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# Acoustic identification of five insectivorous bats by their echolocation calls in the Sahelian zone of Far North Cameroon

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# Abstract

**Background:** Despite their abundance and ecological importance, bats are under significant threat worldwide. There is little information about their distribution, roosting, and habitat requirement for most species, making assessing which species is threatened or in need of special conservation measures difficult. The knowledge gap may partly be due to limitations of the old methods of studying bats which mainly involved capture/or observational techniques.

**Material and methods:** In order to evaluate the potential of identifying insectivorous bats by their echolocation calls in the Sahelian zone of northern Cameroon, 65 bats belonging to five species were captured using standard mist netting: *Mops condylurus, Chaerephon major, Mops niveiventer, Scotophilus dinganii,* and *Scotophilus leucogaster.* The bats were identified by using morphometric measurements. An Anabat SD1 detector was later used to record echolocation calls of each individual bat in flight after it was hand-released. The sonogram of each individual bat was analyzed using Analook and categorized into two call types (frequency modulation and frequency modulation/ quasi constant frequency) in order to develop a library of bat reference calls that could be used for a qualitative acoustic survey and species identification. Discriminant function analysis (DFA) was applied to search phase calls of the 65 individual bats in order to evaluate the potential for classifying calls into five species groups. Seven parameters calculated from each search phase call were used to classify calls.

**Results:** Bats where place into two groups according to the structure of calls: FM bats (*Mops condylurus*, *Chaerephon major*, *Mops niveiventer*) and FM/QCF bats (*Scotophilus dinganii* and *Scotophilus leucogaster*). The DFA resulted in a correct overall classification of 69.7%.

**Conclusion:** This preliminary study showed that DFA of call parameters is a feasible method that can be used to identify insectivorous bats in the region by their echolocation calls.

Keywords: Echolocation, Maroua, Bats, Cameroon, Anabat SD1 detector, Sonogram

# Background

Bats (Order Chiroptera) have their greatest diversity in the tropics (Willig, Kaufman, & Stevens, 2003). They provide key ecosystem services such as plant pollination, seed dispersal, insect population regulation (Kalka, Smith, & Kalko, 2008; Kunz, Torrez, Bauer, Lobova, & Fleming, 2011; Lobova, Geiselman, & Mori, 2009) and serve as excellent bioindicator taxa for monitoring environmental

health (Jones, Jacobs, Kunz, Willig, & Racey, 2009; Medellin, Equihua, & Amin, 2000; Ochoa 2000). Yet, despite their importance, many populations of bat species are declining worldwide (Mickleburgh, Hutson, & Racey, 1992; Racey, 1998). Schipper et al. (2008) pointed out that globally, a quarter of all bat species are considered threatened mainly as a consequence of extensive habitat loss and roost disturbance. One of the greatest and least appreciated threats to bats is the lack of basic information about many species (Mickleburgh et al., 1992). Indeed, very little information is known about species requirement and distribution (Hutson, Mickleburgh, & Racey, 2001);



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this in turn makes assessing which species is in need of immediate conservation measures difficult.

In Cameroon, bats are rarely considered in biodiversity conservation plans mainly because of the lack of basic information on their distribution, ecology, and behavior.

Such information about bats in Cameroon still remains limited in spite of major recent contributions by Bakwo Fils (2009, 2010a, 2010b, 2014), Bakwo Fils, Bol, and Tchuenguem Fohouo (2012), Bakwo Fils et al. (2014), Bol, Guieké, and Tsala (2011), Cosson (1995); Hassanin (2014), Lebreton, Bakwo Fils, Takuo, and Diffo (2014) and Sedlàcek, Horak, Riegert, Reif, and Horàcek (2006), which led to the detection of species new to the country. Despite these contributions, much is still to be known concerning bat habitat and feeding ecology. Moreover, all the available data is based on traditional capture methods of surveying bats (e.g., mist nets and harp traps). O'Farrell (1997, p. 1), pointed out that "not all bat species and not all individuals within a species are susceptible to capture."

Acoustic surveys using bat detectors are increasingly being used in addition to these traditional methods (Jones et al., 2009; O'Farrell & Miller, 1999; Ochoa, O'Farrell, & Miller, 2000). According to O'Farrell (1997), acoustic detection has been shown to be a powerful supplement to standard capture methods to identify many insectivorous bats that are underrepresented in field inventories. Indeed, bats of the families Rhinolophidae, Hipposideridae, and some subfamilies of Vespertilionidae are very skilled at avoiding mist nets because of their high versatility inflight and efficient echolocation calls (Kingston, Francis, Akbar, & Kunz, 2003). Acoustic detection depends on the use of ultrasonic bat detectors to monitor echolocation calls produced by insectivorous bats. These detectors are widely used to study habitat use by bats (Krusic, Yamasaki, Neefus, & Pekins, 1996; Law, Anderson, & Chidel, 1999; Vaughan, Jones, & Harris, 1997). Detectors often reveal the presence of more species at a site than capture techniques (Kuenzi & Morrison, 1998; Murray, Britzke, Hadley, & Robbins, 1999; O'Farrell & Gannon, 1999) and can be deployed in a much wider variety of locations than capture techniques (O'Farrell, Miller, & Gannon, 1999). Bat detectors can also be used to accurately identify bat (Britzke, Murray, Heywood, & Robbins, 2002; O'Farrell et al., 1999; Parsons & Jones, 2000). Detectors, unlike netting and trapping, do not involve handling and thus minimize disturbance. However, before acoustic methods can be used for inventories, a library of authenticated calls is required to describe the echolocation call characteristics of each species present in a particular area so that, when calls of unknown bats are recorded in the field, they can be compared with these reference calls. Acoustic survey has never been used to Page 2 of 9

characterize and identify echolocation calls of bats anywhere in Cameroon. This explains the total absence of echolocation reference call libraries.

The main purpose of this study was therefore to use the Anabat SDI detector, which is a frequency division detector, to record, identify, and describe the calls of the most abundant insectivorous bats of Sahelian zone of northern Cameroon, notably *Mops condylurus, Chaerephon major, Mops niveiventer, Scotophilus dinganii*, and *Scotophilus leucogaster* (Bol et al., 2011) and also to evaluate the potential for acoustic identification by discriminant function analysis (DFA) using restricted sample sizes. This preliminary study will provide the basis for future acoustic monitoring studies in the region and elsewhere in the country.

# Methods

## Study site

The study was conducted in the town of Maroua capital of the Far North Region of Cameroon. The town is situated in the Sudano-Sahelian zone of the country between latitudes 10° and 13° north and longitudes 13° and 15° east (Yengue & Callot, 2002). The region has the Sudano-Sahelian (semi-arid) climate characterized by variations in climatic elements (Bourtrais et al., 1984). There are two alternating seasons: a long dry season that runs from October to May and a short rainy season from June to September. The mean annual rainfall is about 600–900 mm and mostly recorded in the months of July and August. The average temperature is about 28 °C but can attain a maximum of 45 °C in the months of March and April (Yengue & Callot, 2002).

#### Mist netting and identifications of bat species

Bats were sampled for 18 non-consecutive nights between July 2012 and January 2013. Eight capture sites comprising foraging, drinking, and roost sites were selected in the study area based on prior knowledge of bats activities (Table 1). The geographical positions of each site were noted using a hand-held GPS (Garmin eTrex). Free-flying bats were captured using two mist nets with four shelves ( $9 \times 2.60$  m, 16-mm mesh size) that were tied between 4-m-long poles. The nets were deployed from 6 pm to about midnight. Each time a bat flew into the net, it was carefully untangled by an observer and identified to species level using morphometric measurements (Hayman & Hill, 1971; Robbins, De Vree, & Van Cakenberghe, 1985; Rosevear, 1965). After proper identification, the bats were released for validation of echolocation calls.

## Acoustic recording of echolocation calls

Echolocation calls of 16 Chaerephon major, 12 Mops condylurus, 10 Mops niveiventer, 12 Scotophilus leucogaster, and 19 Scotophilus dinganii were recorded in flight using the Anabat SD 1 bat detector (Titley

Number of individuals per								
Sites	А	В	С	D	E	F	G	Н
Altitude (m)	404	402	403	420	401	403	403	414
Capture nights	04	04	03	01	01	01	03	01
GPS coordinates	10° 35' 38.5" N 14° 20' 15.2' E	10° 35' 47.7" N 14° 18' 54.1" E	10° 35′ 90.2″ N 14° 20′ 25.0″ E	10° 34' 24.5" N 14° 16' 54.6" E	10° 35' 39.8" N 14° 17' 18.5" E	10° 35' 90.2" N 14° 20' 25.0" E	10° 35' 58.8" N 14° 18' 44.4" E	10° 35′ 37.2″ N 14° 17′ 19.5″ E
Habitat types	Drinking site	River bed	Drinking site	Roost site	Roost site	farmland	Drinking site	Roost site
Molossidae								
Chaerephon major (n = 23)	-	-	22	01	-	-	-	-
Chaerephon nigri (n = 01)	-	01	-	-	-	-	-	-
Chaerephon pumilus (n = 06)	01	02	01	-	-	-	02	-
Mops condylurus (n = 12)	01	-	-	11	-	_	-	-
Mops niveiventer (n = 10)	-	-	-	10	-	_	-	-
Vespertilionidae								
Nycticeinops schilieffenii (n = 01)	-	-	01	-	-	-	-	-
Pipistrellus nanus (n = 01)	-	-	-	-	-	01	-	-
Pipistrellus nanulus (n = 01)	-	01	-	-	-	-	_	-
Pipistrellus inexpectatus (n = 01)	-	-	-	-	-	01	-	-
Scotoecus hirundo (n = 02)			01	-	-	-	01	-
Scotophilus dinganii (n = 20)	05	12	03	-	-		05	
Scotophilus leucogaster (n = 17)	10	03	03	01	-	-	-	-
Rhinolophidae								
Rhinolophus fumigatus (n = 01)	-	-	-	-	-	-	01	-
Total individuals ( $n = 96$ )	16	20	30	23	0	02	09	0
Total species ( $n = 13$ )	03	06	06	04	0	01	04	0

Table 1 Bat species recorded in eight survey areas in Maroua, Cameroon (numbers in parentheses refer to captured individuals)

A: Pont vert; B: Pont rouge; C: Pont jaune; D: Pont Makabay; E: Collège de l'espoir; F: Mizao 1; G: Mizao; H: Pont palar. Including characteristic of different habitats investigated, altitude, and GPS coordinates

Electronics) after the bats were hand-released. All hand-releases and recordings were performed not far from where the bats were caught. The recordings were made with the detector held at the same height as the bat. The calls were recorded by an observer, who stood at about 20 m from the point of release. The detector was turned on as soon as the bat took off from the hands of the person releasing it. If possible, bats were followed on foot for some distance by the person holding the Anabat detector. The sensitivity of the detector was adjusted to the maximum sensitivity in order to record calls of very low intensity.

## Analysis of echolocation calls

The structures of recorded call were analyzed using Analook software (version 4.8). One of the last echolocation calls in each sequence considered to be a search phase call and which allowed for selection of variables with confidence was selected (Betts, 1998). Calls were filtered before measurement using the "filter" command in Analook designed to exclude echoes and unwanted background noise. Additional cleaning was done using the "off dot" in Analook. This was done to ensure that selected variables could be measured with confidence.

The following variables were measured from each selected sonogram by Analook: maximum frequency (Fmax), minimum frequency (Fmin), mean frequency (Fmean), frequency at the knee (Fk), time at knee (Tk), characteristic frequency (Fc), time at characteristic frequency (Tc), duration (Dur), initial slope (Si), and characteristic slope (Sc); frequency is given in kilohertz, time in millisecond, and slopes expressed in octave per second (octave/s). Based on the sonograms, bats were grouped into two call categories: frequency modulation types (FM) and frequency modulation followed by quasi constant frequency (FM/QCF). The discriminant function analysis was performed using seven variables as predictors of membership to five-bat species group (grouping variable). The predictors were Fmax, S1, Sc, Fmin, Fmean, Fc, and Tk. The variables Dur, Tk, and Tc failed the tolerance test and were not included in the analysis. The calls selected for classification were considered to belong to search phase.

## Statistical procedures

For the five species form which calls were recorded, descriptive statistics were calculated for each bat species. Subsequently, a discriminant function analysis (DFA) was performed to assign echolocation calls to species (Russo & Jones, 2002). The analysis determines which variables separate the species using discriminant functions (Digby & Kempton, 1987). Eigenvalues indicate the strength of the functions in differentiating one group from another. Wilk's lambda values were obtained with a multivariate analysis of variance (MANOVA) to provide a measure of the relative discriminating power of each variable, the lower value, the higher, and the discriminating power of the variable (Rodríguez-San Pedro & Simonetti, 2013). The significance level of lambda is determined from the distribution of chi-square (Parsons & Jones, 2000). To obtain a graphical representation of the separation of groups based on their discriminant functions, we plotted the group centroids with 95% confidence limits for separate functions and the canonical discriminant functions. For each species, descriptive statistics (mean  $\pm$  SE) were calculated (Rodríguez-San Pedro & Simonetti, 2013). All of these analyses were performed using the software SPSS 17.0.

# Results

### Qualitative analysis of echolocation calls

Eight sites were sampled within the Maroua area. Mist nets were monitored for 108 net hours and resulted in 96 captures of 13 species, representing seven genera in three families (Vespertilionidae, Molossidae, and Rhino-lophidae). The predominant species were *Chaerephon major* (n = 23), *Scotophilus dinganii* (n = 20), and *Scotophilus leucogaster* (n = 17), *Mops condylurus* (n = 12), *Mops niveiventer* (n = 10) (Table 1).

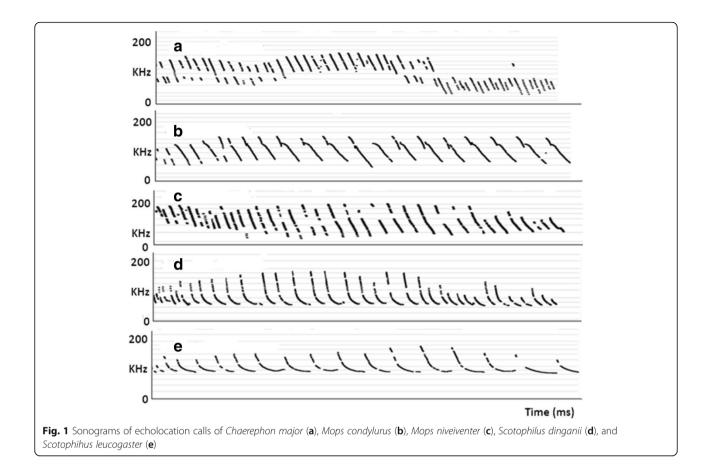
Species in which single or very few individuals were captured were not included in the DFA. This involved eight of the 13 captured species. The five bat species were Chaerephon major, Mops condylurus, Mops niveiventer, Scotophilus leucogaster, and Scotophilus dinganii. We analyzed a total of 172 call files from which the best quality calls were selected for further analysis. Descriptive statistics of calls from all species recorded are shown in Table 2. The echolocation call of Chaerephon major is a frequency modulation (FM) call, which is characterized by steep, linear pulses of highly variable frequencies and a slight leftward leaning (Fig. 1a). The call of Mops condylurus is a FM call. Pulses are steep and linear and leftward leaning (Fig. 1b). Echolocation call of Mops niveiventer is FM call and is made up of broadband, steep, near vertical pulses of variable frequencies (Fig. 1c). The echolocation call of *Scotophilus dinganii* is a FM/QCF call. The pulses are mostly broadband, curved with an initial steep FM sweep that ends with a QCF (Fig. 1d). The echolocation call of Scotophilus leucogaster is an FM/QCF call. Pulses are broadband,

					<sup>-</sup> insectivorous					

Family/species	Call structure	Ν	Fmax	Fmin	Fmean	Fk	Fc	Duration	Tk	Tc
Molossidae										
Chaerephon major	FM	16	39.3 ± 6.9	37.4 ± 6.7	$36.4 \pm 6.7$	38.2 ± 7.3	33.7 ± 7.3	$1.66 \pm 0.40$	0.35 ± 0.	1.46 ± 0.36
Mops condylurus	FM	12	37.8 ± 3.8	34.2 ± 4.9	36.4 ± 4.5	38.2 ± 4.7	33.67 ± 5.1	$0.78\pm0.89$	$0.003 \pm 0.1$	0.75 ± 0.75
Mops niveiventer	FM	10	37.7 ± 2.9	34.1 ± 2.6	35.9 ± 2.8	37.6 ± 2.7	34.1 ± 2.6	$0.74\pm0.10$	$0.00 \pm 0.1$	0.71 ± 0.65
Vespertilionidae										
Scotophilus dinganii	FM/QCF	19	$58.5 \pm 8.9$	$52.6 \pm 6.2$	55.6 ± 7.5	$58.5 \pm 8.9$	$52.7 \pm 6.2$	$0.88 \pm 0.92$	$0.03\pm0.06$	$0.86\pm0.84$
Scotophilus leucogaster	FM/QCF	12	55.7 ± 4.3	50.6 ± 3.0	53.2 ± 3.6	55.7 ± 4.3	50.7 ± 3.0	$0.81 \pm 0.23$	$0.00\pm0.01$	0.80 ± 0.21

The table shows mean ± standard deviation and the range of the eight time and frequency parameters. Recordings were made with ANABAT bat detectors and analyzed with ANALOOK software. Initial slope of call (S1) and slope at the flattest section of the call (Sc) were delibrately excluded from the descriptive statistics. Frequency parameters were measured in kilohertz and time parameters in milliseconds (ms). Based on the sonograms, bats were grouped into two categories: frequency modulation types (FM) and frequency modulation followed by quasi constant frequency types (FM/QCF)

N number of bats in sample, *Fmax* maximum frequency of the call, *Fmin* minimum frequency of the call, *Fmean* mean frequency of the call,  $F_{K}$  frequency at the knee,  $F_{C}$  characteristic frequency, *Dur* duration of the call,  $T_{K}$  time into the call when  $F_{K}$  is reached, and  $T_{C}$  time into the call when  $F_{C}$  is reached



curved with an initial FM sweep that ends with a QCF sweep (Fig. 1e).

# Quantitative analysis

## Discriminant function analysis

Four discriminant functions were produced. The first discriminant function accounted for 58.2% of the total variation between bat species and the second 40.3% (Table 2). Only the first two functions were important because they accounted for 98.5% of the total variation. The first function has canonical correlation of 0.916 (Table 3).

The four canonical discriminant functions obtained for the species groups gave a combined  $\chi^2$  (28) = 205.604, p < 0.05 (Table 4). It indicated that the function as a whole is significant and the discriminant function does better than chance at separating the bat calls into species groups. After removal of the first function, there was still a strong association between bat species and predictors,  $\chi^2$  (18) = 97.084, p < 0.05(Table 4). After removal of the second function,  $\chi^2$ (10) = 7.572, p = 0.671, there was no longer a strong association between bat species and predictors (Table 3). The first function (Wilks' lambda = 0.31) has the greatest discriminatory ability in classifying calls in different species groups (Table 4). Overall, 69.7% of the echolocation calls were correctly classified into the different species of bats, exceeding the value for classification based on chance (33.3%) (Table 5). At the individual group level, 52.6% of calls of *Scotophilus dinganii* were correctly classified, 75.5% of *Scotophilus leucogaster*, 70.0% of *Mops niveiventer*, 55.6% of *Mops condylurus*, and 93.8% of *Chaerephon major* (Table 5).

About 47.4% of calls of *Scotophilus dinganii* were misclassified as calls of *S. leucogaster* while 25.0% of calls of *Scotophilus leucogaster* were misclassified as belonging to *Scotophilus dinganii*. For *Mops niveiventer*, 30.0% of their calls were misclassified as belonging to *Mops condylurus*. For *Chaerephon major*, 6.3% of their calls were classified as belonging to *Scotophilus dinganii* (Table 5).

The plot of mean canonical scores and the canonical discriminant functions (Fig. 2) demonstrates that the species are well separated in multidimensional space. It can be observed that function 1 mostly separate the calls from *Mops condylurus* and *Mops niveiventer* which are two closely related species from those of *Scotophilus dinganii* and *Scotophilus leucogaster* which are also closely related. Then, function 2 mostly separates *Mops* species and *Scotophilus* species from *Chaerephon major*.

**Table 3** Relative power of discriminant functions. The first two functions are the most important and account for about 98.5% of the total variation

Function	Eigen value	% of variance	Cumulative %	Canonical correlation
1	5.216	58.2	58.2	0.916
2	3.615	40.3	98.5	0.885
3	0.098	1.1	99.6	0.298
4	0.036	0.4	100.0	0.186

First four canonical discriminant functions were used in the analysis

# Discussion

The Anabat frequency division bat detector is one of a number of ultrasonic devices used to detect and identify bat species based on the identity of their echolocation calls. Although acoustic detectors permit the sampling of bats in a larger area than traps or nets, species with low-intensity vocalization may not adequately be represented because echolocation calls of low intensity weaken rapidly in the atmosphere as such cannot be detected from afar (O'Farrell & Gannon, 1999). Moreover, before acoustic detection can be used in an area to identify bats, reference collections of bat echolocation calls need to be established. The use of the Anabat ultrasound detector to record echolocation calls of bats in the Sahelian region represents a start toward establishing a comprehensive library of reference echolocation calls of bats in Cameroon.

In our study, we recorded echolocation calls of most abundant insectivorous bats present in the sahelian region of Far North Cameroon (Mops condylurus, Chaerephon major, Mops niveiventer, Scotophilus dinganii, and Scotophilus leucogaster) (Bol et al., 2011), using an Anabat ultrasound detector after they were hand-release. We later performed a discriminant function analysis (DFA) to classify echolocation calls into five species groups. The echolocation calls were correctly classified to species with 69.7% accuracy using cross validation DFA. The sonograms were also viewed and revealed differences in call structure among the different species. Overall, the level of accurate classification of calls into species group by DFA was reasonably high. However, classification rates at species level were comparatively lower when compared to similar studies

**Table 4** Wilks' lambda table shows that the first two functions have the highest discriminatory ability in classifying calls into species group

species group				
Test of function(s)	Wilks' lambda	Chi-square	Df	Sig.
1 through 4	0.031	205.604	28	0.000
2 through 4	0.191	97.804	18	0.000
3 through 4	0.880	7.572	10	0.671
4	0.966	2.069	4	0.723

(Hughes et al., 2011; Kofoky et al., 2009; Rodríguez-San Pedro & Simonetti, 2013). The relative poor performance of the DFA may be attributed to the use of fewer individual of each species. It can also be attributed to the fact that handling and release of bats affect their call structure. Artificial neural networks (Parsons, 2001) and synergetic recognition algorithms (Obrist, Boesch, & Flückiger, 2004) are two other methods that can be applied for acoustic identification of species. Although ANNs could perform better than DFA, they are not trivial to use (Parsons & Jones, 2000). They are the most computationally intensive techniques and take the longest amount of time to train (Armitage & Ober, 2010). Synergetic algorithms require highly sophisticated software and large storage memory (Obrist et al., 2004). Because the ultimate aim of this study was to devise an objective identification tool to be used by other researchers in the region, a standard statistical approach such as the quadratic DFA remains the preferred method, as it is relatively simple and easily accessible.

The three genera clearly differed from each other in multivariate space. Figure 2 shows that the groups centroids of Scotophilus dinganii (forearm length 49-57 mm) and Scotophilus leucogaster (forearm 43-51 mm) are almost on the same spot, indicating that call parameters of these morphologically similar species are almost the same. Their calls differ from the calls of *Mops condylurus* (forearm 45–50 mm) and Mops niveiventer (forearm 44-47 mm), which are species that are smaller than the Scotophilus species but are morphologically similar to each other. The calls of Chaerephon major (forearm 42-44 mm) are distinct from the Scotophilus species and the Mops species. This confirms that closely related species, with similar morphology and/or ecology may show convergent evolution of their call features and hence similarities in their echolocation calls (Papadatou, Butlin, & Altringham, 2008; Parsons & Jones, 2000).

To the best of our knowledge, echolocation calls for Mops niveiventer, Chaerephon major, and Scotophilus leucogaster presented here are the first published records of these species using the Anabat detector in Africa. Mops condylurus and Scotophilus dinganii have been studied in other parts of Africa using the Anabat and their echolocation call parameters published. The two Scotophilus species in this study emitted typical broadband, curved FM/QCF calls with an initial steep FM sweep that ends with a QCF. All FM/QCF bats are expected to forage mainly in open spaces (Vaughan et al., 1997) because FM/QCF calls are suitable for use in open environments with some obstacles (Simmons, Fenton, & O'Farrell, 1979). Scotophilus dinganii recorded calls with a maximum frequency of  $58.5 \pm 8.9$  kHz, minimum frequency of  $52.6 \pm 6.2$  kHz, frequency at the knee of

	Species	Predicted group membership							
		S. dinganii	S. leucogaster	M. niveiventer	M. condylurus	C. major			
%	S. dinganii	52.6	47.4	0.0	0.0	0.0	100.0		
	S. leucogaster	25.0	75.0	0.0	0.0	0.0	100.0		
	M. niveiventer	0.0	0.0	70.0	30.0	0.0	100.0		
	M. condylurus	0.0	11.1	33.3	55.6	0.0	100.0		
	C. major	6.2	0.0	0.0	0.0	93.8	100.0		

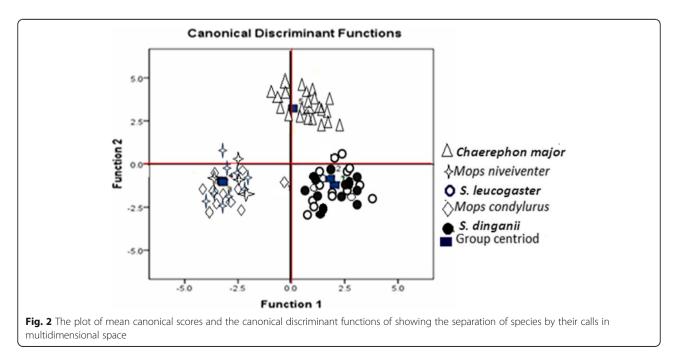
Table 5 Classification table showing the percentage of calls correctly classified into species groups

69.7% of original grouped cases correctly classified

 $58.5 \pm 8.9$  kHz, and characteristic frequency of  $52.7 \pm$ 6.2 kHz (Table 1). These frequency parameters were generally higher than those recorded by Jacobs, and Walker (2007) in Kwazulu-Natal; Barclay, Schoeman and Waddington (2011) in Durban; Taylor, Sowler, Schoeman, and Monadjem (2013) and Linden et al. (2014) in Soutpansberg; and Taylor et al. (2013) at Farm Welgevonden in South Africa. Sonograms of all molossids bats indicate that their echolocation calls are broadband FM calls (Table 2). This implies that many of them are expected to forage in the open or at the edge of forests, using shorter duration signals that are well suited broadband for three-dimensional target localization and for separating figures and ground (Simmons, 1973). Mops condy*lurus* recorded calls with Fmax of  $39.2 \pm 6.9$  kHz, Fmin of  $34.2 \pm 6.7$  kHz, Fk of  $38.2 \pm 7.3$  kHz, and Fc of 38.67 ± 7.3 kHz (Table 2). These frequency parameters were generally higher than those recorded by Linden et al. (2014); Monadjem, Taylor, Cotterill, and Schoeman (2010); Naidoo, Mackey, and Schoeman (2011); Schoeman and Waddington (2011); and Taylor et al. (2013) in Republic of South Africa and Taylor et al. (2013) in Swaziland. These results suggest a geographical variation in echolocation calls of these two species. Similar geographic variation in calls were reported for other species by Thomas, Bell, and Fenton (1987). This variation can be attributed to differences in techniques of recording and analyzing calls, notably the hand-release method used during the study as opposed to recordings made in a flight tent or in an open space. It might also be due to the habitat differences (clutter or open) or the distance from the detector and humidity of air (Kalko & Schnitzler, 1993). Intraspecific variation of echolocation calls can also be caused by differences in sex (Jones, Gordon, & Nightingale, 1992) and age (Masters, Raver, & Kazial, 1995).

### Conclusions

Despite the fact that our study was based on a limited sample sizes ( $N \le 20$  for all species), the study



demonstrates that a quantitative method (DFA) could potentially provide an effective technique to acoustically sample bats in the region, but before this can be practical the echolocation calls of these five species need to be recorded in different habitat types such as clustered and open spaces in order to take into account geographical variation of echolocation calls. The study also provides evidence that acoustic techniques can play a significant role in complementing traditional capture techniques.

#### Abbreviations

ANN: Artificial neural network; DFA: Discriminant function analysis; Dur: Duration; Fc: Characteristic frequency; Fk: Frequency at the knee; FM: Frequency modulation; Fmax: Maximum frequency; Fmean: Mean frequency; Fmin: Minimum frequency; GPS: Global Positioning System; MANOVA: Multivariate analysis of variance; QCF : Quasi constant frequency; Sc: Characteristic slope; Si: Initial slope; Tc: Time at characteristic frequency; Tk: Time at knee;  $\chi^2$ : Chi-square

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#### Availability of data and materials

Data sharing is not applicable to this article because datasets were generated or analyzed during the current study.

#### Authors' contributions

BFEM, AMM, DET, and TJL designed and performed this work. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

The authors declare that this work was carried out within an appropriate ethical framework.

#### **Competing interests**

The authors declare that they have no competing interests.

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