Analysis of the echolocation calls and morphometry of a population of *Myotis chiloensis* (Waterhouse, 1838) from the southern Chilean temperate forest

Gonzalo Ossa¹, José Tomás Ibarra¹, Kathrin Barboza², Felipe Hernández¹,
Nicolás Gálvez¹, Jerry Laker¹ and Cristián Bonacic¹

¹Laboratorio Fauna Australis, Programa de Recursos Naturales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. Vicuña Mackenna 4860, Macul, Casilla 306 – 22, Santiago, Chile.

²Centro de Estudios en Biología Teórica y Aplicada, Programa para la Conservación de los Murciélagos de Bolivia. Box 9641, La Paz, Bolivia.

Abstract

G. Ossa, J.T. Ibarra, K. Barboza, F. Hernández, N. Gálvez, J. Laker, and C. Bonacic. 2010. Analysis of the echolocation calls and morphometry of a population of *Myotis chiloensis* (Waterhouse, 1838) from the southern Chilean temperate forest. *Cien. Inv. Agr.* 37(2): 131-139. Echolocation is characteristic of bats of the suborder Microchiroptera. Though recent studies of echolocation calls in Latin America have generated significant advances in knowledge about distribution, habitat use and ecology of bats, the recording and analysis of bat calls is barely known in Chile. As a first step in studies on the ecology of the endemic Chilean myotis bat (*Myotis chiloensis*), we carried out morphometric measures and analyzed echolocation calls in a rural site near Pucón (39°15'S 17°W) in the Araucanía Region of southern Chile. During January 2009, we obtained 22 records from captured and 75 records from flying individuals. The analysis of calls in searching phase showed that the terminal frequency for this species is 43.4 ± 1.2 kHz, with a mean duration of 2.1 ± 1.0 ms and an interval between pulses of 77.5 ± 16.9 ms. The calls are FM – QCF, as is characteristic for the family Vespertilionidae. The contribution of new morphometric data from captured and released individuals indicates differences from previous studies. The records and acoustic analysis establishes a baseline for more detailed future ecological investigation of this and other bat species in Chile.

Key words: *Myotis chiloensis*, echolocation, morphometrics, bats, Chiroptera, temperate rainforest, Chile.

Introduction

Chiropterans (bats) are known to provide significant services to agriculture, especially for controlling pest insects, pollinators and seed

dispersers (Wickramasinghe *et al.*, 2004; Cleveland *et al.*, 2006; Federico *et al.*, 2008). In Chile, knowledge of bats is largely based on individuals captured in mist nets (Solís, 2008). However, this method shows a noticeable bias for species of the Phyllostomidae family and other low flying species (Siles and Terán, 2007; Barboza *et al.*, 2006).

sonic echolocation calls that the Microchiroptera use for night-flight guidance (Kalko and Schnitzler, 1993; Kunz and Racey, 1998). Echolocation was first described by Griffin (1938), and extensively updated by Korine and Kalko (2001).

Bats emit pulses of ultrasound and compare these pulses with patterns of echoes generated by the environment. These echoes rebound off solid objects or prey, providing detailed information about distance, shape, size, etc., and bats change the frequency, amplitude and time of their calls depending on the precision required (Neuweiler, 2000; Schnitzler and Kalko, 2001; Schnitzler *et al.*, 2003). Bat species have developed different types of calls, according to the different environments they inhabit, the prey available and the characteristic development of each species and their particular auditive systems (Kalko and Schnitzler, 1989; Kalko and Schnitzler, 1993; Korine and Kalko, 2001).

The frequency range, shape and duration of the calls varies between species (Murray *et al.*, 2001). The calls are usually characterised as three phases (Neuweiler, 2000; Schnitzler *et al.*, 2003; Siles and Terán, 2007): a) Search calls: to detect prey, with a narrow frequency range and a longer duration; b) Approximation calls, with increased frequency range and shorter duration; and c) Calls of terminal phase (*Feeding Buzz*), heard when a bat attempts to capture prey, with decreased frequency range and minimum duration (Kalko and Schnitzler, 1989). This last phase is not observed in bats that obtain their food from the ground, like some *Myotis* spp., as in this case, they are guided by the sounds emitted by the prey (Arlettaz *et al.*, 2001). The terminal phase is typical of species specialised in hunting airborne prey (Siemers and Schnitzler, 2004).

Monitoring of bat echolocation calls requires equipment able to record ultrasound in audible formats, using time expansion and/or heterodyne conversion (Walsh and Catto, 2004). The analysis of these calls is helpful for the recording known species (Ahlén and Baagoe, 1999), the determination of new species that are difficult to capture with traditional methods (LaVal and Rodríguez-H., 2002), the development of studies on the ecology of foraging behaviour (Kalko

and Schnitzler, 1989; McCracken, 1996), and activity patterns (Barboza *et al.*, 2006; Barboza *et al.*, 2009), among others.

In Chile, 11 bat species have been described, all belonging to the suborder Microchiroptera, with six of them belonging to the Vespertilionidae family, of which two belong to the genus *Myotis* (Canals and Cattán, 2008; Iriarte, 2008). The members of this family are small, presenting a face free from visible ornaments as leafed nose (Vargas, 2007), uropatagium or a well-developed tail membrane, and have a mainly insectivorous diet (Moya *et al.*, 2007).

Myotis chiloensis (Waterhouse, 1838) is found in shrubland, savannah and temperate rainforest environments of Chile (extending from the Region of Coquimbo to Cape Horn) and Argentina (in the provinces of Chubut and Neuquén). It bat with the most southerly distribution recorded to date (Mann, 1978; Galaz *et al.*, 2009; Galaz and Yáñez, 2006). During daytime, it hides in a wide range of sites, such as bark fissures, the roofs and walls of houses and rocky caves (Iriarte, 2008). Like the rest of the species described in Chile, its echolocation calls have not until now been recorded (Solís, 2008).

A study was developed to record and analyze the ultrasound calls emitted by *M. chiloensis* in a rural zone of the temperate forests, as a first step in creating an acoustic data base to support the monitoring and study of bats in Chile. In addition, new morphometric data are presented obtained from live individuals, which are compared to morphometric data from previous studies.

Materials and methods

Acoustic calls and morphometrics of *M. chiloensis* were studied during the summer of 2009, in a rural sector of the Pucón *comuna* (39°15' S - 17°45' O), IX Region of Araucanía, Chile.

The study site was located in a zone of horticultural cultivation and fruit trees set in a predominantly wooded matrix. The area is

adjacent to the high-elevation El Cañi Nature Sanctuary, which includes 500 ha of native forest of four species of *Nothofagus* and *Araucaria araucana* (MacAdam *et al.*, 1996). The area has a high rainfall temperate climate with Mediterranean influence, where the precipitation is distributed through the whole year, reaching its peak during the winter period. The annual average temperature is 9.7 °C (Di Castri and Hajek, 1976).

A small colony of bats in the roof space of a house was identified. Two mist nets were set up, (3 and 6 m long), at 1 m from the soil and at a distance of 2 m from the house during a period of 5 days, for a 2-hour period per night. In all, we carried out a total capture effort of 90 net linear metre hours. The captured bats were placed in cloth bags and were determined as *M. chiloensis*, using the key proposed by Galaz and Yáñez (2006). Then, morphometric measurements were made of nine individuals captured, with a 0.1 cm caliper and a 100 g spring balance scale, according to the methodology used by Mitchell-Jones and McLeish (2004).

For the recording of echolocation calls, the method chosen by Barboza *et al.* was used, (2006). A Pettersson D240X bat detector was used (Pettersson Elektronik AB, Uppsala) in

10X-expanded mode, connected to a Edirol professional digital recorder by Roland R-09, set to record WAV audio files. To record flight calls, one person would set a bat free, while another, standing 5m away, would record the bat calls in search phase. Recorded calls were analyzed later using Avisoft SASLab Pro 4.51 Software (R. Spetch, Germany), using a 44.1 KHz sampling frequency, FFT 256 length, Hamming type window and 75% of overlapping. The best quality files recorded from the individuals captured in the nets were used for the calls characterization.

Results

We captured 12 bats using the mist nets, of which 11 were found to be *M. chiloensis*, the other was identified as *Tadarida brasiliensis*. Capture effort for *M. chiloensis* was 0.12 captured individuals / H/m red.

Morphometrics

The average values $\pm$ DS, in mm, for: total length (T.L.), tail length (T.L.), foot length (L.Pp.), ear (E), tragus (T), forearm length (F.L.), uropatagium length (L.U.) and weight (P) are detailed in

Table 1. Comparison between the morphometric measurements collected and those reported in other *Myotis chiloensis* studies.

Measures	Ossa <i>et al.</i> , (collected data) ($\mu \pm$ DS)	Galaz and Yáñez, 2006 (μ)	Canals and Cattan, 2008 (Range)	Canals <i>et al.</i> , 2001 ($\mu \pm$ DS)	Mann, 1978 (Range)
Number of individuals	9	-	-	49	-
Total length (mm)	71.7 $\pm$ 6.2	86.3	95 – 98	-	94 – 97
Tail length (mm)	28.7 $\pm$ 4.1	38.4	43	36.3 $\pm$ 6.0	42 – 44
Foot length (mm)	7.2 $\pm$ 0.9	-	-	-	-
Ear (mm)	9.5 $\pm$ 1.9	12.3	-	-	-
Tragus (mm)	4.8 $\pm$ 1.0	-	-	-	-
Forearm length (mm)	38.5 $\pm$ 1.6	37.3	-	38.2 $\pm$ 1-4	36 – 40
Uropatagium (mm)	28.7 $\pm$ 4.1	-	-	-	-
Weight (g)	6.4 $\pm$ 0.7	6.58 $\pm$ 0.5	8	-	-

Table 1. Additionally, the morphometric values reported in previous studies for *M. chiloensis* in Chile are presented.

Acoustic monitoring

A total of 22 audio files were obtained from the 11 captured individuals. A further 75 files were recorded of wild bats at the capture site. The 12 files with the best recording quality were used for the analysis of the captured individuals. These calls include the search, approximation and terminal phases (Figure 1).

The analysis of the echolocation calls in search phase is presented in Figure 2. This analysis

showed an average frequency of 43.4 ± 1.2 kHz, with a duration of 2.1 ± 1.0 ms and interval between pulses of 77.5 ± 16.9 ms. Calls are composites of both FM (Frequency modulation) and QCF (Quasi-Constant Frequency). In the initial FM phase, a wide variation in the frequency is observed over a short period of time. During the second QCF phase, the call remains in a very limited frequency range for a longer period of time.

Discussion

This study provides new morphometric information and, for the first time in Chile, the echolocation calls of *M. chiloensis* are described and analyzed.

Figure 1. Complete sonogram of a *Myotis chiloensis* call - Search phase, Approach phase and Terminal phase (or Feeding buzz). The analysis used Software Avisoft SASLab Pro 4.51 (R. Spetch, Germany), 44.1 kHz sampling frequency, 256 FFT length, Hamming window and 75% overlap.

Figure 2. Analysis of a typical *Myotis chiloensis* search phase call. The terminal frequency value is 43.0 kHz, and its duration is 2.0 ms. The figure explain the two components of the call (FM and QCF).

Morphometrics

Measurements of total length and tail length obtained from the captured individuals were outside ranges published by other studies (Canals and Cattán, 2008; Galaz and Yáñez, 2006; Canals *et al.*, 2001; Mann, 1978). This could occur because the values described by those studies were obtained from embalmed individuals, and thus may have experienced some variation as a result (Torres-Mura, personal communication). Alternatively, the variations might be due to geographical variation in this species (Canals *et al.*, 2001), or indeed to the difficulty of taking accurate morphometric measurements from live bats in the field, especially when the measurements include the uropatagium (in which the tail of these species is embedded). The standard deviation of the measurements of total length (71.1 ± 6.2), tail length (28.7 ± 4.1) and uropatagium length (28.7 ± 4.1) indicates the high variability in the measurements. Likewise, the values obtained for the forearm length (38.5 ± 1.6) and weight of the individuals (6.4 ± 0.7), are similar to the values reported by other authors, such as Canals *et al.* (2001) (L.A. 38.2 ± 1.4), Galaz and Yáñez (2006) (L.A. 37.3; P. 6.58 ± 0.5) and Mann (1978) (L.A. 36-40). These measurements are easier in the field and have lower standard deviation.

Some data are reported in this study, such as right foot length (7.2 ± 0.9) and tragus length (4.8 ± 1.0) that have not been described in literature. They represent new morphometric records for this species.

The implication of the differences recorded in the morphometric data is that either there exists more than one subspecies or that there is incompatibility between the *in vivo* measurements and the measurement of dead, preserved specimens. Accordingly, the development of a protocol that allows standardizing the measurements is still necessary, to make reliable comparisons throughout the country.

Acoustic monitoring

Calls in search phase were used for acoustic analyses (Fenton and Bell, 1981; Parsons and Jones, 2000; Kalko and Aguirre, 2007), because they represent 90% of the calls emitted by bats during foraging (Fenton *et al.*, 1977).

The calls for *M. chiloensis* were determined to be of FM – QCF type, which are characteristic of species foraging in environments with obstacles like forest borders or clearings (Kalko and Schnitzler, 1993; Schnitzler and Kalko, 2001; Kalko and Aguirre, 2007). These bats capture flying insects and have the agility to navigate between obstacles (Schnitzler and Kalko, 2001). The FM component allows bats to detect objects precisely, as long as they are not very small (Schnitzler *et al.*, 2003). FM is used to navigate, measure distances and avoid obstacles, achieving a high resolution of objects and surfaces (Kalko and Aguirre, 2007). On the other hand, the quasi-constant frequency corresponds to an adaptation of the component of constant frequency (CF), which is used to differentiate insects from dense vegetation. The quasi-constant frequency is used to locate insects in open environments, away from obstacles (Schnitzler and Kalko, 2001). The calls with CF component are long (>30 ms), in comparison to those QCF (<20 ms), and both rely on the beating of the insect's wings to produce an "acoustic mirror" (Kalko and Aguirre, 2007). The strength of the echo will depend on

the angle of the insect wings relative to the direction of the bat (Kalko and Aguirre, 2007). As the CF component is extended over time, the echo contains several flapping cycles of the insect, indicating its position and speed (Kalko and Aguirre, 2007).

Only the terminal frequency of *M. chiloensis* was measured in this study, as Vespertilionidae and other families using FM components are easier to recognize through the lowest frequency calls, specifically at the end of the FM component (Fenton and Bell, 1981). This is because the lowest frequencies are less subject to atmospheric attenuation (Kalko and Schnitzler, 1989). Therefore, a bat with FM type calls passing at seven meters from the microphone will have an initial frequency lower than one bat passing at three meters (Fenton and Bell, 1981).

A negative relation has been described between the frequency of the calls in search phase and the size of the bat (Jones, 1999). The small *M. chiloensis*, weighing around 6 g, operates at high frequency.

Conclusions

The acoustic analysis allowed us to describe the distinctive characteristics of the calls of *M. chiloensis* and has improved our ability to identify this species reliably in the field. We were able to record some basic information about behavioural ecology, observing it to hunt low around vegetation.

Use of ultrasound detectors opens the possibility to record new species flying higher and that are still undetected by traditional methods (Barboza *et al.*, 2009). The creation of a complete ultrasound library of the bat species present in the country is still necessary. Once established, we will be able to make a more complete record of the distributions of the different chiropterans present in Chile.

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Resumen

G. Ossa, J.T. Ibarra, K. Barboza, F. Hernández, N. Gálvez, J. Laker y C. Bonacic. 2010. Análisis de las llamadas de ecolocación y morfometría de una población de *Myotis chiloensis* (Waterhouse, 1838) del bosque templado del Sur de Chile. Cien. Inv. Agr. 37(2): 131–139.

El sistema de ecolocación es característico de los murciélagos del suborden Microquiróptera. A pesar de que el estudio de este sistema ha generado significativos avances en el conocimiento de la distribución, uso de hábitat y ecología de murciélagos en Latinoamérica, el registro y análisis de las llamadas de las especies de Chile aún es incipiente. Con el objetivo de ampliar el conocimiento actual de *Myotis chiloensis*, se realizaron mediciones morfométricas y análisis de sus llamadas de ecolocación en un sector rural periférico a la ciudad de Pucón (39°15'S 17°W), Región de la Araucanía, sur de Chile. En enero de 2009, fueron obtenidas 22 grabaciones de llamadas de individuos capturados y 75 llamadas de individuos en vuelo. El análisis de las llamadas en fase de búsqueda indica que la frecuencia promedio característica para esta especie es 43.4 ± 1.2 kHz, con una duración promedio de 2.1 ± 1.0 ms y un intervalo entre pulsos de 77.5 ± 16.9 ms. Los componentes que se observan en las llamadas de búsqueda para esta especie, son de Frecuencia Modulada (FM) y Frecuencia casi Constante (QCF), las que son características de la familia Vespertilionidae. El aporte de nuevos datos morfométricos de los individuos capturados y liberados en el área indican diferencias respecto a estudios anteriores. Los registros y análisis acústicos de este murciélago endémico del sur de Sud América, generan nuevas oportunidades para el estudio de su ecología y la de otras especies en Chile.

Palabras clave: *Myotis chiloensis*, ecolocación, morfometría, murciélagos, Chiroptera, bosques templados, Chile.

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