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The reassessment of the threatened status of the Indian endemic Kolar Leaf-nosed Bat *Hipposideros hypophyllus* Kock & Bhat, 1994 (Mammalia: Chiroptera: Hipposideridae)

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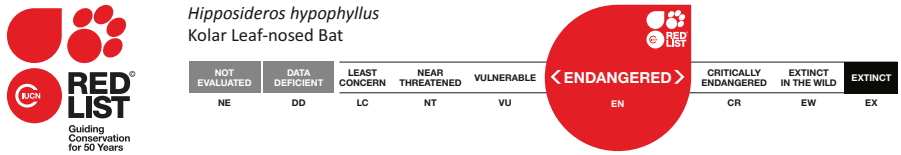
Abstract: The Kolar Leaf-nosed Bat *Hipposideros hypophyllus* Kock & Bhat, 1994, endemic to Kolar District, Karnataka, India was listed as 'Endangered' in the IUCN Red List of Threatened Species due to its restricted distribution and continuing decline in the quality of its habitat. The species has not been sighted or collected since its initial collection in the years 1983 and 1985 wherein eight individuals were collected from Therahalli and 41 individuals were collected from Hanumanhalli, respectively. Based on recent observations and collections from the type locality, we provide information about its distribution, threats, phylogenetic position and conservation status. We also provide an updated conservation assessment of this species following the IUCN Red List categories.

Keywords: Critically Endangered, Hanumanhalli, *Hipposideros hypophyllus*, Kolar.



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INTRODUCTION

Hipposideros hypophyllus Kock & Bhat, 1994 belongs to the *bicolor* species group of the family *Hipposideridae* Gray, 1813 represented by 13 species in South Asia (Srinivasulu & Srinivasulu 2012). This taxon is unique among the *bicolor* species group in possessing a single pair of supplementary leaflets.

From Hanumanhalli, the type locality, and Therahalli, situated about 19km west of Hanumanhalli, 41 individuals and eight individuals respectively of hipposiderids of unknown identity were collected as a part of serological studies designed to investigate the role of bats in Japanese encephalitis virus transmission in the years 1983 and 1985. These were misidentified as *Hipposideros pomona* based on similarities in skull morphology (Banerjee et al. 1988; Bhat & Jacob 1990; Sreepada et al. 1993). The specimens were later re-examined and described as *Hipposideros hypophyllus* by D. Kock & H.R. Bhat in 1994.

After these initial collections this species was not sighted or collected (see Molur & Srinivasulu 2008 for further details). Hence, based on the assumption that this species is restricted to <500km^2 and known from only two locations, this species was listed as 'Endangered' in the IUCN Red List of Threatened Species (Molur & Srinivasulu 2008). Surveys were recommended to validate the taxonomy, distribution and the population status of the species and suggestions were made to urgently identify and protect key roosting sites of this species (Molur & Srinivasulu 2008).

Based on recent sightings and collection of vouchers we provide updated information about the distribution, population size, threats and phylogenetic position of the species and propose an updated Red List status for this endemic species.

MATERIALS AND METHODS

Study area

Kolar District is located in south-eastern Karnataka in peninsular India. The general vegetation of the district is that of tropical dry deciduous forests and tropical thorn forest types (Champion & Seth 1968). The district was formerly known for its vast gold mines. The general topography is hilly, with hills (958–1120 m) made up of large boulders, interspersed with agriculture fields, scrub jungles and low granitic hills. Caves and cave systems are present among the boulder hills and subterranean caves in the low hills.

Surveys

Surveys were conducted between November and December 2013, and in May 2014 covering the entire area of Kolar District to ascertain the presence of the species. As a part of our surveys, we interviewed locals in order to locate the roost sites of bats. Several probable locations including old temples (dilapidated and occasionally used), caves, subterranean caverns and crevices among hills, and old dilapidated houses were searched intensively for bats. During our searches we found four subterranean caves on a low granite hill at Hanumanhalli, a village located 50km east of Kolar township, the type locality of the Kolar Leaf-nosed Bat. Of the four subterranean caves we found one harbouring bats. At the cave as well as in the surrounding areas we conducted six mist net night surveys. At Therahalli, located 19km west of Hanumanhalli, a subterranean cave was located. Here and in neighbouring boulder hills we conducted nine mist net night surveys.

Specimens examined

BNHS 18363 *Hipposideros hypophyllus*, Hanumanhalli, Kolar District, 04.iii.1985, Paratype, female, coll. H.R. Bhat; NHM.OU.K16.2014, *Hipposideros hypophyllus*, Hanumanhalli, Kolar District, 12.v.2014, male, coll. C. Srinivasulu and Aditya Srinivasulu; NHM.OU.K18.2014, *Hipposideros hypophyllus*, Hanumanhalli, Kolar District, 12.v.2014, male coll. Harpreet Kaur and Tariq Ahmed Shah.

Photograph: NHM.OU.PK1.2013, *Hipposideros hypophyllus*, Hanumanhalli, Kolar District, B. Srinivasulu and Tariq Ahmed Shah.

Morphometry

Two male individuals of the species were captured during one of the mist net night surveys at Hanumanhalli, retained as vouchers and deposited at the Natural History Museum of the Department of Zoology, Osmania University (NHM.OU.K16.2014; NHM.OU.K18.2014). External and cranio-dental measurements of the collected and preserved specimens were taken to the nearest 0.01mm with the help of a digital vernier caliper. The measurements of the specimens are presented in Table 1. The baculum of one of the male specimens (NHM.OU.K16.2014) was prepared by immersing the penis in 5% potassium hydroxide, staining with alizarin red and microdissection. This was subsequently measured using an oculometer.

DNA extraction & Molecular phylogeny

Liver tissues were taken from freshly collected

Table 1. External and cranio-dental measurements (in mm) of voucher specimens (n = 2) of *Hipposideros hypophyllus* collected from Hanumanhalli, Kolar District, Karnataka.

	NHM. OU.K16.2014	NHM. OU.K18.2014
Sex	Male	Male
External		
Forearm length (FA)	39.86	39.89
Head-body length (HB)	40.88	41.72
Ear length (E)	17.72	16.73
Tail length (Tl)	24.27	23.30
Length of hindfoot (Hf)	6.88	6.69
Length of tibia (Tib)	16.78	17.54
Length of 3rd metacarpal (3mt)	30.31	30.09
Length of 4th metacarpal (4mt)	29.63	32.64
Length of 5th metacarpal (5mt)	29.08	29.97
1st phalanx of 3rd metacarpal (1ph3mt)	15.55	16.17
2nd phalanx of 3rd metacarpal (2ph3mt)	15.21	15.33
1st phalanx of 4th metacarpal (1ph4mt)	9.23	10.26
2nd phalanx of 4th metacarpal (2ph4mt)	7.22	8.16
Width of horseshoe	5.77	5.46
Cranio-dental		
Greatest length of the skull (GTL)	17.21	17.37
Condylocanine length (CCL)	14.56	14.92
Condylobasal length (CBL)	14.88	15.26
Zygomatic breadth (ZB)	8.13	8.1
Breadth of braincase (BB)	7.78	7.93
Mandible length (M)	10.04	10.0
Mandibular toothrow (CM_3)	5.85	5.76
Maxillary toothrow (CM^3)	5.34	5.44
Posterior palatal width (M^3-M^3)	5.46	5.77
Anterior palatal width (C^1-C^1)	3.4	3.3

specimens and used for DNA extractions. The tissue was digested at 55^{0}C using an extraction buffer containing 50mM Tris-Hcl, 20mM EDTA, 10% SDS with 15 µl Proteinase K. DNA was then extracted following salting out protocol (Miller et al. 1988) and was re-suspended in nuclease free water. A polymerase chain reaction was performed to amplify mitochondrial cytochrome oxidase subunit I (cox1) gene, using the forward primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). PCR reactions were performed in a 25µl reaction volume wherein 12.5µl of the 2X PCR master mix (Thermo Scientific) was taken to which 1µl forward primer, 1µl reverse primer, 2µl template DNA were added and the final volume then adjusted with nuclease free water. The PCR thermal regime consisted of one cycle of 1 min at 94^{0}C; five cycles of 1 min at 94^{0}C, 1.5 min at 45^{0}C and 1.5 min at 72^{0}C; 35 cycles of 1 min at 94^{0}C, 1.5 min at 50^{0}C and 1 min at 72^{0}C and a final cycle of 5 min at 72^{0}C (Hebert et al. 2003). The PCR products were then outsourced for sequencing. The BLAST tool (Altschul et al. 1990) was used to analyze the integrity of the sequence. The sequence was submitted to NCBI GenBank (accession number KM069426). We retrieved additional sequences for other related species from NCBI GeneBank database (http://www.ncbi.nlm.nih.gov/). GenBank accession numbers for the sequences used for the analysis are provided in Table 2. Species belonging to the family Vespertilionidae were used as outgroups in the analysis. Sequences were aligned using MUSCLE (Edgar 2004), and a molecular phylogenetic analysis was performed using the MEGA 6.0 (Tamura et al. 2013). A best fit model for nucleotide substitution was selected from 24 models using MEGA 6.0 (Tamura et al. 2013) based on the minimum Bayesian Information Criterion (BIC) value (Nei & Kumar 2000). Hasegawa-Kishino-Yano (1985) model with gamma distribution and invariant sites (HKY+G+I, BIC = 4695.88, lnL = -2047.00, G = 0.12, I = 0.00) was obtained as a best fit model. The phylogenetic tree was constructed using a Maximum Likelihood (ML) method and its reliability was estimated using bootstrap values run for 1000 iterations.

RESULTS AND DISCUSSION

Description (Image 1)

A medium-sized bat with forearm length ranging between 39.86–39.89 mm. Comparable in size with *Hipposideros durgadasi* with which it shares its roost at Hanumanhalli (Kaur et al. 2014). Ears tall (16.73–17.72 mm) and ear conch with 10 transverse ridges, the bottom six ridges distinctly bifurcated towards the outer border. Body colour fawn to golden to pale grey depending upon the time of the year. In winter we observed the individuals to be deep golden-coloured with fat deposits, while in summer the bats were dark grey to pale grey all over. The area surrounding the noseleaf is pigmented dark as are the ears that are dark in comparison to the rest of the body. The anterior leaf is cup-shaped with a slight median fold which curves slightly inward when viewed from the underside of the noseleaf and possesses a single wave on the lateral margin. Nostrils well-developed, the narial lappets do not cover the nostrils and are present more toward the lateral surface of the nostrils. The single well developed

Table 2. Details of COI sequences of *Hipposideros* species of *bicolor* species group used for the phylogenetic analysis

Species	GenBank Accession Number	Location
Hipposideros cervinus	HM540360	Malaysia, Johor, Kuala Jasin, Endau Rompin National Park
Hipposideros cervinus	HM540354	Malaysia, Sabah, Gomantong Caves
Hipposideros cineraceus	HM540494	Laos, Vientiane, Vang Vieng, Near Ban Nam Pe
Hipposideros cineraceus	HM540486	Viet Nam, Quang Ninh, Minh Hoa, Ke Bang
Hipposideros dyacorum	HM540516	Malaysia, Sabah, Sepilok Forest Reserve
Hipposideros dyacorum	HM540515	Malaysia, Kelantan, Gua Musang
Hipposideros doriae	HM540514	Malaysia, Pahang, Kuala Lompat
Hipposideros khaokhouayensis	HM540535	Laos, Vientiane, Vang Vieng, Near Ban Nam Pe
Hipposideros khaokhouayensis	HM540534	Laos, Vientiane, Vang Vieng, Near Ban Nam Pe
Hipposideros halophyllus	HM540531	Malaysia, Perlis, Kg. Bukit Jerneh
Hipposideros galeritus	HM914937	Viet Nam, Dong Nai, Tan Phu District, Cat Tien National Park
Hipposideros galeritus	HM540530	Laos, Champasak, Nong, Approx 5 km W of km20, Dong Kanthung
Hipposideros pomona	HM540555	Myanmar
Hipposideros pomona	HM540607	Laos, Champasak, 5kmW Of `Kilometre 20`, Dong Kanthung Region
Hipposideros rotalis	HM540616	Laos, Vientiane, Vang Vieng, Near Ban Nathao
Hipposideros rotalis	HM540614	Laos, Vientiane, Phou Khao Khouay - Tak Leuk
Hipposideros ridleyi	HM540613	Malaysia, Pahang, Kuala Lompat
Hipposideros ridleyi	HM540612	Malaysia, Pahang, Kuala Lompat
Hipposideros ruber	JF444162	Cote d'Ivoire, Parc National De Mont Peko, 6 km W Of Sibabli
Hipposideros ruber	JF444152	Cote d'Ivoire, Parc National De Mont Peko, 6 km W Of Sibabli
Hipposideros caffer	JF444149	Cote d'Ivoire, Parc National De Mont Peko, 6 km W Of Sibabli
Hipposideros beatus	JF444148	Cote d'Ivoire, Parc National De Mont Peko, 6 km W Of Sibabli
Hipposideros beatus	JF443884	Cote d'Ivoire, Parc National De Tai, Institute D'Ecologie Tropicale
Hipposideros bicolor 131 KHz	HM540340	Malaysia, Negeri Sembilan, Pasoh Forest Reserve
Hipposideros bicolor 131 KHz	HM540338	Malaysia, Pahang, Krau Wildlife Reserve
Hipposideros bicolor 142 KHz	HM540380	Malaysia, Negeri Sembilan, Pasoh Forest Reserve
Hipposideros bicolor 142 KHz	HM540378	Malaysia, Pahang, Krau Wildlife Reserve
Hipposideros cf. *ater*	HM540368	Indonesia, Jawa Barat, Cibodas
Hipposideros cf. *ater*	HM540367	Malaysia, Sabah, Tabin Wildlife Reserve
Pipistrellus javanicus	HM914966	Viet Nam, TP Ho Chi Minh, Thanh Da
Pipistrellus coromandra	GU684804	Viet Nam, Lam Dong, Lac Duong Dist., Bi Doup-Nui Ba national park

pair of supplementary leaflets start from underneath the anterior leaf extending beyond the anterior leaf, narrow and end on the upperlip. A small frontal sac is present behind the posterior leaf. Internarial septum broad at the base and tapering proximally. Intermediate noseleaf with two pairs of vibrissae (one pair of vibrissae reported by Kock & Bhat 1994) on the lateral surface and exhibits a slight bulge in the front. The intermediate leaf is much smaller than the anterior and posterior leaves. Posterior noseleaf broader than the anterior noseleaf, short with three vertical septa dividing it into four cells the lateral ones narrower than the central pair. Wings are attached to the ankles. The last vertebra of the tail extends beyond the tail membrane.

The first upper premolar (pm^2) is minute in comparison to the second upper premolar (pm^4), in the toothrow due to which the canine and the second upper premolar are separated by a gap. The last upper molar (m^3) is reduced in comparison to the second upper molar (m^2). The first lower premolar (pm_2) is almost 2/3rd the height of the second lower premolar (pm_4). The zygoma are slender and the sagittal crest is not very prominent in frontal region, absent posteriorly (Image 2).

The baculum (Image 3), broader at the base and

Image 1. Kolar Leaf-nosed Bat *Hipposideros hypophyllus* Kock & Bhat 1994, not collected, live male specimen at type locality, Hanumanhalli, Kolar District, Karnataka, India.

Image 2. Skull and mandible of *Hipposideros hypophyllus* (NHM.OU.K16.2014)
A - skull dorsal view; B - skull occlusal view; C - skull lateral view; D - mandible occlusal view; E - mandible lateral view. (Scale 10mm)

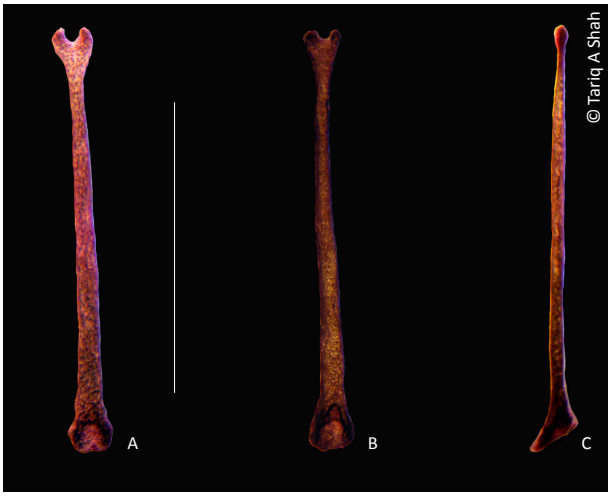

Image 3. Baculum of *Hipposideros hypophyllus* (NHM.OU.K16.2014) A - dorsal view; B - ventral view; C - lateral view (Scale 2mm)

Image 4. The mouth of the subterranean cave at Hanumanhalli—the only known roost of *Hipposideros hypophyllus*.

Table 3. Bat species netted and released during mist net night surveys in Hanumanhalli and Therahalli, Kolar District, Karnataka

Survey Time		Nov 2013	Dec 2013	May 2014
No. of mist net night surveys		**2**	**1**	**3**
Hanumanhalli (no. of individuals netted)				
1.	*Hipposideros speoris*	6	1	6
2.	*Hipposideros fulvus*	4	0	2
3.	*Hipposideros hypophyllus*	4	0	4
4.	*Hipposideros durgadasi*	2	2	8
5.	*Rhinopoma hardwickii*			1
Therahalli (no. of individuals netted)				
No. of mist net night surveys		**4**	**2**	**3**
1.	*Hipposideros durgadasi*	0	0	4

Table 4. Emergence times of bat species from Hanumanhalli and Therahalli, Kolar District, Karnataka

	Species	Emergence time (in hr)
Hanumanhalli (12.v.2014)		
1.	*Hipposideros speoris*	1816
2.	*Hipposideros fulvus*	1837
3.	*Hipposideros hypophyllus*	1848
4.	*Hipposideros durgadasi*	1855
5.	*Rhinopoma hardwickii*	1853
Therahalli (13.v.2014)		
1.	*Hipposideros durgadasi*	1859

gradually tapering towards the bifid apex, is straight-sided and 2.5mm long. Ventrally, the base possesses a concavity.

Distribution

The Kolar Leaf-nosed Bat is endemic to Karnataka, India and has been known from two localities, namely, Hanumanhalli and Therahalli in Kolar District (Kock & Bhat 1994; Molur & Srinivasulu 2008). Presently based upon mist net night surveys and collections in both areas, we found that this species is conspicuously absent from Therahalli and is restricted to a single subterranean cave in Hanumanhalli, the type locality.

Population estimates

We roughly estimate the population of *Hipposideros hypophyllus* to be between 150–200 individuals in Hanumanhalli, based on counts of emerging bat numbers, and extrapolating a ratio of individuals mist-netted at the cave entrance (Table 3). During our surveys we found the species to be restricted to the type locality and it has most probably become locally extinct in Therahalli.

Habitat and ecology

We found *Hipposideros hypophyllus* roosting in inaccessible narrow subterranean granite caves (Image 4). It shared its roost with *H. speoris*, *H. fulvus* (cohabitants reported earlier, present observation), *H. durgadasi* (see Kaur et al. 2014); earlier reported as *H. cineraceus* see Bhat & Jacob 1990; Kock & Bhat 1994), and *Rhinopoma hardwickii* (present observation). Each species of bat had its own time of emergence (Table 4) and were found to forage in the vicinity of the roosting site. Surveys carried out in the winter months showed that the bats foraged less and very few individuals of all the four species of hipposiderids emerged from their

roost for foraging leading to the assumption that bats here enter torpor during this period corroborated by our observations on bats in neighbouring districts. We observed pregnant and lactating females during the late summer surveys, thus we did not collect any females.

Phylogenetic position

The Model test suggested that the best fit nucleotide substitution model was the Hasegawa-Kishino-Yano (1985) model with gamma distribution and invariant sites (HKY+G+I, BIC = 4695.88, lnL = -2047.00, G = 0.12, I = 0.00). The resultant Maximum Likelihood (ML) tree shows *Hipposideros hypophyllus* nested within the clade of *galeritus* subgroup of *bicolor* species group (Fig. 1). This is corroborated by the morphological characters of the species which differs from individuals of the *bicolor* subgroup in possessing supplementary leaflets, a broad, short skull with an elevated rostral profile, and karyologically in possessing intercalary C-bands in two of the submetacentric autosomal pairs in addition to the pericentric bands (Sreepada et al. 1993; Kock & Bhat 1994). Additionally, our results concur with recent phylogenetic analysis of hipposiderids of Southeast Asia that shows *H. galeritus* and *H. cervinus* form a clade of *galeritus* subgroup separate from the clade of *bicolor* subgroup (Murray et al. 2012).

Threats and conservation

The hill on which the subterranean cave roost is located is granitic in nature. Here and in adjoining areas illegal granite stone extraction is occurring (Image 5). On the same hill we came across two more roosting sites in subterranean caverns that were abandoned two years ago by bats due to fires lit for easier extraction of the stone. The mining activity has perilously progressed to within 50–60 m from the only known roost of the Kolar Leaf-nosed Bat. Based on updated information on the distribution and threats (this paper) we propose a new conservation status of the species (Appendix 1). We also propose that urgent steps to mitigate habitat degradation is initiated.

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Image 5. View of the habitat on the granitic hill at Hanumanhalli, Kolar District, Karnataka, India showing habitat destruction due to stone quarrying.

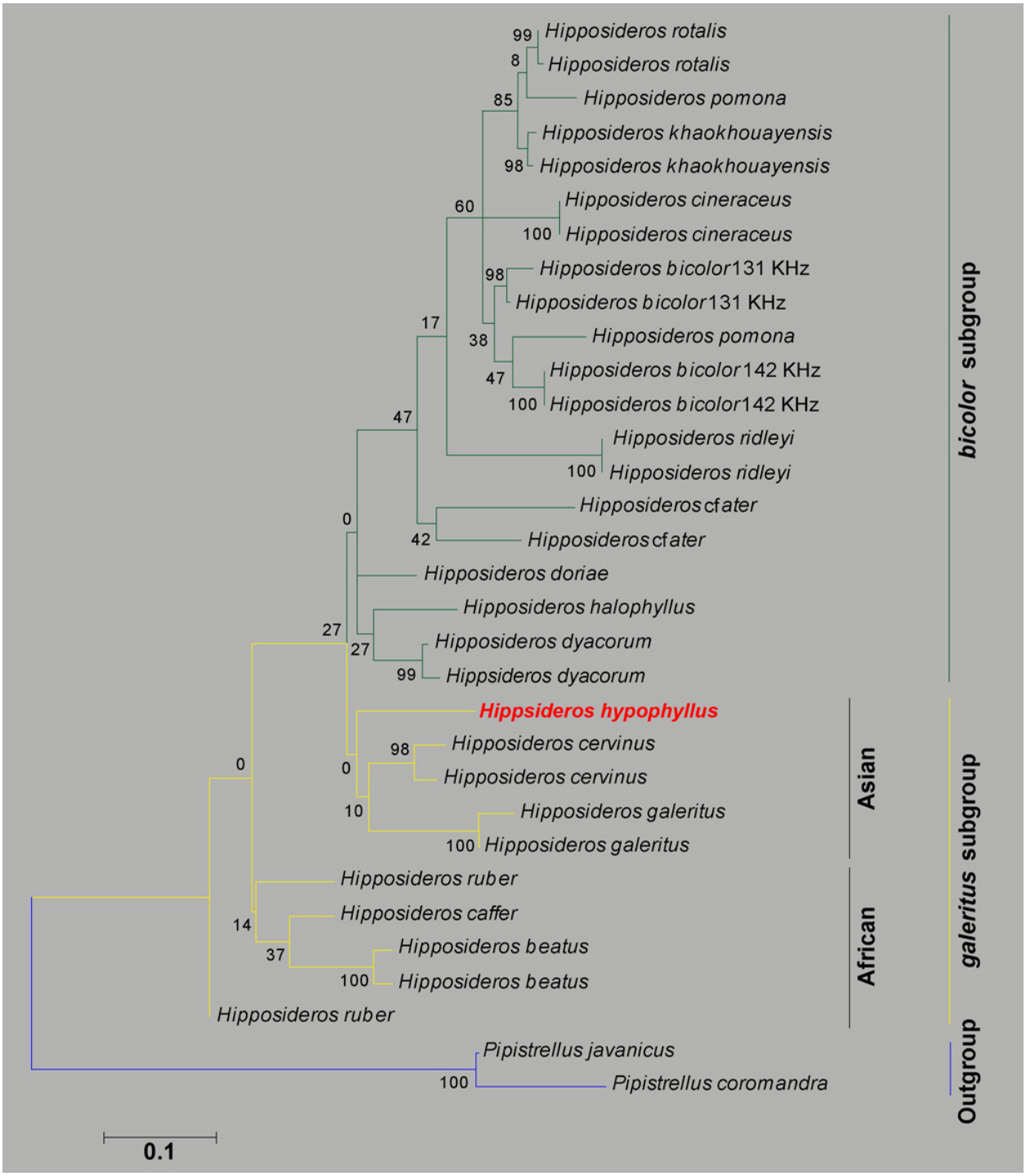

Figure 1. Phylogenetic position of *Hipposideros hypophyllus* inferred using the Maximum Likelihood (ML) method employing the HKY+G+I model. Bootstrap values were calculated from 1000 bootstrap iterations, values are shown to the left of each node. Members of *Pipistrellus* species are used as the outgroup.

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Appendix 1. Proposed Red List Status for *Hipposideros hypophyllus*

Current Status: Endangered B1ab(iii)+2ab(iii)

Proposed Status: Critically Endangered B1ab(iii)+2ab(iii)

Taxonomy
Scientific name: *Hipposideros hypophyllus*
Species authority: Kock & Bhat, 1994
Common names: Kolar Leaf-nosed Bat
Synonyms: *Hipposideros pomona* (in part)
Taxonomic notes: Belongs to *galeritus* subgroup of *bicolor* species group.

Geographic range
Range description: Endemic to India, Karnataka, Kolar District. The estimated (approximate) current extent of occurrence (EOO) is less than 100km^2 and the known area of occupancy (AOO) is less than 10km^2.
Countries: India (state of Karnataka)

Habitat and Ecology
Habitat and Ecology: Known to inhabit narrow, inaccessible subterranean cave and sharing its roost with other species of bats namely *Hipposideros durgadasi*, *Hipposideros speoris*, *Hipposideros fulvus* and *Rhinopoma hardwickii*.

Threats
Major Threats: On-going illegal granite mining and stone quarrying occur perilously close to the roost at the type locality thereby altering the quality and nature of the habitat. Unknown threats, most probably habitat alteration and human use have resulted in the extirpation of the species from Therahalli and also other subterranean caverns in the type locality.

Population
Population: No information on the population status. The estimated number of individuals in the only known roost is between 150-200 individuals.
Population trend: Declining, inferred from the non-availability of the species in other similar habitats where the species previously occurred.

Conservation
Conservation action: No conservation actions are currently in place. There is an urgent need to prohibit stone quarrying adjacent to the only known roosting site of this species. Education and awareness programs need to be carried out among the general public and also among the decision makers, for the protection of this species. Immediate action needs to be taken by according protected status to *Hipposideros hypophyllus* under the Indian Wildlife (Protection) Act.

Assessment Information
Red List category and criteria: Critically Endangered B1ab(iii)+2ab(iii)
Justification: *Hipposideros hypophyllus* is assessed as Critically Endangered because it is restricted in distribution to only one subterranean cave at the type locality Hanumanhalli, and an estimated area of occupancy (AOO) of less than 10km^2. There is also a continuing decline in the quality of its habitat and threat to its roost due to illegal granite mining.

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Author Contribution: All authors contributed equally in field work. BS studied the paratype. HK, TAS, GD worked on the fresh voucher specimens, HK and BS extracted and sequenced the gene, CS and AS performed the molecular analysis. BS and CS designed and wrote the paper.

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