

# Molecular evidence for the taxonomic status of *Hemidactylus brookii* group taxa (Squamata: Gekkonidae)

Aaron M. Bauer<sup>1</sup>, Todd R. Jackman<sup>1</sup>, Eli Greenbaum<sup>1,2</sup>, Anslem de Silva<sup>3</sup>,  
Varad B. Giri<sup>4</sup> & Indraneil Das<sup>5</sup>

<sup>1</sup>Department of Biology, Villanova University, USA

<sup>2</sup>Department of Biological Sciences, University of Texas at El Paso, USA

<sup>3</sup>15/1 Dolosbage Road, Gampola, Sri Lanka

<sup>4</sup>Herpetology Section, Bombay Natural History Society, Mumbai, India

<sup>5</sup>Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, Kota Samarahan, Malaysia

*Hemidactylus brookii* has one of the widest distributions and, arguably, one of the most confused taxonomic histories of any gekkonid lizard. Nuclear (RAG1 and PDC) and mitochondrial (ND2, cytb) DNA sequence data were employed to examine relationships among a sample of putative *H. brookii*, including a topotypical specimen from Borneo. Two clades were recovered, one consisting of specimens from Borneo (Sarawak), Myanmar, Peninsular Malaysia and Karnataka, southwestern India, and another of specimens from Sri Lanka, Mauritius and Kerala, southwestern India. Both clades are well supported and deeply divergent from one another, whereas genetic variation within each clade is limited. None of the analytical approaches used recovered a well-supported monophyletic *H. brookii sensu lato*. Near uniformity of *H. brookii sensu stricto* in East Asia suggests that this species has spread to this region relatively recently. The name *H. parvimaculatus* Deraniyagala 1953 is available for the Sri Lankan clade and this form should be treated as a valid species. Existing data cannot be used to distinguish whether this species has colonized Sri Lanka from South India or vice versa. The Palghat Gap provides a candidate barrier to gene flow between *H. brookii* and *H. parvimaculatus*. Although the identity of *H. brookii* complex geckos in East Asia and Sri Lanka appears resolved, the situation in India and Pakistan remains complex and thorough revisionary work, coupled with phylogenetic studies, is needed to determine species boundaries in this region.

**Key words:** *H. parvimaculatus*, India, Sri Lanka, phylogeny, taxonomy

## INTRODUCTION

The systematic status of *Hemidactylus brookii* has long been confused. For most of the last 100 years it has been considered a polytypic species with a nearly pan-tropical distribution (Smith, 1935; Loveridge, 1947; Wermuth, 1965; Kluge, 1969; Röslér, 2000). Kluge (1969) argued on morphological grounds that the New World “brookii”, *H. b. haitianus* and *H. b. leightoni*, formed with *H. palaichthus* a natural radiation in the Americas. He also resurrected the use of *H. brookii angulatus* for the African members of the group, which had been considered subspecifically identical with those of tropical Asia. However, he did not comment specifically on the Asian forms themselves, the group to which the name *brookii* is, in fact, linked. Carranza & Arnold (2006), in a molecular phylogeny of *Hemidactylus*, demonstrated that *H. b. haitianus* (elevated to full specific status along with *H. leightoni* by Powell et al., 1996) and *H. b. angulatus* were members of an African clade, distinct from Asian *H. brookii*, and that at least *H. palaichthus* belonged to yet another major clade within the genus. Thus, of the non-Asian subspecies of *H. brookii* recognized by Kluge (1969), *H. leightoni* is a valid species restricted to northern South America (Rivas, 2002), *H. angulatus* is probably a

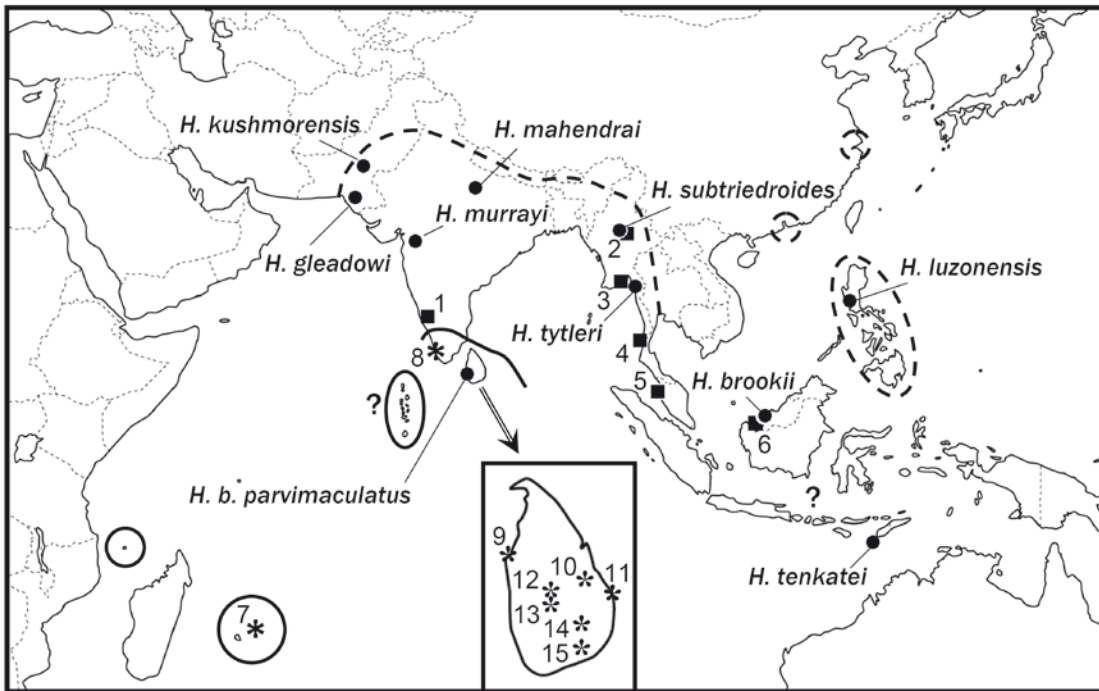
complex of species, all restricted to Africa (Carranza & Arnold, 2006) and *H. haitianus* is a valid species of the *angulatus* group, occurring both in West Africa and in the West Indies, where it was probably introduced within historical times (Weiss & Hedges, 2007).

At present at least eight names are in the synonymy of Asian *H. brookii* (Brown & Alcalá, 1978; Zug et al., 2007): *Gecko tytleri* Tytler 1865, *H. kushmorensis* Murray 1884, *H. gleadowii* Murray 1884, *H. murrayi* Gleadow 1887, *H. tenkatei* Lidth de Jeude 1895, *H. subtriadroides* Annandale 1905, *H. luzonensis* Taylor 1915, and *H. brookii parvimaculatus* Deraniyagala 1953. Recently Zug et al. (2007) have suggested that an eighth nomen, *H. mahendrai* Shukla 1983, previously regarded as a valid Indian species, may also be based on *H. brookii*.

The majority of these names have long been consigned to synonymy, although Kästle (2002) recognized *H. b. subtriadroides* as a valid species in northern Myanmar and adjacent northeast India, and *H. b. parvimaculatus* has been regarded as valid by most Sri Lankan authors (e.g. Deraniyagala, 1953; Manamendra-Arachchi, 1997; de Silva, 1998; Wickramasinghe & Somaweera, 2002, 2008; Somaweera, 2005; Ziesmann et al., 2007). The extent of the range of “*H. brookii*” remains uncertain and ostensibly extends from Pakistan (Khan et al., 1999;

**Table 1.** List of samples used in this study. ADS = Anslem de Silva field series, AMB = Aaron M. Bauer field series, CAS = California Academy of Sciences, JFBM = James Ford Bell Museum, University of Minnesota, St Paul, JS = Jay Sommers (Kansas City), LLG = L. Lee Grismer field series, ZRC = The Raffles Museum of Biodiversity Research, National University of Singapore. ADS and AMB specimens are pending accession in the National Museum of Sri Lanka, Colombo. Localities for *H. brookii* and *H. parvimaculatus* are plotted in Figure 1.

Sample (map location)	Museum no.	Locality	GenBank accession numbers			
			cyt <i>b</i>	ND2	RAG1	PDC
<i>Hemidactylus brookii</i> (1)	Carranza & Arnold (2006)	India, Karnataka, Subrahmnnya	DQ120276	—	—	—
<i>Hemidactylus brookii</i> (2)	CAS 206638	Myanmar, Mandalay Division	EU268407	EU268375	EU268314	EU268344
<i>Hemidactylus brookii</i> (3)	CAS 208159	Myanmar, Yangon	GQ375294	GQ458052	GQ375312	GQ375306
<i>Hemidactylus brookii</i> (3)	Carranza & Arnold (2006)	Myanmar, Yangon Division, Mingalardan	DQ120274	—	—	—
<i>Hemidactylus brookii</i> (4)	CAS 229632	Myanmar, Tanintharyi Division	GQ375295	GQ458051	GQ375313	GQ375307
<i>Hemidactylus brookii</i> (5)	LLG 6755	Malaysia, Pulau Pinang, Empangon Air Hitam	EU268398	EU268366	EU268305	EU268320
<i>Hemidactylus brookii</i> (5)	LLG 6754	Malaysia, Pulau Pinang, Empangon Air Hitam	EU 268397	EU268365	EU268304	EU268334
<i>Hemidactylus brookii</i> (6)	ZRC 2.6167	Malaysia (Borneo), Sarawak, Loagan Bunut National Park	GQ375293	GQ458050	GQ375314	GQ375305
<i>Hemidactylus parvimaculatus</i> (7)	Carranza & Arnold (2006)	Mauritius	DQ120272	—	—	—
<i>Hemidactylus parvimaculatus</i> (8)	Carranza & Arnold (2006)	India, Kerala, Kollam	DQ120273	—	—	—
<i>Hemidactylus parvimaculatus</i> (9)	AMB 7466	Sri Lanka, Mampuri, 7°59'38"S, 79°44'33"E	GQ375292	GQ458056	GQ375311	GQ375304
<i>Hemidactylus parvimaculatus</i> (10)	AMB 7432	Sri Lanka, Tempitiya, 7°35'26"S, 81°25'38"E	GQ375300	—	—	—
<i>Hemidactylus parvimaculatus</i> (11)	ADS 36	Sri Lanka, Kartivu, 7°22'35.6"S, 81°58'59.0"E	GQ375291	GQ458053	GQ375310	GQ375303
<i>Hemidactylus parvimaculatus</i> (12)	AMB 7480	Sri Lanka, Matale, 7°31'48"S, 80°37'39"E	GQ375298	—	—	—
<i>Hemidactylus parvimaculatus</i> (12)	AMB 7427	Sri Lanka, Matale, 7°31'48"S, 80°37'39"E	GQ375299	—	—	—
<i>Hemidactylus parvimaculatus</i> (13)	AMB 7475	Sri Lanka, Kandy, 7°15'36"S, 80°37'11"E	GQ375290	GQ458055	GQ375309	GQ375302
<i>Hemidactylus parvimaculatus</i> (14)	AMB 7424	Sri Lanka, Dehikindagama, 6°56'00"S, 81°17'17"E	GQ375296	—	—	—
<i>Hemidactylus parvimaculatus</i> (15)	AMB 7426	Sri Lanka, Gonaganara, 6°36'53"S, 81°16'13"E	GQ375297	—	—	—
<i>Hemidactylus frenatus</i>	LLG 6745	Malaysia, Pulau Pinang, Empangon Air Hitam	EU268390	EU268358	EU268297	EU268327
<i>Hemidactylus frenatus</i>	LLG 4871	Malaysia, Pahang, Bukit Bakong	GQ375289	GQ458049	GQ375308	GQ375301
<i>Hemidactylus frenatus</i>	CAS 214157	Myanmar, Mandalay Division	DQ120281	—	—	—
<i>Hemidactylus frenatus</i>	CAS 212900	Myanmar, Ayeyarwarde Division, Mwe Hauk	DQ120280	—	—	—
<i>Hemidactylus imbricatus</i>	JS 11	Pakistan (captive specimen)	EU268385	EU268353	EU268292	EU268322
<i>Hemidactylus imbricatus</i>	JFBM 2	Pakistan (captive specimen)	EU268383	EU268354	EU268293	EU268323
<i>Hemidactylus reticulatus</i>	AMB 5730	India, Tamil Nadu, Vellore	EU 268410	—	—	EU268347



**Fig. 1.** Map of Asia illustrating the type localities (black circles) of the taxa in the *Hemidactylus brookii* group (see text for discussion of current synonymies), and the ingroup sampling for this study (black squares = *H. brookii* sensu stricto; asterisks = *H. parvimaculatus*). Numbered localities correspond to those listed in Table 1. Dashed line represents the approximate limits of distribution of *H. brookii* in mainland Asia based on literature records. Approximate areas of occurrence in the Philippines, Hong Kong/Macau, and Zhejiang Province, China are also enclosed in dashed lines. The distribution of *H. brookii* in the Indo-Australian Archipelago is patchy and poorly documented. Solid line across south India indicates the approximate northern limit of *H. parvimaculatus*. The identity of *H. brookii* group taxa in most of south India needs confirmation and it is possible that both *H. brookii* and *H. parvimaculatus* co-occur in some regions. Insular areas of occurrence of *H. parvimaculatus* (Comoro Islands, Mascarene Islands, and perhaps the Maldives) are enclosed within solid lines.

Khan, 2006) and possibly Iran (Anderson, 1999; although only records west of the Indus Valley are considered to be within the natural range, Zug et al., 2007) in the west to Borneo (Bartlett, 1895), the Philippines (Brown & Alcalá, 1978), and possibly Pulau Roti (near Timor) (Lidth de Jeude, 1895) in the east (Fig. 1). Localities in China (including Hong Kong and Macau) are scattered and localized and almost certainly represent relatively recent introductions (Romer, 1978; Karsten et al., 1986; Zhao & Adler, 1993; Chan et al., 2006). Indeed, records to the east of Myanmar are few and scattered (Denzer & Manthey, 1991; Bauer et al., 2002) and are limited chiefly to areas of human activity (Bartlett, 1895). Only in India and immediately neighbouring countries is *H. brookii* considered to be both widespread and common, and even there, there is no agreement as to whether it occurs virtually countrywide (Smith, 1935; Tikader & Sharma, 1992; Daniel, 2002; Sharma, 2002), or is restricted to northern areas (Das, 2002; Das & de Silva, 2005), although regardless it is often considered to have achieved its broad distribution partly through human agency (Murthy, 1990; Daniel, 2002). Certainly there are several species represented in India that are currently masquerading under the

name *H. brookii* (Mahony, 2009). A revision of all South Asian members of this complex is required and several teams of researchers are currently engaged in addressing this issue from both phylogenetic and alpha systematic perspectives.

The types of *H. brookii* were reported by Gray (1845) to derive from “Australia” and “Borneo”. However, the species does not occur in Australia (Bauer & Henle, 1994) and consequently the type locality was independently restricted to “Borneo” by both Smith (1935) and Pope (1935), although even this has been questioned (Shelford, 1901). However, the recent rediscovery of *H. brookii* from Loagan Bunut National Park, Sarawak, western Borneo (Das & Jensen, 2006; Das & Sukumaran, 2007), confirms the species’ presence there and provides an opportunity to compare topotypical material with putative *H. brookii* from elsewhere in Asia in order to determine what “real” *H. brookii* is and where it occurs. Thus, as part of a broader study of the relationships of tropical Asian *Hemidactylus* (Bauer et al., 2010), we employed a molecular systematic approach to this question in order to provide a basis for further, more extensive efforts to unravel the Gordian knot of *H. brookii* taxonomy.

**Table 2.** Primers used in this study.

Primer	Gene	Reference	Sequence
PHOF2	PDC	Bauer et al. (2007)	5'-AGATGAGCATGCAGGAGTATGA-3'
PHOR1	PDC	Bauer et al. (2007)	5'-TCCACATCCACAGCAAAAACTCCT-3'
L4437b	Met tRNA	Macey et al. (1997)	5'-AAGCAGTTGGGCCCATAACC-3'
L5002	ND2	Macey et al. (1997)	5'-AACCAAACCCAACTACGAAAAAT-3'
ND2f101	ND2	Greenbaum et al. (2007)	5'-CAAACACAAACCCGRAAAAT-3'
ND2r102	ND2	Greenbaum et al. (2007)	5'-CAGCCTAGGTGGGCGATTG-3'
Trpr3a	Trp tRNA	Greenbaum et al. (2007)	5'- TTTAGGGCTTTGAAGGC-3'
H5934a	COI	Macey et al. (1997)	5'- AGRGTGCCAATGTCTTTGTGRTT-3'
R13	RAG1	Groth and Barrowclough (1999)	5'- TCTGAATGGAAATTCAAGCTGTT-3'
R18	RAG1	Groth and Barrowclough (1999)	5'-GATGCTGCCTCGGTCGGCCACCTTT-3'
RAG1 F700	RAG1	Bauer et al. (2007)	5'-GGAGACATGGACACAATCCATCCTAC-3'
RAG1 R700	RAG1	Bauer et al. (2007)	5'-TTTGTACTGAGATGGATCTTTTGTGCA-3'

MATERIALS AND METHODS

Sampling

Carranza & Arnold (2006) demonstrated that Asian *H. brookii* complex species were members of a tropical Asian clade of *Hemidactylus*. More specifically, they found *H. brookii* to be in a subclade also including *H. frenatus* and *H. flaviviridis* and supported by some morphological features by Zug et al. (2007). Bauer et al. (2008, 2010) demonstrated that the closest relationships of *H. brookii* group geckos lie with a clade of chiefly terrestrial *Hemidactylus*, including *H. imbricatus* (formerly *Teratolepis fasciata*), *H. reticulatus*, *H. gracilis*, *H. albofasciatus* and probably the recently described *H. sataraensis* (Giri & Bauer, 2008). On this basis we used *H. imbricatus*, *H. frenatus* and *H. flaviviridis* as successively more distant outgroups for our analyses. Analysed specimens of *H. brookii* came from Peninsular Malaysia, Borneo, Myanmar, and Sri Lanka and previously published sequence data allowed us to incorporate additional material from India and Mauritius into our analyses (Table 1, Fig. 1).

Molecular methods

Genomic DNA was isolated from 95–100% ethanol-preserved tail or liver samples with the Qiagen DNeasy tissue kit (Valencia, CA, USA). We used double-stranded PCR to amplify 3101 aligned bases of mitochondrial (ND2, cytb) and nuclear (RAG1, PDC) gene sequence data with 12 different published primers (Table 2).

Amplification of 25 µl PCR reactions was executed on an Eppendorf Mastercycler gradient thermocycler. Amplification of genomic DNA occurred with an initial denaturation step of 95 °C for 2 min, followed by denaturation at 95°C for 35 s, annealing at 50 °C for 35 s, and extension at 72 °C for 95 s with four seconds added to the extension per cycle for 32 cycles for mitochondrial DNA and 34 cycles for nuclear DNA. When necessary, annealing temperatures were adjusted to increase or decrease specificity on a case by case basis, and products were

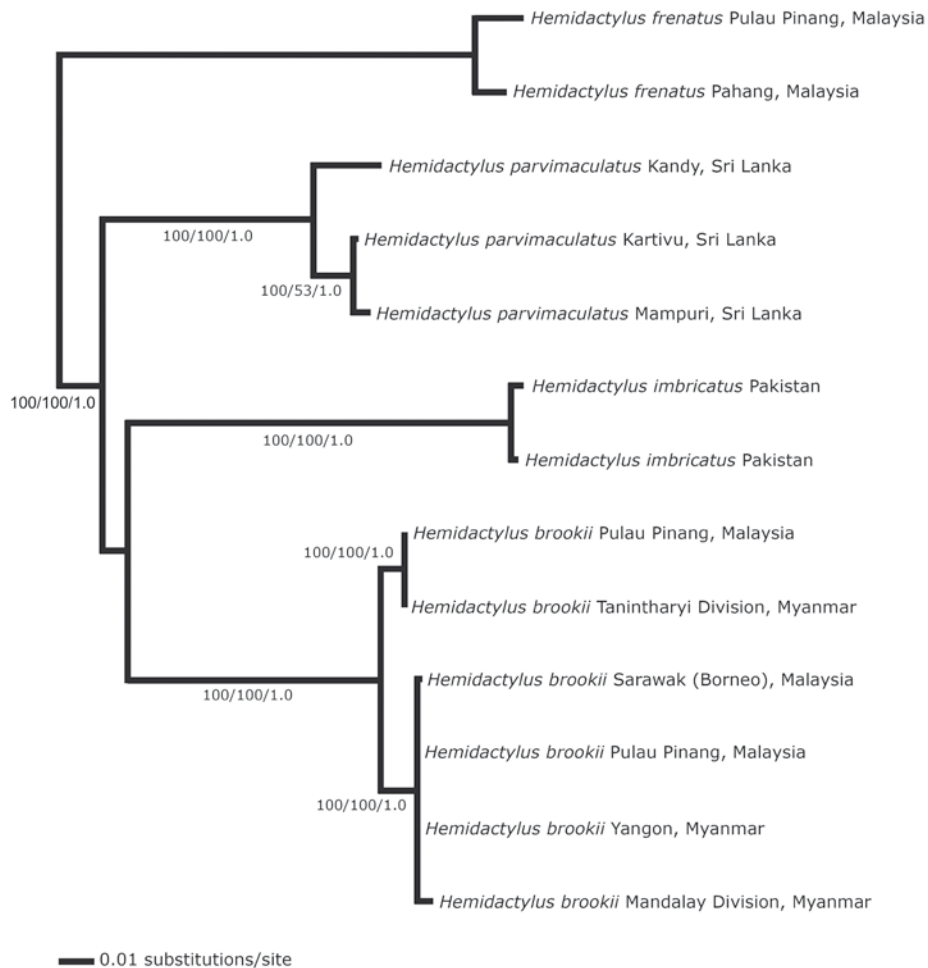
visualized with 1.5% agarose gel electrophoresis. Target products were purified with AMPure magnetic bead solution (Agencourt Bioscience) and sequenced with either the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) or the DYEnamic™ ET Dye Terminator Kit (GE Healthcare). Sequencing reactions were purified with CleanSeq magnetic bead solution (Agencourt Bioscience) and analysed with an ABI 3700 automated sequencer. The accuracy of sequences was ensured by incorporating negative controls and sequencing complementary strands.

Phylogenetic analyses

Sequences were aligned by eye using SeqMan™, and protein-coding genes were translated to amino acids with MacClade (Maddison & Maddison, 1992) to confirm conservation of the amino acid reading frame and check for premature stop codons. Phylogenetic relationships among the samples were assessed with maximum parsimony and Bayesian optimality criteria. Data from all four genes (RAG1, PDC, ND2 and cytb) were combined for a single analysis. A second data set incorporating cytb data only was also investigated, permitting us to incorporate GenBank sequences of additional *H. brookii* from the work of Carranza & Arnold (2006).

Maximum parsimony (MP) analyses were conducted in PAUP\*4.0b10 (Swofford, 2002). The heuristic search algorithm was used with the following conditions: 25 random addition replicates, tree bisection-reconnection (TBR) branch swapping, zero-length branches collapsed to yield polytomies, and gaps treated as missing data. Each base position was treated as an unordered character with four alternate states. We used nonparametric bootstraps (1000 pseudoreplicates unless stated otherwise) to assess node support in resulting topologies. The Akaike Information Criterion (AIC) in ModelTest 3.06 (Posada & Crandall, 1998) was used to find the model of evolution that best fitted the data for subsequent maximum-likelihood (ML) and Bayesian inference (BI) analyses. In the





**Fig. 2.** Phylogenetic relationships among members of the *Hemidactylus brookii* clade based on the combined analysis of the mitochondrial genes ND2 and cyt *b*, and the nuclear genes RAG1 and PDC. Maximum likelihood tree topology and branch lengths with support values reported in the format: maximum likelihood bootstraps/maximum parsimony bootstraps/Bayesian posterior probabilities.

ML analysis of the combined data, the GTR +  $\Gamma$  + I model was used with the most parsimonious tree to estimate the parameters, and the same conditions as the parsimony search were used to find the ML tree. For the cytochrome *b* only analysis with expanded taxon sampling, the GTR +  $\Gamma$  model was used for likelihood and Bayesian analyses.

Partitioned Bayesian analyses were conducted with MrBayes 3.1 (Ronquist & Huelsenbeck, 2003) with default priors. Separate models for each gene and codon position of protein-coding genes were estimated (Brandley et al., 2005). A total of 10 partitions were made: RAG1, 3 codons; PDC, 3 codons, ND2 and cytochrome *b*, 3 codon positions; and mitochondrial tRNAs. Analyses were initiated with random starting trees and run for 2,000,000 generations; Markov chains were sampled every 100 generations. Convergence was checked by plotting likelihood scores against generation, and 112 trees were discarded as “burn in”. Two separate analyses with two independent chains were executed to check for convergence of log-likelihoods in stationarity (Huelsenbeck & Ronquist, 2001). Both analyses ended with the standard deviation of split frequencies less than 0.01 (0.002 for both).

## RESULTS

For the expanded taxon sampling analysis of cytochrome *b* data alone, the likelihood analysis produced a single tree with  $-\ln L = 1490.9$ . This tree was identical in topology to the Bayesian majority rule consensus, except for nodes with posterior probabilities of less than 0.50, making the likelihood tree one of the possible resolutions of the Bayesian trees. A parsimony analysis resulted in four most parsimonious trees with 236 steps. The combined likelihood analysis (RAG1, PDC, ND2, cyt*b*) produced a single tree with  $-\ln L = 9083.46$ . This tree was also one of the possible resolutions of the Bayesian tree where posterior probabilities were less than 0.50. The parsimony analysis of the combined data produced four equally parsimonious trees with 1014 steps.

In the combined analysis (Fig. 2) *Hemidactylus brookii* from Borneo is virtually identical to specimens from Pulau Pinang, Peninsular Malaysia and Yangon and the Mandalay Division of Myanmar and together these specimens form a well-supported clade with 100% bootstrap support (ML, MP) and a Bayesian posterior



**Fig. 3.** Phylogenetic relationships among members of the *Hemidactylus brookii* clade based on the analysis of the *cyt b* mitochondrial gene. Maximum likelihood tree topology and branch lengths with support values reported in the format: maximum likelihood bootstraps/ maximum parsimony bootstraps/ Bayesian posterior probabilities.

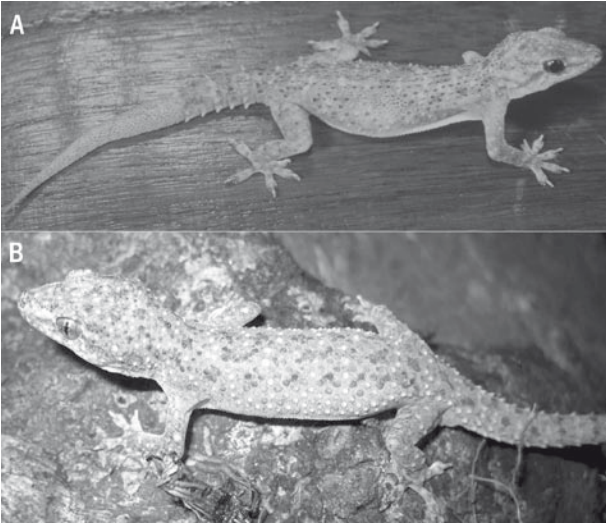
probability of 1.0. In turn this clade is sister to a pair of specimens from the Tanintharyi Division, Myanmar and another specimen from Pulau Pinang. All six of these specimens comprise a well-supported *H. brookii sensu stricto* clade that is deeply divergent from a well-supported clade of *H. brookii parvimaculatus* from Sri Lanka and from *H. imbricatus*. Although weakly supported, our ML analysis found *H. b. brookii* and *H. imbricatus* to be sister taxa, whereas our MP analysis retrieved *H. b. parvimaculatus* and *H. imbricatus* as sister taxa, also with weak support. The monophyly of the *H. brookii sensu lato* clade (*H. brookii* + *H. parvimaculatus* + *H. imbricatus*) was strongly supported in all analyses. An analysis of the nuclear data alone (RAG1 and PDC, not shown) recovered the same relationships as an analysis of the mitochondrial DNA alone and the combined data, except that there is insufficient variation to resolve any of the relationships within *H. b. brookii*.

With expanded taxon sampling, the *cytb* data alone (Fig. 3) likewise retrieve a well-supported *Hemidactylus brookii* clade comprising an unresolved trichotomy of well-supported and deeply divergent *H. brookii*, *H. parvi-*

*maculatus*, and *H. imbricatus* + *H. reticulatus* lineages. Within *H. brookii* an additional Yangon area specimen is identical to the Bornean topotype, and a specimen from Subrahmnyna [presumably Subrahmanya], Karnataka, India as sister to the remainder of the clade as a whole. In *H. b. parvimaculatus*, an additional specimen from Kollam, Kerala, India is nearly identical to a specimen from Kandy, Sri Lanka and these together are sister to all remaining Sri Lankan samples and a specimen from Mauritius. Sequence divergence (uncorrected %) within each of the two *brookii* clades is 0.1–1.7% and divergence between them is 10.7–11.9%.

DISCUSSION

Assuming that the population of *H. brookii* occurring in Sarawak (Fig. 4A) today is the same from which the syntypes (BMNH 1947.3.6.47–49) were derived, we can confidently state that true *H. brookii* occurs not only in Borneo, but in Peninsular Malaysia, both northern and southern Burma, and in Karnataka, southwestern India. Another species occurs in the southwest Indian state of



**Fig. 4.** Life photographs of (A) *Hemidactylus brookii* from Loagan Bunut National Park, Sarawak, Malaysia (Borneo), and (B) *Hemidactylus parvimaclatus* from Tissamaharama, Southern Province, Sri Lanka. Note the larger, more closely-spaced tubercles in *H. parvimaclatus*.

Kerala, throughout Sri Lanka, and in Mauritius. The distribution of this second species can be further surmised based on the work of Vences et al. (2004) and Rocha et al. (2005) who, using 16s mtDNA, found little difference between Sri Lankan and Mauritian specimens and those from Reunion, Rodrigues and the Comoro Islands.

Poor sampling prevents us from determining whether the divergent haplotypes occurring in both peninsular Malaysia and Myanmar represent native haplotype diversity and thus probable long-term presence, or if they reflect multiple colonizations from different source areas. Given the small size of Pulau Pinang and its extensive commercial and cultural connections to other countries, particularly India, we suspect that the latter explanation applies in this case. The haplotype diversity in more isolated Myanmar is more likely to reflect real population substructuring. The near identity of the Bornean sample with those from Pinang, Yangon and Mandalay, respectively 1200, 2300 and 2750 km distant, strongly suggests that Bartlett (1895) was correct in his assumption that toptypical *H. brookii* represents a translocated population from the southeast Asian mainland.

The second species of “*H. brookii*” is clearly that for which Deraniyagala (1953) erected the name *H. brooki* [sic] *parvimaclatus*. Deraniyagala (1953) and many subsequent authors (e.g. de Silva et al., 2000; Rösler, 2000; Das, 2001; Wickramasinghe & Somaweera, 2002) have considered this to be an endemic Sri Lankan form. It has been distinguished from the nominotypical mainland Asian form on the basis of its smaller dark dorsal markings and less pronounced dorsal tubercles, although neither of these features appears to be reliable (see Figure 4). Samples from the Comoro Islands and Mascarenes are likewise very similar to Sri Lankan specimens, but

not identical, nor identical to one another, suggesting that these distant island groups, which have certainly been populated, at least in part, through human agency (Vences et al., 2004), have probably received colonists representing several related haplotypes. The single sample from Kerala likewise is not identical to any of the other samples, but differs very little. Either south India or Sri Lanka is plausible as a source area for the Mascarenes and Comores as there is, and has long been, extensive commerce, and thus ship and, more recently, air traffic between these regions. Wickramasinghe & Somaweera (2008) reported that this species had been found on ships and floating logs, attesting to its invasive potential. In the absence of greater sampling in both India and Sri Lanka it is not possible to determine if Sri Lanka was itself colonized from India or if the Kerala population represents an invasion of the mainland from Sri Lanka. Likewise, it is not currently possible to determine the extent of this species’ occurrence on the Indian mainland, or if *H. brookii* and *H. parvimaclatus* occur in sympatry anywhere.

The taxonomic implications of this work are that at least two full species should be recognized within what has long been considered a single, but polytypic, *Hemidactylus brookii*. The name *H. brookii* is restricted to the species occurring in East Asia and in parts of India (including Karnataka, but probably most of the subcontinent; Fig. 1). Although it is not possible, without careful examination of the surviving type material, to unambiguously assign other names to the subjective synonymy of *H. brookii*, it seems likely, given the occurrence of *H. brookii* in widely separated parts of Myanmar (Zug et al., 2007), that *Gecko tytleri* – type locality “Moulmein” [= Mawlamyine, Myanmar] is referable to this taxon. The type of *H. subtrioides* is also from Myanmar, and its type locality, “Tsagain, Upper Burma” (currently, Sagaing, northern Myanmar), is only a few kilometres from our northernmost sample from the Mandalay Division. Thus, we strongly suspect that this name also belongs in the synonymy of *H. brookii*. The status of specimens from far northern Myanmar and Arunachal Pradesh, India (the area depicted by Kästle [2000] in his range map of *H. b. subtrioides*) is unknown as no recent material has been obtained from this region (Zug et al., 2007). *Hemidactylus tenkatei* Lidth de Jude 1895 – type locality “Rotti” [= Pulau Roti, Indonesia], which, if truly from Roti, probably represents, like the Bornean population, a translocation from elsewhere in southeast Asia, is thus also almost certainly a synonym of *H. brookii sensu stricto*. The same is probably true of *H. luzonensis* Taylor 1915 – type locality “Manila, Philippine Islands”, which, according to Brown & Alcalá (1978), is identical to the Bornean types of *H. brookii*.

The case of the second species is somewhat more complex. Although the name *H. brookii parvimaclatus* – type locality “Colombo” [Sri Lanka] is clearly applicable to this form, its presence on the Indian mainland leaves open the possibility that one of several other older names based on peninsular Indian types might have priority. However, as all remaining names in synonymy of *H. brookii sensu lato* (Zug et al., 2007) are associated with specimens from either Pakistan: *H. kushmorensis* – type locality “Bhaner



[= Bhanar], Upper Sind frontier”, *H. gleadowi* – type locality “Rantha forest in Sind, (Jerruck division)”, or the Indian peninsula north of Kerala: *H. murrayi* – type locality “Pimpri and Garvi, in the ‘Dangs’” [Gujarat], *H. mahendrai* – type locality “suberbs [sic] of district Kanpur of Uttar Pradesh” we think it likely that none of these apply to this species and at least tentatively regard the correct name for this taxon as *Hemidactylus parvimaculatus*. The identity of the other names remains in question. They may well refer to *H. brookii sensu stricto*, but one or more may apply to distinctive members of the *H. brookii* species complex.

Based on these taxonomic conclusions, *Hemidactylus parvimaculatus* is probably the only member of the *H. brookii* complex occurring in Sri Lanka, Mauritius, Rodrigues, Reunion and the Comoro Islands (Fig. 1). An *H. brookii* group gecko, presumably *H. parvimaculatus*, also occurs on the Maldives (Laidlaw, 1903; Phillips, 1958). *Hemidactylus brookii sensu stricto* occurs from Myanmar eastwards through Indochina, the Malay Peninsula, parts of China and on scattered islands of the Indo-Australian Archipelago, including Borneo. Specimens from Pakistan are also ostensibly referable to *H. brookii* but require a detailed revision as several names currently in synonymy are based on Pakistani material and some “*H. brookii*” from adjacent Rajasthan have proved to be specifically distinct (Bauer et al., 2010) and referable to an entirely different major clade of *Hemidactylus* (*sensu* Carranza & Arnold, 2006). In India both *H. brookii* and *H. parvimaculatus* are demonstrably present, adding to an already extremely diverse *Hemidactylus* fauna (Giri & Bauer, 2008; Giri, 2008; Giri et al., 2009). In light of the recognition of the specific distinctness of *H. parvimaculatus*, as well as the discovery of new members of the *H. brookii* complex in peninsular India (Mahony, 2009), a critical review and revision of all Indian “*brookii*” is desperately needed in order to determine the number of species present, their geographic distributions, and their morphological and biological characteristics. Virtually all names in the synonymy of *H. brookii* are associated with inadequate diagnoses and a thorough morphological investigation across the entire subcontinent, preferably coupled with phylogenetic and phylogeographic studies, will be needed to clarify the taxonomy of the group. The difficulties in identifying and diagnosing taxa at the species level for all of the species previously considered to be *H. brookii* shows that molecular sequence data will be crucial in the future to complement and confirm species boundaries in this group of morphologically similar species.

A lack of key data, both morphological and molecular, from peninsular Indian *H. brookii* group taxa precludes a critical evaluation of alternative hypotheses regarding the history of the spread of both *H. brookii sensu stricto* and *H. parvimaculatus*. However, we predict that Indian *H. brookii* will exhibit high haplotype diversity and that the low diversity and broad distribution of a single haplotype in East Asia reflects recent expansion out of India. Near genetic uniformity of seven of eight Sri Lankan samples of *H. parvimaculatus* from across the island is suggestive that the species recently colonized from India, but in the absence of additional south Indian samples colonization

in the opposite direction cannot be rejected. However, the apparent restriction of *H. parvimaculatus* to extreme southern India + Sri Lanka raises the possibility that the Palghat (Palakkad) Gap, a low pass in the Western Ghats of Kerala and western Tamil Nadu that has been implicated as a barrier to gene flow in organisms as diverse as frogs (Bossuyt et al., 2004; Biju & Bossuyt, 2009) and elephants (Vidya et al., 2005), may have played a role in cladogenesis of the *H. brookii* group of geckos. Lineage divergence within *H. parvimaculatus* is more likely related to factors intrinsic to Sri Lanka and/or South India than to the current water barrier between the two countries. The Palk Strait, which today is only 20 km wide and 10 m deep, is only a recent feature, as there were prolonged periods of connection during the Pleistocene. During the last glacial maximum (about 20,000 ybp) sea levels were approximately 120 m lower than today, yielding a 140 km wide land bridge. Such connections existed for at least half of the last half million years and were present as recently as 10,000 years ago (Rohling et al., 1998). Further research will clarify if *H. parvimaculatus* is another component of the endemic herpetofauna of Sri Lanka that has, until recently, been grossly underestimated (Batuwita & Bahir, 2005; Pethiyagoda, 2005; Manamendra-Arachchi & Pethiyagoda, 2005), or if, like the smaller islands of the Indian Ocean, Sri Lanka is a recent recipient of another successful Indian colonizer.

## ACKNOWLEDGEMENTS

Permission to conduct research in Sri Lanka was kindly provided by the Director General, Department of Wildlife Conservation. We also thank the Conservator General of Forests for his approval. We thank Tony Gamble (JFBM), Robert C. Drewes and Jens V. Vindum (CAS), Jay Sommers (Kansas City) and L. Lee Grismer (LaSierra University) for the loan of tissue samples. Tom Kennedy assisted in the laboratory, and Christopher C. Austin participated in field work in Sri Lanka and Karen Jensen and Jeet Sukumaran in Sarawak. This research was supported by grant DEB 0515909 from the National Science Foundation of the United States.

## REFERENCES

- Anderson, S.C. (1999). *The Lizards of Iran*. Ithaca: Society for the Study of Amphibians and Reptiles.
- Bartlett, E. (1895). The crocodiles and lizards of Borneo in the Sarawak Museum, with descriptions of supposed new species, and the variation of colours in the several species during life. *Journal of the Straits Branch of the Royal Asiatic Society* 1895(28), 73–96.
- Batuwita, S. & Bahir, M.M. (2005). Description of five new species of *Cyrtodactylus* (Reptilia: Gekkonidae) from Sri Lanka. *Raffles Bulletin of Zoology*, Supplement 12, 351–380.
- Bauer, A.M., de Silva, A., Greenbaum, E. & Jackman, T.R. (2007). A new species of day gecko from high elevation in Sri Lanka, with a preliminary phylogeny of Sri Lankan *Cnemaspis* (Reptilia: Squamata: Gekkonidae). *Mitteilungen aus dem Museum für Naturkunde in Berlin. Zoologische*



- Reihe 83 (Supplement), 22–32.
- Bauer, A.M., Giri, V.B., Greenbaum, E., Jackman, T.R., Dharne, M.S. & Shouche, Y.S. (2008). On the systematics of the gekkonid genus *Teratolepis* Günther, 1869: another one bites the dust. *Hamadryad* 33, 13–27.
- Bauer, A.M. & Henle, K. (1994). *Das Tierreich* 109. *Gekkonidae. Part I, Australia and Oceania*. Berlin: Walter de Gruyter Publishers.
- Bauer, A.M., Jackman, T.R., Greenbaum, E., Giri, V. & De Silva, A. (2010). South Asia supports a major endemic radiation of *Hemidactylus* geckos. *Molecular Phylogenetics and Evolution*. doi:10.1016/j.ympev.2010.06.014.
- Bauer, A.M., Pauwels, O.S.G. & Sumontha, M. (2002). *Hemidactylus brookii brookii*. Distribution. *Herpetological Review* 33, 322.
- Biju, S.D. & Bossuyt, F. (2009). Systematics and phylogeny of *Philautus* Gistel, 1848 (Anura, Rhacophoridae) in the Western Ghats of India, with descriptions of 12 new species. *Zoological Journal of the Linnean Society* 155, 374–444.
- Bossuyt, F., Meegaskumbura, M., Beenaerts, N., Gower, D.J., Pethiyagoda, R., Roelants, K., Mannaert, A., Wilkinson, M., Bahir, M.M., Manamendra-Arachchi, K., Ng, P.K.L., Schneider, C.J., Oommen, V.O. & Milinkovitch, M.C. (2004). Local endemism in the Western Ghats–Sri Lanka biodiversity hotspot. *Science* 306, 479–481.
- Brandley, M.C., Schmitz, A. & Reeder, T.W. (2005). Partitioned Bayesian analysis, partition choice, and the phylogenetic relationships of scincid lizards. *Systematic Biology* 54, 373–390.
- Brown, W.C. & Alcalá, A.C. (1978). *Philippine Lizards of the Family Gekkonidae*. Dumaguete City: Silliman University Natural Science Monograph Series No. 1.
- Carranza, S. & Arnold, E.N. (2006). Systematics, biogeography, and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 38, 531–545.
- Chan, S.K.F., Cheung, K.-S., Ho, C.-Y., Lam, F.-N. & Tang, W.-S. (2006). The geckos of Hong Kong. *Hong Kong Biodiversity* 13, 1–9.
- Daniel, J.C. (2002). *The Book of Indian Reptiles and Amphibians*. Mumbai: Bombay Natural History Society.
- Das, I. (2001). Biodiversity and biogeography of the herpetofauna of Southern Asia. In *An Overview of the Threatened Herpetofauna of South Asia*, 1–38. Bambaradeniya, C.N.B. & Samarasekara, V.N. (eds). Colombo: IUCN Sri Lanka and Asia Regional Biodiversity Programme.
- Das, I. (2002). *A Photographic Guide to the Snakes and Other Reptiles of India*. London: New Holland Publishers, London.
- Das, I. & de Silva, A. (2005). *A Photographic Guide to the Snakes and Other Reptiles of Sri Lanka*. London: New Holland Publishers.
- Das, I. & Jensen, K.A. (2006). The herpetofauna of Loagan Bunut. In *Scientific Journey Through Borneo: Loagan Bunut. A Scientific Expedition on the Physical, Chemical, Biological, and Sociological Aspects. Kuala Lumpur and Kota Samarahan: Peat Swamp Forest Project, UNDP/GEF funded (MAL/99/G31)*, 131–154. Tuen, A.A., Sayok, A.K., Toh, A.N. & Noweg, G.T. (eds). Kuching: Sarawak Forest Department and Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak.
- Das, I. & Sukumaran, J. (2007). Confirmation of *Hemidactylus brookii* Gray, 1845 from Borneo. *Gekko* 5(1), 18–20.
- Denzer, W. & Manthey, U. (1991). A nominal checklist of the lizards inhabiting Peninsular Malaysia and Singapore. *Raffles Bulletin of Zoology* 39, 309–322.
- Deraniyagala, P.E.P. (1932). The Gekkonoidea of Ceylon. *Spolia Zeylanica* 16, 291–310, pls LVIII–LXIV.
- Deraniyagala, P.E.P. (1953). *A Colored Atlas of Some Vertebrates from Ceylon. Volume Two. Tetrapod Reptilia*. Colombo: Ceylon National Museums.
- de Silva, A. (1998). *The Sauria (Lizards and Varanids) of Sri Lanka: A Checklist and an Annotated Bibliography*. Colombo: Department of Wildlife Conservation/GEF/UNDP/FAO.
- de Silva, A., Bauer, A.M., Goonewardene, S., Drake, J., Nathaniel, S. & de Silva, P. (2005) [2006]. Some observations on the geckos inhabiting the Knuckles Massif. *Lyriocephalus* 6, 125–133, pl. 8.
- de Silva, A., Molur, S. & Walker, S. (eds). (2000). *Conservation Assessment and Management Plan, CAMP Report for Amphibians and Selected Taxa of Reptiles of Sri Lanka*. Colombo: Conservation Breeding Specialist Group, Sri Lanka.
- Giri, V. (2008). A new rock dwelling *Hemidactylus* (Squamata: Gekkonidae) from Maharashtra, India. *Hamadryad* 32, 25–33.
- Giri, V. & 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. *Journal of Herpetology* 43, 385–393.
- Gray, J.E. (1845). *Catalogue of the Specimens of Lizards in the Collection of the British Museum*. London: British Museum (Natural History).
- Greenbaum, E., Bauer, A.M., Jackman, T.R., Vences, M. & Glaw, F. (2007). A phylogeny of the enigmatic Madagascan geckos of the genus *Uroplatus* (Sauria: Gekkonidae). *Zootaxa* 1493, 41–51.
- Groth, J.G. & Barrowclough, G.F. (1999). Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. *Molecular Phylogenetics and Evolution* 12, 115–123.
- Huelsenbeck, J.P. & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.
- Kartsen, S.J., Lau, M. W.-N. & Bogadek, A. (1986). *Hong Kong Amphibians and Reptiles*. Hong Kong: Urban Council.
- Kästle, W. (2002). Family Gekkonidae (geckos). In *Amphibians and Reptiles of Nepal. Biology, Systematics, Field Guide*, 674–691, 697–722. Schleich, H.-H. & Kästle, W. (eds). Ruggell, Liechtenstein: A.R.G. Gantner Verlag K.G.
- Khan, M.S. (2006). *Amphibians and Reptiles of Pakistan*. Malabar, Florida: Krieger Publishing Company.
- Khan, W.A., Ahmad, M.S. & Ali, M.A. (1999). Lizards of southern Waziristan, NWFP, Pakistan. *Acta Scientifica* 9, 125–134.
- Kluge, A.G. (1969). The evolution and geographical origin of the New World *Hemidactylus mabouia*–*brookii* complex (Gekkonidae, Sauria). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 138, 1–78.
- Laidlaw, F.F. (1903). Amphibia and Reptilia. In *The Fauna and*

- Geography of the Maldive and Laccadive Archipelagoes, Volume 1*, 119–122. Gardiner, J.S. (ed.). Cambridge: Cambridge University Press.
- Lidth de Jeude, T.W. van (1895). Reptiles from Timor and neighboring islands. *Notes, Leiden Museum* 16, 119–127.
- Loveridge, A. 1947. Revision of the African lizards of the family Gekkonidae. *Bulletin of the Museum of Comparative Zoology at Harvard College* 98, 1–469, pls 1–7.
- Macey, J.R., Larson, A., Ananjeva, N.B., Fang, Z. & Papenfuss, T.J. (1997). Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution* 14, 91–104.
- Maddison, W.P. & Maddison, D.R. (1992). *MacClade Version 3: Analysis of Phylogeny and Character Evolution*. Sunderland, Massachusetts: Sinauer Associates.
- Mahony, S. (2009). A new species of gecko of the genus *Hemidactylus* (Reptilia: Gekkonidae) from Andhra Pradesh, India. *Russian Journal of Herpetology* 16, 27–34.
- Manamendra-Arachchi, K. (1997). Gecko! *Sri Lanka Nature* 1, 45–55.
- Manamendra-Arachchi, K. & Pethiyagoda, R. (2005). The Sri Lankan shrub-frogs of the genus *Philautus* Gistel, 1848 (Ranidae: Rhacophorinae), with description of 27 new species. *Raffles Bulletin of Zoology*, Supplement 12, 163–303.
- Murthy, T.S.N. (1990). A field book of the lizards of India. *Records of the Zoological Survey of India, Occasional Papers* 115, 1–122.
- Pethiyagoda, R. (2005). Exploring Sri Lanka's biodiversity. *Raffles Bulletin of Zoology*, Supplement 12, 1–4.
- Phillips, W.W.A. (1958). Observations on the fauna of the Maldive Islands. Part IV—Amphibians and reptiles. *Journal of the Bombay Natural History Society* 55, 217–220.
- Pope, C.H. (1935). *The Reptiles of China. Turtles, Crocodilians, Snakes and Lizards. Natural History of Central Asia, Vol. 10*. New York: American Museum of Natural History.
- Powell, R., Henderson, R., Adler, K. & Dundee, H. (1996). An annotated checklist of West Indian amphibians and reptiles. In *Contributions to West Indian Herpetology: A Tribute to Albert Schwartz*, 51–93, pls 1–8. Powell, R. & Henderson, R.W. (eds). Ithaca: Society for the Study of Amphibians and Reptiles.
- Posada, D. & Crandall, K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Rivas, G. (2002). Geographic distribution. *Hemidactylus leightoni*. *Herpetological Review* 33, 148.
- Rocha, S., Carretero, M.A. & Harris, D.J. (2005). Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro Islands. *Molecular Phylogenetics and Evolution* 35, 292–299.
- Rohling, E.J., Fenton, M., Jorissen, F.J., Bertrand, P., Ganssen, G. & Caulet, J.P. (1998). Magnitudes of sea-level lowstands of the past 500,000 years. *Nature* 394, 162–165.
- Romer, J.D. (1977) [1978]. Reptiles new to Hong Kong. *Journal of the Hong Kong Branch of the Royal Asiatic Society* 17, 232–234.
- Ronquist, F. & Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Rösler, H. (2000). Kommentierte Liste der rezent, subrezent und fossil bekannten Geckotaxa (Reptilia: Gekkonomorpha). *Gekkota* 2, 28–153.
- Sharma, R.C. (2002). *The Fauna of India and the Adjacent Countries. Reptilia, Volume II (Sauria)*. Kolkata: Zoological Survey of India.
- Shelford, R.W.C. (1901). A list of the reptiles of Borneo. *Journal of the Straits Branch of the Royal Asiatic Society* 1901(35), 43–68.
- Smith, M.A. (1935). *The Fauna of British India, Including Ceylon and Burma. Reptilia and Amphibia. Vol. II – Sauria*. London: Taylor and Francis.
- Somaweera, R. (2005). A bark gecko (*Hemidactylus leschenaultii*) preying on a wolf snake (*Lycodon striatus sinhaleyus*). *Gekko* 4(2), 8–10.
- Somaweera, R. & Wickramasinghe, M. (2006). Geckos on the move: how humans have influenced the distribution of *Hemidactylus* geckos in Sri Lanka. *Abstracts of the 11th Biological Sciences Graduate Congress, 15<sup>th</sup>–17<sup>th</sup> December, 2006*. Thailand: Chulalongkorn University.
- Swofford, D.L. (2002). *PAUP\*. Phylogenetic Analysis Using Parsimony (\*and other methods)*. Sunderland, Massachusetts, Sinauer.
- Taylor, E.H. (1953). A review of the lizards of Ceylon. *University of Kansas Science Bulletin* 35, 1525–1585.
- Tikader, B.K. & Sharma, R.C. (1992). *Handbook of Indian Lizards*. Calcutta: Zoological Survey of India.
- Vences, M., Wanke, S., Vietes, D.R., Branch, W.R., Glaw, F. & Meyer, A. (2004). Natural colonization or introduction? Phylogeographical relationships and morphological differentiation of house geckos (*Hemidactylus*) from Madagascar. *Biological Journal of the Linnean Society* 83, 115–130.
- Vidya, T.N.C., Fernando, P., Melinck, D.J. & Sukumar, R. (2005). Population differentiation within and among Asian elephant (*Elephas maximus*) populations in southern India. *Heredity* 94, 71–80.
- Weiss, A.J. & Hedges, S.B. (2007). Molecular phylogeny and biogeography of the Antillean geckos *Phyllodactylus wirshingi*, *Tarentola americana*, and *Hemidactylus haitianus* (Reptilia, Squamata). *Molecular Phylogenetics and Evolution* 45, 409–416.
- Wermuth, H. (1965). *Liste der rezenten Amphibien und Reptilien, Gekkonidae, Pygopodidae, Xantusiidae. Das Tierreich* 80. Berlin: Walter de Gruyter.
- Wickramasinghe, M. & Somaweera, R. (2002). Distribution and current status of the endemic geckos of Sri Lanka. *Gekko* 3(1), 2–13.
- Wickramasinghe, M. & Somaweera, R. (2008). Changes in the distribution ranges of the Sri Lankan *Hemidactylus* geckos. *Gekko* 5(2), 44–60.
- Zhao, E. & Adler, K.K. (1996). *The Herpetology of China*. Oxford, Ohio: Society for the Study of Amphibians and Reptiles.
- Ziesmann, S., Janzen, P. & Klaas, P. (2007). Die Vielfalt der Geckos. *Draco* 30, 38–44.
- Zug, G.R., Vindum, J.V. & Koo, M.S. (2007). Burmese *Hemidactylus* (Reptilia, Squamata, Gekkonidae): taxonomic notes on tropical Asian *Hemidactylus*. *Proceedings of the California Academy of Sciences, Fourth Series* 58, 391–408.

Accepted: 10 August 2009