Echolocation calls of four species of leaf-nosed bats (genus *Hipposideros*) from central peninsular India

Bats comprise nearly a quarter of the total mammalian fauna in India[1]. They are threatened with extinction due to human apathy, myths and superstitions, loss and degradation of habitat and loss of foraging habitats. Bats provide essential economically significant ecosystem services such as pollinating the night-blooming flowers, keeping in check the populations of insect pests of agriculture and human importance, and also forest regeneration due to seed dispersal[2].

Insectivorous bats use echolocation to orient and forage for flying insects in the dark and also for social communication[3–6]. Acoustic surveys represent a powerful tool to assess bat distribution, and habitat preferences, and are also widely applicable in the monitoring and conservation schemes[7]. Echolocation calls among bats vary both inter- and intra-specifically and can be governed due to various factors such as location of the roost, habitat, time and stage of foraging, proximity of other conspecifics and gender[8]. The echolocation calls of bats are species-specific and vary according to the type of information they seek[9–12]. Factors such as these influence the structure of the echolocation calls rendering identification difficult. Multivariate analyses, especially discriminant function analysis, come in handy for accurate species identifications[13]. Recent acoustic studies[14,15] in the Western Ghats, India have relied upon discriminant function analysis for the successful classification and identification of echolocation calls of bats.

Leaf-nosed bats (family Hipposideridae) produce echolocation calls containing constant frequency (CF) segments which are of higher pitch compared to calls produced by other species of bats, with the exception of the horseshoe bats[16]. The CF calls of the leaf nosed bats have a frequency-modulated component which involves a downward frequency dip at the end of the CF component.

India is home to 15 species of leaf-nosed bats with one species under the genus *Asellia* Gray, 1838, one species under the genus *Coelops* Blyth, 1848, and 13 species under the genus *Hipposideros* Gray, 1831 (refs 1, 17). In this communication, we provide the echolocation call characteristics of four species of leaf-nosed bats from central peninsular India and using discriminant function analysis we try to understand based on their call characteristics if any niche separations exist among the species. We also report the echolocation calls of *Hipposideros lankadiva* Kelaart 1850 from India.

Surveys were conducted in the districts of Hyderabad and Khammam, Telangana and in the districts of Kolar, Tumkur and Bellary, Karnataka to locate the roosting sites of bats. Mist nets were used to capture and identify the species and such roosting sites were marked for further acoustic sampling. From such sites, single-species roosting sites of Schneider's leaf-nosed bat *Hipposideros speoris* (Schneider, 1800), Fulvus leaf-nosed bat *Hipposideros fulvus* Gray, 1838, Cantor's leaf-nosed bat *Hipposideros galeritus* Cantor, 1846 and Indian leaf-nosed bat *Hipposideros lankadiva* Kelaart, 1850 were identified and echolocation calls were recorded as the bats emerged out and foraged in the vicinity, using ANABAT SDI bat detector (Titley Electronics, Australia). The recorded calls were analysed with AnalookW software which uses a logarithmic scale of frequency (0–200 kHz) with appropriate lines of reference, and frequency–time graphs were produced. Each call was defined as an individual, discrete vocal pulse and the parameters measured were maximum frequency (F_{max}), minimum frequency (F_{min}), mean frequency (F_{mean}), frequency at the knee (F_k), time at the knee (T_k), quality of the knee (Q_k), characteristic frequency (F_c), time at characteristic frequency (T_c), duration (Dur), initial slope (S_i) and characteristic slope (S_c). Frequencies are measured in kilohertz, time in millisecond, and slope as octaves per second. Maximum frequency, minimum frequency, duration (time in milliseconds from the beginning to the end of the call) and characteristic frequency were considered for the present analysis. F_{min} and F_{max} are affected by 'noise' generated through the recording process above and below call pulses. The mark-off points option was used to delete these points. Only the calls that were clearly defined were used for the analysis. Linear discriminant analysis (DA) was performed to check whether the species form distinct clusters based on call parameters[18]. DA attempts to model the difference between the classes of data by extracting factors that maximize inter-class variation and minimize intra-class variations. The null hypothesis, which states that the mean vectors of the four species are equal, was tested using Wilks' lambda[19]. We calculated Mahalanobis distances[19] among the individuals and computed Fisher's distances to check if the clusters formed by the species are significantly different from each other. Since multiple tests were done on the data, Bonferroni correction was applied for Fisher's distances. Statistical analysis was performed using the freeware PAST[20].

A total of 3090 calls were recorded, of which those with good clarity and very less noise were selected for each species – *H. speoris* ($n = 469$), *H. fulvus* ($n = 184$), *H. galeritus* ($n = 10$) and *H. lankadiva* ($n = 427$), and analysed using AnalookW software. Mean, standard deviation and coefficient of variation of each parameter of each call for each species were also calculated.

The echolocation call characteristics and the frequency – time graphs of the calls of the four hipposiderid species are as shown in Figure 1. The results of analysis of the parameters of each call for all the four species show that that the mean characteristic frequency of *H. fulvus* was 155.69 ± 2.09, followed by *H. speoris* (138.88 ± 4.02), *H. galeritus* (112.48 ± 3.65) and *H. lankadiva* (78.33 ± 5.28), while maximum frequency values ranged from 82.96 ± 1.53 in *H. lankadiva* to 156.93 ± 0.46 in *H. fulvus*, and minimum frequency values ranged from 62.96 ± 4.72 in *H. lankadiva* to 133.98 ± 11.71 in *H. fulvus*. The duration of calls of *H. lankadiva* was greatest among the four species recorded (8.87 ± 3.47), while the shortest call duration was that of *H. fulvus* (3.00 ± 1.02) (Table 1).

ANOVA suggested that the four call parameters were significantly different ($P < 0.0001$ in all the cases) for the four bat species. DA extracted three factors, with the first two factors explaining 99.99% of the total variation in the data.

The four bat species were significantly different from each other (Wilks' lambda = 0.006, $F_{12,2866} = 1426.96$, $P < 0.0001$) and were separated on the first DA axis (Figure 2). F_{max}, F_{min} and F_c were positively correlated, while Dur was negatively correlated, with the first axis indicating that the four species *H. lankadiva*, *H. galeritus*, *H. speoris* and *H. fulvus* can be separated based on the increasing values in the first three characters and decreasing value of the fourth character (Figure 2). As the species formed significantly distinct clusters, it is evident that there is complete niche separation in the call structure of the four species. Our analysis also suggests that if more comprehensive data on call structures of all bat species are generated, an identification key based on the call analysis is possible. However, it is also essential to do an analysis of population variation in the call structures so as to understand the possibility of call plasticity and presence of cryptic species before the calls can be used for generating identification keys.

Table 1. Echolocation call parameters of the four leaf-nosed bat species (genus *Hipposideros*) from Telangana and Karnataka, India

Species	F_{max}	F_{min}	F_c	Dur	*N*
Hipposideros speoris	141.28 ± 3.66 (131.15–148.15) 0.026	115.27 ± 8.99 (86.96–140.35) 0.078	138.88 ± 4.02 (126.98–145.45) 0.029	6.09 ± 1.21 (1.17–11.04) 0.198	469
Hipposideros fulvus	156.93 ± 0.46 (156.86–160.00) 0.003	133.98 ± 11.71 (88.89–153.85) 0.087	155.69 ± 2.09 (145.45–156.86) 0.013	3.00 ± 1.02 (1.01–5.82) 0.341	184
Hipposideros galeritus	114.46 ± 0.93 (112.68–115.94) 0.008	99.30 ± 2.07 (97.56–103.90) 0.020	112.48 ± 3.65 (102.56–114.29) 0.031	4.81 ± 0.46 (3.86–5.27) 0.090	10
Hipposideros lankadiva	82.96 ± 1.53 (74.07–93.33) 0.018	62.96 ± 4.72 (47.34–80.00) 0.075	78.33 ± 5.28 (57.97 ± 85.11) 0.067	8.87 ± 3.47 (2.13–18.20) 0.391	427

The values presented are mean ± standard deviation, range in parentheses and coefficient of variation included in three rows for each species and each parameters.

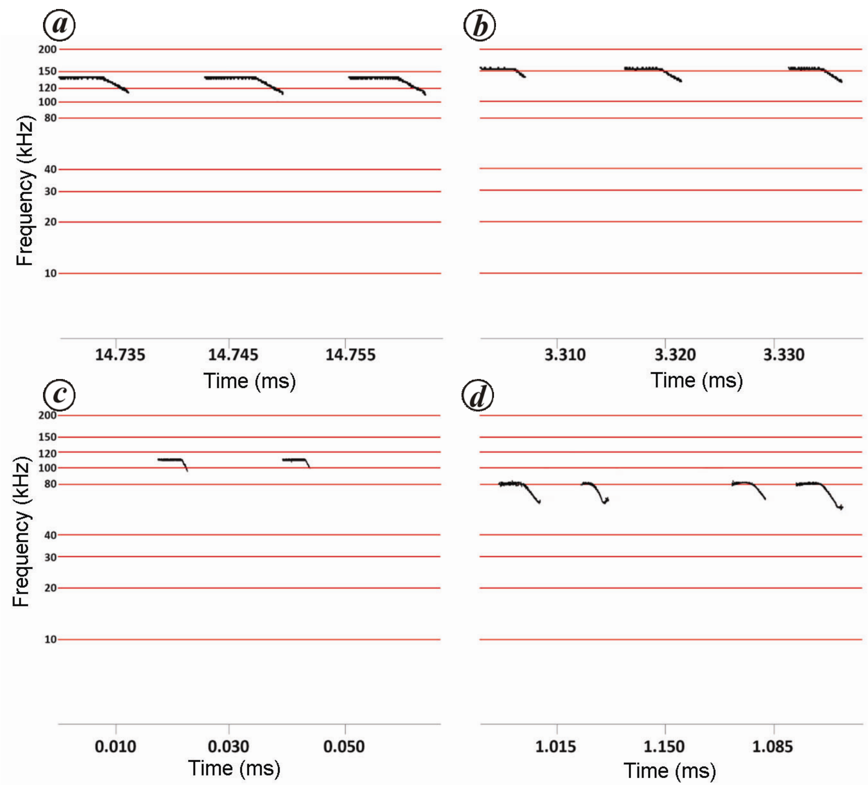

Figure 1. Frequency–time graphs of echolocation calls of four species of leaf-nosed bats (genus *Hipposideros*) from central peninsular India. ***a***, Schneider's leaf-nosed bat *Hipposideros speoris*; ***b***, Fulvus leaf-nosed bat *Hipposideros fulvus*; ***c***, Cantor's leaf-nosed bat *Hipposideros galeritus*; ***d***, Indian leaf-nosed bat *Hipposideros lankadiva*.

The echolocation calls of all the four species of leaf-nosed bats were of typical constant frequency-frequency modulated (CF-FM) type with a short CF component and an equally short or sometimes a tapering FM tail. Studies have shown that there exists habitat and geographic variation in echolocation call frequency among bats[21,22], as is evidenced by the echolocation calls of *H. fulvus* and *H. speoris* recorded during the present study. The analysis of the calls of these two species of bats showed that *H. fulvus* called in a range 145.45–156.86 kHz, and *H. speoris* called in a range 126.98–145.45 kHz, while the study by Jones *et al.*[23] in Madurai in Tamil Nadu showed that *H. fulvus* called at a frequency of 157 kHz and *H. speoris* called at a frequency of 138 kHz.

The echolocation call analysis of *H. lankadiva*, the largest of the leaf-nosed bats in peninsular India considered during the present study, showed that this species called at a considerably low frequency between 57.97 and 85.11 kHz. The call exhibited a comparatively shorter CF component and a longer tapering FM component, suggesting that the bat uses such calls to hunt in narrow spaces. The roosting site of this bat is located in the deep recesses of a dilapidated fort with narrow openings, explaining the short CF component and comparatively longer FM component in the call. Studies on *H. lankadiva* from Sri Lanka[24] show variation in call frequency with those recorded from the present study. The Indian and Sri Lankan populations of *H. lankadiva* are geographically isolated and need further in-depth comparative studies to understand intra-specific and any subspecies level differences.

The maximum frequency of the echolocation call of *H. galeritus* recorded

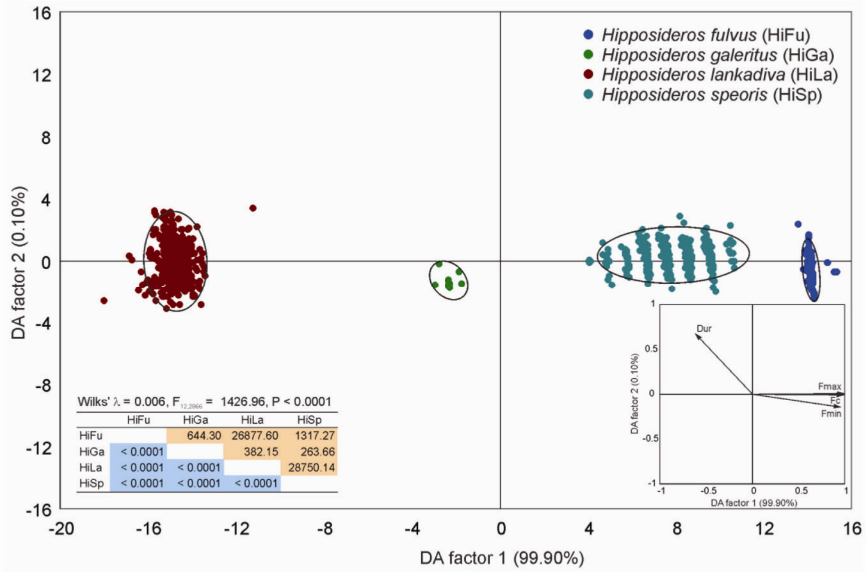

Figure 2. Linear discriminant analysis of four hipposiderid bat species based on call parameters. (Inset, left) Test statistics with Fisher's distances in red cells and *P* values in blue cells. (Inset, right) Factor correlations on the first two axes. Values in parentheses indicate percentage of variation explained by the DA factor. Ellipse depicts 95% confidence intervals.

in Cambodia[25] was 100.7 ± 1 (98.5–102.5 kHz), while during the present study it was observed in the range 112.68–115.94 kHz with an average of 114.46 ± 0.93 kHz. Furthermore, the call characteristics of the species in our study differed from those recorded in Kudremukh National Park, Karnataka with respect to all the four parameters analysed, indicating a clear differentiation of the Western Ghats population and those from other regions studied by us. The variations could be indicative of call plasticity due to ecological separation and a possibility of the presence of a species complex that needs confirmation through further detailed morphological, acoustic and molecular investigations.

More studies are needed towards acoustic identification of bats of India. Studies into collating the acoustic signatures of bats in peninsular India would help identify the echolocation call characteristics of bat species present in the country and resolve the status of many cryptic species.

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