

## Correspondence

### Vocalizations of *Limnomedusa macroglossa* (Amphibia: Anura: Alsodidae)

VINICIUS GUERRA<sup>1,2</sup>, RODRIGO LINGNAU<sup>3</sup>, BRUNO DE SOUZA BARRETO<sup>4</sup>,  
AXEL KWET<sup>5</sup> & ROGÉRIO PEREIRA BASTOS<sup>2</sup>

<sup>1</sup>) Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais (PEA),  
Universidade Estadual de Maringá (UEM), Av. Colombo, 5790, 87020-900, Maringá, PR, Brazil

<sup>2</sup>) Laboratório de Herpetologia e Comportamento Animal, Departamento de Ecologia, Instituto de Ciências Biológicas,  
Universidade Federal de Goiás, Campus Samambaia, Av. Esperança, s/n, 74690-900 Goiânia, GO, Brazil

<sup>3</sup>) Universidade Tecnológica Federal do Paraná, Campus Francisco Beltrão,  
Linha Santa Bárbara, s/n, 85601-970, Francisco Beltrão, PR, Brazil

<sup>4</sup>) Laboratório de Modelagem Ecológica, Coordenação de Ciência da Terra e Ecologia, Museu Paraense Emílio Goeldi,  
Av. Perimetral, 1901, Terra Firme, 66077530, Belém, Pará, Brasil

<sup>5</sup>) Haldenstr. 28, 70736 Fellbach, Germany

Corresponding author: VINICIUS GUERRA, e-mail: [vinicius.guerrabatista@gmail.com](mailto:vinicius.guerrabatista@gmail.com)

Manuscript received: 8 June 2017

Accepted: 31 July 2017 by JÖRN KÖHLER

The anuran family Alsodidae is currently composed of 30 species in three genera: *Alsodes*, *Eupsophus* and *Limnomedusa* (FROST 2017). The species of the monotypic genus *Limnomedusa* is distributed throughout northeastern Argentina, adjacent Paraguay, Uruguay and southern Brazil (states of Paraná, Santa Catarina, and Rio Grande do Sul) (KWET et al. 2010). *Limnomedusa macroglossa* (DUMÉRIL & BIBRON, 1841) is a medium-sized frog (average snout-vent length in males 46.7 mm and in females 52.9 mm; KAEFER et al. 2009) with a dorsal skin that is covered by large warts and elongated tubercles. The dorsum is brown or grey, with a light-coloured triangular spot on the snout and a dark spot between and behind the eyes (STRANECK et al. 1990, KWET et al. 2010). *Limnomedusa macroglossa* has been considered a threatened species in Paraguay (MOTTE et al. 2009), whereas in Brazil, it is listed as ‘critically endangered’ in the state of Paraná and ‘endangered’ in the state of Santa Catarina (SEGALLA & LANGONE 2004, CONSEMA 2011). However, *L. macroglossa* is classified as ‘least concern’ in global and Brazilian lists of threatened species (IUCN 2016, BRB 2016).

*Limnomedusa macroglossa* is terrestrial and occurs widely in open habitats and forests where it lives on the ground near streams on basalt or rocky outcrops (GUDYNAS & GEHRAU 1981, KWET et al. 2010). The most comprehensive study on the reproductive biology of this species was that of KAEFER et al. (2009), who provided detailed in-

formation on the reproductive season, reproductive mode, number and diameter of eggs, calling period, calling site, and breeding habitat. The eggs ( $396 \pm 65.8$  eggs per clutch; KAEFER et al. 2009) are laid during the months of September, October and November in flood pools and temporary ponds along rivers and small rocky streams, and the tadpoles grow up in both ponds and streams (KAEFER et al. 2009). Despite this information on its reproductive biology and even published audio recordings of calls in guides from Argentina and Brazil (STRANECK et al. 1990, KWET & MÁRQUEZ 2010), quantitative parameters of the advertisement call of *L. macroglossa* have yet to be described. Herein, we provide the first description of the quantitative parameters of two different calls of *L. macroglossa*.

A calling male *L. macroglossa* (Fig. 1) was recorded on 10 October 2013 in the municipality of Três Barras do Paraná, state of Paraná, Brazil ( $-25.4061^\circ$ ;  $-53.1173^\circ$ ). The calls were recorded at a sampling rate of 22 kHz and 16-bit resolution (wav file format) using a Tascam recorder coupled to a Sennheiser ME66 microphone positioned at about 50 cm from the calling male. Air temperature was measured with a digital thermo-hygrometer (precision  $\pm 1^\circ\text{C}$ ). The voucher specimen was collected and deposited in the Amphibian Collection of the Universidade Tecnológica Federal do Paraná, Francisco Beltrão (RLUTF 918). Recordings were deposited in the Fonoteca Zoológica (FonoZoo – the animal sound library of the Museu Na-

cional de Ciencias Naturales, Madrid, Spain; 10038). Additional call recordings were also obtained from FonoZoo (Supplementary Table S1). Some of these vocalizations were found published in an acoustic guide containing calls of amphibian species from southern Brazil and Uruguay (KWET & MÁRQUEZ 2010). Analyses of all recordings of *L. macroglossa* were performed at sampling frequencies of 22 kHz and 16-bit resolution. For each recording, data on site, date, hour and air temperature at the time of recording were compiled (Supplementary Table S1). Two specimens were deposited in the collection of the Museu de Ciência e Tecnologia (MCT) at the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) (MCT 2566, 5607).

Calls were analysed on a personal computer using Raven Pro software, version 1.5, 64-bit version (Bioacoustic Research Program 2013), with the following settings: window size = 256 samples; window type = Hann; overlap = 50%; DFT size = 256 samples, grid spacing = 188 Hz. Sound figures were produced using the Seewave package version 1.6.4 (SUEUR et al. 2008) of the R platform, version 3.3.2, 64-bit version (R Development Core Team 2016). Seewave settings employed were: window name (Fast Fourier Transformation) = Hanning window function; window length = 256 samples; overlap = 80%.

Terminology and definitions in call descriptions follow KÖHLER et al. (2017). We analysed 20 advertisement calls from each individual ( $n = 120$  calls analysed,  $n = 6$  individuals), and 22 aggressive calls from four males (see Table 1, Supplementary Table S1). Five call parameters were measured from the oscillogram: call duration (s), pulse number, pulse duration (s), inter-call interval (s), and call rate (calls/minute). Spectral parameters were obtained by selecting two variables in Raven 1.5: (1) 90% bandwidth (Hz), where the two parameters (upper and lower frequency) included the lowest and highest frequencies, mi-



Figure 1. Adult male *Limnomedusa macroglossa* from Três Barras do Paraná, Paraná State, Brazil. Photo by R. LINGNAU.

nus the upper and lower 5% of the frequency band formed by the energy distribution; and (2) dominant frequency (Hz). The number of harmonics and the frequency of each harmonic of the analysed calls were measured from spectrograms. Measurements are presented as the mean  $\pm$  standard deviation (minimum–maximum) or mode.

Recorded males of *L. macroglossa* called from the ground, sitting on flooded soil or rocks, or partially submerged at the edge of rivers or ponds. Air temperatures of analysed recordings ranged from 17–24°C. Most recorded males emitted two different calls: an advertisement call and an aggressive call. Numerical parameters of these calls are presented in Table 1.

Advertisement calls (Fig. 2) were composed of a single pulsed note with harmonic structure and emitted alone or in groups of two to seven calls (Table 1). The number of harmonics ranged from two to six, but calls with three harmonics were most common; the dominant frequency was

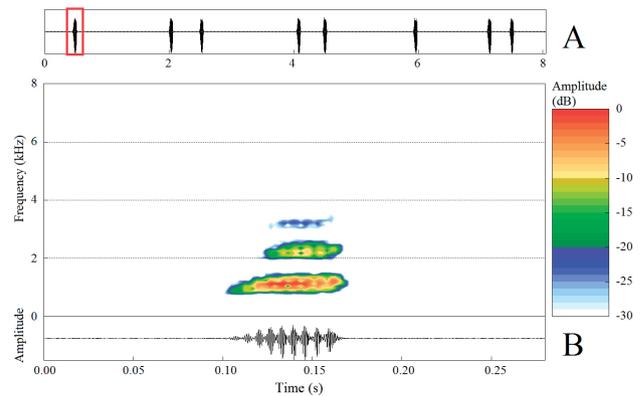


Figure 2. Advertisement call of *Limnomedusa macroglossa* from Rio Guarani, Três Barras do Paraná, Brazil. (A) Oscillogram of a series of advertisement calls; (B) spectrogram (above) and oscillogram (below) of the advertisement call highlighted with a red outline (air temperature = 21.6°C). Voucher specimen RLUTF 918. Voucher recording: FonoZoo 10038.

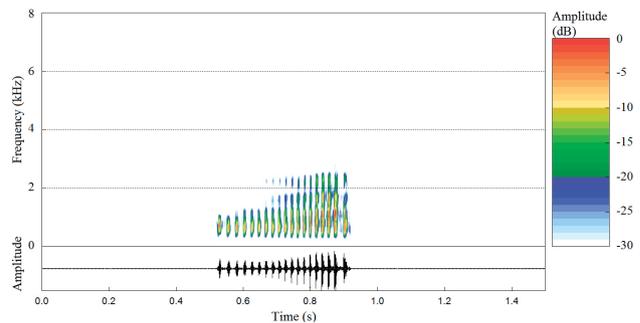


Figure 3. Aggressive call of *Limnomedusa macroglossa* from near Tacuarembó, Uruguay. Spectrogram (above) and oscillogram (below) (air temperature = 17°C). Unvouchered specimen. Voucher recording: FonoZoo 17300.

Table 1. Call parameters of vocalizations of adult males *Limnomedusa macroglossa* summarized from various recordings. Measurements are presented as the mean  $\pm$  standard deviation (minimum–maximum) or mode (for pulse number and call group). Air temperature during recordings varied between 17 and 24°C.

Parameter	Advertisement call (n = 120 calls analysed, n = six males)	Aggressive call (n = 22 calls analysed, n = four males)
Call duration (s)	0.07 $\pm$ 0.01 (0.05–0.09)	0.40 $\pm$ 0.03 (0.35–0.45)
Pulse number	6 (4–9)	19 (15–29)
Pulse duration (s)	0.01 $\pm$ 0.00 (0.01–0.03)	0.01 $\pm$ 0.00 (0.01–0.03)
Silence interval between calls (s)	0.55 $\pm$ 0.74 (0.04–6.56)	1.20 $\pm$ 0.46 (0.57–2.15)
Call rate (s)	91.17 $\pm$ 42.12 (46–152)	3.32 $\pm$ 1.04 (1–4)
Call group	1 (1–7)	–
Dominant frequency (Hz)	1002.01 $\pm$ 92.04 (861.30–1205.90)	884.84 $\pm$ 201.00 (689.10–1205.90)
Upper frequency (Hz)	1633.67 $\pm$ 387.45 (1033.60–2325.60)	1887.11 $\pm$ 350.55 (1033.60–2239.50)
Lower frequency (Hz)	735.01 $\pm$ 103.49 (516.80–947.50)	555.95 $\pm$ 91.03 (516.80–861.30)
Harmonic number	3 (2–6)	3 (2–3)
Harmonic 2 (Hz)	1949.49 $\pm$ 221.35 (1550.40–2239.50)	1660.03 $\pm$ 146.03 (1378.10–1894.90)
Harmonic 3 (Hz)	2910.57 $\pm$ 403.58 (2067.20–3445.30)	2385.91 $\pm$ 245.35 (2239.50–2928.50)
Harmonic 4 (Hz)	4193.03 $\pm$ 290.21 (3617.60–4651.20)	–
Harmonic 5 (Hz)	5135.69 $\pm$ 69.46 (4995.70–5168.00)	–
Harmonic 6 (Hz)	6263.09 $\pm$ 249.25 (5857.00–6546.10)	–

in the first harmonic (Fig. 2, Table 1). The advertisement call was emitted by all males of *L. macroglossa* recorded, and was the most frequently heard call. Advertisement calls emitted alone were the most common vocalizations (34.2% of all calls), followed by calls emitted in groups of two (30% of all calls); groups of seven calls were recorded only once (0.8% of all calls).

Aggressive calls comprised a pulsed note with harmonic structure (Fig. 3). This call exhibited a gradually increasing amplitude and a decreasing frequency modulation towards the end of the call. Males emitted this call only sporadically. Aggressive calls possessed two or three well-defined harmonics and higher values of call duration, pulse number, pulse duration, and upper frequency than the advertisement call (see Table 1 for complete numerical values).

*Limnomedusa macroglossa* emits two different call types that we refer to as an advertisement call and an aggressive call, respectively (sensu TOLEDO et al. 2015, KÖHLER et al. 2017). Aggressive calls were emitted by males in response to advertisement calls from other males. Since there are no previous reports on territoriality or physical combat in *L. macroglossa* (KAEFER et al. 2009, LANGONE & PRIGIONI 1985) further studies should be conducted to confirm its aggressive function. The vocal behaviour of males of *L. macroglossa* seems to comprise the frequent alternating emission of both call types to attract females on the one, and in a context of inter-male aggressiveness on the other hand.

Vocalization by *L. macroglossa* was first reported in a book accompanying a cassette tape with anuran recordings from Argentina (STRANECK et al. 1990), but parameters of these calls were not provided. The first sonogram provided

on page 47 in STRANECK et al. (1990) seems to represent the vocalization that we identified as the advertisement call, whereas the sonogram below refers to what we consider the aggressive call.

Vocalizations of males play a key role during the reproductive period for most anuran species (WELLS 2007), and accurate quantitative descriptions of anuran calls may provide important information for taxonomic and phylogenetic studies by uncovering relationships between taxa (e.g., GOICOECHEA et al. 2010). *Limnomedusa* is a monotypic genus and comparisons with calls of related species are limited. As with other species of the family Alsodidae, the advertisement call of *L. macroglossa* is composed of a single harmonic note, although call duration is shorter and dominant frequency is lower than in the calls of most species of *Eupsophus* (FORMAS 1989, FORMAS & BRIEVA 1994, NUÑEZ et al. 2012). The spectral structure (without a series of sidebands) and temporal structure (pulsed call) of the *L. macroglossa* vocalizations also differ from the call structure in *Eupsophus*, thus apparently not corroborating a close relationship between *Limnomedusa* and the *Eupsophus*–*Alsodes* clade. The advertisement call of *L. macroglossa* seems to be more similar to the calls of some species of the family Cycloramphidae (Supplementary Table S3). On the other hand, closely related species may have completely different call structures, as is the case of *Boana riojana* and *Boana marianitae* (KÖHLER et al. 2017), and a different call structure does not necessarily contradict close relationships. Our bioacoustic data support those of BLOTTO et al. (2013), who reported on the molecular phylogenetics of Alsodidae and suggested that the inclusion of *Limnomedusa* within Alsodidae should be considered provisional (see FROST 2017).

## Acknowledgements

VG would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and RPB the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 308204/2013-1) for providing scholarships. In addition, we are grateful to the Fonoteca Zoológica, Madrid, for permitting the use of some of their recordings. RL acknowledges CNPq for support (Process number 478511/2011-6) and AK acknowledges CNPq/PADCT/CIAMB for support (Process number CIAMB# 01/97-03/02).

## References

- Bioacoustics Research Program (2013): Raven Pro: Interactive Sound Analysis Program (version 1.5) [Computer software]. – The Cornell Lab of Ornithology, Ithaca, NY. Available at <http://www.birds.cornell.edu/raven>.
- BLOTTO, B. L., J. J. NUÑEZ, C. A. ÚBEDA, W. C. WHEELER, & J. FAIVOVICH (2013): Phylogenetic relationships of a Patagonian frog radiation, the *Alsodes* + *Eupsophus* clade (Anura: Alsodidae), with comments on the supposed paraphyly of *Eupsophus*. – *Cladistics*, **29**: 113–131.
- BRB – Brazil Red Book of Threatened Species of Fauna (2016): Instituto Chico Mendes de Conservação da Biodiversidade. – Available at [http://www.icmbio.gov.br/portal/images/stories/comunicacao/publicacoes/publicacoes-diversas/dcom\\_sumario\\_executivo\\_livro\\_vermelho\\_ed\\_2016.pdf](http://www.icmbio.gov.br/portal/images/stories/comunicacao/publicacoes/publicacoes-diversas/dcom_sumario_executivo_livro_vermelho_ed_2016.pdf).
- CONSEMA – Conselho Estadual Do Meio Ambiente – (2011): Lista de Espécies da Fauna Ameaçadas de Extinção no Estado de Santa Catarina. – Available at <http://ignis.org.br/lista>.
- FORMAS, J. R. (1989): A new species of *Eupsophus* (Amphibia: Anura: Leptodactylidae) from Southern Chile. – *Proceedings of the Biological Society of Washington*, **102**: 568–576.
- FORMAS, J. R. & L. BRIEVA (1994): Advertisement calls and relationships of Chilean frogs *Eupsophus contulmoensis* and *E. insularis* (Amphibia: Anura: Leptodactylidae). – *Proceedings of the Biological Society of Washington*, **107**: 391–397.
- FROST, D. R. (2017): Amphibian Species of the World: an Online Reference. Version 6.0. – American Museum of Natural History, New York, USA. Electronic Database available at <http://research.amnh.org/herpetology/amphibia/index.html>, accessed at 11 01 2017.
- GOICOECHEA, N., I. DE LA RIVA & J. M. PADIAL (2010): Recovering phylogenetic signal from frog mating calls. – *Zoologica Scripta*, **39**: 141–154.
- GUDYNAS, E. & A. GERHAU (1981): Notas sobre la distribución y ecología de *Limnomedusa macroglossa* (Dumeril & Bibron, 1841) en Uruguay (Anura, Leptodactylidae). – *Iheringia*, **60**: 81–99.
- IUCN – International Union for Conservation of Nature, Conservation International & Nature Serve (2004): *Limnomedusa macroglossa*. – The IUCN Red List of Threatened Species. Version 2016-3. Available at [www.iucnredlist.org](http://www.iucnredlist.org).
- KAEFER, I. L., C. BOTH & S. Z. CECHIN (2009): Breeding biology of the rapids frog *Limnomedusa macroglossa* (Anura: Cycloramphidae) in southern Brazil. – *Journal of Natural History*, **43**: 1195–1206.
- KÖHLER, J., M. JANSEN, A. RODRÍGUEZ, P. J. R. KOK, L. F. TOLEDO, M. EMMRICH, F. GLAW, C. F. B. HADDAD, M.-O. RÖDEL & M. VENCES (2017): The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. – *Zootaxa*, **4251**: 1–124.
- KWET, A. & R. MÁRQUEZ (2010): Sound guide of the calls of frogs and toads from southern Brazil and Uruguay, double CD and booklet. – Fonoteca, Madrid.
- KWET, A., R. LINGNAU & M. DI-BERNARDO (2010): Pró-Mata: Amphibians of the Serra Gaúcha, South of Brazil. – Brasilien-Zentrum, University of Tübingen, Alemanha.
- LANGONE, J. A. & C. M. PRIGIONI (1985): Observaciones sobre una puesta de *Limnomedusa macroglossa* (Anura: Leptodactylidae). – *Actas de las Jornadas de Zoología del Uruguay*, **1**: 83–86.
- MOTTE, M., K. NÚÑEZ, P. CACCIALI, F. BRUSQUETTI, N. SCOTT & A. L. AQUINO (2009): Categorización del estado de conservación de los Anfibios y Reptiles de Paraguay. – *Cuadernos de herpetología*, **23**: 5–18.
- NUÑEZ, J. J., F. E. RABANAL & J. R. FORMAS (2012): Description of a new species of *Eupsophus* (Amphibia: Neobatrachia) from the Valdivian Coastal Range, Southern Chile: an integrative taxonomic approach. – *Zootaxa*, **3305**: 53–68.
- TOLEDO, L. F., I. A. MARTINS, D. P. BRUSCHI, M. A. PASSOS, C. ALEXANDRE, C. F. B. HADDAD (2015): The anuran calling repertoire in the light of social context. – *Acta Ethologica*, **18**: 87–99.
- R Development Core Team (2016): R: A language and environment for statistical computing, Version 3.3.2. – Available at <http://www.R-project.org>.
- SEGALLA, M. V. & J. A. LANGONE (2004): Anfíbios. – pp. 537–577 in: MIKICH, S. B. & R. S. BÉRNILS (eds): Livro Vermelho da Fauna Ameaçada no Estado do Paraná. – Curitiba. Instituto Ambiental do Paraná.
- STRANECK, R., E. V. de OLMEDO & G. R. CARRIZO (1990): Catálogo de voces de anfibios argentinos. – LOLA, Buenos Aires, 130 pp.
- SUEUR, J., T. AUBIN & C. SIMONIS (2008): Seewave: a free modular tool for sound analysis and synthesis. – *Bioacoustics*, **18**: 213–226.
- WELLS, K. D. (2007): *The Ecology and Behavior of Amphibians*. – The University of Chicago Press, Chicago.

## Supplementary material

Supplementary Table S1. Information on the calls recorded and used.

Supplementary Table S2. Advertisement call parameters of species of Alsodidae and *Cycloramphus*.