



## Short Communication

# Molecular phylogeny of *Hemidactylus* geckos (Squamata: Gekkonidae) of the Indian subcontinent reveals a unique Indian radiation and an Indian origin of Asian house geckos

Rohini Bansal, K. Praveen Karanth \*

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

## ARTICLE INFO

## Article history:

Received 10 February 2010

Revised 9 June 2010

Accepted 14 June 2010

Available online 18 June 2010

## Keywords:

Commensal

Dispersal-vicariance analysis

Mitochondrial DNA

Nuclear markers

Out-of-India

## ABSTRACT

Represented by approximately 85 species, *Hemidactylus* is one of the most diverse and widely distributed genera of reptiles in the world. In the Indian subcontinent, this genus is represented by 28 species out of which at least 13 are endemic to this region. Here, we report the phylogeny of the Indian *Hemidactylus* geckos based on mitochondrial and nuclear DNA markers sequenced from multiple individuals of widely distributed as well as endemic congeners of India.

Results indicate that a majority of the species distributed in India form a distinct clade whose members are largely confined to the Indian subcontinent thus representing a unique Indian radiation. The remaining *Hemidactylus* geckos of India belong to two other geographical clades representing the Southeast Asian and West-Asian arid zone species. Additionally, the three widely distributed, commensal species (*H. brookii*, *H. frenatus* and *H. flaviviridis*) are nested within the Indian radiation suggesting their Indian origin. Dispersal-vicariance analysis also supports their Indian origin and subsequent dispersal out-of-India into West-Asian arid zone and Southeast Asia. Thus, Indian subcontinent has served as an important arena for diversification amongst the *Hemidactylus* geckos and in the evolution and spread of its commensal geckos.

© 2010 Elsevier Inc. All rights reserved.

## 1. Introduction

Geckos of the genus *Hemidactylus* are one of the most widely distributed and species-rich groups of gekkonids. With over 85 species from world over, the genus *Hemidactylus* accounts for 10% of the total number of species in the family Gekkonidae (Kluge, 2001; Feng et al., 2007). They are found in a range of ecological conditions from wet tropical forest to arid deserts and are distributed throughout the tropical Asia, Africa, and the New World. These geckos appear to have reached South America by natural transmarine colonisation (Kluge, 1969; Carranza and Arnold, 2006 and references therein), which is supported by their life history traits such as eggs with calcareous shell and adhesive glutinous substance, ability to withstand prolonged exposure to seawater (Smith, 1935; Kluge, 1969). Additionally they are able to survive without food and water for long periods by utilising the fat reserves in their tail (Smith, 1935; Kluge, 1969). These attributes have probably contributed to near global distribution of some *Hemidactylus* geckos. The Indian subcontinent harbours three such widely distributed species, namely, *H. brookii*, *H. flaviviridis* and *H. frenatus*.

A recent species level phylogeny of *Hemidactylus* based on mitochondrial cytochrome *b* and 12S rRNA genes by Carranza and Arnold (2006) inferred that this genus consists of five different geographic clades viz. tropical Asian clade, *H. angulatus* clade, Arid clade, *H. mabouia* clade and African-Atlantic clade. The tropical Asian clade comprised of two separate clusters, one constituting the widely distributed species (*H. flaviviridis*, *H. brookii* and *H. frenatus*) and the other one constituting Southeast Asian species (*H. platyurus*, *H. bowringii*, *H. karenorum* and *H. garnotii*). However, their study included only 30 species of *Hemidactylus*, which does not account for even half of the total estimated diversity of the genus. Additionally, only nine of the 28 currently known species from the Indian subcontinent were included in their phylogeny. More importantly, none of the endemic Indian species were analysed in this study. In contrast, another phylogenetic study based on one mitochondrial and four nuclear genes by Bauer et al. (2008) showed that the so-called tropical Asian clade is not monophyletic. In their study, species from the tropical Asia formed two distinct clades; one representing species from India and the other consisting of species from Southeast Asia. Nevertheless, these two clades were not sister groups. Here again, most Indian species have not been included.

Smith (1935) listed 17 species of *Hemidactylus* geckos from Indian subcontinent. These included; *H. bowringii*, *H. brookii*,

\* Corresponding author. Fax: +91 80 23601428x2280.  
E-mail address: [karanth@ces.iisc.ernet.in](mailto:karanth@ces.iisc.ernet.in) (K.P. Karanth).

*H. depressus*, *H. flaviviridis*, *H. frenatus*, *H. garnotii*, *H. giganteus*, *H. gracilis*, *H. karenorum*, *H. leschenaultii*, *H. maculatus*, *H. persicus*, *H. prashadi*, *H. reticulatus*, *H. subtriedrus*, *H. triedrus* and *H. turcicus*. Since then, additional species have been added to the growing list of *Hemidactylus* geckos of India including, *H. albofasciatus*, *H. anamallensis*, *H. imbricatus*, *H. mahendrai*, *H. platyurus*, *H. porbandarensis*, *H. scabriceps* (see Giri and Bauer, 2008 and the references therein).

Recently, four new species, *H. aaronbaueri* (Giri, 2008), *H. sataraisensis* (Giri and Bauer, 2008), *H. treutleri* (Mahony, 2009) and *H. gujaratensis* (Giri et al., 2009) were described from India. Thus, currently at least 28 species of *Hemidactylus* geckos have been recognised from the Indian subcontinent. Amongst these species, at least three are widely distributed in India and their distributions also extend to the other parts of the tropical Old World (Daniel, 2002). These include: *Hemidactylus frenatus*, distributed predominantly in South India and Southeast Asia; *H. flaviviridis*, distributed from the shores of Red sea to much of India and *H. brookii*, distributed throughout the tropical Asia. These three species also occur commensally with humans and are commonly called “house geckos”. House geckos have been unwittingly introduced into Central and South America, Australia, and islands around the world due to anthropogenic activity (Carranza and Arnold, 2006 and the references therein). The other species are mostly confined to Indian subcontinent, except a few species from northeast India, which are also known from neighbouring countries.

As mentioned earlier, the molecular phylogeny of the genus *Hemidactylus* by Carranza and Arnold (2006) used only nine of the 28 species from the Indian subcontinent. Bauer et al. (2008), added three endemic species from Indian subcontinent, but the focus of their work was on the phylogenetic position of *H. imbricatus* (previously known as *Teratolepis fasciatus*). Including the remaining species, particularly the endemic species from India, in the phylogeny is important to test the monophyly of the tropical Asian group (Zug et al., 2007; Giri and Bauer, 2008).

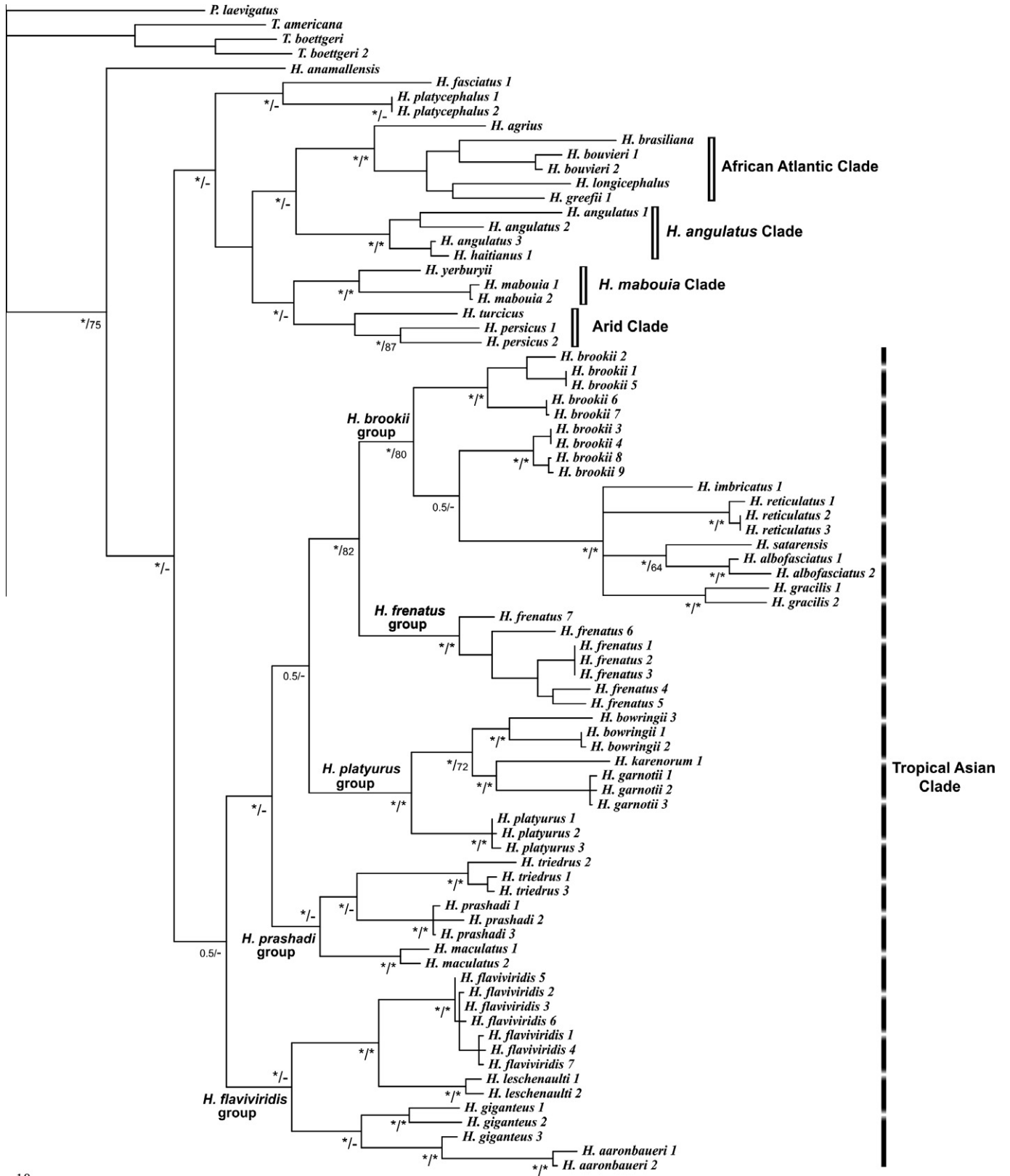
In the present study, 21 out of the 28 species found in the Indian subcontinent have been included with increased sampling of widely distributed *Hemidactylus* species as well as multiple samples of most of the endemics. The mitochondrial DNA (mtDNA) phylogeny was generated using the same two genes as Carranza and Arnold (2006). Additionally, for a subset of these species two nuclear markers were sequenced to generate the nuclear DNA (nDNA) phylogeny. These phylogenies were in turn used to test various phylogenetic hypotheses including, monophyly of species restricted to the Indian subcontinent (Indian clade), monophyly of species that are distributed predominantly in Southeast Asia (Southeast Asian clade), monophyly of species distributed in tropical Asia (tropical Asian clade). The study also attempts to resolve the evolutionary origin and biogeography of commensal house geckos, *H. brookii*, *H. frenatus* and *H. flaviviridis*.

**Table 1**

Details of the specimens for which the nuclear and mitochondrial markers were sequenced in the current study.

Sample name	Voucher number	Locality	GenBank Accession numbers			
			Cyt <i>b</i>	12S	RAG-1	PDC
<i>Cyrtodactylus fasciatus</i>	CES091196	Kemtpy road, Mussoorie, Uttarakhand, India	–	–	HM622351	HM622366
<i>Hemidactylus aaronbaueri</i> 1 <sup>a</sup>	CES/08/022	Pune, near Ghatgh, Maharashtra, India	HM595640	HM595676	–	–
<i>Hemidactylus aaronbaueri</i> 2 <sup>a</sup>	CES/08/016	Peth Fort, Raigadh Dist., Maharashtra, India	HM595641	HM595677	HM622352	HM622367
<i>Hemidactylus albofasciatus</i> 1 <sup>a</sup>	CES/07/038	Malvan, Sindhudurg Dist., Maharashtra, India	HM595642	HM595678	–	–
<i>Hemidactylus albofasciatus</i> 2 <sup>a</sup>	CES/08/018	Malvan, Sindhudurg Dist., Maharashtra, India	HM595643	HM595679	–	–
<i>Hemidactylus anamallensis</i> <sup>a</sup>	CES/08/029	Vadiyoor, Eravikulam, Tamil Nadu, India	HM595644	HM595680	HM622353	HM622368
<i>Hemidactylus bowringii</i> 4	CES/08/008	Sikkim, India	–	–	HM622354	HM622369
<i>Hemidactylus brookii</i> 5	CES/06/036	Tumkur, Karnataka, India	HM595645	HM595681	–	–
<i>Hemidactylus brookii</i> 6	CES/06/078	WII, Dehradun, Uttarakhand, India	HM595646	HM595682	–	–
<i>Hemidactylus brookii</i> 7	CES/06/175	Jammu, Jammu and Kashmir, India	HM595647	HM595683	–	–
<i>Hemidactylus brookii</i> 8	CES/06/119	Amasebailu, Karnataka, India	HM595648	HM595684	–	–
<i>Hemidactylus brookii</i> 9	CES/06/080	Palakkad, Kerala, India	HM595649	HM595685	HM622355	HM622370
<i>Hemidactylus flaviviridis</i> 3	CES/08/021	Bagalkot, Karnataka, India	HM595650	HM595686	–	–
<i>Hemidactylus flaviviridis</i> 4	CES/07/019	Lucknow, Uttar Pradesh, India	HM595651	HM595687	–	–
<i>Hemidactylus flaviviridis</i> 5	CES/06/173	Jammu, Jammu and Kashmir, India	HM595652	HM595688	–	–
<i>Hemidactylus flaviviridis</i> 6	CES/06/122	Pune, Maharashtra, India	HM595653	HM595689	–	–
<i>Hemidactylus flaviviridis</i> 7	CES/06/071	Hospet, Karnataka, India	HM595654	HM595690	–	–
<i>Hemidactylus frenatus</i> 7	CES/07/035	Athirapalli, Valparai, Tamil Nadu, India	HM595655	HM595691	HM622356	HM622371
<i>Hemidactylus giganteus</i> 1 <sup>a</sup>	CES/07/013	Nandi Hills, near Bangalore, Karnataka, India	HM595656	HM595692	–	–
<i>Hemidactylus giganteus</i> 2 <sup>a</sup>	CES/08/013	Hampi, Karnataka, India	HM595657	HM595693	HM622357	HM622372
<i>Hemidactylus giganteus</i> 3 <sup>a</sup>	CES/07/034	Devarayanadurga, Tumkur, Karnataka, India	HM595658	HM595694	HM622358	HM622373
<i>Hemidactylus gracilis</i> 1 <sup>a</sup>	CES/08/024	Kolhapur, Maharashtra, India	HM595659	HM595695	–	–
<i>Hemidactylus gracilis</i> 2 <sup>a</sup>	CES/07/039	Pune, Maharashtra, India	HM595660	HM595696	HM622359	HM622374
<i>Hemidactylus leschenaultii</i> 1 <sup>a</sup>	CES/07/003	Chennai, Tamil Nadu, India	HM595661	HM595697	–	–
<i>Hemidactylus leschenaultii</i> 2 <sup>a</sup>	CES/07/041	Chidambaram, Tamil Nadu, India	HM595662	HM595698	HM622360	–
<i>Hemidactylus maculatus</i> 1 <sup>a</sup>	CES/07/005	Ramnagar, Karnataka, India	HM595663	HM595699	–	–
<i>Hemidactylus maculatus</i> 2 <sup>a</sup>	CES/08/028	Nilgiri Hills, Tamil Nadu, India	HM595664	HM595700	HM622361	HM622375
<i>Hemidactylus persicus</i> 2	CES/08/027	Nabh Dongar, Jaisalmer, Rajasthan, India	HM595665	HM595701	HM622362	HM622376
<i>Hemidactylus platyurus</i> 5	CES/08/025	Kalimpong, West Bengal, India	–	–	HM622363	HM622377
<i>Hemidactylus prashadi</i> 1 <sup>a</sup>	CES/07/037	Ratnagiri, Maharashtra, India	HM595666	HM595702	–	–
<i>Hemidactylus prashadi</i> 2 <sup>a</sup>	CES/06/170	Seeta Nadhi, Udipi, Karnataka, India	HM595667	HM595703	–	–
<i>Hemidactylus prashadi</i> 3 <sup>a</sup>	CES/07/040	Castle Rock, Karnataka, India	HM595668	HM595704	HM622364	HM622378
<i>Hemidactylus reticulatus</i> 1 <sup>a</sup>	CES/07/016	Pavgada, Karnataka, India	HM595669	HM595705	–	–
<i>Hemidactylus reticulatus</i> 2 <sup>a</sup>	CES/06/024	Nandi Hills, Near Bangalore, Karnataka, India	HM595670	HM595706	–	–
<i>Hemidactylus reticulatus</i> 3 <sup>a</sup>	CES/06/025	Nandi Hills, Near Bangalore, Karnataka, India	HM595671	HM595707	–	–
<i>Hemidactylus sataraisensis</i> <sup>a</sup>	CES/08/010	Chalakewadi, Satara District, Maharashtra, India	HM595672	HM595708	–	–
<i>Hemidactylus triedrus</i> 1 <sup>a</sup>	CES/07/007	Ramnagar, Karnataka, India	HM595673	HM595709	HM622365	HM622379
<i>Hemidactylus triedrus</i> 2 <sup>a</sup>	CES/07/011	Devanahalli, near Bangalore, Karnataka, India	HM595674	HM595710	–	–
<i>Hemidactylus triedrus</i> 3 <sup>a</sup>	CES/07/023	Atigulipura, C. nagar, Talvadi, Karnataka, India	HM595675	HM595711	–	–

<sup>a</sup> Species restricted to Indian subcontinent.



**Fig. 1.** Maximum parsimony tree based on the combined mitochondrial dataset consisting of cyt b and 12 S rRNA genes. Bayesian posterior probabilities and parsimony bootstrap support are shown at the base of the nodes. ‘\*/’ corresponds to Bayesian posterior probability of one and parsimony bootstrap support of  $\geq 90$ , ‘-’ corresponds to bootstrap support of  $\leq 50$ . Posterior probability of  $< 1$  and bootstrap values between 51 and 89 are mentioned at the nodes. The vertical bars indicate the five major clades reported by Carranza and Arnold (2006). The Tropical-Asian clade is highlighted by dashed vertical bar and is further divided into species groups which are labelled along the branches.

## 2. Materials and methods

### 2.1. Taxon sampling

A total of 39 individuals from 16 species of *Hemidactylus* were collected opportunistically across India. Tissue samples from tail clippings were stored in 95% ethanol. Species were identified in accordance with the keys provided by Smith (1935) and, Giri and Bauer (2008).

Samples collected for the study and their sources are listed in Table 1. For the remaining species, sequences were downloaded from GenBank (see Supplementary information).

### 2.2. DNA extraction, PCR amplification and sequencing

Extraction of total genomic DNA was carried out from tissue sample following standard phenol chloroform isoamyl alcohol method described in Sambrook and Russell (2001). Mitochondrial genes cytochrome *b* (cyt *b*) and 12S ribosomal RNA (12S rRNA) have been shown to be useful in resolving the phylogeny of *Hemidactylus* geckos (Carranza and Arnold, 2006). To derive the mtDNA phylogeny of Indian *Hemidactylus* geckos, these two markers were sequenced from multiple specimens per species using primers and conditions described in Carranza and Arnold (2006). Two nuclear markers, Recombination Activation Gene 1 (RAG-1) and Phosducin (PDC) were used to derive the nDNA phylogeny of Indian *Hemidactylus* geckos. These markers have been shown to be useful in resolving the phylogeny of *Hemidactylus* geckos at the level of deeper nodes (Bauer et al., 2008). Here, we sequenced both of these markers, using primers and conditions described in (Bauer et al., 2008), from a subset of samples such that multiple species from every well supported clade in the mtDNA tree were included.

All PCR amplifications were carried out in 25  $\mu$ l reaction volume, with 1.5 units of Taq DNA Polymerase (Bangalore Genei, Bangalore, India), 0.25 mM of dNTP's (Bangalore Genei), 2.0 mM of MgCl<sub>2</sub> (with the exception of 3.0 mM MgCl<sub>2</sub> for *H. leschenaultii*), 1  $\mu$ l of 0.5 mg/ml of BSA, 0.1  $\mu$ M (Sigma) of each primer and 40 ng of DNA. Primer details and thermocycler conditions used for the mitochondrial and nuclear genes are provided in the Supplementary information section. PCR products were purified using QIAquick PCR Purification kit (Qiagen) and sequences were obtained commercially from MWG Biotech Pvt. Ltd. (Bangalore).

### 2.3. Phylogenetic analysis

Phylogenetic analysis was carried out on two separate datasets constituting mitochondrial and nuclear genes. Published sequences of *Hemidactylus* taken from Carranza and Arnold (2006) and Bauer et al. (2008) are listed in Table 1 of Supplementary information. The sequences for each gene were aligned separately using ClustalW 1.6 (Thompson et al., 1994) integrated in software MEGA

(Tamura et al., 2007), using default parameters. Cytochrome *b* sequences were translated into amino acids prior to analysis and they did not show any stop codons suggesting that all are functional copies. Congruence between cyt *b* and 12S rRNA datasets of the mitochondrial genes and between RAG-1 and PDC datasets of the nuclear genes was checked using partition homogeneity test in PAUP\* version 4.0b10 (Swofford, 2002). Since, null hypothesis of congruence could not be rejected ( $P = 0.01$ ) in both the cases, the alignments were combined to yield the final mitochondrial (cyt *b*, 12S rRNA) and nuclear (RAG-1 and PDC) datasets. The alignments are available at TreeBASE (Piel et al., 2009) with submission IDs 10676 and 10677. The nuclear and mitochondrial datasets could not be combined because the downloaded sequences for these two datasets were from different papers and therefore were generated from different specimens of the same species.

The nuclear and mitochondrial datasets were subjected to the following phylogenetic analyses. Maximum parsimony (MP) tree was estimated using heuristic search algorithms in PAUP\* with tree bisection–reconnection branch swapping and 1000 replicates of random addition option. Parsimony bootstrapping using 1000 replicates was performed to assess nodal support using the above heuristic search criteria (except with simple addition option). Nucleotide substitution model that best fits each dataset and the model parameters were estimated using Akaike information criterion implemented in the program MODELTEST version 3.7 (Posada and Crandall, 1998) in conjunction with PAUP\*. Akaike information criteria is better suited for model selection in phylogenetics that likelihood ratio test (Posada and Buckley, 2004). The chosen model with the estimated parameters were used to derive the maximum likelihood (ML) tree in PAUP\*, along with the above MP heuristic search settings. Gaps were treated as missing data in all analyses.

Bayesian analysis was implemented in MrBayes version 3.1 (Ronquist and Huelsenbeck, 2003) using the chosen model with uniform priors. The program was run for 10<sup>7</sup> 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, determined in Tracer version 1.4.1 (Rambaut and Drummond, 2007), were discarded as burn-in. The remaining trees were imported into PAUP\* to generate a majority-rule consensus tree and to derive posterior probabilities for each node. Datasets were subjected to multiple Bayesian analysis to confirm concordance across runs.

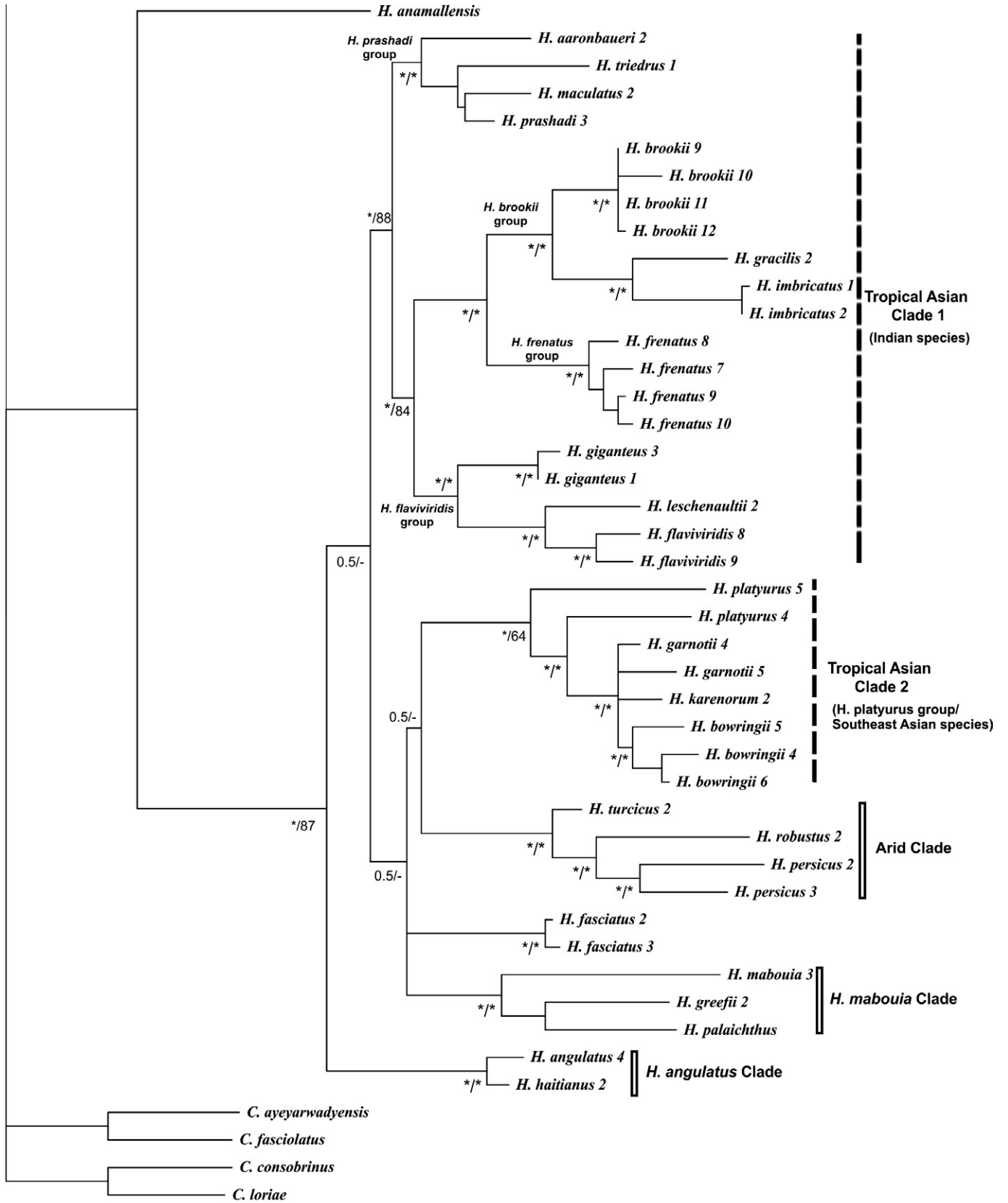
### 2.4. Hypothesis testing

Three different phylogenetic hypotheses were tested; monophyly of species restricted to the Indian subcontinent, Southeast Asia and tropical Asia. To this end, constrained trees that are consistent with these scenarios were first constructed manually in the newick format. These constrained trees were used to derive alternate likelihood trees consistent with each scenario in PAUP\*. The

**Table 2**  
Results of Shimodaira–Hasegawa test. Topological hypotheses were derived from the phylogenetic relationships suggested in various studies (see text for details). The likelihood trees are the best trees generated with mitochondrial and nuclear dataset in the present work.

No.	Topological hypothesis	–ln L	Diff. –ln L	P
<i>Mitochondrial dataset</i>				
1	Likelihood tree	14728.64453	Best tree	–
2	Tropical-Asian species monophyletic Carranza and Arnold (2006)	14728.19558	0.55105	0.665 <sup>a</sup>
3	Within Tropical-Asian clade, species from Indian subcontinent and Southeast Asia form sister clades Carranza and Arnold (2006)	14732.86028	5.21573	0.343 <sup>a</sup>
<i>Nuclear dataset</i>				
1	Likelihood tree	6633.01300	Best tree	–
2	Tropical-Asian species monophyletic Carranza and Arnold (2006)	6637.95925	4.94624	0.130 <sup>a</sup>

<sup>a</sup>  $P > 0.05$ .



**Fig. 2.** Maximum parsimony tree based on the combined nuclear dataset consisting of RAG-1 and PDC genes. Bayesian posterior probabilities and parsimony bootstrap support are shown at the base of the nodes. ‘\*/’ corresponds to Bayesian posterior probability of 1 and parsimony bootstrap support of  $\geq 90$ , ‘-’ corresponds to bootstrap support of  $\leq 50$ . Posterior probability of  $< 1$  and bootstrap values between 51 and 89 are mentioned at the nodes. The vertical bars indicate the four of the five major clades reported by Carranza and Arnold (2006). The Tropical-Asian clade is highlighted by dashed vertical bar and is further divided into species groups which are labelled along the branches.



likelihood score of these alternate trees were compared with the best tree by implementing 1000 rell bootstrap replicates of Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) in PAUP\*.

### 2.5. Biogeographic analysis

To better understand the evolutionary origin and dispersal of house geckos of the Indian subcontinent, part of the phylogenetic tree, that contained *H. brookii*, *H. frenatus* and *H. flaviviridis*, was subjected to dispersal–vicariance analysis in the programme DIVA (Ronquist, 1997). In this analysis, terminal nodes were assigned to Indian subregion, Southeast Asia (Indochinese and Sundaic subregions), and West-Asian arid zone based on the current distribution of various species. The West-Asian arid zone here corresponds to the area extending from Pakistan to Israel. The subregion designations are as per Corbet and Hill (1992). The program was run with default setting and the ancestral areas for various internal nodes as well as the number of dispersal events were estimated using the parsimony criteria.

### 3. Results

In this study the total sequence length for the two mitochondrial genes, *cyt b* (303 bp) and 12S (399 bp), was 702 bp and for the two nuclear genes, RAG-1 (1039 bp) and PDC (395 bp), was 1434 bp. The best fit models for the mitochondrial and nuclear datasets were GTR + I + G and TrN + G, respectively. In the case of the mitochondrial dataset, the three tree building methods yielded very similar tree topologies with minor differences. The differences were at the base of the tree where the bootstrap support is very low indicating that the relationships are not very robust at deeper nodes. Fig. 1 shows a strict consensus of 241 equally parsimonious trees requiring 3321 changes. In this tree the endemic *H. anamallensis* is basal to all *Hemidactylus* and the remaining species fall into two large groups; one consisting of the species from tropical Asia (tropical Asian clade) and the other constituting representatives from the remaining clades reported by Carranza and Arnold (2006). However, the parsimony bootstrap supports for these two clades are very low.

The tropical Asian clade in turn consists of five well supported groups: *H. flaviviridis* group (*H. aaronbaueri*, *H. flaviviridis*, *H. giganteus*, *H. leschenaultii*); *H. brookii* group (*H. albofasciatus*, *H. brookii*, *H. gracilis*, *H. imbricatus*, *H. reticulatus*, *H. sataraisensis*); *H. frenatus* group (*H. frenatus*); *H. platyurus* group (*H. bowringii*, *H. garnotii*, *H. karenorum*, *H. platyurus*); *H. prashadi* group (*H. maculatus*, *H. prashadi*, *H. triedrus*). Within the tropical Asian clade, *H. platyurus* group, which consists of species distributed predominantly in Southeast Asia, is nested within a group consisting of species from the Indian subcontinent. Thus, the species from Indian subcontinent and those from Southeast Asia are not reciprocally monophyletic. However, tropical Asian clade was not retrieved by ML and Bayesian analyses; here the *H. flaviviridis* group branched with the non-Asian species (see Supplementary information). Nevertheless, these trees were not significantly different from the ML tree where *H. flaviviridis* group was constrained to branch with the tropical Asian clade (Table 2). All the tree building methods also supported the monophyly of various species in these five species groups of the tropical Asian clade with the exception of two species. *Hemidactylus giganteus* is paraphyletic with respect to *H. aaronbaueri* in the *H. flaviviridis* group. In the *H. brookii* group, *H. brookii* is paraphyletic with respect to other members of this group.

All the tree building methods generated nearly identical trees for the nuclear dataset. Fig. 2 shows a strict consensus of 36 equally parsimonious trees requiring 736 changes. In this tree the endemic *H. anamallensis* was again basal to all other *Hemidactylus* geckos. The

remaining species formed two separate clades. One includes the species distributed predominantly in the Indian subcontinent (Indian clade). This clade is well supported with high posterior probability and bootstrap, whereas the second clade received poor support and consisted of the remaining groups described by Carranza and Arnold (2006) as well as the *H. platyurus* group of our mitochondrial tree. Thus in the nuclear tree, the tropical Asian group is not monophyletic. Nevertheless a likelihood tree, wherein the Southeast Asian *H. platyurus* group was constrained to be sister to Indian clade, was not significantly different from the best tree (Table 2). The Indian clade in turn consists of four well supported species groups, *H. flaviviridis*, *H. brookii*, *H. frenatus* and *H. prashadi* groups. These groups were also retrieved in our mitochondrial tree. One significant discord between the mitochondrial and nuclear dataset is with respect to the phylogenetics position of *H. aaronbaueri*. In the mitochondrial tree it branches within *H. flaviviridis* group, whereas in the nuclear tree it branches with the *H. prashadi* group. The dispersal–vicariance analysis generated a single optimal reconstruction for the ancestral areas of various internal nodes (see Supplementary information). The analysis suggested three independent dispersal events of the house geckos from India. These dispersal events included dispersal of *H. flaviviridis* to the West-Asian arid zone and dispersal of *H. brookii* and *H. frenatus* to Southeast Asia.

### 4. Discussion

The molecular data presented here supports the monophyly of species restricted to the Indian subcontinent (Indian clade). This clade is strongly supported in the nuclear tree, whereas in the mitochondrial tree the *H. platyurus* group, which consists of species distributed predominantly in Southeast Asia, is nested within the Indian clade. Nevertheless, the likelihood score of the mitochondrial tree, in which the Southeast Asia and Indian species are constrained to be reciprocally monophyletic, is not significantly different from the best tree (Table 2). Based on these results, following species can be assigned to the Indian clade: *H. aaronbaueri*, *H. albofasciatus*, *H. brookii*, *H. flaviviridis*, *H. frenatus*, *H. giganteus*, *H. gracilis*, *H. imbricatus*, *H. leschenaultii*, *H. maculatus*, *H. prashadi*, *H. reticulatus*, *H. sataraisensis* and *H. triedrus*.

Interestingly, *H. anamallensis*, which is endemic to the high altitudes of Western Ghats of south India, does not fall in the Indian clade. This species is basal to all *Hemidactylus* in both the nuclear as well as mitochondrial trees. Thus, *H. anamallensis* appears to be “genetically” distinct from all *Hemidactylus* and its allocation to this genus, by Bauer and Russel (1995), needs to be re-examined.

The most likely sister group of the Indian clade appears to be the group constituting the *Hemidactylus* species from Southeast Asia (*H. platyurus* group in our tree). As mentioned above, the mitochondrial tree supports their close relationship, but, in the nuclear tree, these two clades are not sister groups. Nevertheless, the likelihood score of the nuclear tree, wherein these two clades are constrained to be sister groups, is not significantly different from the best tree (Table 2). More sampling of the Southeast Asian *Hemidactylus* needs to be undertaken for a robust test of this scenario.

In this study 21 out of the 27 *Hemidactylus* geckos of the Indian subcontinent were used (excluding *H. anamallensis*) and a majority of these (at least 15 species) form a distinct Indian clade. Thus, the Indian subcontinent harbours a unique radiation of *Hemidactylus* geckos. The widely distributed house geckos are nested with this Indian radiation strongly suggesting their Indian origin. This scenario is supported by the ancestral area reconstruction in DIVA (see Supplementary information). According to the DIVA analysis the ancestral areas of distribution of house geckos was the Indian region. *Hemidactylus flaviviridis* dispersed out-of-India and expanded its range to the West-Asian arid zone whereas *H. brookii* and *H. frenatus* dispersed to Southeast Asia. Thus, there have been

three out-of-India dispersals of *Hemidactylus* geckos. Similar scenarios of multiple long distance dispersals across landmasses have also been reported in other commensal species of *Hemidactylus* geckos (Carranza and Arnold, 2006 and references therein). Nevertheless, it must be pointed out that the sampling of house geckos in this study was largely confined to the Indian subcontinent but type localities of some of them are outside of this region. Thus, for a rigorous test of this “out-of-India” hypothesis and for nomenclature consistency, these species need to be sampled from throughout their range including topotypical samples from the type locality. It must be noted that peninsular India has served as a “biotic ferry” that introduced Gondwanan elements into Asia, but these out-of-India dispersal events have occurred in the early Tertiary (Roy and Karanth, 2009). In contrast the *Hemidactylus* radiation and subsequent dispersal of some lineages appears to be relatively younger, additionally some species have undergone recent human-mediated dispersals (Carranza and Arnold, 2006 and references therein).

The remaining *Hemidactylus* geckos of India belong to two other clades. These include *H. persicus* and *H. turcicus* of the Arid clade described by Carranza and Arnold (2006) and the *H. platyurus* group of Southeast Asia. Thus, the *Hemidactylus* geckos of the Indian subcontinent are represented by three different lineages, one of which is largely restricted to India and has radiated into at least 15 species, while species from the other two lineages have extended their ranges into the eastern and western fringes of this landmass. The Indian clade is likely to harbour more species. This is because at least six other species also restricted to Indian subcontinent have not been included in this study. These are *H. depressus*, *H. gujaratensis*, *H. mahendrai*, *H. porbandarensis*, *H. scabriceps*, *H. subtriadrus* and *H. treutleri*. The phylogenetic positions of these species need to be evaluated. However, the validity of *H. mahendrai*, has recently been questioned recently (see Giri and Bauer, 2008). Additionally, in the mitochondrial tree two species, *H. giganteus* and *H. brookii*, in the Indian clade are not monophyletic. This in turn suggests that there might be many cryptic species within the Indian clade. Nevertheless, the paraphyly of *H. brookii* is not well supported in the mitochondrial tree and in the nDNA tree this species is monophyletic. Thus our dataset does not support the splitting of the so-called *H. brookii* into multiple species.

The phylogeny of *Hemidactylus* geckos of the Indian subcontinent supports a unique Indian radiation consisting of a majority of species from this landmass. The widely distributed and commensal house geckos (*H. brookii*, *H. frenatus* and *H. flaviviridis*) are nested with this clade suggesting their Indian origin. These species might have dispersed out-of-India and colonised parts of West-Asian arid zone and Southeast Asia. Additionally, commensalism amongst the Indian *Hemidactylus* geckos has evolved independently in three different lineages. Altogether these results suggest that the Indian subcontinent has served as an important arena for diversification amongst the *Hemidactylus* geckos and in the evolution and spread of its commensal geckos.

## Acknowledgments

We are very grateful to Varad Giri of Bombay Natural History Society for providing us with the samples of many endemic species. C.R. Akarsh, M.S. Chaitra, Aniruddha Dutta Roy, Chetan Nag and Ishan Agarwal also provided us with samples from different locations in India. Thanks to Ishan Agarwal and Varad Giri for their

comments on the manuscript. This research was supported by Grant from Ministry of Environment and Forest, Government of India. We are thankful to Karnataka Forest Department for collection permits.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.06.008.

## References

- Bauer, A.M., Russel, A.P., 1995. The systematic relationships of *Dravidogecko anamallensis* (Günther 1875). Asiatic Herpetol. Res. 6, 30–35.
- Bauer, A.M., Giri, V.B., Greenbaum, E., Jackman, T.R., Dharme, M.S., Shouche, Y.S., 2008. On the systematics of the gekkonid genus *Teratolepis* Günther, 1869: another one bites the dust. Hamadryad 33, 13–28.
- Carranza, S., Arnold, E.N., 2006. Systematics, biogeography, and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. Mol. Phylogenet. Evol. 38, 531–545.
- Corbet, G.B., Hill, J.E., 1992. The Mammals of the Indomalayan Region: A Systematic Review. Oxford University Press, New York. pp. 1–12.
- Daniel, J.C., 2002. The Book of Indian Reptiles and Amphibians. Oxford University Press, Mumbai.
- Feng, J., Han, D., Bauer, A.M., Zhou, K., 2007. Interrelationships among Gekkonid geckos inferred from mitochondrial and nuclear gene sequences. Zool. Sci. 24, 656–665.
- Giri, V.B., 2008. A new rock-dwelling *Hemidactylus* (Squamata: Gekkonidae) from Maharashtra, India. Hamadryad 32, 25–33.
- Giri, V.B., Bauer, A.M., 2008. A new ground-dwelling *Hemidactylus* (Squamata: Gekkonidae) from Maharashtra, with a key to the *Hemidactylus* of India. Zootaxa 1700, 21–34.
- Giri, V.B., Bauer, A.M., Vyas, R., Patil, S., 2009. New species of rock-dwelling *Hemidactylus* (Squamata: Gekkonidae) from Gujarat, India. J. Herpetol. 43 (3), 385–393.
- Kluge, A.G., 1969. The Evolution and Geographical Origin of the New World *Hemidactylus mabouia brookii* Complex (Gekkonidae, Sauria). Miscellaneous Publications, Museum of Zoology, University of Michigan, p. 138.
- Kluge, A.G., 2001. Gekkotan lizard taxonomy. Hamadryad 28, 1–209.
- Mahony, S., 2009. A new species of gecko of the genus *Hemidactylus* (Reptilia: Gekkonidae) from Andhra Pradesh, India. Russ. J. Herpetol. 16 (1), 27–34.
- Piel, W.H., Chan, L., Dominus, M.J., Ruan, J., Vos, R.A., Tannen, V., 2009. Treebase v. 2: A database of phylogenetic knowledge. e-Biosphere.
- Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9), 817–818.
- Rambaut, A., Drummond, A., 2007. Tracer v1.4. Available from <http://beast.bio.ed.ac.uk/>.
- Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.
- Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
- Roy, A.D., Karanth, K.P., 2009. The out-of-India hypothesis: what do molecules suggest? J. Biosci. 34, 687–697.
- Sambrook, J., Russell, D., 2001. Preparation of genomic DNA from mouse tails and other small samples. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 6.23–6.27.
- Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.
- Smith, M.A., 1935. The Fauna of British India, Including Ceylon and Burma, Reptilia and Amphibia, vol. II—Sauria. Taylor and Francis, London.
- Swofford, D.L., 2002. PAUP: Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.10b. Sinauer Associates, Sunderland, MA.
- Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.
- Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acids Res. 22, 4673–4680.
- Zug, G.R., Vindum, J.V., Koo, M.S., 2007. Burmese *Hemidactylus* (Reptilia, Squamata, Gekkonidae): taxonomic notes on Tropical Asian *Hemidactylus*. Proc. Calif. Acad. Sci. 4 (58), 387–405.