Scientific contributions

Update on the distribution of "Chiroptera sp1" in the southern and eastern parts of La Réunion Island based on acoustic surveys

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Keywords : *Scotophilus*, range expansion, acoustic identification.

La Réunion Island (2512 km^2) is a tropical volcanic island of recent origin (2–3 million years ago) in the western Indian Ocean, located to the east of Madagascar (780 km) and close to Mauritius (210 km). Despite the fact that only two species of insectivorous bats are currently recognized to occur on the island – the endemic molossid *Mormopterus francoismoutoui* Goodman *et al*., 2008, and Afro-Malagasy Region emballonurid *Taphozous mauritianus* Geoffroy, 1818 – recent acoustic surveys revealed a distinct acoustic type, which can be related to a third species (BARATAUD and GIOSA, 2013). The third form, that we refer to herein as Chiroptera sp1, might be the Pale House Bat, *Scotophilus borbonicus* Geoffroy, 1803, previously reported on La Réunion (GEOFFROY, 1803; MAILLARD, 1862; VINSON, 1868; MAC-AULIFFE, 1902; HILL, 1980; CHEKE and DAHL, 1981) and inferred to have occurred at higher altitudes until its presumed extinction in the late 19th century (CHEKE and HUME, 2008). The species was subsequently reported from Madagascar (DORST, 1947, but see GOODMAN *et al.*, 2005). On La Réunion, MAILLARD (1862) called it the "upland bat" and noted that it "lives generally in the forests and is also found in the coastal zone". Based on acoustic measurements, a recent study (PRIÉ *et al.*, 2016) provided strong evidence to associate the newly found Chiroptera sp1 to the genus *Scotophilus*, by performing a comparison between *M. francoismoutoui*, Chiroptera sp1 and its presumed congener *S. robustus* from Madagascar. PRIÉ *et al.* (2016) also aimed to discuss the uncertain taxonomic status of *S. borbonicus*, as the remaining specimen allocated to this species is in poor condition (GOODMAN *et al.*, 2005). According to PRIÉ *et al.* (2016), Chiroptera sp1 is presumed to inhabit the northwestern part of the island in zones associated with semi-dry forest. This conclusion was based on data primarily from this portion of the island and a few additional records from southern and eastern areas.

If indeed Chiroptera sp1 represents a third insectivorous bat species for La Réunion, it is important to have a clear understanding of its distribution to ensure effective management and conservation programs. While the taxon in question has yet to be captured, echolocation calls (BARATAUD and GIOSA, 2013) provide the only current basis for its identification. The aim of the current study is to (i) update information on the distribution of Chiroptera sp1 based on new acoustic records collected in the southern and eastern parts of the island and (ii) present new inferences on its ecology in order to maximize the chance of capturing the species in the near future.

MATERIAL AND METHODS

We used Song Meters SM2BAT+ (Wildlife Acoustics) with omnidirectional microphones to record bat echolocation calls. Weatherproof microphones (SMX-II, Wildlife Acoustics) were placed at least 2 m above the ground, in open flyways, and away from clutter. We placed these devices randomly in the eastern and southern portions of the island, taking into account access and insurance of the recording equipment. Song meters in WAV mode were set to save echolocation sequences of one or more calls during 5 s. This basic sampling unit of 5 s SM2Bat recording could contain data of one or more species. Parameters necessary for species identification are provided in BARATAUD and GIOSA (2013). Chiroptera sp1 is known to have a narrow bandwidth (quasi-constant frequency, QCF) bounded between 29 and 31 kHz (BARATAUD and GIOSA, 2013; PRIÉ *et al.*, 2016). Due to some overlap between the acoustic repertories of the common *Mormopterus francoismoutoui* and Chiroptera sp1, we used a three-step process to rigorously discriminate these two taxa. First, we employed Scan'R software (Binary Acoustic Technology Inc.) to filter out most of *M. francoismoutoui*, *T. mauritianus* and parasitic signals (mostly insects and terrestrial mammals such as *Rattus* spp.). A custom-made calibration was carried out to sharpen the call selection criteria, specifically on trigger level, minimum and maximum frequency cutoff, and minimum duration. Second, all calls that passed the species filters were confirmed manually by the author and M. Barataud, who has extensive experience analyzing bat echolocation data. Acoustic measurements were conducted with BatSound 4.2.1 software (Pettersson Elektronik AB, Sweden) to confirm that the calls belonged to the Chiroptera sp1 repertory. The calls were digitized (sampling rate 96 kHz) and processed through a Fast Fourier Transformation (FFT) using a Hanning Window for spectrograms (FFT size of 512 points). As recommended in BARATAUD (2015), for individual calls, manual measurements were performed associated with the power spectrum, specifically a FFT window of 256 points for quasi-constant frequency (QCF) pulses or 512 points for frequency modulated (FM) or flat-ended FM (FM-QCF) pulses. For each measured call, four parameters were determined and compared to the dichotomous key provided in BARATAUD and GIOSA (2013) to discriminate Chiroptera sp1 from *T. mauritianus* and *M. francoismoutoui*: peak frequency (Fmax), minimum frequency (Fmin), bandwidth (BW), maximum frequency (MF), and interpulse intervals (IPI). These parameters have been shown to separate acoustic differences between bat species in La Réunion (BARATAUD and GIOSA, 2013). Elevation, time of signal, and number of total files recorded per location were also noted. Third, as recommended in BARCLAY and BRIGHAM (2004), the distribution of our selected Chiroptera sp1 echolocation calls was confirmed with a reference library kindly provided by M. Barataud and recorded from captured *M. francoismoutoui* equipped with light sticks and identified flying individuals of Chiroptera sp1 recorded in 2009 and 2012 (see BARATAUD and GIOSA, 2013; PRIÉ *et al.*, 2016) on La Réunion. Calls falling inside the set of values determined from the reference library were considered as belonging to Chiroptera sp1 and those falling in the overlap between *M. francoismoutoui* and Chiroptera sp1 acoustic repertories were classified as potential but unconfirmed signals.

RESULTS AND DISCUSSION

From 49 different locations in eastern and southern La Réunion, 779 hours of recordings and 82,036 call files were collected over 66 nights from February 2015 to March 2017 (see Appendix 1, Figure 1). After the three filtering steps detailed in methods section above, 16 call files of Chiroptera sp1 were confirmed from six localities. An additional 41 call files were classified as probable Chiroptera sp1, providing another 12 probable localities.

As confirmed in previous studies (BARATAUD and GIOSA, 2013; PRIÉ *et al.,* 2016), Chiroptera sp1 emitted single harmonic pulses consisting in most cases of a narrowband quasi-constant frequency component (QCF) and occasionally FM-QCF pulses (four out the 16

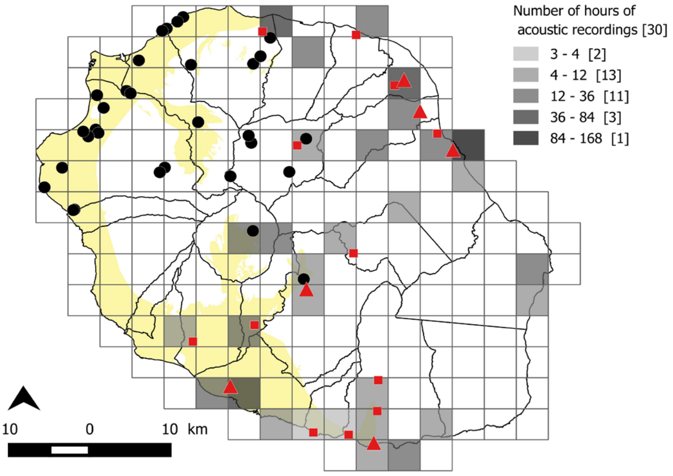

Figure 1. Distribution of Chiroptera sp1 and search effort on La Réunion. Black dots: previous data collected from PRIÉ *et al.* (2016); red triangles: new data collected specifically in the context of this study from eastern and southern areas; red squares: potential Chiroptera sp1 calls collected during the current study; yellowish areas indicates the semi-dry forest zone.

Table 1. Acoustic parameters (in kHz and msecs [IPI]) from the 16 confirmed Chiroptera sp1 call files collected in the eastern and southern parts of La Réunion.

	FM-QCF pulses		QCF pulses	
	n	***Mean (±SD)***	***n***	***Mean (±SD)***
MF		48.45 (±9.26)		31.18 (±1.69)
Fmin		32.87 (±1.89)		29.62 (±0.53)
Fmax	4	33.29 (±1.64)	12	29.82 (±0.41)
BW		15.59 (±7.67)		1.56 (±1.96)
IPI		173.25 (±78.80)		217.42 (±96.24)

confirmed Chiroptera sp1 calls). In Table 1, a summary of confirmed call acoustic measurements is presented.

All confirmed Chiroptera sp1 call sequences were recorded early in the night (from 6:30 PM to 8:43 PM) or predawn (from 5:21 AM to 5:46 AM). After adding data of calls attributed to Chiroptera sp1 from the northern part of the island (PRIÉ *et al.,* 2016), the same temporal trend is confirmed, although predawn activity is slightly extended to 6:10 AM. When considering the complete dataset, including probable Chiroptera sp1 calls, only four of the 64 call files fell outside the activity period mentioned above (11:25 PM, 2:47 AM, 3:10 AM, 4:27 AM). Chiroptera sp1 was confirmed at elevations ranging from 20 m to 1,360 m, with 70% of all probable and confirmed calls from sea-level to 90 m, although the highest volume of recordings, almost 90% (see Appendix 1), was made above 500 m. When including confirmed localities from PRIÉ *et al.* (2016), elevations ranged from sea-level to 1,900 m (Figure 2).

The acoustic data reported here extend the known limit distribution of Chiroptera sp1 on La Réunion, including eastern and southern portions of the island, as well as the eastern windward coast (Figure 1), providing evidence that this form is not restricted to dry and semi-dry environments, as previously proposed (PRIÉ *et al.*, 2016). Understanding the distribution and nightly activity of Chiroptera sp1 on La Réunion is a critical first step towards identifying its habitat requirements, its subsequent capture, and associating a species name with this taxon. The notable scarcity of signals matching the acoustic signature of Chiroptera sp1 suggests that it might fly high enough to exceed the microphone detection range or is rare compared to *M. francoismoutoui* and, to a lesser extent, *T. mauritianus*. For call files that were recorded at the edge of the microphone detection range, the signal to noise ratio was in some cases sufficiently high to obscure resolution for possible positive identification of Chiroptera sp1. Detector height may introduce biases to ground-based ultrasound survey, potentially resulting

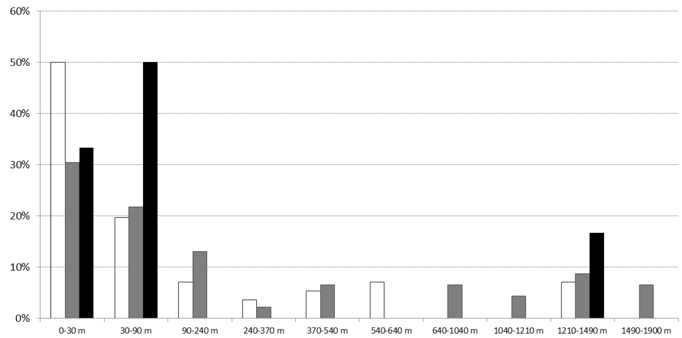

Figure 2. Elevational range of collected calls of Chiroptera sp1. White bars: relative percentage of all confirmed and probable calls obtained during the current study; gray bars: relative percentage of all confirmed calls from the current study and PRIÉ et al. (2016); black bars: relative percentage of all confirmed calls from the current study.

in false absences and unrepresentative activity levels (STATON and POULTON, 2012). Consequently, foraging height preferences of Chiroptera sp1 should be investigated in more detail, as this factor might explain why this species is only recorded at early and late night: as already suggested in BARATAUD and GIOSA (2013), this taxon may have a different flight behavior during the middle portion of the night as compared to early night and predawn hours. Survey protocols to specifically search for Chiroptera sp1 should include the use of detectors placed in different vertical positions, for example at ground level to up to 30 m (using different types of towers or even buildings in urban areas), as suggested by COLLINS and JONES (2009). While Chiroptera sp1 was diagnosed primarily at lower elevations (Figure 2), its presence also at higher altitudes was confirmed. Considering the larger amount of data collected in this current study was from the coastal portion of the island, it might be useful to augment surveys in the uplands, from where the species was originally considered to occur (MAILLARD, 1862; MAC AULIFFE, 1902). Finally, our results suggest the need to conduct more systematic ecological studies on this taxon, determine the best means for its capture, and if it represents *Scotophilus borbonicus*. This species is considered as critically endangered (HUTSON *et al.*, 2001) and has been tentatively confirmed from Madagascar based on older specimens (GOODMAN *et al.,* 2005).

ACKNOWLEDGMENTS

I kindly thank Michel Barataud for providing considerable support to conduct this study from the start and for very helpful comments on the manuscript. I am also grateful to all the individuals who allowed me to sample bat ultrasounds from their property: Floriane Desruisseaux, Chloé and Loick Mahler, Dominique Hoareau, Aude Baratin, Bénédicte Grondin-Rodier, the ROCS company. Finally, I wish to thank Steve Goodman and Victor Van Cakenberghe who provided valuable guidance on earlier versions of this manuscript.

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Submitted: 15 April 2017
Accepted: 23 April 2017
Managing Editor: V. Van Cakenberghe

Appendix 1.

Location of bat acoustic inventory stations. PC: probable calls; CC: confirmed calls; NC: unconfirmed presence of Chiroptera sp1. Number of recorded hours (NRH): *n*=779; Total number of monitoring nights (TNMN): *n*=66; Total number of files (TNF): *n*=82036). X and Y in UTM40S.

Date	Commune	Locality	Elevation (m)	NRH	X	Y	TNMN	TNF	Chiroptera sp1
26/02/2015	Petite Ile	Grande Anse Beach	0	2	349329	7636215	1	221	
26/02/2015	Petite Ile	Grande Anse Beach	0	2	349329	7636448	1	238	PC
11/05/2015	Bras-Panon	Liberia forest - Kiosque	680	12	357343	7674281	1	3770	
11/05/2015	Bras-Panon	Liberia forest - Parking	640	12	356619	7673974	1	195	
16/12/2015	Le Tampon	Bois Court	1360	12	348451	7654701	1	187	CC
16/12/2015	Saint-Pierre	Grand Bois	130	12	346509	7638535	1	3265	
16/12/2015	Saint-Louis	Plaine du Gol	150	12	334264	7647950	1	511	PC
09/01/2016	Saint-Joseph	Langevin	70	12	360900	7635104	1	49	
09/01/2016	Saint-Joseph	Manapany	20	3	353723	7635920	1	666	PC
10/01/2016	Sainte-Marie	Duparc	80	12	345550	7687570	1	3349	
05/02/2016	Saint-André	Cité Lamarck	110	36	359469	7680990	1	8538	PC
22/03/2016	Saint-Pierre	ZAC Canabady	80	12	341570	7641130	1	2243	
30/03/2016	Saint Denis	Chaudron	20	12	342731	7688867	1	1533	
30/03/2016	Saint Denis	Chaudron	20	12	342744	7688862	1	731	
30/03/2016	Saint Denis	Chaudron	20	12	342810	7688929	1	1101	
12/07/2016	Saint-Philippe	Cap Méchant	40	12	365940	7635924	1	3336	
12/07/2016	Sainte-Rose	Piton Sainte-Rose	90	24	378093	7658433	2	239	
13/07/2016	Bras-Panon	Rivière du Mât-les-Hauts	140	24	358933	7679325	2	770	
26/07/2016	Entre-Deux	Ravine Citron	490	24	341976	7650058	1	1855	PC

Date	Commune	Locality	Elevation (m)	NRH	X	Y	TNMN	TNF	Chiroptera sp1
17/08/2016	Le Tampon	Grand Bassin	690	12	347880	7656910	1	343	
23/08/2016	Saint-André	Near Jumbo Score	80	12	360654	7681696	1	1846	
23/08/2016	Saint-André	Near Jumbo Score	80	12	360969	7681731	1	1664	
27/09/2016	Saint-Pierre	Industrial area n°4 - Cliffs	60	12	338945	7642191	1	254	CC
27/09/2016	Saint-Pierre	Industrial area n°4 - Savanna	60	12	338893	7641720	1	90	
27/09/2016	Saint-Pierre	Pont Ravine des Cabris	40	12	338483	7642013	1	1278	
30/09/2016	Saint-Benoît	Patu de Rosemont High School	20	36	366762	7672672	3	1481	CC
30/09/2016	Saint-Benoît	Patu de Rosemont High School	20	36	366719	7672629	3	1247	
30/09/2016	Saint-Benoît	Patu de Rosemont High School	20	24	366765	7672687	3	5575	PC
25/11/2016	Saint-André	Near Jumbo Score	80	12	360654	7681696	1	1508	CC
25/11/2016	Saint-André	Near Jumbo Score	80	12	360891	7681754	1	338	
04/01/2017	Salazie	Mare à Citron	640	12	347300	7673240	1	1760	PC
11/01/2017	Saint-Pierre	Industrial area n°4 - Cliffs	60	12	338945	7642191	1	453	CC
11/01/2017	Saint-Pierre	Industrial area n°4 - Savanna	60	12	338893	7641720	1	197	
11/01/2017	Saint-Pierre	Pont Ravine des Cabris	40	12	338483	7642013	1	3769	
06/02/2017	Saint-Denis	Moufia	70	12	342970	7687940	1	170	PC
15/02/2017	Sainte Marie	Beaumont	300	24	347420	7684500	2	2310	
15/02/2017	Bras Panon	City center	50	24	362650	7677680	2	745	CC
20/02/2017	Saint-Benoît	Patu de Rosemont High School	20	24	366762	7672672	2	2860	CC
20/02/2017	Saint-Benoît	Patu de Rosemont High School	20	16	366765	7672687	2	7667	
20/02/2017	Saint-Benoît	Patu de Rosemont High School	20	24	366719	7672629	2	1321	
13/03/2017	Saint-Joseph	Langevin	70	12	360900	7635104	1	2024	
13/03/2017	Saint-Joseph	Piton Babet	20	12	356869	7634877	1	1670	CC
17/03/2017	Sainte-Suzanne	City center	30	24	354671	7687498	2	4641	PC
19/03/2017	Plaine des Palmistes	Col de Bellevue	1490	12	354332	7659328	1	1477	PC
19/03/2017	Plaine des Palmistes	Pyramide	890	12	359286	7664198	1	120	
20/03/2017	Saint-Benoît	Le Bourbier	50	24	364804	7674767	2	233	
23/03/2017	Sainte-Rose	Bois Blanc	130	12	377174	7655788	1	19	

Date	Commune	Locality	Elevation (m)	NRH	X	Y	TNMN	TNF	Chiroptera sp1
23/03/2017	Sainte-Anne	City center	10	12	369426	7668491	1	1234	
23/03/2017	Sainte-Rose	Enclos	80	12	375881	7652029	1	61	
23/03/2017	Saint-Joseph	Lit Rivière des Remparts	170	12	357321	7638971	1	589	PC
23/03/2017	Saint-Joseph	Lit Rivière des Remparts - Cressonière	350	12	357416	7642943	1	295	PC

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