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

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*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, cyt b) DNA sequence data were employed to examine relationships among a sample of putative *H. brookii*, including a toptotypic 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; Rösler, 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).


The majority of these names have long been consigned to synonymy, although Kästle (2002) recognized *H. b. subtriedroides* 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>
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<th>Location</th>
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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.
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 & Alcala, 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.

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.
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

<table>
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ML analysis of the combined data, the GTR + Γ + 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 + Γ 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 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
probability of 1.0. In turn this clade is sister to a pair of specimens from the Taninthary 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 cyt b 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. parvimaculatus*, and *H. imbricatus + H. reticulatus* lineages. Within *H. brookii* an additional Yangon area specimen is identical to the Bornean topotype, and a specimen from Subrahmanya [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

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**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.
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 the topotypical 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] parvimaculatus. Deraniyagala (1953) and many subsequent authors (e.g. de Silva et al., 2000; Rössler, 2000; Das, 2001; Wickramasinghe & Somaweera, 2002) have considered this to be an endemic Sri Lankan form. It has been distinguished from the nonotypical 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. parvimaculatus occur in sympathy 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. subtriodoides 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. subtriodoides is unknown as no recent material has been obtained from this region (Zug et al., 2007). Hemidactylus tenkatei Lidth de Jeude 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 & Alcala (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 parvimaculatus – 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. kashmoresis – type locality “Bhaner

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**Fig. 4.** Life photographs of (A) Hemidactylus brookii from Loagan Bunut National Park, Sarawak, Malaysia (Borneo), and (B) Hemidactylus parvimaculatus from Tissamaharama, Southern Province, Sri Lanka. Note the larger, more closely-spaced tubercles in H. parvimaculatus.
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from peninsular India this group of morphologically similar species. shows that molecular sequence data will be crucial in the absence of additional south Indian samples colonization that the species recently colonized from India, but in the low diversity and broad distribution of a single haplotype brookii H. parvimaculatus critical evaluation of alternative hypotheses regarding the identifying and diagnosing taxa at the species level for phylogenetic and phylogeographic studies, will be needed across the entire subcontinent, preferably coupled with diagnoses and a thorough morphological investigation synonymy of Hemidactylus brookii, 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.

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