Journal of Mammalogy, 99(4):965–973, 2018
DOI:10.1093/jmammal/gyy070

The acoustic gymnastics of the dwarf dog-faced bat (*Molossops temminckii*) in environments with different degrees of clutter

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Molossops temminckii is the only species of the Molossidae capable of exploring background-cluttered space. We tested the hypothesis that *M. temminckii* uses the clutter rejection strategy in cluttered environments and determined whether the echolocation calls of this species show large-scale geographic variation. We recorded echolocation calls of 10 individuals of *M. temminckii* using 4 treatments with different degrees of clutter. We compared individuals recorded in Venezuela with individuals recorded in Brazil to verify geographic variation in calls. In the cluttered environments, *M. temminckii* emitted short downward frequency-modulated calls with short pulse intervals. In the uncluttered environments, *M. temminckii* emitted long upward frequency-modulated calls with longer intervals and shorter bandwidth. The decreased pulse duration and interval in cluttered environments support the hypothesis that *M. temminckii* uses the clutter rejection strategy. Additionally, we found that in Brazil, *M. temminckii* emits shorter calls with higher minimum frequency compared with bats recorded in Venezuela, indicating large-scale geographic variation, probably because of environmental factors.

Molossops temminckii é a única espécie de Molossidae com capacidade de explorar áreas com obstáculos de fundo. Nesse trabalho testamos a hipótese do uso da estratégia de rejeição de obstáculo em ambientes fechados, e determinamos se há variação geográfica de larga escala nos chamados de ecolocalização de *M. temminckii*. Gravamos 10 indivíduos, em 4 tratamentos, com diferentes graus de obstáculos. Comparamos os chamados de ecolocalização gravados na Venezuela e Brasil para verificar a variação geográfica. Em ambientes com obstáculos, *M. temminckii* emite chamados curtos, de frequência modulada descendente, com intervalos curtos entre os chamados. Nos ambientes abertos, emite chamados longos, de frequência modulada ascendente, com intervalos mais longos e menor largura de banda. A menor duração e o menor intervalo em ambientes fechados suportam a hipótese que *M. temminckii* utiliza a estratégia de rejeição de obstáculo. No Brasil, *M. temminckii* emite chamados mais curtos, com frequência mínima mais alta, em comparação com os morcegos gravados na Venezuela. Esse fato indica variação geográfica de larga escala e fatores ambientais provavelmente contribuem para essa variação.

Key words: bat echolocation, Brazil, Cerrado, Molossidae, vocal plasticity, vocal repertoire

The echolocation mechanism of a bat species is adapted to its environment and foraging behavior (Schnitzler and Kalko 2001). Bats of the family Molossidae (free-tailed bats) are identified by the tail that extends beyond the uropatagium and by long and narrow wings adapted to rapid flight but with little maneuverability (Freeman 1981; Norberg and Rayner 1987). These bats forage in open areas, generally above the tree canopy, and emit long-duration calls characterized by

constant frequency (CF) or quasi-constant frequency (QCF—Schnitzler and Kalko 2001; Jung et al. 2014). Their family displays high plasticity in their acoustic repertoire (Fenton 1984; Schnitzler and Kalko 2001), as has been observed in the dwarf dog-faced bat, *Molossops temminckii* (Guillén-Servent and Ibáñez 2007).

Molossops temminckii is a small molossid bat (weight = 6 g; forearm = 30 mm), characterized by separate triangular ears and a smooth face (Freeman 1981; Gregorin and Taddei 2002). It is distributed throughout South America, from Venezuela to Uruguay (Simmons 2005). The species feeds primarily on insects of the orders Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, and Orthoptera (Freeman 1981; Ibáñez and Ochoa 1985). The bats hunt the insects in flight (aerial insectivores) in areas with an intermediate level of clutter (Guillén-Servent and Ibáñez 2007), such as at forest edges or between widely spaced trees. An unusual feature of this bat's acoustic repertoire is the ability to emit upward frequency-modulated (UFM) calls, practicing true vocal gymnastics when emitting the final part of the echolocation calls in the search phase, with a frequency greater than the initial frequency (Guillén-Servent and Ibáñez 2007). Compared with other members of the family Molossidae, *M. temminckii* has a low wing loading and aspect ratio, which leads to slower flight but greater maneuverability (Freeman 1981; Norberg and Rayner 1987).

The structure of a bat echolocation call is directly related to the degree of clutter where it forages (Schnitzler and Kalko 2001; Denzinger et al. 2016). Calls can also vary in the presence of conspecifics or other bat species (Ubernickel et al. 2012), according to the task being performed (Mora et al. 2011), and with geographic factors (Barclay et al. 1999; Gillam and McCraken 2007). Previous studies have described the importance of vocal plasticity in the acoustic repertoire of bat species and how calls vary according to the degree of clutter in the flight environment (Kalko and Schnitzler 1993; Biscardi et al. 2004; Xu et al. 2008). To account for clutter and avoid overlap between emitted calls and returning echoes, bats decrease pulse duration and intervals between pulses; these adaptations are consistent with the clutter rejection strategy described by Kalko and Schnitzler (1993).

By decreasing the pulse duration and the interval between pulses, bats can keep a clutter-free window because they avoid overlaps with the echo that must return to the animal before a second pulse is emitted (Kalko and Schnitzler 1993). Different bat families use this strategy, which appears to be the most effective way for low-duty-cycle bats to navigate cluttered environments. In addition to the degree of clutter, the structure of the environment together with climatic conditions appear to influence call characteristics and lead to geographic variation, as has been demonstrated for *Molossus molossus* (O'Farrell et al. 1999; Mora et al. 2004), *Eptesicus fuscus* (Surlykke and Moss 2000; Murray et al. 2001; Rodriguez and Mora 2006), *Lasiurus borealis*, *Perimyotis* (formerly *Pipistrellus*) *subflavus*, and *Myotis grisescens* (Murray et al. 2001). Jiang et al. (2015) reported that genetic drift, cultural drift, ecological selection, sexual selection, and social selection are important factors influencing geographic variation in vocalization. Geographic variation in acoustic signals can lead to reproductive isolation and acoustic speciation (Wilkins et al. 2013; Jiang et al. 2015), emphasizing the need for a better understanding of geographic variation in bat echolocation calls.

The aim of this study was to determine whether *M. temminckii* uses a clutter rejection strategy and to understand how the species adapts its echolocation calls to the degree of clutter in the environment. To that end, we recorded the calls of individuals in environments with different degrees of clutter. In addition, we compared our results with data from the literature to determine if large-scale geographic variation exists between the echolocation calls of *M. temminckii* from Brazil and Venezuela.

Materials and Methods

Study site.—We conducted this study in the central plateau region of Brazil, located in the Cerrado biome, Federal District, Brazil. The climate of the region is tropical humid, with an Aw savanna climate subtype according to the Köppen climate classification, with a mean rainfall of 1,500 mm and temperature of 18°C to 28°C (Alvares et al. 2013). The region has 2 well-defined seasons: the rainy season (October to March) is hot and humid, and the dry season (April to September) is cold and dry (Alvares et al. 2013). In this study, bats were captured in areas of Cerrado sensu stricto, which is characterized by widely spaced, mid-size, twisted trees in the presence of scattered larger woody plants and shrubs in a grassy matrix, without a continuous canopy (Felfili et al. 1994).

Capture of individuals.—We captured 10 individuals (5 females and 5 males) of *M. temminckii* with mist nets between September 2013 and April 2014. We captured 2 of the males at the Águas Emendadas Ecological Station (15°32′29.87″S, 47°34′23.83″W) a few minutes after sunset as they exited riparian forest (probably from shelter) toward the surrounding Cerrado sensu stricto. We captured 5 females and 2 males in the APA Gama Cabeça de Veado, close to the International Airport of Brasília (15°53′16.87″S, 47°56′39.12″W) during the first 2 h after sunset, and 1 male on private property in Planaltina (15°36′32.51″S, 47°36′48.13″W), a few minutes after sunset. We observed some *M. temminckii* individuals as they flew above the smaller trees, approximately 3 m above the ground, and identified them with the help of a real-time ultrasound detector (Pettersson Ultrasound Detector D240x; Petterson Elektronik AB, Uppsala, Sweden). These bats flew below and between the largest trees in the area, such as those of the genus *Vochysia*, which are approximately 8 m tall.

We placed the captured individuals in cloth bags and transported them to the vivarium of the University of Brasília. We kept bats in the vivarium for 2 days under controlled conditions with access to water and food ad libitum until we made the recordings. The room in the vivarium was regulated to keep the natural photoperiods. After the recordings, we released all individuals with the exception of 1 male and 1 female, which were deposited in the Chiroptera Collection of the University

of Brasília (CCUNB) as voucher specimens (CCUNB 882 and 895, respectively). We fed the individuals with *Tenebrio* larvae before their release at the capture site. We followed ASM guidelines (Sikes et al. 2016) and carried out this study under authorization from SISBIO (27719) and approval by the ethics committee on the use of animals of the University of Brasília (CEUA-UnB) (111378/2014).

Recordings.—We recorded echolocation calls as.WAV files using a Song Meter SM2BAT (Wildlife Acoustics, Maynard, Massachusetts) ultrasonic bat detector in stereo with 2 SMX-US (Wildlife Acoustics) omnidirectional microphones and a sampling frequency of 192 kHz. We recorded *M. temminckii* in both open and closed environments, with 2 treatments for each type of environment: the building corridor and tent represented closed environments (high degree and intermediate degree of clutter, respectively), and the zip-line and hand release represented open environments (low clutter). We recorded 4 males in the corridor of the Department of Zoology at the University of Brasília (2.5 × 3 × 50 m; width, height, and length, respectively), and 6 individuals (4 females and 2 males) in a tent constructed with shade cloth (3 × 3 × 2.5 m; length, width, and height, respectively). We assume our experimental treatments offer similar challenges that the species may face in cluttered spaces and reflect the kinds of foraging areas that *M. temminckii* may use. We recorded 8 individuals (2 males and 6 females) with the zip-line method according to the procedure described in Szewczak (2000). This method consists in permitting a bat to fly while attached to a 3-m length of elastic cord pulled over its head. The elastic cord is connected to a 50-m monofilament line about 1 m above the ground, which enables bats to achieve a steady mode of flight before recording calls. We also recorded 2 males following hand release at the capture site. We selected call sequences with at least 6 pulses and with the best aspect-noise ratio for analysis.

Acoustic parameters.—From each recorded pulse, we measured the following 6 acoustic parameters: pulse duration (ms), measured from the beginning to the end of the pulse; pulse interval (ms), measured from the beginning of one pulse to the beginning of the next pulse; maximum frequency (kHz), which corresponded to the final pulse frequency for UFM calls and was measured at the beginning of the pulse for downward frequency-modulated (DFM) calls; minimum frequency (kHz), measured at the beginning of the pulse for UFM calls and at the end of the pulse for DFM calls; peak frequency, which corresponded to the maximum intensity frequency in the middle of the pulse; and bandwidth, which corresponded to the difference between the maximum frequency and the minimum frequency. We analyzed only the fundamental harmonic of each call, even though a second weaker harmonic was present in some UFM and DFM calls. We analyzed data using Avisoft-SAS Lab Pro version 5.1.05.30 (Avisoft Bioacoustics, Berlin, Germany), and processed calls with automatic separation of the elements with −20 dB of maximum amplitude, using a 3-threshold algorithm. We plotted these pulses simultaneously on the spectrogram, which shows the relationship between frequency, time, and intensity, and on the oscillogram, which is a temporal digitization of the recordings that represents pulse intensity and time. We constructed spectrograms from 512 fast Fourier transforms using a Hamming window function with 93.75% overlap between consecutive fast Fourier transforms and frame length of 100%.

Statistical analyses.—We analyzed UFM and DFM calls separately because they differ regarding initial frequency (minimum in UFM calls and maximum in DFM calls) and final frequency (maximum in UFM calls and minimum in DFM calls). We analyzed differences in the acoustic parameters according to treatment (open environment, zip-line and hand release; closed environment, corridor and tent) to test whether the clutter rejection strategy applies to *M. temminckii* and determine how it adapts its echolocation calls to the degree of clutter in the environment. We performed multivariate analysis of variance (MANOVA) tests separately for UFM calls and DFM calls. We evaluated differences in acoustic parameters between treatments by analysis of variance (ANOVA) and performed a Tukey test to identify parameters that differed significantly. Multiple comparisons by ANOVA were followed by the Bonferroni correction.

We used linear discriminant analysis (LDA) to visualize the spatial separation of treatment groups for 30 UFM pulses and 30 DFM pulses for each of the 4 treatments. We calculated the mean and *SE* of all pulses for each bat in each treatment. To identify different sonotypes of *M. temminckii*, we used a *K*-means cluster analysis. Because the *K*-means cluster analysis requires a predetermined interval for analysis, we grouped all analyzed calls and visually defined a minimum of 3 groups and a maximum of 19 groups of calls. We then ran other repetitions with *K*-means tests increasing the minimum limit of groups until we reached the best number of call groups.

We identified potential geographic variation in search calls of *M. temminckii* by comparing data from Brazil and data from the study of Guillén-Servent and Ibáñez (2007) from Venezuela (14 individuals—50 pulses/sequences). We performed the Welch modified 2-sample *t*-test to compare the 6 parameters measured for the search phase in both studies. Guillén-Servent and Ibáñez (2007) conducted their study in floodplains, characterized by many open areas and few, widely spaced forest patches (El Frío Biological Station; 7°48.8′N, 68°53.99′W), approximately 3,480 km from where the individuals were captured. In this study, we recorded calls in a dry, nonflooded area, within Cerrado biome, whereas in Venezuela, Guillén-Servent and Ibáñez (2007) recorded calls in areas that are seasonally flooded, providing a more humid environment. We performed all analyses in R version 3.2.3 (R Development Core Team 2012), with a significance level of $P = 0.05$.

Results

We analyzed 1,464 pulses from 64 call sequences (Fig. 1) of *M. temminckii*. Acoustic parameters differed between the 2 types of environment and between treatments for both UFM and DFM pulses (Table 1). For UFM pulses, pulse duration, pulse interval, and peak frequency differed between environments.

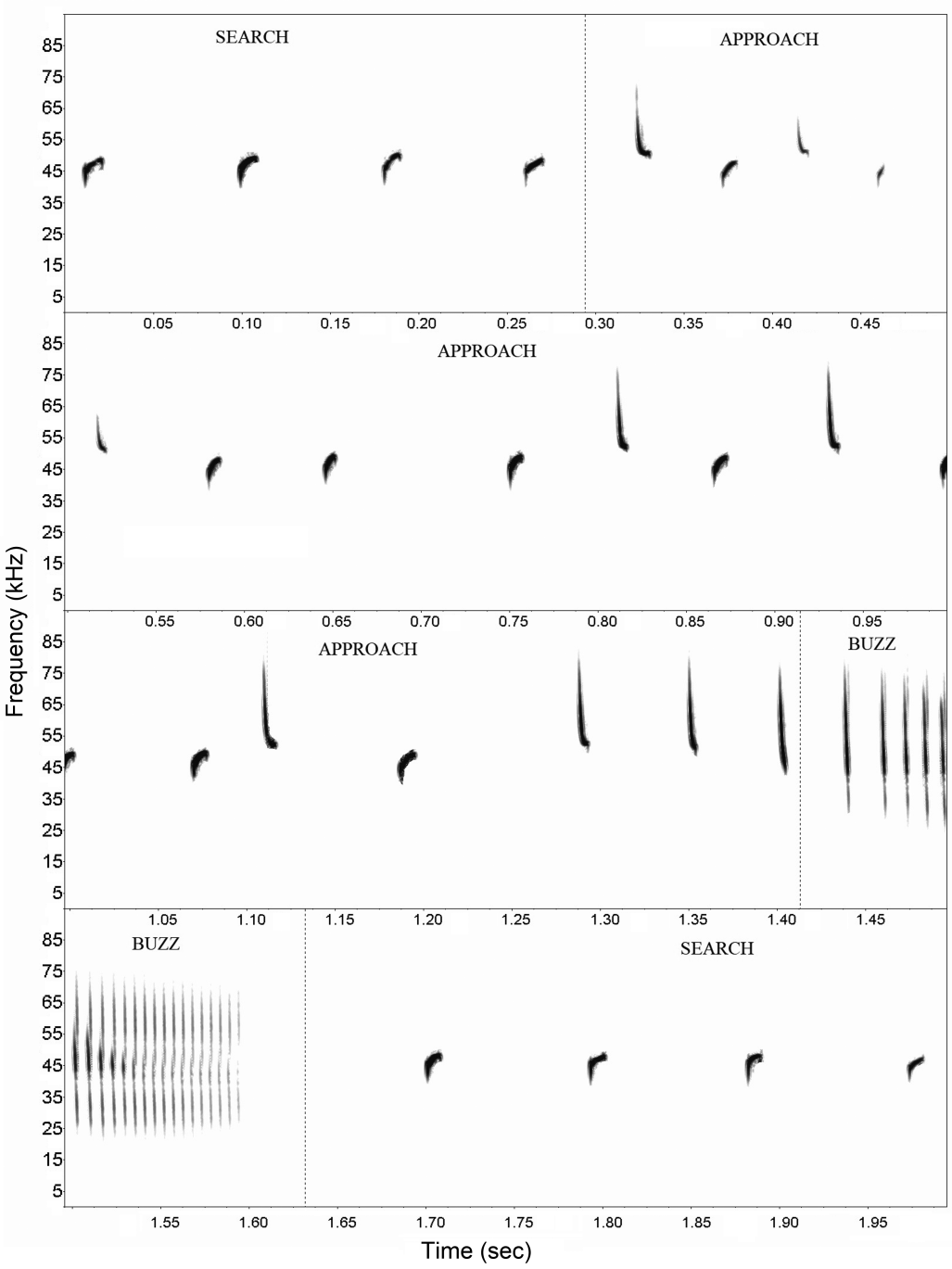

Fig. 1.—Sequence of echolocation behavior of *Molossops temminckii* showing search phase, approach phase, and buzz phase recorded in Cerrado sensu stricto in the Cerrado biome, Federal District, Brazil.

For DFM pulses, pulse interval, minimum frequency, and bandwidth differed between environments. However, the parameters analyzed did not differ when comparing treatments within the same environment. Similarly, the acoustic characteristics of emitted pulses did not differ significantly between the cluttered environment treatments (corridor and tent) or between the open environment treatments (zip-line and hand release; Fig. 2).

With *K*-means cluster analysis, we separated the 1,464 calls into 10 sonotypes (Fig. 3). We identified 5 DFM sonotypes with a short pulse duration, short pulse interval, and large bandwidth (approximately 40 kHz). These calls were more frequently used for indoor navigation (corridor and tent; Figs. 4A and 4B). We identified 5 UFM sonotypes with an initial frequency-modulated (FM) portion and increased duration in the QCF portion, resulting in longer pulse duration and interval. The UFM pulses were most commonly used for navigation in open environments (zip-line and hand release; Figs. 4C and 4D), and had a lower bandwidth (zip-line: 6.92 ± 0.12; hand release: 7.76 ± 0.12) compared with the closed environments, with a maximum 8.86 ± 0.41 kHz observed in the corridor. When the

Table 1.—Parameters of echolocation call pulses of *Molossops temminckii* recorded in closed and open environments, in the Cerrado biome, Federal District, Brazil, and results of analysis of variance (ANOVA) between treatments. Results are expressed as mean ± *SE*. Different superscript letters represent significant differences in mean values ($P < 0.05$; Tukey test). DFM = downward frequency-modulated; freq. = frequency; UFM = upward frequency-modulated. Sample sizes for each treatment are in parentheses.

	Closed environments		Open environments		ANOVA
UFM calls	Corridor (30)	Tent (202)	Zip-line (353)	Hand release (142)	*P*
Duration, ms ($F_{3,722} = 7.668$)	3.99 ± 0.35^{ab}	3.45 ± 0.07^{a}	5.01 ± 0.07^{b}	4.11 ± 0.11^{ab}	0.003
Interval, ms ($F_{3,722} = 30.338$)	61.76 ± 4.33^{a}	96.27 ± 3.58^{ab}	149.55 ± 4.91^{c}	118.63 ± 4.52^{bc}	0.001
Min freq., kHz ($F_{3,722} = 59.248$)	44.06 ± 0.43^{a}	47.36 ± 0.16^{a}	45.59 ± 0.14^{a}	43.98 ± 0.20^{a}	0.055
Max freq., kHz ($F_{3,722} = 64.387$)	52.92 ± 0.21^{a}	53.56 ± 0.08^{a}	52.51 ± 0.05^{a}	51.74 ± 0.14^{a}	0.08
Peak freq., kHz ($F_{3,722} = 100.69$)	51.30 ± 0.18^{ab}	51.91 ± 0.09^{a}	50.95 ± 0.06^{ab}	49.38 ± 0.15^{b}	0.004
Bandwidth, kHz ($F_{3,722} = 24.72$)	8.86 ± 0.41^{a}	6.20 ± 0.13^{a}	6.92 ± 0.12^{a}	7.76 ± 0.12^{a}	0.1
DFM calls	Corridor (321)	Tent (242)	Zip-line (117)	Hand release (57)	*P*
Duration, ms ($F_{3,776} = 47.122$)	2.05 ± 0.02^{a}	1.90 ± 0.04^{a}	2.47 ± 0.08^{a}	3.23 ± 0.15^{a}	0.11
Interval, ms ($F_{3,776} = 76.123$)	47.48 ± 0.97^{a}	46.90 ± 1.54^{a}	84.87 ± 4.61^{b}	99.66 ± 4.69^{b}	0.002
Min freq., kHz ($F_{3,776} = 17.672$)	40.08 ± 0.27^{a}	44.26 ± 0.43^{a}	46.74 ± 0.48^{b}	43.31 ± 0.51^{ab}	< 0.0001
Max freq., kHz ($F_{3,776} = 58.612$)	66.30 ± 0.21^{a}	68.01 ± 0.29^{a}	64.50 ± 0.37^{a}	58.82 ± 0.63^{a}	0.04
Peak freq., kHz ($F_{3,776} = 64.235$)	52.99 ± 0.22^{a}	55.70 ± 0.26^{a}	54.20 ± 0.20^{a}	45.62 ± 0.81^{a}	0.15
Bandwidth, kHz ($F_{3,776} = 41.583$)	26.21 ± 0.34^{a}	23.75 ± 0.51^{a}	17.75 ± 0.69^{b}	16.51 ± 0.45^{b}	< 0.0001

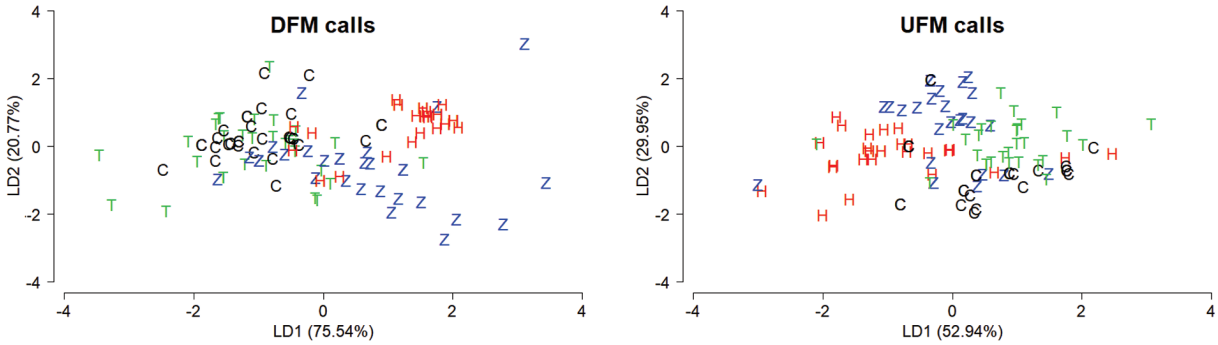

Fig. 2.—Results of linear discriminant analysis (LDA) of a random sample of 30 upward frequency-modulated (UFM) pulses per treatment, with 75.54% data grouping in the x-axis, and 30 downward frequency-modulated (DFM) pulses per treatment, with 52.94% data grouping in the x-axis, for *Molossops temminckii* recorded in the Cerrado biome, Federal District, Brazil. LDA shows the spatial separation of the 2 types of environment for the UFM and DFM pulses: C (corridor) and T (tent) represent cluttered environments, and Z (zip-line) and H (hand release) represent uncluttered environments.

same kind of pulse occurred in 2 different environments, they were different mainly in their pulse duration, pulse interval, and bandwidth.

A comparison of search calls between *M. temminckii* in Brazil and Venezuela (Guillén-Servent and Ibáñez 2007) revealed significant differences between all acoustic parameters (Table 2). Pulses in Brazil were significantly shorter and more spaced than in Venezuela (call duration: 4.11 ± 0.11 ms in Brazil and 7.8 ± 1.6 ms in Venezuela, $t_{49.16} = 16.29$, $P < 2.2 \times 10^{-16}$; interval: 118.63 ± 4.52 ms in Brazil and 97 ± 29.9 ms in Venezuela, $t_{49.79} = -5.09$, $P = 5.4 \times 10^{-6}$). Moreover, pulses were wider in Venezuela than in Brazil (bandwidth: 24.6 ± 9.6 kHz in Venezuela and 7.76 ± 0.12 kHz in Brazil, $t_{49.01} = 12.40$, $P < 2.2 \times 10^{-16}$).

Discussion

The calls of *M. temminckii* differed according to the degree of clutter in the environment, which is consistent with the clutter rejection strategy described by Kalko and Schnitzler (1993). When navigating in cluttered environments, *M. temminckii* decreased the pulse duration and interval to maintain a clutter-free window between the emitted pulse and the returning echo. To avoid echo overlap, *M. temminckii* used DFM calls with increased bandwidth to acquire detailed information on the environment. This behavior has also been observed for *Myotis nigricans* (Siemers et al. 2001), *Nycticeius cubanus* (Mora et al. 2005), *E. fuscus* (Surlykke and Moss 2000), *Phyllops falcatus* (Macias et al. 2005), *Rhinolophus ferrumequinum* (Xu et al. 2008), and *Myotis daubentonii* (Fawcett and Ratcliffe 2015). The clutter rejection strategy appears to be used by a variety of bat families (e.g., Molossidae, Vespertilionidae, Phyllostomidae, and Rhinolophidae) to avoid collision during flight in closed environments.

Bats of the family Molossidae display adaptations to flight in areas with little or no clutter because they typically emit CF and QCF calls that match their morphological adaptations of long (high aspect ratio) and narrow (high wing loading) wings (Freeman 1981; Norberg and Rayner 1987). The adaptations that *M. temminckii* implements to navigate in cluttered

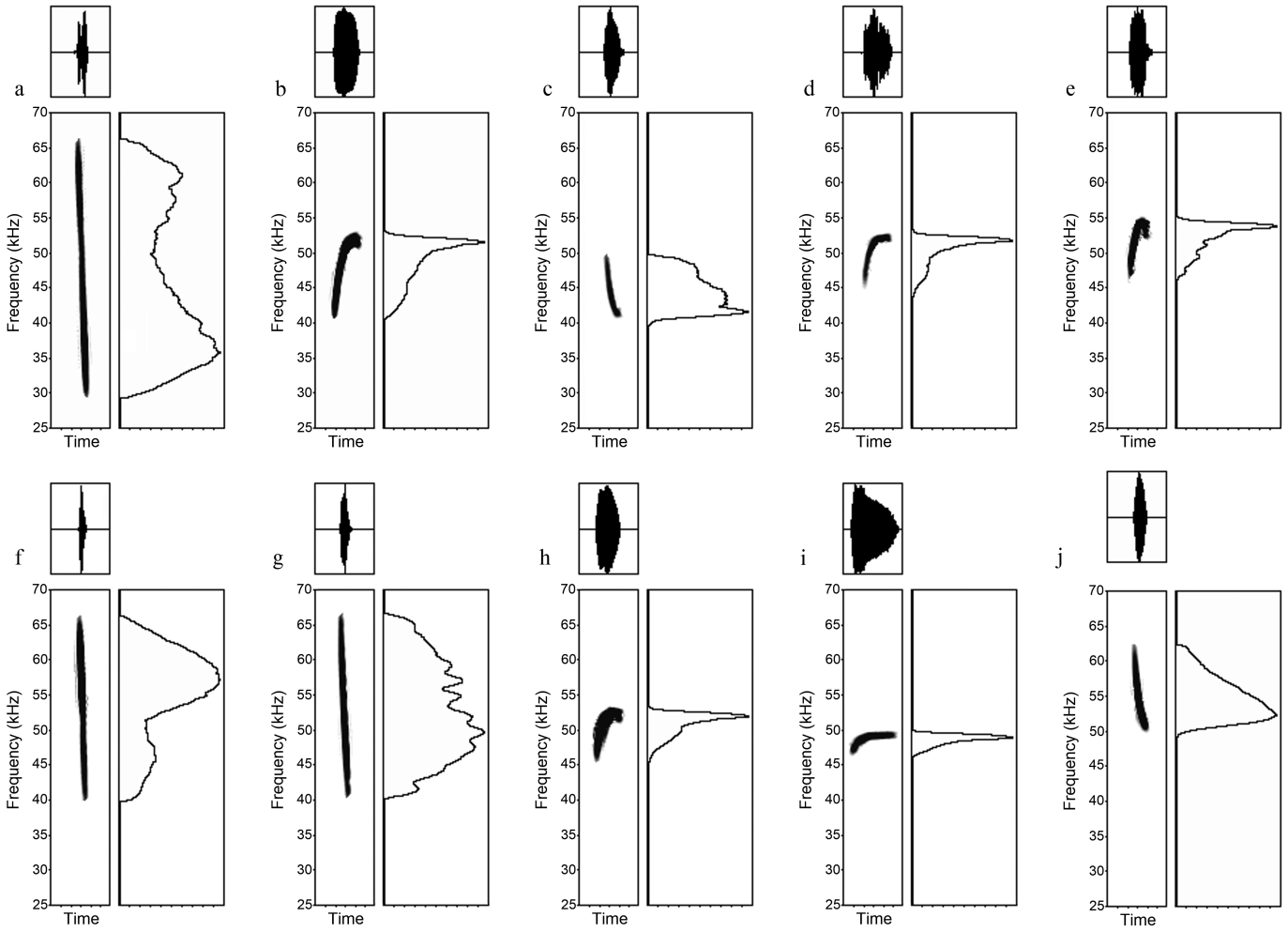

Fig. 3.—Results of *K*-means cluster analysis of *Molossops temminckii* echolocation calls recorded with sample rate of 192 kHz for all pulses in the Cerrado biome, Federal District, Brazil: power spectrum (right), oscillogram (top), and spectrogram (left).

environments, such as increased bandwidth, provide a better analysis of the environment, details about obstacles (Schnitzler and Kalko 2001), and information on possible prey such as texture, speed, and size (Simmons and Stein 1980; Schnitzler and Kalko 2001), which suggests that the strategy that *M. temminckii* uses during the approach and buzz phases is similar to the strategy used to deal with clutter.

The calls of *M. temminckii* are similar to those recorded for bats of the family Vespertilionidae, which forage in environments with a high degree of clutter (Kalko and Schnitzler 1993). The FM portion of the pulse is important for acquiring details on the environment and prey, whereas the QCF portion undergoes less atmospheric attenuation and propagates longer, increasing the clutter detection distance (Schnitzler and Kalko 2001). Thus, *M. temminckii* is able to avoid clutter and search for signs of prey with the same pulse (Simmons et al. 1978; Guillén-Servent and Ibáñez 2007).

The sonotypes of *M. temminckii* recorded in this study are similar to those recorded in Venezuela (Guillén-Servent and Ibáñez 2007), where the UFM pulses had a peak frequency of approximately 50 kHz. Peak frequency appears to be the best parameter for identifying the species in Brazil and Venezuela. The pulses of the echolocation call sequence (search, approach, and buzz phases; Fig. 1) reflect a large part of the acoustic repertoire that we accessed when recording *M. temminckii* in this study. For example, pulses in the corridor, which had the highest degree of clutter, were similar to the buzz phase, which occurs when the bat is very close to its prey. In contrast, the pulses analyzed in the tent, which provides an intermediate level of clutter, were similar to the approach phase, which occurs when the bat is further away from prey and less echo is present. In open environments (zip-line and hand release), the pulses we recorded were similar to the search phase.

In comparison to the work of Guillén-Servent and Ibáñez (2007), we identified an additional sonotype of *M. temminckii*. This sonotype, which is UFM and found in the zip-line environment, showed a longer pulse duration, smaller FM portion, with another QCF portion, greater than that observed for *M. temminckii* in Venezuela. As the degree of clutter increases, the bat adapts echolocation call pulses by applying the clutter rejection strategy. The separation of sonotypes is to some extent subjective, and the new sonotype identified in the present study likely reflects the greater number of cluttered

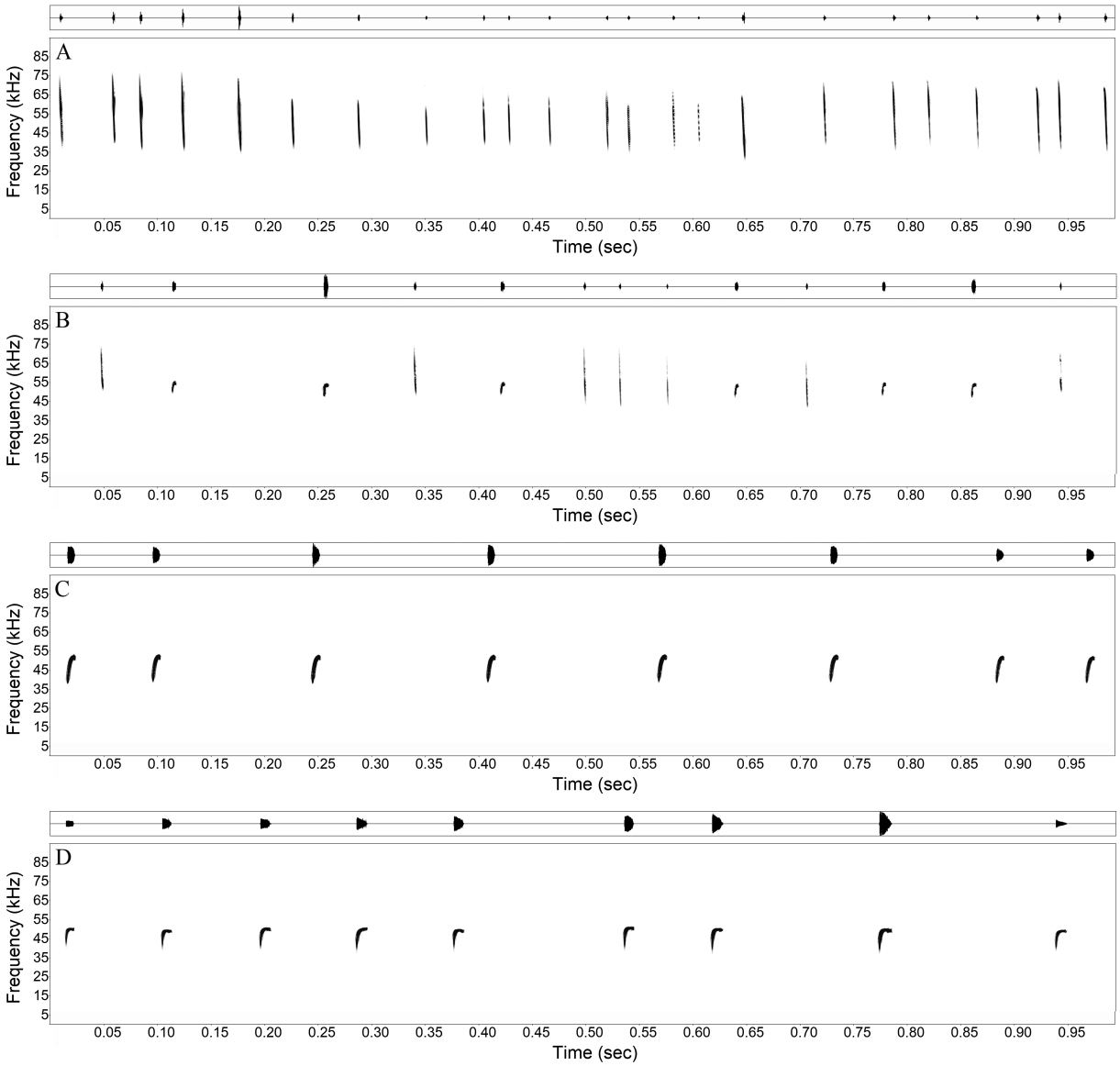

Fig. 4.—Oscillograms and spectrograms of *Molossops temminckii* echolocation calls in open and cluttered environments, ranging from high clutter (A, corridor) and intermediate clutter (B, tent) to open environments (C, zip-line; D, hand release) recorded in the Cerrado biome, Federal District, Brazil.

Table 2.—Acoustic parameters of search phase echolocation pulses of *Molossops temminckii* recorded by Guillén-Servent and Ibáñez (2007) in Venezuela and recordings in the Cerrado biome, Federal District, Brazil, and results of Welch modified 2-sample *t*-tests between sites. Results are expressed as mean ± *SD*. freq. = frequency; *n* = number of pulses.

	Venezuela (n = 50)	Brazil (n = 142)	t	$d.f.$	P
Duration, ms	7.8 ± 1.6	4.11 ± 0.11	16.29	49.16	$< 2.2 \times 10^{-16}$
Interval, ms	97 ± 29.9	118.63 ± 4.52	–5.09	49.79	5.4×10^{-6}
Min freq., kHz	40.4 ± 3.5	43.98 ± 0.20	–7.23	49.11	2.9×10^{-9}
Max freq., kHz	50.4 ± 1.5	51.74 ± 0.14	–6.31	49.30	7.7×10^{-8}
Peak freq., kHz	50.4 ± 1.4	49.38 ± 0.15	5.14	49.40	4.7×10^{-6}
Bandwidth, kHz	24.6 ± 9.6	7.76 ± 0.12	12.40	49.01	$< 2.2 \times 10^{-16}$

treatments (representing high and intermediate degrees of clutter) in which *M. temminckii* was recorded. Because this species displays great vocal plasticity, recordings in a variety of environments are essential for accurate acoustic identification (Barclay and Brigham 1991; Biscardi et al. 2004). In addition, a detailed description of the recording site is necessary to avoid misidentification.

Molossops temminckii is able to independently alter the FM and QCF portions of its calls. Because of its vocal plasticity, *M. temminckii* is well adapted to a range of environments,

including the heterogeneous vegetation of the savanna and the Cerrado sensu stricto, as shown by Guillén-Servent and Ibáñez (2007) and our study, respectively.

We observed significant differences between the search pulses recorded in Venezuela and those recorded in Brazil in all acoustic parameters of UFM pulses, which supports the idea of geographic variation in *M. temminckii* echolocation calls. This difference highlights the importance of obtaining a local or regional reference for the acoustic repertoire of a species for accurate identification. This variation likely occurs through the adaptation of bats to their environments (Mora et al. 2004), as well as learning or genetic differences between populations (Jiang et al. 2015).

Climatic conditions also may play a role in this geographic variation. Previous studies have suggested that sensory systems adapt to local environments, as described by the sensory drive hypothesis (Endler 1992; Jacobs et al. 2017), and the variation in echolocation calls may be due to climatic factors such as humidity and temperature (Jacobs et al. 2017). Atmospheric attenuation is an important environmental characteristic that alters sound propagation; this attenuation increases as the frequency of emitted pulses increases and the environment becomes more humid (Lawrence and Simmons 1982). Because the Venezuelan recordings were made in flooded open area patches in the middle of the Amazon forest (humid environment), and the recordings of the present study were made in the Brazilian Cerrado (dry environment), these differences in humidity likely account for some of the observed differences in acoustic parameters. However, geographic variation in bat echolocation calls is not well understood; therefore, further studies are needed.

Our results show that the UFM search phase pulses of *M. temminckii* recorded in Brazil on hand release (natural environment) had shorter duration but higher minimum frequency than those reported by Guillén-Servent and Ibáñez (2007) for *M. temminckii* in Venezuela (Table 2). Calls with longer durations and lower minimum frequencies are used by high-flying bats (Jensen and Miller 1999). Tall Amazon forest trees force the bats to fly higher, even in the open savanna areas, than they would in Cerrado sensu stricto, which has smaller trees that are widely and nonuniformly spaced. These differences in canopy height may therefore contribute to the observed geographic variation in *M. temminckii* calls.

A previous study reported that phylogenetic proximity also influences echolocation calls of Molossidae bats (Jung et al. 2014). The difference observed between calls of *M. temminckii* from Brazil and Venezuela (approximately 3.5 kHz) is slightly smaller than that observed between cryptic species of *Pteronotus* recorded in South America (approximately 6 kHz) (De Thoisy et al. 2014). Results of molecular analyses suggest that there are more *Pteronotus* species than previously described (Pavan and Marroig 2016). Similarly, the Venezuelan and Brazilian *M. temminckii* may represent 2 subspecies because of the great geographic distance reflected in its echolocation calls, as has been described in previous studies (Law et al. 2002; Jiang et al. 2015). Sensory drive (i.e., adaptations to local environments) is another factor that appears promote speciation (Boughman 2002); however, further morphological and molecular studies are needed to confirm this hypothesis.

Taken together, our results along with the data presented by Guillén-Servent and Ibáñez (2007) contribute to a more complete description of the acoustic repertoire of *M. temminckii*. Clutter rejection strategy appears to be an effective method to navigate in cluttered environments; however, further studies are necessary to better understand factors promoting vocal plasticity in bats, how these animals use the environment, and how these adaptations confer evolutionary advantages in a constantly changing environment.

Acknowledgments

We express our gratitude to the Brazilian National Council for Scientific and Technological Development (CNPq), which granted a scholarship to DFR (166314/2017-0), TFO (140285/2018-0), and a fellow grant to LMSA (309299/2016-0) and supported our fieldwork through the "Cerrados do Planalto Central - Estrutura, dinâmica e processos ecológicos" PELD project. We thank the 3 anonymous reviewers and the editors who improved our manuscript a lot with their comments.

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Submitted 8 March 2018. Accepted 14 June 2018.

Associate Editor was Jorge Ortega.