | Wayanad, Tamil Nadu, India    | KC73517 | KC735104 | KC735090 | KC735095 |
| *Hemidactylus angulatus*       | MVZ 245438     | Nigeria, Togo Hills, Nkawanta | HQ426540 | - | EU268306 | EU268336 |
| *Hemidactylus angulatus 1*     | E1708.15       | Kajiado District, Rift valley, Kenya | - | DQ120412 | - | - |
| *Hemidactylus bowringii*       | CES 08008      | Sikkim, India                | -     | -              | HM622354    | HM622369 |
| *Hemidactylus brooki* 2        | CES 06080      | Palakkad, Kerala, India      | KC735118 | HM595685 | HM622355 | HM622370 |
| *Hemidactylus fasciatus 2*     | -              | Rabí, Gabon                   | -     | -              | EU268309    | EU268339 |
| *Hemidactylus frenatus 2*      | CES 07035      | Athirapalli, Valparai, Tamil Nadu, India | KC735119 | KC735105 | HM622371 | HM622356 |
### Table 1. Cont.

| Sample name                  | Voucher Number | Locality                                      | C-mos          | 12S rRNA        | RAG-1          | PDC        |
|------------------------------|----------------|-----------------------------------------------|----------------|----------------|----------------|------------|
| Hemidactylus giganteus       | CES 07013      | Nandi Hills, near Bangalore, Karnataka, India | KC735120       | KC735106       | -              | -          |
| Hemidactylus giganteus       | CES 08013      | Hampi, Karnataka, India                      | -              | -              | HM622357       | HM622372   |
| Hemidactylus graniticolous   | CES 08028      | Nilgiri Hills, Tamil Nadu, India              | -              | -              | HM622361       | HM622375   |
| Hemidactylus greelli         | CAS 219044     | Praia da Mutamba, Sao Tome Island, Sao Tome and Principe | HQ426542      | -              | EU268308       | EU268338   |
| Hemidactylus greelli         | E7014.4        | Principe, Sao Tome and Principe               | -              | DQ120414       | -              | -          |
| Hemidactylus haitanus        | AMB 4189       | Dominican Republic (1), Santo Domingo        | HQ426543       | -              | -              | -          |
| Hemidactylus haitanus 1      | Hhaits5        | Matanzas, Matanzas province, Cuba            | -              | DQ120388       | EU268311       | EU268341   |
| Hemidactylus haitanus 2      | CAS 198442     | near Santo Domingo, National Dist., Dominican Republic | -              | -              | EU268307       | EU268337   |
| Hemidactylus mabouia         | E609.20        | Lake Nabugabo, Masaka District, Uganda       | -              | DQ120377       | -              | -          |
| Hemidactylus mabouia         | MCZ R-184446   | Limpopo Province, South Africa               | -              | -              | EU268300       | EU268330   |
| Hemidactylus mabouia         | JME 1864       | Wundanyi, Kenya                              | HQ426546       | -              | -              | -          |
| Hemidactylus maculatus       | BNHS 1516      | Zirad, Raigadh dist., Maharashtra, India     | -              | -              | HM559707       | HM559674   |
| Hemidactylus palaiichthus    | LSUMZ H-12421  | Roraima State, Brazil                        | -              | -              | EU268307       | EU268337   |
| Hemidactylus persicus 2      | CES 08027      | Nabh Dongar, Jaisalmer, Rajasthan, India     | KC735121       | KC735107       | HM622362       | HM622376   |
| Hemidactylus platyrurus 2    | CES 08025      | Kalimpong, West Bengal, India               | -              | -              | HM622363       | HM622377   |
| Hemidactylus robustus        | MVZ 248437     | 40 km South of Mipur Sakro, Thatta District, Pakistan | -              | EU268315       | EU268345       |            |
| Hemidactylus turcicus        | LSUMZ H-1981   | Baton Rouge, Louisiana, USA                  | -              | EU268299       | EU268329       |            |
| Homonota fasciata            | TG 00085       | Paraguay                                     | -              | EU293629       | EU293697       |            |
| Lepidodactylus lugubris      | AMB 4111       | Kirimati, Kiribati                           | -              | -              | EFS34812       | EFS34853   |
| Lialis burtonis              | TG 00078       | Provinci Papua, Indonesia                    | -              | EF534782       | EF534822       |            |
| Narudasia festiva            | AMB 3243       | Narudas, Namibia                             | -              | EFS34808       | EFS34850       |            |
| Nephurus millii              | AMB 499        | Western Australia, Australia                 | -              | EFS34780       | EFS34820       |            |
| Oedura marmorata             | AMS 143861     | Queensland, Australia                        | -              | EFS34779       | EFS34819       |            |
| Paradelma orientalis         | QM-J56089      | 20 km N Capella, Queensland, Australia       | -              | HQ426304       | HQ426215       |            |
| Phelsuma madagascariensis    | FG/MV 2002.797 | Manongarivo, Madagascar                      | -              | EFS34811       | AB081507       |            |
| Phyllodactylus xantii        | ROM 38490      | Baja California Sur, Mexico                  | -              | EF534807       | EF534849       |            |
| Phyllodactylus xantii        | ROM 38490      | Baja California Sur, Mexico                  | -              | EFS34807       | EFS34849       |            |
| Pristurus catteri            | TG 00083       | Yemen                                        | -              | EFS34803       | EFS34845       |            |
| Pygopus nigriceps            | AMB 53         | Northern Territory, Australia                | -              | EF534783       | EF534823       |            |
| Rhoptropus boultoti          | CAS 214713     | Twyfellfontein, Namibia                      | -              | EFS34810       | EFS34852       |            |
| Sphaerodactylus elegans      | YPM 14795      | Florida, USA                                 | -              | EFS34787       | EFS34828       |            |
| Tarentola Americana          | MVZ 241223     | 13 km E of Pilon, Granma Province, Cuba      | -              | HQ426332       | HQ426243       |            |
| Teratoscincus raborowskii    | TG 00070       | China                                        | -              | EFS34799       | EFS34841       |            |
| Thecadactylus salmoensis     | KU 214929      | Cuzco Amazonico, Madre de Dios, Peru          | -              | EU293644       | EU293711       |            |

Sequences generated by the authors have accession numbers starting with KC. For a complete list of C-mos and 12S rRNA sequences see Feng et al. [25].

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Materials and Methods

Sample collection and DNA sequencing

Genera that are purported to be closely related to *Hemidactylus* such as *Cyrtodactylus*, *Cyrtopodian*, *Geckoella* as well as *H. anamallensis* were collected opportunistically from across India (table 1). Total DNA was extracted from the tail clippings stored in absolute alcohol following standard proteinase K protocol [24]. Three nuclear, C-mos, recombination activation gene (*RAG-1*) and phosducin (*PDC*), and one mitochondrial marker, 12S ribosomal RNA (*12S rRNA*), were PCR amplified from the above samples. All PCR amplifications were carried out in 25 µl reaction volume, with 1.5 unit of Taq DNA polymerase (Bangalore Genei, Bangalore, India), 0.25 mM of dNTP's (Bangalore Genei), 2.0 mM of MgCl2, 1 ul of 0.5 mg/ml of BSA, 0.1 mM (Sigma) of each primer and 40 ng of DNA. Primer combinations and thermocycler conditions are given in supporting information (tables S1 & S2). PCR products were purified using QIAquick PCR Purification kit (Qiagen) and sequences were obtained commercially from Eurofins Biotech Pvt. Ltd. (Bangalore, India).

For the remaining genera of the family Gekkonidae, sequences were downloaded from GenBank (table 1). Percent sequence generated for this study: C-mos 30%, 12S rRNA 20%, RAG-18%, PDC 8%.

Phylogenetic analyses

The sequences generated here were combined with published sequences to derive two different datasets. First, to determine the
The phylogenetic position of *H. anamallensis* within Gekkonidae, the sequences generated by us were added to a combined dataset of the nuclear *C-mos* and mitochondrial 12S rRNA genes generated by Feng et al. [25]. To clarify the position of *H. anamallensis* within the clade consisting of *Hemidactylus* and other closely related genera: RAG-1 and PDC datasets generated by Bauer et al. [13], Gamble et al. [17] and Bansal and Karanth [15] were used. In both the above datasets representatives from all the five clades of the *Hemidactylus* radiation were included. These sequences were aligned using ClustalW 1.6 [26] in the software MEGA v. 4.1 [27], using default parameters. These two datasets were then subjected to maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses. The two datasets could not be combined because there was a lack of overlap in sequence data between them. The *C-mos*+12S rRNA dataset generated by Feng et al. [25] had sequences largely for family Gekkonidae, thus this dataset was useful in inferring the position of *H. anamallensis* within Gekkonidae radiation. However RAG-1 + PDC dataset generated by Gamble et al. [17] had representatives of all the closely related families of Gekkonidae and therefore was useful in molecular dating (see below). Furthermore, in the case of RAG-1+ PDC extensive sequence data was available for *Hemidactylus* from previous works by Bauer et al. [13], and Bansal and Karanth [15]. Thus this dataset was also useful in clarifying the position of *H. anamallensis* within the clade consisting of *Hemidactylus* and other closely related genera.

The MP tree was derived through a heuristic search in in PAUP* version 4.0b10 [28] with tree bisection–reconnection branch swapping and 10 replicates of random addition options. Here transversions were weighted based on empirically determined transition/transversion ratios. Supports for various nodes were evaluated through 1000 replicates of bootstrapping in parsimony analysis. Phylogenetic inference using ML algorithm was also performed in PAUP with the substitution model chosen by MODELTEST [29] and tree bisection–reconnection branch swapping and 10 replicates of random addition options. Since PAUP does not allow for partitioning the dataset for ML search, another ML tree was derived in RAxML [30] wherein the dataset was partitioned. Bayesian analysis was run in Mr. Bayes version 3.1 [31] using the mixed model (see supporting information for partitioning scheme) with variable priors for 10⁷ generations with four chains, wherein sampling was undertaken for every 100 generations. All sample points before the stage when the Markov chain reached a stable likelihood value were discarded as burn-in determined in Tracer v 1.4.1 [32]. The remaining trees were imported into PAUP* to generate a majority-rule consensus tree and to derive posterior probabilities for each node. Gaps were treated as missing data for all analyses.

**Analysis of insertions and deletions (indels) in *C-mos* gene**

*C-mos* is a proto-oncogene that encodes the protein serine/threonine kinase that regulates meiotic maturation in germ cells [33]. It is a single-copy gene that lacks introns and repetitive elements. Insertions and deletions in *C-mos* have been reported to be uncommon [34]. However, Han et al. [35] reported a 21 bp deletion in *C-mos* that was shared by some gekkonids. Additionally, our preliminary analysis suggested that members of the *Hemidactylus* radiation shared a 9 bp insertion. Given that indels are quite rare in coding regions, such changes could be used as phylogenetically informative characters for determine the position of *H. anamallensis*. Thus we checked the *C-mos* alignment for the presence of these indels in *Hemidactylus* (including *H. anamallensis*) and other related genera.

**Molecular dating**

The RAG-1 and PDC dataset (1439 characters) was also used to determine the divergence dates among *H. anamallensis*, *Hemidactylus* and other closely related genera. Independent calibrations from previously published studies [17–19] were used to constrain nodes in the divergence date analyses. Two out of five calibrations used in the previous studies were excluded from further analysis by the fossil cross- validation method used by Gamble et al. [17]. The excluded calibrations were (i) the minimum age of *Paralaelurus orientalis*/ *Pygopus nigropes* split, using the fossil *Pygopus hortulanus*, (ii) the maximum calibrations were (i) the minimum age of *Primaderma nesori* [36] was used to constrain the Helodermatidae/ Anguidae split (exponential distribution, mean 3.0, offset 99.0), (ii) Two amber preserved specimens of *Sphaerodactylus* spp. [37,38] were used to constrains the node constituting *Sphaerodactylus* species (exponential distribution, mean 5.0, offset 23.0), (iii) The split of *Tantucus senecus- Tantucus rubroocellatus* [39] which was purported to have occurred due to Tein Shan-Pamir uplift in western China, 10 Ma [40,41] (Normal distribution, mean 10.0, SD 0.5).

The dataset was partitioned into two genes (RAG-1 1044 bp, PDC 395 bp) and the model of sequence evolution as mentioned in supporting information (table S3) was applied to both the partitions. Given that a strict clock model of molecular evolution is purported to be biologically unrealistic [42] a relaxed molecular clock model with uncorrelated lognormal distribution and Yule process tree prior (as recommended for species level phylogenies) were used. These analyses were undertaken in the program BEAST v. 1.6.1 [43]. Base frequencies were estimated in BEAST, and gamma distribution categories were set to four. A default setting for substitution rate was used. The program was run for 5 x 10⁶ generations. Tracer v 1.4.1 [32] was used to determine convergence and effective sample sizes for the run.

**Supporting Information**

**Figure S1** Bayesian estimates of dates based on RAG-1 and PDC dataset. Bootstrap supports and Bayesian posterior probabilities are shown at the base of the nodes. Grey bars indicate the credible intervals. K-T indicates Cretaceous-Tertiary boundary and I/A indicates the date of collision of India with Asian plate. (TIF)

**Table S1** List of Primers used. (DOC)

**Table S2** Thermo cycler profile used for amplification of genes. (DOCX)

**Table S3** Partitioning scheme and model of sequence evolution for the genes in the datasets. The datasets were partitioned according to the genes in both MrBayes and RAxML. (DOCX)

**Table S4** Estimated ages (in Myr) of the nodes and the corresponding 95% CI for the nodes labelled in figure S1. (DOCX)

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Author Contributions

Conceived and designed the experiments: RB KPK. Performed the experiments: RB. Analyzed the data: RB KPK. Contributed reagents/materials/analysis tools: RB KPK. Wrote the paper: RB KPK.