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Molecular data in conjunction with morphology help resolve the *Hemidactylus brookii* complex (Squamata: Gekkonidae)

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Abstract Molecular data are increasingly being used to resolve cryptic species complexes; however, subsequent formal species description and taxonomic revisions often remain incomplete. Given that most species are described based on morphology-based alpha taxonomy, one cannot resolve nomenclatural issues of species complexes without the aid of morphology. In this study, we examined the taxonomic status of a long-known human commensal and species complex, *Hemidactylus brookii*. To this end, samples of *H.* cf. *brookii* and related species were collected across India. We analyzed molecular as well as morphological data to resolve the taxonomy of this species complex. Seven deeply divergent, well-supported clades were recovered using the mitochondrial phylogeny, five of which were also retrieved in the nuclear tree. One of these consists of five morphologically distinct species of ground-dwelling *Hemidactylus*. The genetic distances across each clade of putative species of *H. brookii* sensu lato were comparable to that between morphologically distinct species of ground-dwelling *Hemidactylus*. Meristic characters such as number of precloacal-femoral pores, number of non-pore bearing scales interrupting the series of pored scales, dorsal pholidosis, and presence/absence of divided lamellae can be used to distinguish these putative species from each other. However, morphological characters of *H. brookii* sensu stricto did not correspond to any of the putative species studied. The study also revealed that the "*H. brookii* complex" in India includes two commensal species, *Hemidactylus parvimaculatus* and *Hemidactylus murrayi*. Furthermore, these two lineages have independently acquired adaptations that could have assisted them in exploiting human habitat. An identification key to diagnose species within this complex and rest of the *Hemidactylus* in India is proposed.

Keywords Cytochrome *b* · RAG1 · Cryptic species · Invasive species · Phylogeny

Introduction

Cryptic species are defined as "…. discrete species that are difficult, or sometimes impossible, to distinguish morphologically and thus have been incorrectly classified as a single taxon" (Beheregaray and Caccone 2007). Difficulty in distinguishing these species morphologically often results in confused taxonomy, with multiple species clubbed together as a species complex. A substantial portion of biodiversity is made up of cryptic species (Bickford et al. 2007; Pfenninger and Schwenk 2007), and therefore, identifying these units is crucial, given that species are the fundamental units used to assess biodiversity and understand patterns in ecology (Isaac 2004). Cryptic species have been found across varied taxonomic groups, including well-studied taxa like mammals (Brown et al. 2007; Olivieri et al. 2007), and across various biogeographic regions (Pfenninger and Schwenk 2007). Although the existence of cryptic species has been known

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for a long time, the number of studies identifying and delimiting cryptic species has grown exponentially only in the last 30 years with advancements in molecular tools (Pfenninger and Schwenk 2007). However, one cannot resolve nomenclatural issues of species complexes without the aid of morphology. This is because most species were described before the advent of molecular tools on the basis of morphology-based alpha taxonomy and the type specimens of these are usually unsuitable or inaccessible for molecular work. Therefore, morphological data are the connecting link between the specimens sampled during a molecular study and type specimens (Schlick-Steiner et al. 2007). Especially in cases of organisms with confused taxonomic history, there is a need to thoroughly examine the morphology of specimens used in the phylogeny and refer to type specimens before assigning them to a particular species.

Hemidactylus brookii is one such species complex and has been a recent topic of interest due to its complex taxonomic history (Bauer et al. 2010a; Mahony 2011). However, lack of molecular data and detailed description of the type specimens made resolving its taxonomy difficult. Gray in 1845 described *H. brookii* from Australia and Borneo, but the type locality was later determined to be limited to Borneo (Mahony 2011). This was followed by descriptions of other species across South and Southeast Asia (e.g., Murray 1884; Gleadow 1887), Africa (Hallowell 1854), and South America (Boulenger and A 1911) that were subsequently synonymized with *H. brookii* (Kluge 1969; Meerwarth 1901; Bauer et al. 2010a; Smith 1935). *H. brookii* was thought to be distributed pan-tropically until 2006, when Carranza and Arnold's study demonstrated that the African and South American "*H. brookii*" belonged to very different lineages and these were designated as different species. Currently, the range of *H. brookii* is limited to Asia. There were several synonyms of *H. brookii* from the region—*Gecko tytleri*, *Hemidactylus gleadowi*, *Hemidactylus kushmorensis*, *Hemidactylus luzonensis, Hemidactylus murrayi, Hemidactylus subtriedroides*, and *Hemidactylus tenkatei*. Bauer et al. (2010a) postulated that some synonyms of *H. brookii* might in fact be distinct species. Rösler and Glaw (2010) elevated one of these nomina, *H. tenkatei*, to a valid species. Mahony in 2011 reexamined the specimens in the type series of *H. brookii* along with its synonyms - *H. kushmorensis, H. gleadowi*, and *H. subtriedroides* from the Natural History Museum, London. He concluded that *H. kushmorensis* and *H. gleadowi* were distinct species, while considering *H. subtriedroides* a junior synonym of *H. tenkatei*. Meanwhile a new "*brookii* like" species, *Hemidactylus treutleri*, was described from peninsular India (Mahony 2009) which was proposed to be morphologically similar to *H. brookii*.

Bansal and Karanth (2010) demonstrated that the Asian *H. brookii* was not monophyletic. In their phylogenies, *H. brookii* was paraphyletic with respect to a clade consisting of endemic ground-dwelling *Hemidactylus* species. Bauer et al. (2010a) examined the status of *H. brookii* sensu lato from Sri Lanka and few other localities from Asia, including a topotypical sample from Borneo assumed to represent *H. brookii* sensu stricto. These samples were used in a molecular study and the Sri Lankan subspecies *H. brookii parvimaculatus* was elevated to species—*H. parvimaculatus*. It was speculated that *H. brookii* was distributed throughout India and Southeast Asia and the range of *H. parvimaculatus* was hypothesized to be in Sri Lanka, Mauritius, and in India, south of Palghat Gap. This wide distribution of both species was thought to be caused by human-mediated translocation (Bauer et al. 2010a). However, a recent molecular study on *H. tenkatei* from Timor suggests that the topotypical samples of *H. brookii* from the Bauer et al. (2010a) study also represent *H. tenkatei* (Kathriner et al. 2014). While the taxonomic status of the *H. brookii* complex in Southeast Asia and Sri Lanka was resolved to some extent, the status of the *H. brookii* complex within peninsular India remained unknown (Bauer et al. 2010a).

Geckos of the genus *Hemidactylus* are known to be moved around by humans and include at least nine species of human commensals (Carranza and Arnold 2006; Bauer et al. 2010a). Of these, four species—*H. brookii*, *H. parvimaculatus*, *Hemidactylus frenatus*, and *Hemidactylus flaviviridis*—are nested within the endemic Indian *Hemidactylus* radiation and have been purported to have an Indian origin (Bansal and Karanth 2010; Bauer et al. 2010b). Human commensal species are those that exploit human-modified habitats or niches created by humans (Jones et al. 2013) and occur predominantly in and around human habitation. Given their close proximity to humans, these species have been inadvertently and sometimes deliberately translocated by humans (Keller 2007). This aspect of human commensals has been of considerable interest to ecologists (Austin 1999) and a cause of concern to conservation biologists (Banks and Hughes 2012) Introduced species of *Hemidactylus* are known to have caused considerable ecological damage in introduced areas. *H. frenatus* introduced in the Mascarene Islands is thought to be one of the main causes for the extermination of three species and local extinction of three other species of native *Nactus* geckos (Arnold 2000; Cole et al. 2005). Unfortunately, very little is known about the distribution of commensal *Hemidactylus* species in India and their native habitat.

In this study, samples of *H. brookii* sensu lato were collected opportunistically across India and putative species within the *H. brookii* complex were identified using molecular methods. Morphology of these specimens was further examined to identify diagnostic characters for each clade, which was then used to develop a dichotomous identification key (as suggested in Fujita and Leaché 2011). Descriptions of the type specimens of *H. brookii* sensu stricto, *H. kushmorensis*, and *H. gleadowi* by Mahony (2011),

Hemidactylus murrayi by Gleadow (1887), and *H. tenketai* by Kathriner et al. (2014) were then compared using the identification key to name each of the clades. This study highlights the importance of incorporating morphology in a molecular study to resolve cryptic species complexes that have had complicated taxonomic history.

Materials and methods

Taxon sampling

Opportunistic sampling was carried out in various locations in India for *H. brookii* sensu lato and the recently elevated species from this complex along with endemic ground-dwelling *Hemidactylus* (Table 1 and Fig. 1). Entire specimens and, in some cases, only tissue samples were collected. Tail or liver samples were preserved in 100 % alcohol in the field and then stored at −20 °C in the lab. Specimens were fixed in 4 % formalin and preserved in 70 % alcohol. Field identification was based on morphological characters provided in Smith (1935) and Giri and Bauer (2008).

Molecular methods

DNA was extracted from the tissue samples following the phenol chloroform isoamyl alcohol method as per Sambrook and Russell (2001). This DNA extract was then stored at −20 °C for further use. Partial mitochondrial cytochrome *b* gene (cyt *b*, 307 base pairs (bp)) was PCR amplified using primers published in Bauer et al. (2007) and partial nuclear Recombination Activating Gene1 (RAG1, 642 bp) was amplified according to primers published in Groth and Barrowclough (1999) and Bauer et al. (2007). These markers have previously generated well-supported and resolved trees for *Hemidactylus* and *H. brookii* phylogenies (Carranza and Arnold 2006; Bansal and Karanth 2010; Bauer et al. 2010a; Bauer et al. 2010b). PCR amplification, purification, and sequencing were carried out according to Bansal and Karanth (2010). Specimens used in the present study, locations, specimen codes, and GenBank accession numbers for the two genes analyzed are listed in Table 1.

Phylogenetic analyses

The mitochondrial and nuclear data were concatenated, as well as analyzed separately using maximum likelihood and Bayesian methods. Published sequences from Carranza and Arnold (2006), Bansal and Karanth (2010), Bauer et al. (2010a) and Bauer et al. (2010b) were also retrieved from GenBank. For the combined data set, only samples that had both mitochondrial and nuclear data were included. Other representative species were also added from the tropical Asian *Hemidactylus* clades. The *Hemidactylus bowringii* group was used as an outgroup as it was found to be the sister clade to the tropical Asian clade in the combined nuclear and mitochondrial tree in previous studies (Bansal and Karanth 2010; Bauer et al. 2010a; Bauer et al. 2010b). The sequences were aligned in MEGA 5.01 (Tamura et al. 2011) using clustalW. The nucleotide sequences were converted to amino acid sequences to check for sequencing errors and pseudogenes. Dataset was partitioned into two partitions corresponding to the two genes. It was not partitioned further as per codon positions, given the short lengths of the fragments sequenced. PartitionFinder v1.1.1 (Lanfear et al. 2012) was used to find the model of sequence evolution for the two partitions. GTR + G was used as the model of sequence evolution for both the partitions (when the dataset was partitioned based on codon positions, the optimal partitioning scheme suggested by partitionFinder included four partitions. However, the RAxML tree built using this scheme had marginally lower bootstrap values, while the topology remained the same). Phylogenies were constructed using a maximum likelihood (ML) approach in RAxMLGUI (Silvestro and Michalak 2012). A thorough bootstrap was carried out for 1000 reps with 10 ML searches. Bayesian analysis was performed in MrBayes 3.2.1 (Ronquist et al. 2012) with default prior settings. Markov chains were sampled every 500 generations beginning from two randomly generated trees for 8, 000,000 generations until the standard deviation of split frequency was less than 0.005. First 25 % of the trees were discarded as "burn-in". Similar settings were used when analyzing the mitochondrial and nuclear data separately. For the gene tree, only one nucleotide sequence was retained in case of multiple samples having identical sequences. *H. frenatus* was used as an outgroup in these analyses based on the results obtained in the combined phylogeny. Haplotype network was built for the nuclear dataset. A median-joining method (Bandelt et al. 1999) was implemented in the software Network (version 4.6; http://fluxus-engineering.com). Ambiguous sites were coded using the IUPAC nucleotide code for degenerate sites.

We used the Poisson tree processes (PTP) method as an additional line of evidence to identify putative species (Zhnag et al. 2013). The PTP method uses branch lengths as a proxy for number of substitutions per site between two branching event. It relies on the basic assumption that "the number of substitutions between species is significantly higher than the number of substitutions within species" (Zhnag et al. 2013). The model thus searches for transition points on the phylogeny between inter and intra-species branching pattern (Kergoat et al. 2014). This method was implemented on the web server for PTP (available at http://species.h-its.org/ptp/) using the best ML tree resulting from the RAxML analysis (Zhnag et al. 2013). We used this method on the ML tree of the concatenated dataset with the following

Table 1 List of samples used in the study, their specimen numbers, location, Genbank accession numbers, and habitat information. '-'refers to unavailable data

Sample no.	Voucher no.	Locality	Clade/ species	GenBank Accession numbers		Habitat
				Cyt *b*	RAG1	
1	CES09008	Gandagan, Odisha, India 20° 15′ 53.9094″ N 84° 14′ 32.4306″ E	Clade 1 *H. parvimaculatus*	KU720637	KU720682	A
2	CES11020	Polupalli, Tamil Nadu, India 12° 35′ 22.6926″ N 78° 8′ 31.9122″ E	Clade 1 *H. parvimaculatus*	DQ120272 (Carranza and Arnold, 2006)	KU720683	A
3	CES06037	Masinagudi, Tamil Nadu, India 11° 34′ 12.7986″ N 76° 38′ 27.1494″ E	Clade 1 *H. parvimaculatus*	DQ120272	KU720684	–
4	CES08004	Kampalapura, Karnataka, India 12° 49′ 9.8394″ N 77° 2′ 17.88″ E	Clade 1 *H. parvimaculatus*	DQ120272	KU720685	–
5	CES11024	Hassan, Karnataka, India 13° 1′ 9.1194″ N 76° 7′ 27.8394″ E	Clade 1 *H. parvimaculatus*	DQ120272	–	A
6	Hemb22b	Mauritius	Clade 1 *H. parvimaculatus*	DQ120272	–	–
7	CES06180	Coimbatore, Tamil Nadu, India 11° 1′ 0.8394″ N 76° 57′ 20.8794″ E	Clade 1 *H. parvimaculatus*	DQ120272	–	A
8	CES11018	Coimbatore, Tamil Nadu, India 11° 4′ 59.0484″ N 76° 53′ 20.9826″ E	Clade 1 *H. parvimaculatus*	DQ120272	KU720686	A
9	CES06036	Tumkur, Karnataka, India	Clade 1 *H. parvimaculatus*	HM595645 (Bansal and Karanth, 2010)	–	A
10	Hemb1b	Mauritius	Clade 1 *H. parvimaculatus*	DQ120271 (Carranza and Arnold, 2006)	–	–
11	AMB7466	Mampuri, Sri Lanka 7°59′38″S, 79°44′33″E	Clade 1 *H. parvimaculatus*	GQ375292 (Bauer et al. 2010b)	GQ375311	–
12	AMB7424	Dehikindagama, Sri Lanka 6°56′00″S, 81°17′17″E	Clade 1 *H. parvimaculatus*	GQ375296 (Bauer et al. 2010b)	–	–
13	AMB7480	Matale, Sri Lanka 7°31′48″S, 80°37′39″E	Clade 1 *H. parvimaculatus*	GQ375298 (Bauer et al. 2010b)	–	–
14	AMB7426	Gonaganara, Sri Lanka 6°36′53″S, 81°16′13″E	Clade 1 *H. parvimaculatus*	GQ375297 (Bauer et al. 2010b)	–	–
15	ADS36	Kartivu, Sri Lanka 7°22′35.6″S, 81°58′59.0″E	Clade 1 *H. parvimaculatus*	GQ375291 (Bauer et al. 2010b)	GQ375310	–
16	AMB7427	Matale, Sri Lanka 7°31′48″S, 80°37′39″E	Clade 1 *H. parvimaculatus*	GQ375299 (Bauer et al. 2010b)	–	–
17	AMB7432	Tempitiya, Sri Lanka 7°35′26″S, 81°25′38″E	Clade 1 *H. parvimaculatus*	GQ375300 (Bauer et al. 2010b)	–	–
18	CES10015	Rushikulya, Odisha, India 19° 24′ 26.9238″ N 85° 3′ 56.0016″ E	Clade 1 *H. parvimaculatus*	KU720638	KU720687	A
19	CES07025	Attagulipura, Karnataka, India 11° 49′ 45.4794″ N 77° 0′ 20.8794″ E	Clade 1 *H. parvimaculatus*	KU720639	–	A
20	CES06004	Bangalore, Karnataka, India 12° 57′ 36″ N 77° 33′ 36″ E	Clade 1 *H. parvimaculatus*	KU720640	KU720688	A
21	CES06177	Chennai, Tamil Nadu, India 13° 3′ 37.5192″ N 80° 14′ 58.4988″ E	Clade 1 *H. parvimaculatus*	KU720641	KU720689	A
22	CES11027	Poinguinim, Goa, India 14° 58′ 28.9524″ N 74° 5′ 28.5678″ E	Clade 1 *H. parvimaculatus*	KU720642	–	D
23	CES11029	Mollem, Goa, India 15° 22′ 32.502″ N 74° 13′ 36.7278″ E	Clade 1 *H. parvimaculatus*	KU720643	KU720690	D
24	CES10013	Kutugam, Odisha, India 18° 37′ 41.9232″ N 82° 52′ 40.7172″ E	Clade 1 *H. parvimaculatus*	KU720644	KU720691	D
25	CES10011	Araku Valley, Andhra Pradesh, India 18° 14′ 45.9234″ N 82° 59′ 49.1994″ E	Clade 1 *H. parvimaculatus*	KU720645	KU720692	A
26	CES10009	Vizianagaram, Andhra Pradesh, India 18° 7′ 29.64″ N 83° 24′ 11.88″ E	Clade 1 *H. parvimaculatus*	KU720646	KU720693	A
27	CES10012	Majhiguda, Odisha, India 18° 47′ 28.3446″ N 82° 14′ 42.6186″ E	Clade 1 *H. parvimaculatus*	KU720647	KU720694	D
28	E110911	Kollam, Kerala, India	Clade 1 *H. parvimaculatus*	DQ120273 (Carranza and Arnold, 2006)	–	–
29	AMB7475	Kandy, Sri Lanka 7°15′36″S, 80°37′11″E	Clade 1 *H. parvimaculatus*	GQ375290 (Bauer et al. 2010b)	GQ375309 (Bauer et al. 2010b)	–
30	CES11073	Reasi, Himachal Pradesh, India 33° 4′ 41.9592″ N 74° 49′ 52.8054″ E	Clade 3 *H.* cf. *kushmorensis*	KU720648	KU720695	–
32	CES11054	Mandi-Kullu Rd., Himachal Pradesh, India 31° 45′ 21.891″ N 76° 56′ 36.492″ E	Clade 3 *H.* cf. *kushmorensis*	KU720648	–	–
33	CES11057	Kangra-JawalaMukhi Road, Himachal Pradesh, India 32° 1′ 10.8402″ N 76° 14′ 43.5984″ E	Clade 3 *H.* cf. *kushmorensis*	KU720648	KU720696	–
34	CES11070	Lunj-Masrur, Himachal Pradesh, India 32° 6′ 40.0716″ N 76° 9′ 40.3884″ E	Clade 3 *H.* cf. *kushmorensis*	KU720648	KU720697	–

Table 1 (continued)

Sample no.	Voucher no.	Locality	Clade/ species	GenBank Accession numbers		Habitat
				Cyt *b*	RAG1	
35	CES06078	Dehradun, Uttarakhand, India 30° 17′ 2.0394″ N 77° 58′ 28.2″ E	Clade 3 *H.* cf. *kushmorensis*	HM595646 (Bansal and Karanth, 2010)	KU720698	–
36	CES11065	Sujanpur, Himachal Pradesh, India 31° 50′ 3.0516″ N 76° 30′ 28.4616″ E	Clade 3 *H.* cf. *kushmorensis*	KU720648	KU720699	–
37	CES11072	Chamba, Himachal Pradesh, India 32° 33′ 19.782″ N 76° 7′ 37.0452″ E	Clade 3 *H.* cf. *kushmorensis*	KU720649	KU720700	–
38	CES11055	Kangra-Jawalamukhi Road, Himachal Pradesh, India 32° 1′ 10.8402″ N 76° 14′ 43.5984″ E	Clade 3 *H.* cf. *kushmorensis*	KU720650	KU720701	–
39	CES11051	Tattapani-Chaba Road, Himachal Pradesh, India 31° 14′ 30.411″ N 77° 12′ 8.1216″ E	Clade 3 *H.* cf. *kushmorensis*	KU720650	KU720702	–
40	CES11052	Barmana, Himachal Pradesh, India 31° 24′ 46.2312″ N 76° 50′ 6.936″ E	Clade 3 *H.* cf. *kushmorensis*	KU720650	KU720703	–
41	CES11059	Kangra-JawalaMukhi Road, Himachal Pradesh, India 32° 1′ 10.8402″ N 76° 14′ 43.5984″ E	Clade 3 *H.* cf. *kushmorensis*	KU720650	KU720704	–
42	CES09058	Ajmer, Rajasthan, India 26° 26′ 27.9954″ N 74° 45″ 52.5234′ E	Clade 3 *H.* cf. *kushmorensis*	KU720651	–	–
43	CES09004	Baripada, Odisha, India 21° 56″ 10.302′ N 86° 44″ 4.1532′ E	Clade 3 *H.* cf. *kushmorensis*	KU720652	KU720705	–
44	CES06175	Jammu, India	Clade 3 *H.* cf. *kushmorensis*	HM595647 (Bansal and Karanth, 2010)	–	–
45	CES09040	Chotila, Gujarat, India 22° 25′ 52.968″ N 71° 10′ 30.327″ E	Clade 2 *H.* cf. *kushmorensis*	KU720653	KU720706	–
46	CES09052	Mt. Abu, Rajasthan, India 24° 35′ 33″ N 72° 42′ 56.0016″ E	Clade 2 *H.* cf. *kushmorensis*	KU720654	KU720707	–
47	CES07038	Dorle, Ratnagiri, Maharashtra, India	Clade 7 *H. albofasciatus*	HM595642 (Bansal and Karanth, 2010)	KU720708	–
48	CES08018	Malvan, Sindhudurg, Maharashtra, India	Clade 7 *H. albofasciatus*	HM595643 (Bansal and Karanth, 2010)	–	–
49	JFBM2	Pakistan (captive specimen)	Clade 7 *H. imbricatus*	EU268386.1 (Bauer et al. 2010(b))	EU268293 (Bauer et al. 2010(b))	–
50	JS11	Pakistan (captive specimen)	Clade 7 *H. imbricatus*	EU268385 (Bauer et al. 2010b)	EU268292 (Bauer et al. 2010b)	–
51	CES07039	Pune, Maharahstra, India	Clade 7 *H. gracilis*	HM595660 (Bansal and Karanth, 2010)	HM622359 (Bansal and Karanth, 2010)	–
52	CES07016	Pavgada, Karnataka, India	Clade 7 *H. reticulatus*	HM595669 (Bansal and Karanth, 2010)	KU720709	–
53	CES06024	Nandi Hills, Karnataka, India	Clade 7 *H. reticulatus*	HM595670 (Bansal and Karanth, 2010)	–	–
54	CES06025	Nandi Hills, Karnataka, India	Clade 7 *H. reticulatus*	HM595671 (Bansal and Karanth, 2010)	–	–
55	AMB5730	Vellore, Tamil Nadu, India	Clade 7 *H. reticulatus*	EU268410	–	–
56	CES11016	Bagalkot, Karnataka, India 16° 8′ 53.1672″ N 75° 38′ 28.5972″ E	Clade 7 *H. reticulatus*	KU720655	–	–
57	CES08010	Chalakewadi, Maharashtra, India	Clade 7 *H. sataraensis*	HM595672 (Bansal and Karanth, 2010)	–	–
58	CES11036	Chikkabellapur, Karnataka, India 13° 32′ 50.6394″ N 77° 39′ 56.1594″ E	Clade 6 *H.* cf. *gleadowi*	KU720656	KU720710	B
59	CES07031	Ranebennur, Karnataka, India 14° 36′ 53.4234″ N 75° 37′ 11.2692″ E	Clade 6 *H.* cf. *gleadowi*	KU720657	KU720711	B
60	CES06157	Mysore, Karnataka, India 12° 16′ 21.36″ N 76° 37′ 28.56″ E	Clade 6 *H.* cf. *gleadowi*	KU720658	KU720712	–
61	CES11014	Bagalkot, Karnataka, India 16° 8′ 53.1672″ N 75° 38′ 28.5972″ E	Clade 6 *H.* cf. *gleadowi*	KU720659	KU720713	B
62	CES11009	Dapoli, Maharashtra, India 17° 45′ 11.2032″ N 73° 11′ 17.0232″ E	Clade 6 *H.* cf. *gleadowi*	KU720660	KU720714	B
63	CES11003	Ahmednagar, Maharashtra, India 19° 5′ 42.7482″ N 74° 44′ 58.5312″ E	Clade 6 *H.* cf. *gleadowi*	KU720661	KU720715	B
64	CES09051	Iqbalgadh, Gujarat, India 24° 20′ 50.3916″ N 72° 32′ 1.9572″ E	Clade 6 *H.* cf. *gleadowi*	KU720662	KU720716	A

Table 1 (continued)

Sample no.	Voucher no.	Locality	Clade/ species	GenBank Accession numbers		Habitat
				Cyt *b*	RAG1	
65	CES09056	Sadri, Rajasthan, India 25° 11′ 2.7162″ N 73° 27′ 10.4724″ E	Clade 6 *H.* cf. *gleadowi*	KU720662	KU720717	B
66	CES09048	Rampar-Peoni, Gujarat, India 23° 16′ 31.548″ N 69° 10′ 22.512″ E	Clade 6 *H.* cf. *gleadowi*	KU720662	KU720718	B
67	CES11004	Dediyapada, Gujarat, India 21° 31′ 9.12″ N 73° 38′ 43.4394″ E	Clade 6 *H.* cf. *gleadowi*	KU720663	KU720719	B
68	CES06099	Hyderabad, Telangana, India 17° 23′ 6.1584″ N 78° 29′ 12.0156″ E	Clade 6 *H.* cf. *gleadowi*	KU720664	KU720720	–
69	CES06087	Mahabubnagar, Telangana, India 16° 44′ 29.8962″ N 77° 59′ 9.4596″ E	Clade 6 *H.* cf. *gleadowi*	KU720665	KU720721	–
70	CES11031	Badlapur, Maharashtra, India 19° 10′ 14.5488″ N 73° 16′ 57.0606″ E	Clade 4 *H. murrayi*	KU720666	KU720722	A
71	CES11002	Mumbai, Maharashtra, India 18° 55′ 34.4994″ N 72° 49′ 59.8578″ E	Clade 4 *H. murrayi*	KU720667	KU720723	A
72	CES06120	Kota, Karnataka, India 13° 30′ 54″ N 74° 42′ 20.16″ E	Clade 4 *H. murrayi*	KU720667	KU720724	A
73	CES06048	Davangere, Karnataka, India 14° 27′ 58.68″ N 75° 55′ 25.6794″ E	Clade 4 *H. murrayi*	KU720667	KU720725	A
74	CES06032	Shimoga, Karnataka, India 13° 56′ 59.9994″ N 75° 33′ 36″ E	Clade 4 *H. murrayi*	KU720667	KU720726	A
75	ZRC26167	Loagan Bunut National Park, Sarawak, Malaysia (Borneo)	Clade 4 *H. murrayi*	GQ375293 (Bauer et al. 2010b)	GQ375314 (Bauer et al. 2010b)	–
76	CAS206638	Mandalay Division, Myanmar	Clade 4 *H. murrayi*	EU268407 (Bauer et al. 2010b)	EU268314 (Bauer et al. 2010b)	–
77	CAS208159	Yangon, Myanmar	Clade 4 *H. murrayi*	GQ375294 (Bauer et al. 2010b)	GQ375312 (Bauer et al. 2010b)	–
78	LLG6754	Empangon Air Hitam, Pulau Pinang, Malaysia	Clade 4 *H. murrayi*	EU268397.1 (Bauer et al. 2010b)	EU268304 (Bauer et al. 2010b)	–
79	CAS213939	Kyauk Pan Tawn, Mandalay Division, Myanmar	Clade 4 *H. murrayi*	DQ120275 (Carranza and Arnold, 2006)	–	–
80	CAS213515	Mingalardan, Yangon Division, Myanmar	Clade 4 *H. murrayi*	DQ120274 (Carranza and Arnold, 2006)	–	–
81	CES11032	Mumbai, Maharashtra, India 19° 8′ 52.08″ N 72° 52′ 42.6″ E	Clade 4 *H. murrayi*	KU720668	–	A
82	CES09023	Bhagamandala, Karnataka, India 12° 23′ 10.32″ N 75° 31′ 47.6394″ E	Clade 4 *H. murrayi*	KU720669	–	A
83	E110910	Subrahmnya, Karnataka, India	Clade 4 *H. murrayi*	DQ120276 (Carranza and Arnold, 2006)	–	–
84	CES06039	Tiptur, Karnataka, India 13° 15′ 15.1086″ N 76° 28′ 37.653″ E	Clade 4 *H. murrayi*	EU268398	–	A
85	CES06080	Palakkad, Kerala, India	Clade 4 *H. murrayi*	HM595649 (Bansal and Karanth, 2010)	HM622355 (Bansal and Karanth, 2010)	A
86	CES06116	Amasebile, Karnataka, India 13° 35′ 23.9994″ N 74° 45′ 0″ E	Clade 4 *H. murrayi*	EU268398 (Bauer et al. 2010b)	KU720728	A
87	CES06052	Hubli, Karnataka, India 15° 21′ 53.28″ N 75° 7′ 26.04″ E	Clade 4 *H. murrayi*	EU268398	–	A
88	CES07027	Bankapur, Karnataka, India 14° 55′ 0.12″ N 75° 16′ 0.12″ E	Clade 4 *H. murrayi*	EU268398	–	A
89	LLG6755	Pulau Pinang, Empangon Air Hitam, Malaysia	Clade 4 *H. murrayi*	EU268398	EU268305 (Bauer et al. 2010b)	–
90	CES11028	Mollem, Goa, India 15° 22′ 32.502″ N 74° 13′ 36.7278″ E	Clade 4 *H. murrayi*	EU268398	KU720727	A
91	CES06045	ChitraDurga, Karnataka, India 14° 13′ 19.5234″ N 76° 24′ 1.296″ E	Clade 4 *H. murrayi*	EU268398	KU720729	A
92	CES06059	Belgaum, Karnataka, India 15° 51′ 1.296″ N 74° 30′ 16.8084″ E	Clade 4 *H. murrayi*	EU268398	KU720730	A
93	CES11026	Poinguinim, Goa, India 14° 58′ 28.9524″ N 74° 5′ 28.5678″ E	Clade 4 *H. murrayi*	EU268398	KU720731	A
94	CES11021	Dandeli, Karnataka, India 15° 15′ 41.0904″ N 74° 36′ 47.289″ E	Clade 4 *H. murrayi*	KU720670	KU720732	D
95	CES06075	Chikamagalur, Karnataka, India 13° 18′ 44.28″ N 75° 46′ 15.2394″ E	Clade 4 *H. murrayi*	KU720671	–	A
96	CES08042	Naravi, Karnataka, India 13° 7′ 19.3038″ N 75° 8′ 52.7244″ E	Clade 4 *H. murrayi*	KU720672	KU720733	A
97	CAS229632	Tanintharyi Division, Myanmar	Clade 4 *H. murrayi*	GQ375295 (Bauer et al. 2010b)	GQ375313 (Bauer et al. 2010b)	–
98	CES11006	Malshej Ghat, Maharashtra, India 19° 18′ 7.3074″ N 73° 49′ 30.7194″ E	Clade 4 *H. murrayi*	KU720673	KU720734	–
99	CES09042	Junagadh, Gujarat, India 21° 30′ 49.4208″ N 70° 27′ 22.2228″ E	Clade 4 *H. murrayi*	KU720674	KU720735	–

Table 1 (continued)

Sample no.	Voucher no.	Locality	Clade/ species	GenBank Accession numbers		Habitat
				Cyt *b*	RAG1	
100	CES06119	Amasebile, Karnataka, India	Clade 4 *H. murrayi*	HM595648 (Bansal and Karanth, 2010)	–	A
101	CES09047	Balapar, Gujarat, India 23° 18′ 14.7882″ N 69° 3′ 10.1514″ E	Clade 4 *H. murrayi*	KU720675	KU720736	C
102	CES11005	Nasik, Maharashtra, India 19° 59′ 50.8308″ N 73° 47′ 23.2866″ E	Clade 4 *H. murrayi*	KU720676	KU720737	–
103	CES11038	Chikkabellapur, Karnataka, India 13° 33′ 0.72″ N 77° 39′ 41.04″ E	Clade 5: *H. treutleri*	KU720677	–	C
104	CES11040	Chikkabellapur, Karnataka, India 13° 33′ 0.72″ N 77° 39′ 41.04″ E	Clade 5: *H. treutleri*	KU720677	KU720738	C
105	CES09029	Kangudi, Tamil Nadu, India 12° 46′ 7.032″ N 78° 26′ 1.464″ E	Clade 5: *H. treutleri*	KU720678	KU720739	C
106	CES11012	Rishi valley, Andra Pradesh, India 13° 37′ 55.9194″ N 78° 27′ 35.28″ E	Clade 5: *H. treutleri*	KU720679	KU720740	C
107	CES06182	Hampi, Karnataka, India 15° 19′ 59.8794″ N 76° 28′ 0.12″ E	Clade 5: *H. treutleri*	KU720680	KU720741	C
108	CES06108	Hyderabad, Telangana, India 17° 22′ 59.9988″ N 78° 24′ 15.0012″ E	Clade 5: *H. treutleri*	KU720681	KU720742	A
109	LLG6745	Empangon Air Hitam, Pulau Pinang, Malaysia	*H. frenatus*	EU268390 (Bauer et al. 2010b)	EU268297 (Bauer et al. 2010b)	–
110	AMB7420	Sri Lanka, Rathegala	*H. frenatus*	EU268391 (Bauer et al. 2010b)	EU268298 (Bauer et al. 2010b)	–
111	LLG6745	Malaysia, Pulau Pinang, Empangon Air Hitam	*H. frenatus*	EU268390 (Bauer et al. 2010b)	EU268297 (Bauer et al. 2010b)	–
112	CES08013	Hampi, Karnataka, India	*H. giganteus*	HM595657 (Bansal and Karanth, 2010)	HM622357 (Bansal and Karanth, 2010)	–
113	CAS228540	United Arab Emirates, Dubai	*H. flaviviridis*	HM559595 (Bauer et al. 2010b)	HM559693 (Bauer et al. 2010b)	–
114	AMB7443	Sri Lanka, Polonnaruwa	*H. leschenaulti*	HM559601 (Bauer et al. 2010b)	HM559701 (Bauer et al. 2010b)	–
115	CES07040	Castle Rock, Karnataka, India	*H. prashadi*	HM595668 (Bansal and Karanth, 2010)	HM622364 (Bansal and Karanth, 2010)	–
116	BNHS1516	Zirad, Raigad, Maharashtra, India	*H. maculatus*	HM559607 (Bauer et al. 2010b)	HM559707 (Bauer et al. 2010b)	–
117	CES07007	Ramnagar, Karnataka, India	*H. triedrus*	HM595673 (Bansal and Karanth, 2010)	HM622365 (Bansal and Karanth, 2010)	–
118	CAS228109	China, Yunnan Province, Nujang District, Liuku	*H. aquilonius*	EU268406 (Bauer et al. 2010b)	EU268313 (Bauer et al. 2010b)	–
119	CAS222276	Myanmar, Mon State, Kyaihto Township, Kyait Hti Yo Wildlife Sactuary	*H. garnotii*	EU268396 (Bauer et al. 2010b)	EU268303 (Bauer et al. 2010b)	–
120	KU304111	Philippines, Lubang Id., Occidental Mindoro Prov., Lubang Barangay Paraiso	*H. platyurus*	HM559587 (Bauer et al. 2010b)	HM559685 (Bauer et al. 2010b)	–

Individuals were noted to be found in habitat A. city/village mostly on walls inside or outside buildings B. On the ground in open scrub or agricultural fields C. On vertical rock substratum in rocky outcrops D. Forested habitat

parameters: MCMC, 500,000 generations; thinning, 100; burn-in, 0.25; seed, 123, and visually confirmed the convergence of the MCMC chains.

The SH test (Shimodaira and Hasegawa 1999) was performed on the combined dataset to test whether the two lineages of commensals have evolved adaptations independently that allow them to invade human habitation. We considered those species to be commensal which occurred predominantly around human habitation and are rarely found far away from it. Two alternative topologies were compared—the tree obtained from the concatenated data using the ML approach, and, a phylogeny where the commensals, clade 1 (*H. parvimaculatus*) and clade 4 (*H. murrayi*), were constrained to be sister taxa. Constrained phylogeny was constructed using maximum likelihood approach in RAxMLGUI (Silvestro and Michalak 2011) using GTR + G as the model of sequence evolution for both the partitions. One thousand thorough bootstraps were carried out with 10 ML searches. The two topologies were compared in PAUP* v4.0b 10 (Swofford 2003).

Morphology

Phylogenetic analyses were undertaken to identify well-supported clades within the *H. brookii* complex. From the specimens used to obtain the molecular data, a total of 48 specimens were used for morphological analyses such that multiple individuals from all the clades were represented (Table 1). A total of 19 morphometric, six meristic, and other descriptive morphological characters pertaining to enlarged

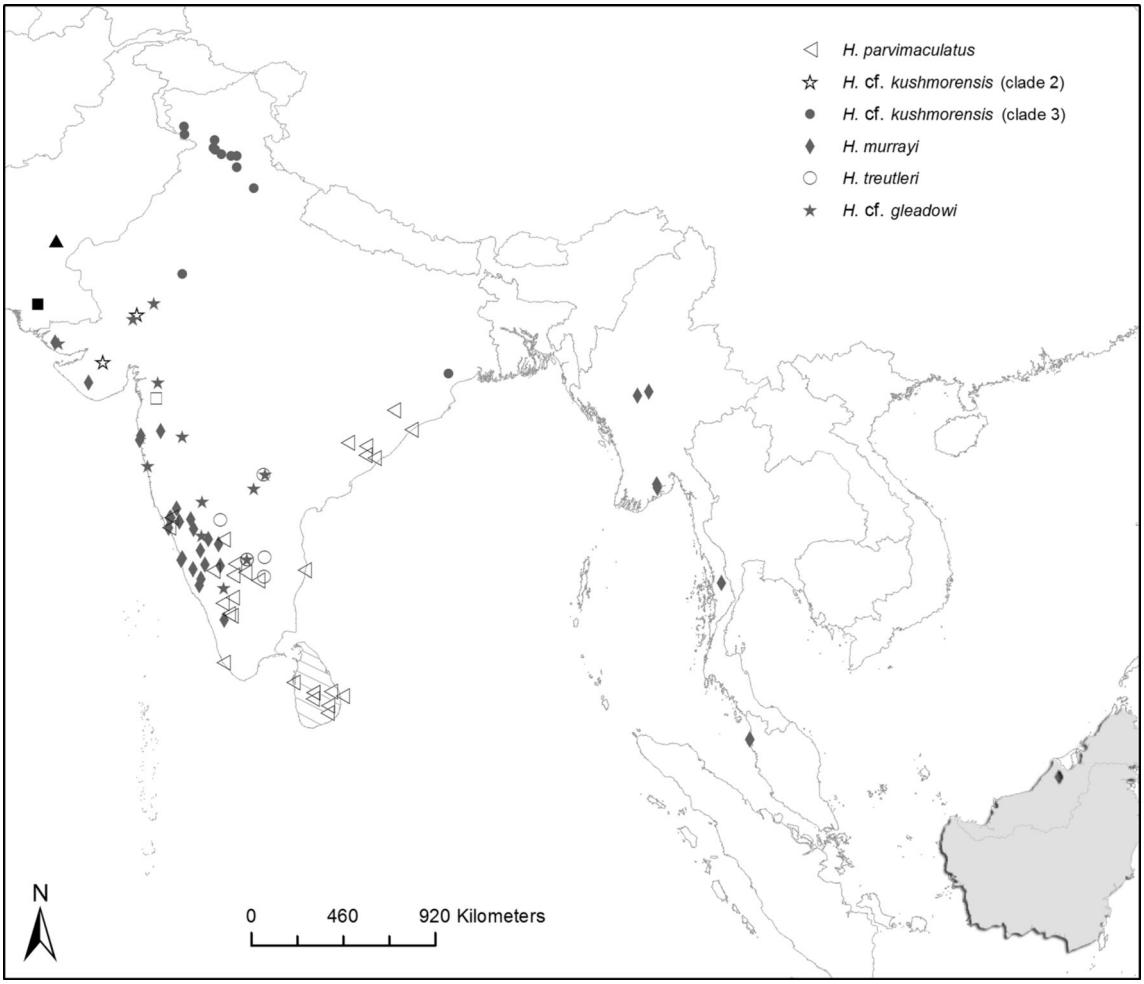

Fig. 1 Map showing the sampling locations and species-assignment of each sample within the *Hemidactylus brookii* complex. Type locality of *H. brookii*, Borneo, is marked in *gray. Open square and closed square* denote the type locality of *H. murrayi* (Pimpri, Gujarat, India), and *H. gleadowi* (Jerruck Division, Pakistan), respectively. *Closed triangle* indicates the type locality of *H. kushmorensis. Hatched lines* show the type locality of *H. parvimaculatus. Hemidactylus parvimaculatus* from Mauritius not shown in the map

dorsal tubercles and lamellae were identified. Scale counts and external observations of morphology were made using a Wild M5 dissecting microscope. Morphometric measurements were taken with a Mitutoyo dial caliper (to the nearest 0.1 mm). The following measurements were noted for each specimen: snout to vent length (SVL, from tip of the snout to vent), trunk length (TRL, distance from the axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hindlimb insertion), maximum body width (BW), crus length (CL, from base of heel to knee), tail length (TL, distance from base of the tail to tail tip), tail width (TW, measured at widest point of tail), head length (HL, distance between the retroarticular process of jaw and snout-tip), head width (HW, maximum width of the head), head height (HH, maximum height of head, from occiput to underside of the jaw), forearm length (FL; from base of the palm to elbow), orbital diameter (OD; greatest diameter of the orbit), nares to eye distance (NE, distance between anterior most point of the eye and nostril), snout to eye distance (SE, distance between anterior most point of the eye and tip of snout), eye to ear distance (EE, distance from anterior edge of the ear opening to posterior corner of the eye), length of ear opening (HE; the maximum length of the ear opening), internarial distance (IN; distance between the nares), interorbital distance (IO, shortest distance between the left and right supraciliary scale rows), mental length (ML, maximum length of the mental scale), first postmental length (1st PML, maximum length of the first post mental scale), first postmental contact region (1st PC, the length of the contact region where first postmentals touch each other) and second postmental length (2nd PML, maximum length of the second post mental). Tail length was not included in the analysis due to many specimens having broken or regenerated tail, and body width was avoided to reduce preservation bias. Meristic characters considered were as follows: number of precloacal-femoral pores on each side, number of scales that lack pores between the two rows of pore-bearing scales, number of rows of enlarged dorsal tubercles at mid-trunk, number of lamellae

on each digit of the forelimb and hindlimb on either side, number of supra and infra labials. Descriptive morphological characters used were size, shape, and pattern of enlarged dorsal tubercles, and lamellae (divided/undivided and oblique/straight transverse series).

Morphological analysis

Morphometric variables were examined for correlation using Pearson's correlation. Data of all the correlated variables was standardized by subtracting the mean from each value and then dividing the result by the standard deviation. This data was then analyzed using principal component analysis (PCA). Following this, principal component 1 (PC1) was analyzed by one-way analysis of variance (ANOVA) to test whether there was a significant difference across the clades given the PC1 eigenvalues. Differences in eigenvalues were further evaluated using Tukey's HSD post hoc comparison to detect clades that were significantly different from each other. All the statistical analyses was carried out in *R* (R Core Team 2013). Subsequently, we identified diagnostic morphological traits for each of the clades retrieved within the complex in the phylogenetic tree. These morphological traits were in turn used to design an identification key.

Results

Molecular phylogeny

The combined phylogeny included 69 individuals of *H. brookii* sensu lato, representative species from the *Hemidactylus* tropical Asian clade, and species from the *bowringii* group (Fig. 2). Maximum likelihood (ML) and Bayesian (BI) approaches retrieved the same seven clades with high bootstrap support and posterior probability value, however some of the higher-level relationships were not well supported in the ML tree. The relationships between these clades did not vary across methods, though there were minor differences within each clade. Among these clades, here named clade 7 corresponds to the morphologically diverse and distinct group of endemic ground-dwelling *Hemidactylus* with five previously described species (*Hemidactylus albofasciatus*, *Hemidactylus gracilis*, *Hemidactylus reticulatus*, *Hemidactylus sataraensis*, and *Hemidactylus imbricatus*).

Mitochondrial sequences of cyt *b* from 108 individuals were analyzed using *H. frenatus* as the outgroup. There was a total of 118 parsimony informative sites and 141 variable sites out of a total of 274 bp. Tree topologies obtained using ML and BI approaches were comparable (Supplementary Fig. 1). The mean *p* distances among clades 1 to 6 were comparable to those between species in clade 7 (Table 2). Nuclear marker RAG1 was analyzed for 75 samples, which had a total of 35 parsimony informative sites out of 68 variable sites. Similar tree topologies were obtained using ML and BI approaches (Supplementary Fig. 2). Except for clades 2 and 3 of the mitochondrial phylogeny, all the other clades were retrieved in the nuclear tree with high bootstrap and posterior probability values. Relationship between these clades differed in the nuclear tree from that of the mitochondrial phylogeny. Similar to mitochondrial data, the mean *p* distances between clades 1, 4, 5, and 6 were comparable to those between species in clade 7 (Table 2).

The haplotype network revealed multiple clusters consisting of related haplotypes and these clusters corresponded to the clades retrieved in the phylogeny. Replacing ambiguous sites with the most likely base did not change the network (Supplementary Fig. 3). The PTP method of species delimitation estimated 14 putative species within the ingroup. Each of the species within the ground-dwelling *Hemidactylus* clade was identified correctly. It also identified clade 1, 2, 3, 4, and 6 as putative species. However, each of the individuals in clade 5 (*H. treutleri*) was indicated as putative species. The likelihood support values for each of the putative species are mentioned in supplementary material 1.

In the SH test, the likelihood score of the best tree (-lnL = 6796.93245) based on concatenated data was significantly higher (SH test, $p < 0.05$) than the tree where the two commensals were constrained to be sister taxa (-lnL = 6895.89739; tree not shown). This suggests that adaptations that could have assisted these geckos in exploiting human habitat have independently evolved at least twice in the *H. brookii* complex.

Morphology

Most morphometric variables were highly correlated (correlation of above 0.6). The least correlated were head height and orbital diameter (0.3362; *p* value = 0.0099). PCA of standardized morphometric data resulted in PC1 explaining 82.2 % of the total variance and PC2 explaining 5.6 %. Plotting the first two principal axes resulted in three clusters corresponding to clade 4, clade 5, and the rest of the *H. brookii* sensu lato (Fig. 3). The loadings of individual morphometric variable in PC1 were all below 0.26 suggesting that all the characters used in this study were equally important to distinguish between clade 4, clade 5, and the rest of the clades. One-way ANOVA of PC1 showed significant difference across the 6 clades ($p < 0.01$). Tukey's HSD post hoc test revealed significant difference (*p* adjusted <0.05) between the following pairs of clades—clade 1 and clade 4, clade 5, and clade 1, clade 5, and clade 2, clade 4, and clade 3, clade 5, and clade 3, and clade 6, and clade 5 (Supplementary table 2).

The meristic data on the number of precloacal-femoral pores (FP) and the number of scales between the two rows of FP was found to be conserved characters within each clade (Table 3). Both these characters when considered together are helpful in distinguishing clade 4 from clade 5 and from the rest of the clades in the *H. brookii* complex (clade 1, 2, 3, and 6). However, when considered together with the dorsal pholidosis—size, shape, and arrangement pattern of enlarged tubercles—all the clades except clade 2 and 3 could be distinguished from each other (ref. Figs. 4 and 5; meristic data in Supplementary Table 3)

Discussion

Phylogenetic analyses indicate that the *H. brookii* complex consists of multiple deeply divergent clades. The mean genetic distance between these clades is comparable to those between previously described morphologically distinct species within clade 7. Furthermore, members of these clades can be diagnosed using certain combinations of morphological characters (but see discussion on clades 2 and 3). Taken together, these results suggest that *H. brookii* is a complex consisting of multiple species. In the following section we discuss each clade in detail.

Clade 1: *H. parvimaculatus*

This clade is represented by 19 individuals from various locations in peninsular India in addition to published *H. parvimaculatus* sequences from Sri Lanka and Mauritius (from Bauer et al. 2010a). This clade is well supported in nuclear, mitochondrial, and combined phylogeny. Individuals of *H. parvimaculatus* sampled during this study were predominantly found in human habitation as opposed to relatively undisturbed areas (see table 1). Therefore, this species appears to be largely a human commensal species. Bauer et al. (2010a) had speculated that *H. parvimaculatus* might be restricted to south of Palghat Gap; however, our survey indicated a much larger range for this species (ref. Fig. 1). On examination of the morphology of these individuals, the number of precloacal-femoral pores (11–17 on either side) and the scales interrupting the two rows of precloacal-femoral pores (1–3) seem to be largely conserved within this clade. This character distinguishes *H. parvimaculatus* from clade 4 and *Hemidactylus treutleri* sensu stricto. It can be distinguished from clade 2 and 3 based on the absence of undivided lamellae and from clade 6 by the presence of smaller subtrihedral tubercles on the mid-dorsum. According to the original description by Deraniyagala (1953), the type specimen was described to have 12 precloacal-femoral pores on the sides, which is consistent with this study, but the number of scales separating the two rows of precloacal-femoral pores was not mentioned in the original description. A more thorough description of the type specimen is necessary to resolve any further nomenclatural issues.

Clade 2 and clade 3: *Hemidactylus* cf. *kushmorensis*

Clades 2 and 3, although not sister to each other, appear indistinguishable from each other with respect to most of the morphological characters examined (Supplementary Table 1 and 3) and do not separate out in the PCA. The only morphological difference between these two clades is the number of femoral pores (Table 3), and the contact of primary postmental with infralabials. The primary postmental scale is strongly in contact with infralabial 1 and weakly with infralabial 2 in clade 2, and in clade 3, it is in contact with only infralabial 1. Their relatively small size, dorsal tubercles, and precloacal-femoral pore pattern resembles the recently elevated species, *H. kushmorensis* Murray (resurrected by Mahony 2011). But, sequences from topotypic samples or type specimens are needed to validate this nomen. Furthermore, additional markers need to be analyzed to confirm the monophyly of this taxon. Clade 2 is deeply divergent and sister to *H. parvimaculatus* in the combined , as well as mitochondrial phylogeny, and clade 3 is sister to *H. parvimaculatus* and clade 2. However, in parsimony and neighbor joining trees, clades 2 and 3 were sister to each other (trees not shown). In the nuclear tree, these clades were not retrieved. Members of clades 2 and 3 are distinct from the rest of the species within the *H. brookii* complex in having small rounded dorsal tubercles and a series of 2–6 undivided lamellae on the 5th toe and sometimes on the other toes.

Clade 4: *H. murrayi*

Clade 4 includes sequences obtained from topotypic specimen of *H. brookii* from Borneo published by Bauer et al. (2010a). It consists of 22 individuals sampled from peninsular India with shallow genetic divergence within the clade, except one sample (CES11005), which shows up to 8 % divergence in mitochondrial data. However, this sample has 0 % divergence in nuclear data from the rest of the samples in this clade. All but one of the samples included in this clade were from human habitation, and this species seems to be predominantly a human commensal (Table 1; Kathriner et al. 2014). Individuals of this species have a series of 6–8 femoral pores on either side with a gap of 5–7 scales between them. The morphological character of femoral pore pattern and the gap between the two rows of femoral pores is conserved within this clade and distinguishes it from the rest of the clades (Table 3). But these characters do not match the type description (redescribed by Mahony 2011) of *H. brookii* sensu stricto. Therefore,

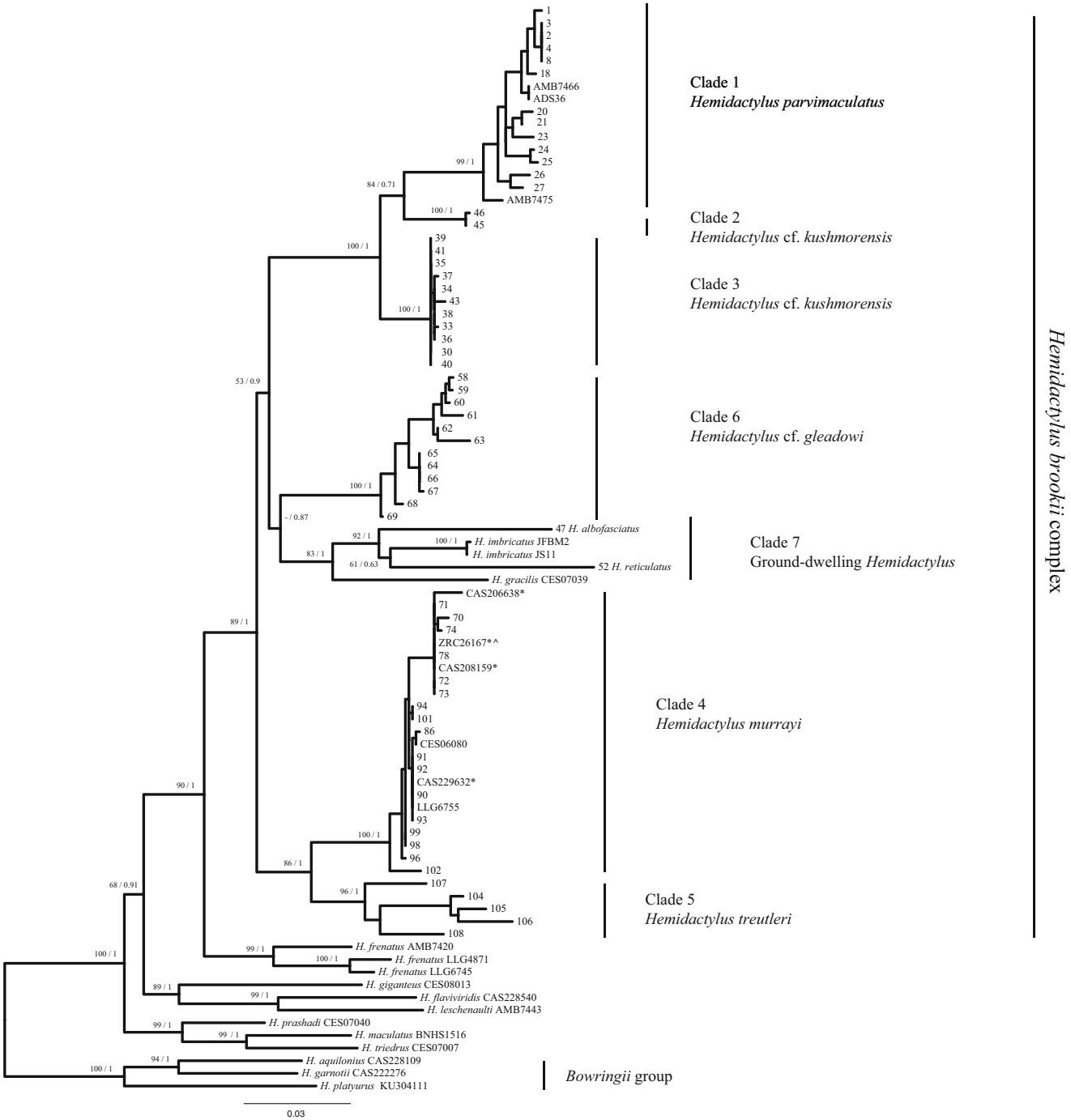

Fig. 2 Maximum Likelihood (ML) tree based on mitochondrial and nuclear data. The values on each node represent ML bootstrap value/ Bayesian posterior probability. Support values below 50/0.5 have been denoted as '-'. Voucher numbers of sequences obtained from NCBI are indicated, and the sequences generated in this study are denoted by serial numbers (ref. table 1). The samples with '*' were considered as *H. tenkatei* by Kathriner et al. (2014) and '^' is to denote sample collected from Borneo by Bauer et al. (2010a)

the specimen considered as *H. brookii* sensu stricto by Bauer et al. (2010a) is probably a different species of human commensal that seems to have recently dispersed to Borneo and other parts of Southeast Asia. In the PCA, clade 4 individuals cluster together and separate out from the other species in the *H. brookii* complex.

The samples studied by Kathriner et al. (2014) as *H. tenkatei* are nested within this clade (CAS206638, ZRC26167, CAS208159, and CAS 229632). *H. tenkatei* was elevated to a valid species based on the examination of the type specimens by Rösler and Glaw (2010). However, specimens of *H. murrayi*, another synonym of *H. brookii*,

Table 2 *p* distance matrix

	H. parvimaculatus Clade 1	*H.* cf. *kushmorensis* Clade 2	*H.* cf. *kushmorensis* Clade 3	*H. murrayi* Clade 4	*H. treutleri* Clade 5	Clade 6 *H.* cf. *gleadowi*	*H. albofaciatus*	*H. imbricatus*	*H. satarensis*	*H. gracilis*	*H. reticulatus*	
H. parvimaculatus Clade 1	–	0.1184	0.1222	0.1702	0.1687	0.1592	0.1709	0.1624	0.1740	0.1679	0.1881	Mean *p*-distance obtained using cyt *b*
H. cf. *kushmorensis* Clade 2	0.0037	–	0.0989	0.1422	0.1495	0.1495	0.1758	0.1346	0.1648	0.1648	0.1967	
H. cf. *kushmorensis* Clade 3	0.0025	0.0012	–	0.1596	0.1573	0.1531	0.1923	0.1456	0.1813	0.1593	0.1896	
H. murrayi Clade 4	0.0143	0.0129	0.0118	–	0.1238	0.1378	0.1923	0.1692	0.1658	0.1745	0.1764	
H. treutleri Clade 5	0.0176	0.0162	0.0151	0.0080	–	0.1359	0.1868	0.1632	0.1484	0.1802	0.1593	
H. cf. *gleadowi* Clade 6	0.0123	0.0110	0.0098	0.0075	0.0107	–	0.1643	0.1319	0.1253	0.1566	0.1559	
H. albofaciatus	0.0143	0.0129	0.0118	0.0141	0.0174	0.0122	–	0.1236	0.1099	0.1593	0.1549	
H. imbricatus	0.0166	0.0153	0.0141	0.0165	0.0198	0.0145	0.0071	–	0.1126	0.1071	0.1324	
H. satarensis	–	–	–	–	–	–	–	–	–	0.1484	0.1099	
H. gracilis	–	–	–	–	–	–	–	–	–	–	0.1495	
H. reticulatus	0.0272	0.0259	0.0247	0.0271	0.0304	0.0251	0.0176	0.0200	–	–	–	

Mean *p* distance obtained using RAG1

The mean genetic distance between each morphologically distinct species within clade 7 is comparable to that across rest of the clades in mitochondrial as well as nuclear data (except clades 2 and 3; See "discussion")

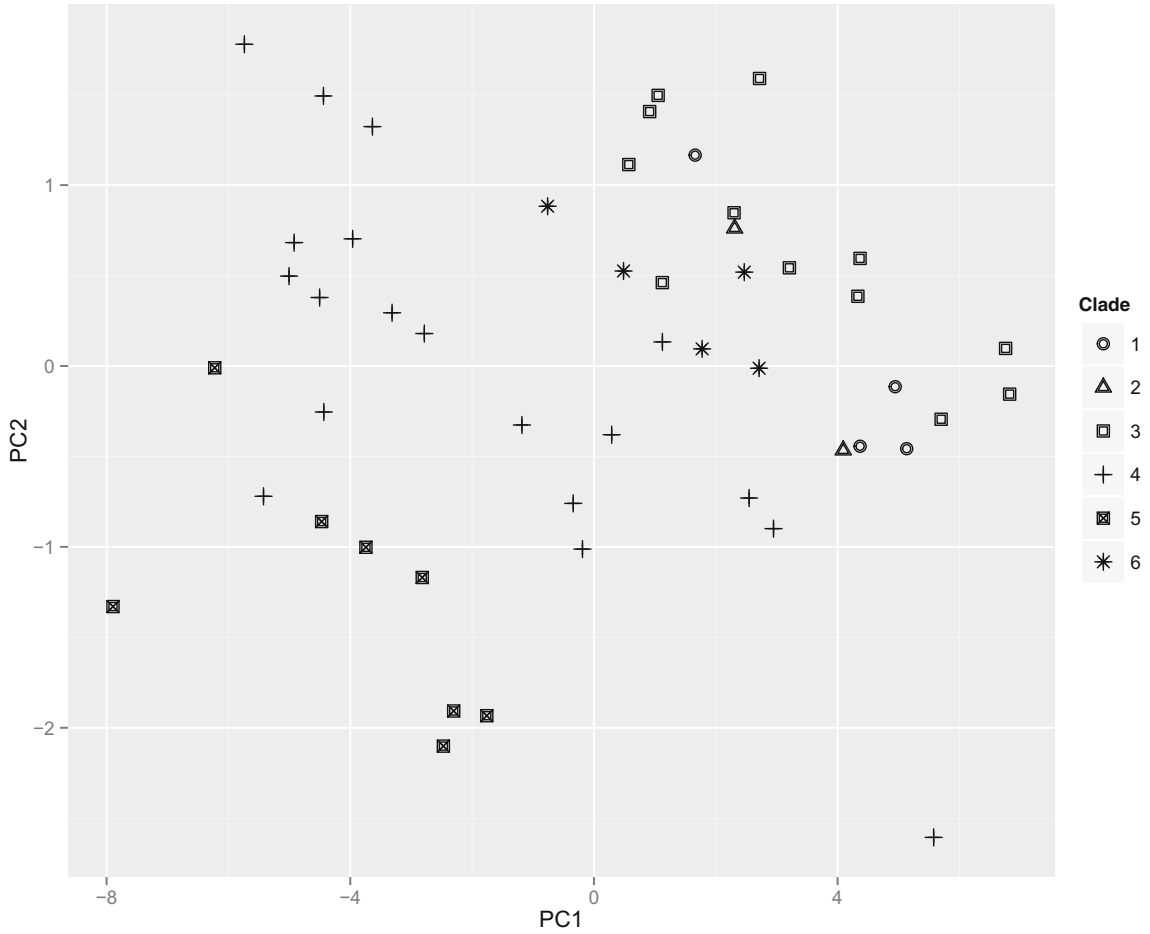

Fig. 3 Principal component analysis using morphometric data of individuals from *Hemidactylus brookii* complex

were not examined in this study. *H. murrayi* was described by Gleadow in 1887, based on fairly detailed morphological descriptions of 24 specimens from Pimpri and Garvi, in Gujarat, India. However, the repository of the type series was not mentioned in the description. *H. murrayi* was described prior to the *H. tenkatei* Lidth de Jeude, 1895, and therefore, *H tenkatei* could be a junior synonym of *H. murrayi* and needs further investigation. Furthermore, the description of *H. murrayi* by Gleadow matches that of the individuals from clade 4. In addition, samples collected from the type locality of *H.*

Table 3 Number of precloacal-femoral pores (FP) and number of non-pore baring scales between the two rows of FP (SBFP), of specimens used in this study compared with the published data on type material

Species	FP Mean (min–max)	SBFP Mean (min–max)	Sample size (*N*)
H. brookii (lectotype) BMNH 1947.3.6.47 Mahony (2011)	13/13	1	1
H. parvimaculatus Clade 1	12.2 (11–17)	2 (1–3)	10
H. cf. *kushmorensis* Clade 2	9	3	2
H. cf. *kushmorensis* Clade 3	12.2 (10–14)	2.4 (1–4)	9
H. kushmorensis (neotype) BMNH 87.9.22.8 Mahony (2011)	10/10	3	1
H. murrayi Clade 4	6.5 (6–8)	6 (5–7)	13
H. murrayi (types) Gleadow (1887)	6–8	>1	8
H. treutleri Clade 5	13.5 (11–16)	8.3 (8–9)	3
H. treutleri (holotype) ZSI 25711 Mahony (2009)	7/7	7	1
Clade 6 *H.* cf. *gleadowi*	12.1 (10–14)	0.5 (0–1)	8
H. gleadowi (neotype) BMHS 84.7.25.8 Mahony (2011)	13/12	1	1

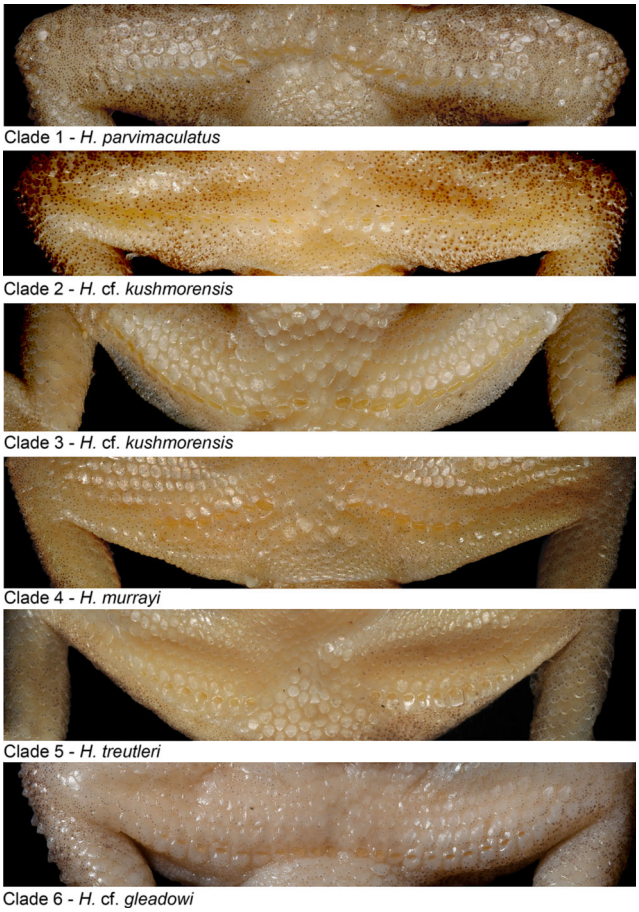

Fig. 4 Precloacal-femoral pore pattern within the *Hemidactylus brookii* complex

murrayi are morphologically identical to the individuals of this clade and the genetic data corroborates this (manuscript in preparation). *Hemidactylus subtriedroides* was another synonym of *H. brookii*, which was later synonymized with *H. tenkatei* (Mahony 2011). While Kathriner et al. (2014) suggest that *H. subtriedroides* is a different species, no molecular data is available to confirm this status.

Clade 5: *H. treutleri*

The six individuals sampled were collected across five different locations including the type locality. All these individuals and several more that were visually identified were found among rocks/boulders in rocky outcrops suggesting that this is a rupicolous species. Morphologically, all individuals of this clade resemble the type description of *H. treutleri* (Mahony 2009) and it was supported by morphometric analysis, which distinguishes this clade from the rest in our study. However, except for a single sample from the type locality (CES06108; female), other samples differ in the number of femoral pores (7 in type versus 11–16 in other samples on either side). This clade is well supported in the nuclear, mitochondrial, and combined phylogenies. Furthermore there is high genetic divergence among samples collected from different locations. One of the reasons for this could be the patchy distribution of rocky outcrops in peninsular India. This naturally fragmented habitat may have led to the greater genetic divergence across individuals from different locations, when compared to the other clades. The other possibility is that this clade represents a species complex. Further sampling will be needed to resolve this issue. However, clade 5 individuals are

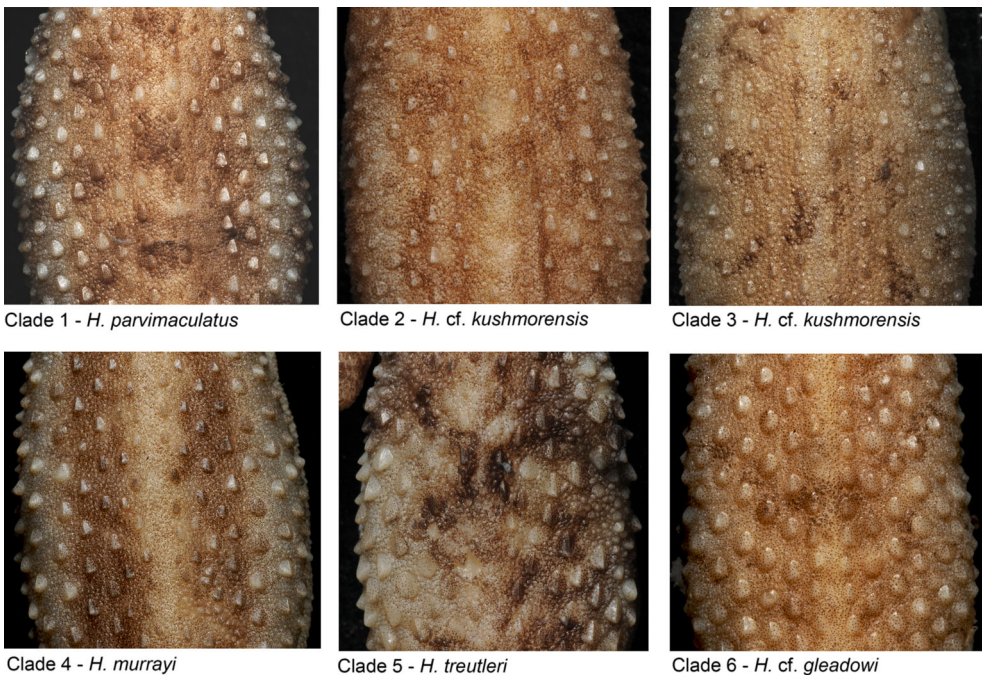

Fig. 5 Enlarged dorsal tubercles on the mid-dorsal of each clade of *Hemidactylus brookii* complex

morphologically distinct from rest of the clades in our study based on the morphometric analysis (Fig. 3). Meristic characters like number of femoral pores and number of scales between the two rows of femoral pores (Table 3), along with tuberculation pattern (Supplementary Table 3) can be used to identify this species in field.

Clade 6: *H.* cf. *gleadowi*

This is one of the most widely distributed species within the complex (excluding the commensals). This species is largely found on the ground in similar habitats as that of other ground-dwelling *Hemidactylus* like *H. reticulatus* and *H. gracilis*. Interestingly, this clade is sister to clade 7 in the mitochondrial phylogeny, which includes the ground-dwelling *Hemidactylus*. Morphologically, this clade is different from clade 4 with respect to the number of precloacal-femoral pores (10–14 vs 7–7 in the latter) and *H. treutleri* sensu stricto in terms of the number of non-pore bearing scales between two rows of pore bearing scales (0–1 vs 7–8 in the latter) and from *H. parvimaculatus*, clade 2 and clade 3, based on the dorsal pholidosis (Fig. 5). Morphologically, this species resembles recently elevated species *H. gleadowi* Murray (redescribed by Mahony 2011). Additionally, one of the samples used in our study from Rampar, Gujarat, was collected around 200 km from the type locality of *H. gleadowi*, Jerruck division across the border in Pakistan (ref. Fig. 1). However, molecular data from topotypic samples or museum specimens would further confirm this nomen for the Indian population.

Clade 7: ground-dwelling *Hemidactylus*

This clade includes five species—*H. albofasciatus*, *H. gracilis*, *H. reticulatus*, *H. sataraensis*, and *H. imbricatus*. All these species are morphologically distinct from each other and the genetic distance across species within this clade is comparable to that across the rest of the clades of *H. brookii* complex (Table 2). Based on the current data, the morphologically diverse group of ground-dwelling geckos seems to be nested within the morphologically conserved clades of the *H. brookii* complex. However, the precise position of this clade within the *H. brookii* radiation is unclear due to the low support. Adding more genetic markers could help validate the phylogenetic position of clade 7 in the phylogeny.

Apart from the considerable genetic distance between the clades of the *H. brookii* complex, the niches also vary across each of these clades. These differences in niches could be one of the causes of lineage divergence. For example, the *H. brookii* complex consists of two species of human commensal geckos (*H. parvimaculatus* and *H. murrayi*), one rock-dwelling (*H. treutleri*) and a ground-dwelling gecko (*H.* cf. *gleadowi*). *H. treutleri*, *H.* cf. *gleadowi*, *H. parvimaculatus*, and *H. murrayi*, are largely distributed in peninsular India. *Hemidactylus parvimaculatus* was noted to have a more eastward distribution, while *H. murrayi* was distributed largely in the West (ref. Fig. 1). *H. treutleri* was noted to co-occur with *H.* cf. *gleadowi* and *H. parvimaculatus* in the same geographical vicinity but restricted to rocky outcrops. On the other hand, *H.* cf. *kushmorensis* (clades 2 and 3) was found in North India and was not found to co-occur with any other species from the *H. brookii* complex. However, details of its habitat preferences could not be established.

Although morphometric characters could not successfully discriminate between all the species, several distinguishing meristic and descriptive features emerge when morphology is examined in the light of molecular data. Diagnostic characters that can be used to distinguish between these species are—size, shape, and arrangement of tubercles on the dorsum, occiput and temporal region, and tail; number of precloacal-femoral pores in combination with the number of non-pore bearing scales separating the pored series; number of undivided lamellae and size of the adult individual. Precloacal-femoral pores are exocrine glands that discharge secretions, which are involved in intraspecific communication (Martín and López 2000; López et al. 2002; López and Martín 2002; López et al. 2003; Martín et al. 2007a; b). Therefore, one would expect such a character to be conserved within a species. We propose a key to identify each of these species and distinguish them from rest of the *Hemidactylus* species from India.

Status of *Hemidactylus brookii* sensu stricto

From this study it is clear that the sample of '*H. brookii*' from Borneo by Bauer et al. (2010a) and thought to be *H. brookii* sensu stricto is a human commensal, *H. murrayi*. The cyt *b* sequence of *H. murrayi* from Borneo is completely identical to sample no. 71 to 74 (ref. Table 1). Furthermore, none of the morphological characters of the clades studied here correspond to *H. brookii* sensu stricto. The pertinent question yet to be addressed is what is the distribution of *H. brookii* sensu stricto? Mahony (2011) mentions that the type series comprises three specimens—specimen BMNH 1947.3.6.47 and BMNH 1947.3.6.48 from Borneo and BMNH 1947.3.6.49 from Australia. Upon examination of the specimens, the author concluded that BMNH 1947.3.6.47 and BMNH 1947.3.6.49 belong to one morphotype, while the other specimen from Borneo belongs to a different morphotype—*H. tenkatei*. Mahony also designated BMNH 1947.3.6.47 as the lectotype of *H. brookii*. Given that *H. murrayi* is also found in Borneo, we think that this is not a case of mistagging, but both these morphotypes are found in Borneo, and the specimen BMNH 1947.3.6.48 represents *H. murrayi*,

while the specimens 1947.3.6.47 and 1947.3.6.49 represent "true brookii".

This study indicates the presence of two human commensals in India, *H. murrayi* and *H. parvimaculatus*. *H. murrayi* seems to have expanded its range eastwards into Southeast Asia—Myanmar, peninsular Malaysia, and East Malaysia, whereas *H. parvimaculatus* has dispersed westward towards Mauritius. Interestingly, these two commensals are not sister to each other. In the tree based on the combined dataset, the commensals branch with non-commensals and these nodes received high supports (Fig. 2). Furthermore, the SH test does not support the sister relationship of the commensals. These results suggest that characters associated with commensalism have evolved independently in the two species.

Key to the *Hemidactylus* Oken of India

Modified after Giri and Bauer (2008). Details on morphological characters newly described species were obtained from original descriptions, *H. treutleri* Mahony (2009), *Hemidactylus graniticolus* Agarwal et al. (2011), *Hemidactylus acanthopholis* Mirza and Sanap (2014), *Hemidactylus yajurvedi* Murthy et al. (2015) and *Hemidactylus hemchandrai* Dange and Tiple (2015) and for *H. murrayi* Gleadow (1887) samples from close to the type locality are referred.

1a.Digits narrow or moderately dilated; a terminal and two or three basal lamellae single, rest deeply notched; length of free distal phalanges of outer four digits less than half the length of their associated subdigital pad.....2

1b.Digits dilated; a terminal and one or two basal lamellae single, rest divided; length of free distal phalanges of outer four digits half or more than half the length of their associated subdigital pad.....7

2a.Scales on back and dorsal aspect of tail granular, intermixed with slightly enlarged tubercles.....3

2b.Scales on back and tail large, pointed, imbricate, keeled 6

3a.Median subcaudal scales without transversely enlarged plates.....4

3b.Median subcaudal scales forming a series of transversely enlarged plates; dorsal granules small, irregular, intermixed with 10 to 12 longitudinal series of more or less oval strongly keeled tubercles; dorsum with quadrangular spots.....*gracilis*

4a.Scales on back granular, intermixed with slightly enlarged tubercles, dorsal aspect of tail flat, not granular, without enlarged tubercles; dorsum without dark reticulations.....5

b.Scales on back and dorsal aspect of tail granular, intermixed with enlarged, conical tubercles; dorsum with dark reticulations .....*reticulatus*

5a.Tail not constricted at base, covered above with flat, imbricate, weakly pointed scales, intermixed with six slightly larger, strongly pointed, weakly keeled, scales on second whorl; back and tail cross-banded with light streaks; maximum SVL 36 mm ..... *albofasciatus*

5b.Tail usually constricted at base, covered above with flat, imbricate, strongly pointed scales, intermixed with 6 much larger, strongly pointed, weakly keeled, scales on second whorl; back with four stripes and transversely arranged spots; maximum SVL 46 mm ..... *sataraensis*

6a.Top of the head and nape covered with granular scales; 5–7 lamellae under fourth toe..... *scabriceps*

6b.Top of the head and nape covered with large, flat, smooth, juxtaposed scales; 9–11 lamellae under fourth toe… ..... *imbricatus*

7a.Scales on back granular, enlarged trihedral, or subtrihedral tubercles absent, tubercles if present, rounded, smooth, or feebly keeled, not in regular longitudinal series.....8

7b.Scales on back granular, intermixed with enlarged, strongly keeled, trihedral, or sub-trihedral tubercles, arranged in more or less regular longitudinal series.....17

8a.Digit I of manus half or less the length of digit II.....9

8b.Digit I of manus more than half the length of digit II..... 12

9a.Cutaneous expansion along the side of the body, digits strongly webbed..... *platyurus*

9b.No cutaneous expansion along the side of the body, digits not strongly webbed 10

10a.Tail weakly depressed, without denticulate lateral edge; male with a continuous series of 26–36 precloacal-femoral pores; 9–10 lamellae under fourth toe.....*frenatus*

10b.Tail strongly depressed, with sharply denticulated lateral edge; males (when present) with medial interruption of precloacal-femoral pore series; 11–13 lamellae under fourth toe.....11

11a. Scales on back composed of uniform small granules; femoral or precloacal pores absent .....*garnotii*

11b.Scales on back granular, intermixed with numerous larger rounded tubercles; males with 18–20 precloacal-femoral pores on each side .... *karenorum*

12a. Tail and sometimes body dorsum with enlarged tubercles 13

12b. Tail and body dorsum lacking enlarged dorsal tubercles 16

13a.Scales on back granular, intermixed with numerous larger rounded tubercles arranged in irregular longitudinal

rows.....
.....14

13b.Scales on back granular, larger rounded tubercles if present, scattered and mostly seen on flanks.....15

14a.Large (maximum SVL* 130 mm); 18–20 rows of irregularly arranged enlarged tubercles; 15–19 femoral pores on each side .....*aaronbaueri*

14b.Medium (maximum SVL* ~ 65 mm); 12–16 rows of irregularly arranged enlarged tubercles; 12–14 femoral pores on each side..... *gujaratensis*

15a.Large (maximum SVL* 98 mm); 10–12 rows of irregularly arranged enlarged tubercles; 10–12 femoral pores on one side ..... *yajurvedi*

15b.Large (maximum SVL* 86 mm); 12–15 rows of irregularly arranged enlarged tubercles; 10–11 femoral pores on one side..... *hemchandrai*

16a.9–11 lamellae under the fourth toe; 10–17 femoral pores on each side..... *leschenaultii*

16b.11–14 lamellae under the fourth toe; 5–7 femoral pores on each side..... *flaviviridis*

17a. 13–15 lamellae under the fourth toe; 18–22 femoral pores on each side; SVL upto 115 mm .....*giganteus*

17b. 9–12 lamellae under fourth toe; 23–28 femoral pores on each side; SVL upto 60 mm..... *…aquilonius*

18a.Males with a series of precloacal pores only..... 19

19b.Males with a series of precloacal-femoral pores.....20

19a.12 to 14 lamellae under the fourth toe; 9 to 13 precloacal pores.....*persicus*

19b.10 to 11 lamellae under the fourth toe; 6 precloacal pores.....*robustus*

20a.Very large (>100 mm SVL*); 9–10 lamellae under first toe.....21

20b.Small to moderately sized (< 85 mm SVL*); 8 or fewer lamellae under first toe.....23

21a.Enlarged dorsal tubercles trihedral, arranged in ~ 20 fairly regular longitudinal series; femoral pores ~ 20 on each side.....22

21b.Dorsal tubercles subtrihedral, arranged in 16–18 fairly regular longitudinal series; femoral pores 23–28, separated by 1–3 pore-less scales.....*graniticolus*

22a.16–19 femoral pores on each side, separated by 5–9 pore-less scales..... *maculatus*

22b.19–21 femoral pores on each side, separated by 13–14 pore-less scales.....*acanthopholis*

23a.Enlarged tubercles on dorsum trihedral or subtrihedral; dorsal pattern with transverse markings or regularly arranged spots.....24

23b.Enlarged tubercles on dorsum trihedral, subtrihedral, conical, or rounded; dorsal pattern with irregularly arranged spots or blotches.....25

24a.Tubercles trihedral; dorsal pattern with bands; 6–14 femoral pores on each side.....*triedrus*

24b.Tubercles subtrihedral; dorsal pattern with spots; 17–20 femoral pores on each side..... *prashadi*

25a.0–4 pore-less scales separating precloacal-femoral pores on either side.....26

25b.4–7 pore-less scales separating femoral pores on either side.....28

26a.Enlarged tubercles small and rounded.....27

26b.Enlarged tubercles trihedral or subtrihedral.....Clade 6 (cf. *gleadowi*)

27a.3 to 4 undivided lamellae below fingers and toes ..... Clades 2 and 3 (cf. *kushmorensis*)

27b.No undivided lamellae below fingers and toes..... ..*parvimaculatus*

28a.Medium (maximum SVL* ~ 60 mm); 5 lamellae on first and 8 on fourth toe.....*murrayi*

28b.Medium (maximum SVL* ~ 70 mm); 6 to 7 lamellae on first and 9 on fourth toe.....*treutleri*

**SVL* Snout-vent length

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Compliance with ethical standards

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