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Source: Acta Chiropterologica, 16(1) : 213-222
Published By: Museum and Institute of Zoology, Polish Academy of Sciences
URL: https://doi.org/10.3161/150811014X683408
Acoustic identification of bats in the southern Western Ghats, India

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Bats play crucial roles in ecosystems, are increasingly used as bio-indicators and are an important component of tropical diversity. Ecological studies and conservation-oriented monitoring of bats in the tropics benefit from published libraries of echolocation calls, which are not readily available for many tropical ecosystems. Here, we present the echolocation calls of 15 species from the Valparai plateau in the Anamalai Hills, southern Western Ghats of India: three rhinolophids (Rhinolophus beddomei, R. rouxi (indorouxii), R. lepidus), one hipposiderid (Hipposideros pomona), nine vespertilionids (Barbastella leucomelas darjelingensis, Hesperoptenus tickelli, Miniopterus fuliginosus, M. pusillus, Myotis horsfieldii, M. montivagus, Pipistrellus ceylonicus, Scotophilus heathii, S. kuhlii), one pteropodid (Rousettus leschenaultii) and one megadermatid (Megaderma spasma). Discriminant function analyses using leave-one-out cross validation classified bats producing calls with a strong constant frequency (CF) component with 100% success and bats producing frequency modulated (FM) calls with 90% success. For five species, we report their echolocation calls for the first time, and we present call frequencies for some species that differ from those published from other parts of the species’ ranges. This exemplifies the need for more local call libraries from tropical regions to be collected and published in order to record endemic species and accurately identify species whose calls vary biogeographically.

Key words: acoustic identification, call library, bats, frequency modulated, constant frequency, multiharmonic, Western Ghats, India, South Asia

INTRODUCTION

Bats are the second most species rich order of mammals, with great ecological diversity, especially in the tropics. They undertake a range of ecosystem services, including seed dispersal, pollination and insect control. A variety of ecologically and commercially important plants rely on bats to some degree as pollinators or seed dispersers (Kunz et al., 2011). Bats are also increasingly used as bioindicators to assess the biodiversity potential of areas and monitor environmental changes (Fenton et al., 1992; Jones et al., 2009; Pedersen et al., 2012), and there is therefore a need for reliable methods for studying bat assemblages.

For many parts of the tropics we lack even the most basic information on the abundance of different bat species, their distribution and habitat requirements. The development of comprehensive survey and monitoring methods is therefore critical for understanding the current status of bats and allowing future monitoring of populations (MacSwiney et al., 2008). The two main methods used for the study of bats are capturing them with mist nets and/or harp traps, or recording their echolocation calls using ultrasound detectors. The use of ultrasound detectors in the tropics has been hampered by the lack of reliable call libraries, which allow identification of bats to genus or species level from their echolocation calls.

Handling bats directly usually allows better species identification than acoustic methods, although some cryptic species are more easily separated by calls (Fenton, 1999), and allows the collection of useful data on the individual bat. However, it is also time-consuming and invasive. Further, it can also lead to biases in sampling as many species fly high above nets, are more agile or are better at detecting nets than others (O’Farrell and Gannon, 1999; Larsen et al., 2007). Habitats such as open fields, large water bodies or tall canopies cannot be easily or effectively sampled using capture methods.
Ultrasound detectors can be used in areas difficult to sample by capture methods, and detect foraging guilds that catching rarely does (Fenton, 1990; MacSwiney et al., 2008). Acoustic transects are easy to standardize and are thus useful for long-term monitoring. However, some species cannot yet be distinguished acoustically, and low intensity echolocators and non-echolocating bats are not accurately represented, particularly in cluttered habitats (Adams et al., 2012). Higher frequency echolocation calls attenuate quickly so are underrepresented; and the type of detector used can also affect which frequencies are recorded, and from what distance (Adams et al., 2012). Ultrasound detectors and catching in combination typically give the most complete inventories and thus they should be used together for surveying and monitoring (Murray et al., 1999; O’Farrell and Gannon, 1999; MacSwiney et al., 2008; Furey et al., 2009).

Given the advantages that ultrasound detectors bring to the study of bats, there have been increased efforts to build call libraries for more regions, especially those facing the gravest threats from habitat loss and conversion (Sedlock, 2001; Furey et al., 2009; Hughes et al., 2010, 2011). Recording calls from as many different localities as possible is important; new species will be identified and recorded, and biogeographic variation in calls can be assessed (Russo et al., 2007; Hughes et al., 2010).

We have started to build an echolocation call library to analyse the calls of an assemblage of bats from the southern Western Ghats of India. The Western Ghats are a mountain range running along the western coast of India. They form one of the eight ‘hottest’ biodiversity hotspots in the world, and are home to a large number of endemic species (Myers et al., 2000). The Western Ghats are the most densely populated of all hotspots with high pressures of habitat loss and degradation due to various human activities (Cincotta et al., 2000; Bawa et al., 2007). As a mountainous tropical area with high levels of endemism, they are likely to be subjected to shifting biotic compositions in the future as mid and high elevation specialists migrate upslope due to global warming, endangering many species whose range will probably contract significantly (LaVal, 2004; Feeley et al., 2013). The Western Ghats are in need of rapid conservation action.

**MATERIALS AND METHODS**

**Study Site**

The study site was the Valparai plateau (c. 220 km²), located in the state of Tamil Nadu in the southern Western Ghats (10.2–10.4°N, 76.8–77.0°E — Fig. 1). The native vegetation is mid-elevation tropical wet evergreen forest of the *Cullenia*...
Extraction of Call Parameters

We extracted call parameters using custom-written software (Scott, 2012; Bellamy et al., 2013). For each call, the following parameters were quantified: i) start frequency — the point at which a signal 12dB above the background noise estimate was encountered; ii) end frequency — where a drop in energy of over 40 dB from the peak energy of the call was seen; iii) call duration — the time in milliseconds between start and end frequencies; iv) frequency of maximum energy (FMAXE) — the frequency containing the maximum energy on a power spectrum (Scott, 2012); and v) bandwidth, obtained by subtracting end frequency from start frequency.

For bats with a strong constant frequency (CF) component to the call (Rhinolophidae and Hipposideridae) only FMAXE and call duration were measured. We used the term constant frequency here to describe a call where the majority of the sound is produced at a single frequency (Fenton, 2013). Constant frequency calls are elsewhere referred to as low duty cycle calls, where frequency modulated (FM) calls that start at a high frequency and sweep down to a low frequency are referred to as low duty cycle calls (Bogdanowicz et al., 1999). For bats making frequency modulated (FM) calls all five measurements were used (Table 1, Figs. 2 and 3).

### TABLE 1. Call parameters for all species

<table>
<thead>
<tr>
<th>Species</th>
<th>Start Frequency (kHz)</th>
<th>End Frequency (kHz)</th>
<th>FMAXE (kHz)</th>
<th>Duration (ms)</th>
<th>Bandwidth (kHz)</th>
<th>Number of recordings</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. pusillus</em></td>
<td>42.81 ± 0.53</td>
<td>41.7–43.3</td>
<td>47.70 ± 13.62</td>
<td>24.7–71.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. lepidus</em></td>
<td>102.31 ± 1.81</td>
<td>97–106.3</td>
<td>25.23 ± 11.38</td>
<td>1.4–51.5</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td><em>R. rouxii</em></td>
<td>60.12 ± 5.21</td>
<td>43.2–83.8</td>
<td>37.65 ± 1.12</td>
<td>33.1–54.0</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td><em>S. kuhlii</em></td>
<td>56.67 ± 2.06</td>
<td>52.4–61.3</td>
<td>45.26 ± 0.76</td>
<td>41.1–44.9</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*First published calls for these species

### Sound Recording and Analysis

Between 2008 and 2013, bats were captured in mist nets and harp traps in forest fragments, coffee, tea and cardamom plantations, along rivers, and at roosts in tunnels and caves. Bats were caught in accordance with Natural England protocol, and their welfare was of the highest priority at all times (http://www.naturalengland.org.uk/Images/wmlg39_tcm6-35872.pdf). Bats were identified to species (Bates and Harrison, 1997, Srinivasulu et al., 2010) and their echolocation calls on release were recorded using a Pettersson D240X ultrasound detector (www.batsound.com) with a sampling rate of 307 kHz and a range of 10–120 kHz recording onto an Edirol R-09 (www.roland.com) digital recorder sampling at 44.1 kHz. The detector was manually triggered four seconds after release to capture 3.4 seconds of calls in 10x time expansion as WAV files.

Up to 10 clear calls with the highest signal to noise ratio were selected from each individual recording and the call parameters were measured using custom-written software (Scott, 2012). For each call parameter, the mean based on up to 10 calls for each individual bat was used for further analysis.

### Automatic Extraction of Call Parameters

For bats with a strong constant frequency (CF) component to the call (Rhinolophidae and Hipposideridae) only FMAXE and call duration were measured. We used the term constant frequency here to describe a call where the majority of the sound is produced at a single frequency (Fenton, 2013). Constant frequency calls are elsewhere referred to as low duty cycle calls, where frequency modulated (FM) calls that start at a high frequency and sweep down to a low frequency are referred to as low duty cycle calls (Bogdanowicz et al., 1999). For bats making frequency modulated (FM) calls all five measurements were used (Table 1, Figs. 2 and 3).
Statistical Analysis

Discriminant function analyses

We used linear discriminant function analysis (L DFA) to classify bat calls from the southern Western Ghats to the species level (R usso and Jones, 2002). L DFA does not require sophisticated software and is available in a variety of statistical software programs, giving it great scope as a mass identification tool for researchers and conservationists in the field (Papadatou et al., 2008). All statistical analyses in this paper were carried out in IBM SPSS Statistics 19 (Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.).

Analyses were carried out separately for rhinolophoid bats producing CF calls and for species producing FM calls. All rhinolophoid (CF) bats were analysed using a linear discriminant function analyses with leave-one-out cross validation, based on FMAXE and call duration. Cross-validation is used to ‘test’ a statistical model to reduce overfitting and give insight into how the model will work on an independent dataset (Stone, 1974). Due to small sample sizes we used leave-one-out cross validation, where one sample is removed and the analysis is performed on all the other data, then validated on the removed sample. This is repeated for as many iterations as there are samples (Stone, 1974). Bats using FM calls with over five sampled individuals (except M. spasma) were grouped and a stepwise Discriminant Function Analysis (DFA) run with the five acoustic variables listed above. As a DFA cannot be performed for groups with less individuals than the number of variables, the two variables that had the lowest Wilk’s λ score and thus contributed the most to the first DFA were identified and a further DFA run with these two variables, which allowed all species with more than two individuals to be included.

All calls were independent, as they were all recorded from different bats. The tolerance values for all of the independent variables are larger than 0.1, so multicollinearity is not occurring in either model. All species showed normal distributions of call parameters apart from Myotis horsfieldii, Pipistrellus ceylonicus, Rousettus leschenaultii, Rhinolophus indorouxi and Scotophilus heathii which had distributions deviating from normal for one or more parameters, mostly due to skewness (as assessed by the Shapiro-Wilks test). Transforming the variables to make them normal and removing outliers did not increase classification success by more than 2% in the FM model (it could not do so in the CF model as classification was 100% with the baseline model) so we used the baseline FM model of untransformed data with outliers. Box’s M statistic was statistically significant on both untransformed and transformed data for both the CF and FM models, so we do not meet the assumption of homogeneity of variance (FM bats \( F = 5.85, \) d.f. = 18, 781.98; CF bats \( F = 6.38, \) d.f. = 6, 1299.69; \( P < 0.001 \) for both). We re-ran both analyses using separate covariance matrices for classification. The classification rate did not change by more than 2% so we used the baseline model with a pooled covariance matrix. Sometimes quadratic classification criteria in DFA are used when the assumption of homogenous variance-covariance matrices is violated; but quadratic DFA does not work as well as linear DFA with small sample sizes ( Friedman, 1989). In our data, the log determinants of the group covariance matrices were very similar, indicating that the violation was not substantial. In neither linear model were species misclassified to the species with highest dispersion, the main problem caused by heterogeneous variance-covariance matrices. We therefore considered linear DFA adequate for the classification of these data.
RESULTS

Discriminant Function Analysis

Constant frequency calls
Using FMAXE and call duration, DFA classified calls to species with 100% success compared with 25% expected from random assignment (overall Wilk’s $\lambda = 0.01$, $P < 0.001$). A stepwise analysis showed that FMAXE was the most important factor in classification (FMAXE: Wilk’s $\lambda = 0.011$, $F_{3, 80} = 2377.60$, $P < 0.001$ — Fig. 2).

Frequency modulated calls
All bats that used a single dominant FM harmonic were grouped for analysis along with $R$. leschenaultii, which uses a low frequency tongue click. This included all non-rhinolophoids other than $M$. spasma, whose calls have an average of four short harmonics, making it easy to separate visually on a spectrogram from other FM bats in the area (Fig. 3).

The data for the five species with over five individuals were analysed using a stepwise DFA with cross validation that showed FMAXE and end frequency to be the most useful predictors (end frequency: Wilk’s $\lambda = 0.037$, $F_{4, 144} = 937.80$, $P < 0.001$; FMAXE: Wilk’s $\lambda = 0.014$, $F_{8, 286} = 265.50$, $P < 0.001$). This had 100% assignment accuracy for $M$. fuliginosus and $R$. leschenaultii, > 90% accuracy for $M$. horsfieldii and $P$. ceylonicus and 56% assignment accuracy for $S$. heathii. A further DFA was run with just FMAXE and end frequency (Fig. 4). This allowed eight species with more than two individuals to be analysed, and classified 89.2% of individuals correctly after cross validation (Wilk’s $\lambda = 0.013$, $P < 0.001$) as compared to 12.5% based on random chance. $Myotis$ montivagus, $M$. pusillus, $R$. leschenaultii and $S$. kuhlii were assigned with 100% accuracy, $M$. fuliginosus with > 90% accuracy, and $M$. horsfieldii, $P$. ceylonicus and $S$. heathii with > 80% accuracy (Table 2).

DISCUSSION

The DFA for species emitting CF calls distinguished 100% of calls correctly. The DFA for the single harmonic FM bats classified calls to species level with ≥90% accuracy, however there is the potential for misclassification of some species in this group as some species had overlapping call parameters. The most difficult species in this assemblage to identify acoustically are likely to be $H$. pomona, whose very high frequency calls attenuate over short distances and so may be less frequently detected (Griffin, 1971); $M$. spasma, which calls very quietly; and $R$. leschenaultii, whose tongue clicks resemble cracking twigs.

![Discriminant function analyses for all FM species with over two individuals](https://bioone.org/journals/Acta-Chiropterologica)
For several species — *B. leucomelas darjelingensis*, *H. tickelli*, *M. montivagus*, *P. ceylonicus*, *S. heathii* — the calls reported here are, we believe, the first published calls from these species. This is the first record of *B. leucomelas darjelingensis* in South India. Finding this bat in a tropical location is surprising as its preferred habitat has been described as ‘Himalayan moist temperate forest and dry coniferous forest areas’, so our record extends the species’ known habitat and range considerably (Benda et al., 2008).

*Hesperoptenus tickelli* and *M. pusillus* were also not thought to live in this area (Korad et al., 2007; Csorba et al., 2008a; Bumrungsri et al., 2008). Korad et al. (2007) list 16 species found between 10°N and 12°N in the Western Ghats that we did not record from there. IUCN range maps suggest that 10 of these may be found in the Valparai area above 800 m a.s.l.: *Pteropus giganteus*, *Hipposideros ater*, *H. fulvus*, *H. speoris*, *Megaladerma lyra*, *Harpichiropha laryus syrurus*, *Pipistrellus coromandus* and *P. tenuis* (Bates and Harrison, 1997; Molur et al., 2008a, 2008b; Csorba et al., 2008b, 2008c, 2008e, 2008f; Francis et al., 2008; Srinivasulu and Molur 2008). Other species noted at this latitude by Korad et al. (2007) and that have been recorded above 800 m a.s.l. are *Rhinolophus luctus*, *Falsistrellus affinis*, *Taphozus theobaldi* and *Tylonycteris pachypus* (Bates and Harrison, 1997; Bates et al., 2008a, 2008b; Csorba et al., 2008d; Walston et al., 2008). The absence of some of these species may be due to the extensive agricultural land use in the area, but we cannot as yet conclusively rule out their presence in the Valparai plateau or surrounding forests. While there is no extensive literature on the habitat requirements of most of these species, *H. ater*, *H. harpia laryus*, *T. theobaldi* and *R. luctus* have all been associated predominantly with undisturbed forest (Bates and Harrison, 1997; Bates et al., 2008a; Csorba et al., 2008c, 2008e; Walston et al., 2008).

### Biogeographic Variation

It is important to record calls from as many locations as possible, as call frequencies can vary geographically within a species (Hughes et al., 2010). Knowing the extent of call variability for a species over a wide geographic area can indicate how widely accurate the existing published call frequencies are. It can also identify populations that might be cryptic species — genetically distinct species with very similar morphological features — or Evolutionarily Significant Units (ESUs),

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**Table 2. DFA with cross validation for species with over two individuals. In cross validation, each case is classified by the functions derived from all cases other than that case.**

<table>
<thead>
<tr>
<th>Predicted Group Membership</th>
<th>M. paludiculus</th>
<th>M. montivagus</th>
<th>R. leschenaultii</th>
<th>S. heathii</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M. paludiculus</strong></td>
<td>29 93.5</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
</tr>
<tr>
<td><strong>M. montivagus</strong></td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
</tr>
<tr>
<td><strong>R. leschenaultii</strong></td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
</tr>
<tr>
<td><strong>S. heathii</strong></td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
</tr>
</tbody>
</table>

**Total**

<table>
<thead>
<tr>
<th>n</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td>100</td>
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</table>
important for conservation decisions (Crandall et al., 2000; Davidson-Watts et al., 2006; Bickford et al., 2007; Frankham, 2010).

Each of the rhinolophoid species recorded here vary in call frequency to some degree across their biogeographic ranges, although there are few data for *R. beddomei* or *R. lepidus* (Fig. 5; Schuller, 1980; Francis and Habersetzer, 1998; Pottie et al., 2005; Struebig et al., 2005; Francis, 2008; Furey et al., 2009; Zhang et al., 2009; Chattopadhyay et al., 2010, 2012; Douangboubpha et al., 2010; Hughes et al., 2010; Soisook et al., 2010). For *R. rouxii* spp. and *H. pomona*, biogeographic variation in call frequency may be due to the existence of cryptic species or subspecies. Cryptic species calling at different frequencies are often found among bats that emit CF calls (Kingston et al., 2001).

Chattopadhyay et al. (2012) characterized two divergent genetic lineages in the South Indian *R. rouxii*, which they consider sibling species; *R. rouxii* (FMAXE 80 kHz) and *R. indorouxii* (FMAXE 90 kHz) (Fig. 5). The bats we recorded resemble the *R. indorouxii* from Tamil Nadu more than the *R. rouxii* from Karnataka or the Sri Lankan *R. r. rubidus* in echolocation frequency and forearm length. The mean forearm (FA) length for adult *R. indorouxii* in this study was 51.1 mm (range 48.4–55.8 mm), which is more similar to the means from the three Tamil sites in Chattopadhyay et al. (51.51, 51.47, and 52.16 mm) than the two sites in Karnataka (47.75 and 50.51 mm) although there is clearly considerable overlap and FA length alone is not diagnostic for this species group.
FMAXE of *H. pomona* varies from 121–140 kHz across its range (Francis and Habersetzer, 1998; Struebig *et al*., 2005; Shek and Lau, 2006; Francis, 2008; Furey *et al*., 2009; Zhang *et al*., 2009; Douangboubpha *et al*., 2010; Hughes *et al*., 2010 — Fig. 5). Sun *et al*. (2009) found genetic divergence sufficient to indicate cryptic speciation between *H. pomona* they caught in China and *H. pomona* GenBank sequences. More work is needed to see whether this divergence correlates with different phonic types. The mean forearm length of adult *H. pomona* in this study was 40.8 mm (range 40–42 mm). In China, FA values for *H. pomona* were similar to those we found and overlapped in all sites: Yunnan 41.5–44.2 mm, Guangdong 40.6–43.0 mm, Hainan 38–42 mm, Hong Kong 40.4–47.1 mm (Shek and Lau, 2006; Zhang *et al*., 2009). In Myanmar FA values were 38.4–42.8 mm (Struebig *et al*., 2005) and in Thailand they were 39.5–44.6 mm (Douangboubpha *et al*., 2010). There is a certain amount of variability here (38–47.1 mm across all studies) but there are as of yet no clear patterns observable relating to potential subspecies. The degree of biogeographic variability in call frequency for *H. pomona* and *R. rouxi* underlines the need for improvements in localized knowledge of rhinolophoid calls across tropical regions. By contrast the call frequencies of FM species varied little geographically, although there was not much biogeographic data available (Jacobs, 1999; Parsons and Jones, 2000; Sedlock, 2001; Dietz, 2005; Pottie *et al*., 2005; Zhang *et al*., 2007; Papadatou *et al*., 2008; Furey *et al*., 2009; Furman *et al*., 2010; Hughes *et al*., 2010, 2011). The often great intraspecific variability and interspecific overlaps in call frequencies means that identifying FM calls may be difficult without prior knowledge of a species’ presence. Recording more bat echolocation calls across the tropics would improve our understanding of the biogeography and ecology of species, and assist in the creation and implementation of evidence-based conservation management plans and long-term monitoring programs.

**ACKNOWLEDGEMENTS**

Funding was provided by NERC (Natural Environment Research Council), University of Leeds, UKIERI (UK India Education and Research Initiative), IUCN Netherlands — Ecosystems Grant Program and CEPF-ATREE Western Ghats Small Grants Program. We thank the Tamil Nadu Forest Department, the Tamil Nadu Electricity Board and managers of Peria Karamalai Tea Company, Bombay Burmah Trading Corporation, Tata Coffee Ltd, Parry Agro Industries Ltd, Altaghat Estate and Thalanar Estate for permissions and local support. We would like to thank the field assistants: Satish Kumar A., Dinesh T., Kannan C., Iyyappan T., Satheesh Kumar S., Pandi, Anand Kumar and Anand Raja for their tireless assistance and good humour. We are also grateful to Swati Sidhu, Dina Nisthar, V. Deepak, Anita Glover, Kadambari V. Deshpande, Christine Singfield, Emma Rigby, Sarah Proctor, Aurelie Laurent, Ruth Angell, Kate Parker and Aditya Malgankar for invaluable help in the field. We thank Christopher Scott for coding the automatic call extraction software used here. Finally, we thank M. Ananda Kumar, Ganesh Raghunathan and T. R. Shankar Raman for logistical support and advice throughout the project and two anonymous referees for their comments which improved the manuscript.

**LITERATURE CITED**


KUNZ, T. H., E. BRAUN DE TORREZ, D. BAUER, T. LOBOVA, and