NORTH-WESTERN JOURNAL OF ZOOLOGY 13 (2): 271-277
Article No.: e161511

http://biozoojournals.ro/nwjz/index.html

A reassessment of the advertisement call of *Ischnocnema parva* (Anura: Brachycephalidae)

Felipe Silva de ANDRADE[1,2,3,*], Isabelle Aquemi HAGA[1] and Ariovaldo Antonio GIARETTA[1]

1. Laboratório de Taxonomia, Sistemática e Ecologia de Anuros Neotropicais. Faculdade de Ciências Integradas do Pontal, Universidade Federal de Uberlândia (UFU), CEP 38304-402, Campus Pontal, Ituiutaba, Minas Gerais, Brasil
2. Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), CP 6109, CEP 13083-970, Campinas, São Paulo, Brasil
3. Programa de Pós-Graduação em Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), CP 6109, CEP 13083-970. Campinas, São Paulo, Brasil
* Corresponding author, F.S. de Andrade, E-mail: felipe_andrade@ymail.com

Received: 11. April 2016 / Accepted: 20. October 2016 / Available online: 24. November 2016 / Printed: December 2017

Abstract. *Ischnocnema parva* is a small species that occurs in the leaf litter within the Atlantic Forest in southeastern Brazil. The advertisement call of this species was described in the early 90's based on an undefined number of males from Estação Biológica da Boracéia in the municipality of Salesópolis, state of São Paulo. In this study, based on data from four localities in two Brazilian states, we re-describe the advertisement call of *I. parva* which consists of a single type of pulsed note emitted sporadically with its fundamental frequency peaking at about 2000 Hz and its dominant frequency peaking at about 4000 Hz. The calls of males from two localities have a higher pulse rate and longer interpulse interval. Compared to our data, the call previously described in literature has a higher pulse rate and lower dominant frequency. In addition, we interpret that the species call has a harmonic structure and a distinct amplitude modulation from the previously reported.

Key-words: Atlantic forest, bioacoustics, Terrarana, taxonomy, vocalization.

Introduction

The Terrarana genus *Ischnocnema* Reinhardt & Lütken comprises 33 species, which occur from Central and southern Brazil to adjacent northern Argentina with most species associated to the coastal Brazilian Atlantic Forest (Canedo & Haddad 2012, Frost 2015). Based on molecular evidence, most *Ischnocnema* species are currently arranged into four species series: *I. guentheri*, *I. lactea*, *I. parva*, and *I. verrucosa* (Canedo & Haddad 2012, Padial et al. 2014). *Ischnocnema manezinho* (Garcia, 1996) and *I. sambaqui* (Castanho & Haddad, 2000) are unassigned to species series (Padial et al. 2014). The *Ischnocnema parva* species series has only three species, whereas other species series include up to 10 species (Canedo & Haddad 2012, Brusquetti et al. 2013, Padial et al. 2014).

Ischnocnema parva (Girard, 1853), *I. pusilla* (Bokermann, 1967), and *I. nanahallux* Brusquetti, Thomé, Canedo, Condez & Haddad, 2013 comprise the *I. parva* species series (Padial et al. 2014). The last two are only known from their type localities (cf. Bokermann 1967, Brusquetti et al. 2013) and no data is available on their advertisement calls. *Ischnocnema parva* was described from the municipality of Rio de Janeiro, state of Rio de Janeiro (RJ), and it is widely distributed in the Atlantic Forest of southeastern Brazil (Siqueira-Jr et al. 2004, Giaretta & Facure 2008, Cruz et al. 2009, Martins et al. 2010, Frost 2015). Brusquetti et al. (2013), based on molecular evidence, suggest that more than one species may be hidden under this name.

Heyer et al. (1990) briefly described the advertisement call of *Ischnocnema parva* from the Estação Biológica de Boracéia, municipality of Salesópolis, state of São Paulo. Herein we describe calls of *I. parva* from several localities in southeastern Brazil, as well as discuss the remarkable differences found between our samples and those previously published. Our study can help future works on revealing the real diversity under the name *I. parva*.

Material and Methods

Bioacoustics

We recorded 26 males and analyzed 91 calls. Fourteen males (48 calls) from the Parque Municipal do Itapetininga, municipality of Atibaia (23°9'4.72''S, 46°31'17.19''W, 1000 m a.s.l.; Mantiqueira mountain range) on 15 October 2013 and 02 November 2015, air temperature 18–22 °C; five males (11 calls) from the municipality of Salesópolis (23°37'52.65''S, 45°55'17.32''W; 860 m a.s.l.; Serra do Mar mountain range) on 28–29 December 2015, 21–23 °C; two males (2 calls) from the municipality of Ubatuba

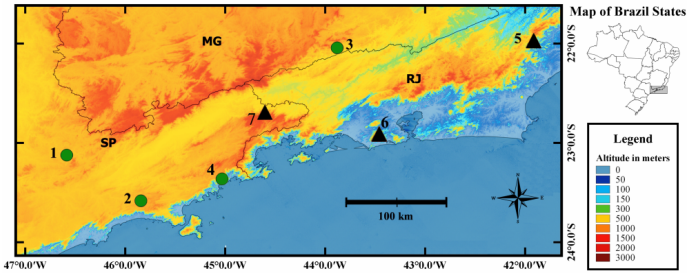

Figure 1. Geographic distribution of the members of the *Ischnocnema parva* species series.

Municipalities labeled: green circles (our samples): 1—municipality of Atibaia (SP), 2—municipality of Salesópolis (SP), 3—municipality of Ubatuba (SP), 4—municipality of Rio Preto (MG); black triangles (type localities): 5—municipality of Santa Maria Madalena (RJ – *I. nanahallux*), 6—municipality of Rio de Janeiro (RJ – *I. parva*), 7—municipality of São José do Barreiro (SP – *I. pusilla*). Brazilian states acronyms: São Paulo = SP, Minas Gerais = MG; and Rio de Janeiro = RJ.

(23°21'52.16''S, 44°58'13.14''W; 165 m a.s.l.; Serra do Mar mountain range) on 20 July 2009 and 29 October 2010, 19 °C; all these localities are in the state of São Paulo (SP), Brazil (Fig. 1). We also recorded five males (30 calls) from the municipality of Rio Preto (21°58'33.001''S, 43°54'10.014''W; 1390 m a.s.l.; Serra Negra mountain range) on 05–06 November 2015, 19–24°C, state of Minas Gerais (MG), Brazil (Fig. 1).

Voucher specimens are deposited in the Frogs Collection of the Universidade Federal de Uberlândia (AAG-UFU), municipality of Uberlândia (MG); and in the amphibian collection of the Museu de Zoologia of the Universidade Estadual de Campinas (ZUEC), municipality of Campinas (SP), under the following numbers: AAG-UFU 5203–5204, 5412; and ZUEC 23212–23214.

Calls were recorded with a ME67/K6 Sennheiser directional microphone connected to a Marantz PMD-670 or Marantz PMD-671, or with a ME66/K6 Sennheiser directional microphone connected to a Boss BR-864, or M-audio Microtrack II. Calls were obtained at sampling rate of 44.1 kHz and sample size of 16 bit. We analyzed calls using Raven Pro 1.5, 64-bit version (Bioacoustics Research Program 2014) with the following settings: window type = Hann; window size = 256 samples; 3 dB filter bandwidth = 248 Hz; brightness = 50%; contrast = 50%; overlap = 85% (locked); DFT size = 1024 samples (locked); grid spacing (spectral resolution) = 43.1 Hz. Temporal traits were measured on oscillograms and the spectral ones on spectrograms. We measured the peaks of fundamental (selecting only the lower band on spectrograms) and others frequency bands through "Peak Frequency (Hz)" function, and the frequency values with 5 and 95% of energy of the call were obtained automatically by the program through its "Frequency 5% (Hz)" and "Frequency 95% (Hz)" functions, respectively. We generated call figures using Seewave v.1.6 package (Sueur et al. 2008) on R version 3.2.3 (R Core Team 2015). Seewave settings were: Hanning window, 85% overlap and 512 points resolution (FFT). The nomenclature and definitions of the acoustic structures follow McLister (1995) (see their Table 2). We calculated means and standard deviations (SD) for each individual and then the overall mean and SD was calculated based on those values (Gerhardt and Huber 2002); whereas the range encompassed the minimum and maximum values for all call sample variation. For each analyzed call, we measured all of its pulse/interpulse intervals.

In order to compare with our samples from Salesópolis, we generated an oscillogram and corresponding spectrogram (with the same settings aforementioned) of advertisement call attributed to *I. parva* by Heyer et al. (1990). The audio file in wav format is available as supplemental material at Frogs of Boracéia: Online Audio Supplement <http://vertebrates.si.edu/herps/frogs_boraceia/list.htm>.

Statistical analysis

We searched for eventual discrimination among populations by applying two functions on R software: (1) "Random Forest" (RF) (radomForest package, Liaw & Wiener 2002), and (2) "dapc" (adegenet package, see Jombart 2008, Jombart et al. 2010). Random Forest algorithm constructs many (e.g. 1000) classification trees using bootstrap samples from the original dataset and then generates classifiers and aggregates results by voting to classes (Breiman 2001); the OOB (out-of-bag) data was used to get an unbiased estimate of the classification error as trees are added to the forest (Breiman 2001).

Traditional Discriminant Analysis (DA) depends on multivariate normality (Pohar et al. 2004) and on a larger number of objects than variables. The multivariate normality assumption was tested through the function "mardiaTest" (MVN package, Korkmaz et al. 2014) on R software and we found that our data were not multivariate normal (details not shown). We applied DA on a few axes (preserving about 95% of the variance) of a Principal Component Analysis, as performed by "dapc", which improves the imbalance between objects and variables (Jombart et al. 2010). The results of "dapc" were evaluated within an exploratory context and to assess their congruence in relation to those from "Random Forest".

We used the following acoustic variables for the statistical analyses: call duration, call rise time, pulses per note, pulse duration, interpulse interval, pulse rate, peak of the dominant frequency, minimum of the dominant frequency, and maximum of the dominant frequency. Considering that both multivariate analyses were highly

Table 1. Measurements of the advertisement call of *Ischnocnema parva* from several localities in southeast Brazil (see text for details). Mean ± SD (amplitude in parenthesis). N = number of specimens recorded (within parenthesis the number of analyzed calls).

	Atibaia	Salesópolis	Rio Preto	Ubatuba
Traits	N = 14 (48)	N = 5 (11)	N = 5 (30)	N = 2 (2)
Call duration (s)	0.5 ± 0.181 (0.210–1.077)	0.39 ± 0.138 (0.216–0.558)	0.699 ± 0.31 (0.183–1.158)	0.605 ± 0.048 (0.571–0.639)
Call rise time (s)	0.384 ± 0.117 (0.087–0.816)	0.348 ± 0.121 (0.205–0.507)	0.483 ± 0.186 (0.163–1.052)	0.625 ± 0.020 (0.611–0.639)
Intercall interval (s)	9.0 ± 5.0 (4.5–24.3)	28.3 ± 19.8 (8.2–55.0)	11.6 ± 8.0 (2.5–28.9)	-
Call rate	7.3 ± 2.7 (2.3–10.8)	3.4 ± 2.9 (1.2–6.7)	7.6 ± 5.2 (3.1–15.1)	-
Number of pulses per note	15.8 ± 5.5 (8.0–35.0)	12.4 ± 3.8 (8.0–17.0)	16.8 ± 6.8 (5.0–27.0)	13.5 ± 0.7 (13.0–14.0)
Pulse duration (ms)	11 ± 3 (3–27)	7 ± 2 (3–12)	14 ± 2 (3–26)	15 ± 3 (7–22)
Interpulse interval (ms)	22 ± 3 (3–27)	26 ± 4 (18–35)	29 ± 3 (13–38)	31 ± 2 (23–36)
Pulse rate (pulses/second)	29.9 ± 3.4 (24.1–33.3)	29.5 ± 2.4 (26.8–32.7)	22.8 ± 0.8 (21.3–24.3)	20.7 ± 0.5 (20.3–21.0)
Peak of dominant frequency (Hz)	3737 ± 177.4 (3359–4091)	3907 ± 97.7 (3797–4078)	3903 ± 251.7 (3574–4350)	3817 ± 38.2 (3790–3844)
Min. dominant frequency (Hz)	3229 ± 173.5 (2391–3445)	3257 ± 314.7 (2297–3609)	3497 ± 222.8 (3273–3919)	3177 ± 254.0 (3000–3359)
Max. dominant frequency (Hz)	4037 ± 172.1 (3747–4565)	4325 ± 228.1 (4078–4828)	4189 ± 232.7 (3876–4522)	4087 ± 54.3 (4048–4125)
Peak fundamental frequency (Hz)	2065 ± 133.4 (1734–2412)	2159 ± 305.6 (1734-2437)	2078 ± 103.4 (1895–2412)	2057 ± 257.7 (1875–2239)

concordant (see results section), we describe the Random Forest results and give a scatterplot of dapc only.

We tested all acoustic variables for statistically significant differences among populations from Atibaia, Salesópolis, and Rio Preto through the Exact Wilcoxon Mann Whitney Rank Sum Test using the package coin (Resampling Statistics, function "wilcox_test", Hothorn et al. 2008) on R; Ubatuba data were not tested due to its low sample size (N = 2 males) and by the evident qualitative concordance to the other samples. As these tests were done between populations pairs, we adjusted the significance levels ("α") considering the number of pairings through the method of Holm (p.adjust function in R). We assumed significance when $p \leq 0.05$.

Results

Call description

During field works we heard hundreds of males and recorded only one call type of *Ischnocnema parva* (Fig. 2), which we assumed was the advertisement call (Wells 2007). All males were calling within the forest and on the leaf litter between 15:32 and 21:00 h; we noticed that the males emit shorter calls at the beginning of their vocal activity. Quantitative variables of the advertisement

Figure 2. Specimens of *Ischnocnema parva* in life from municipality of Atibaia (top) (AAG-UFU 5203) and municipality of Salesópolis (bottom) (AAG-UFU 5412), both state of São Paulo, Brazil.

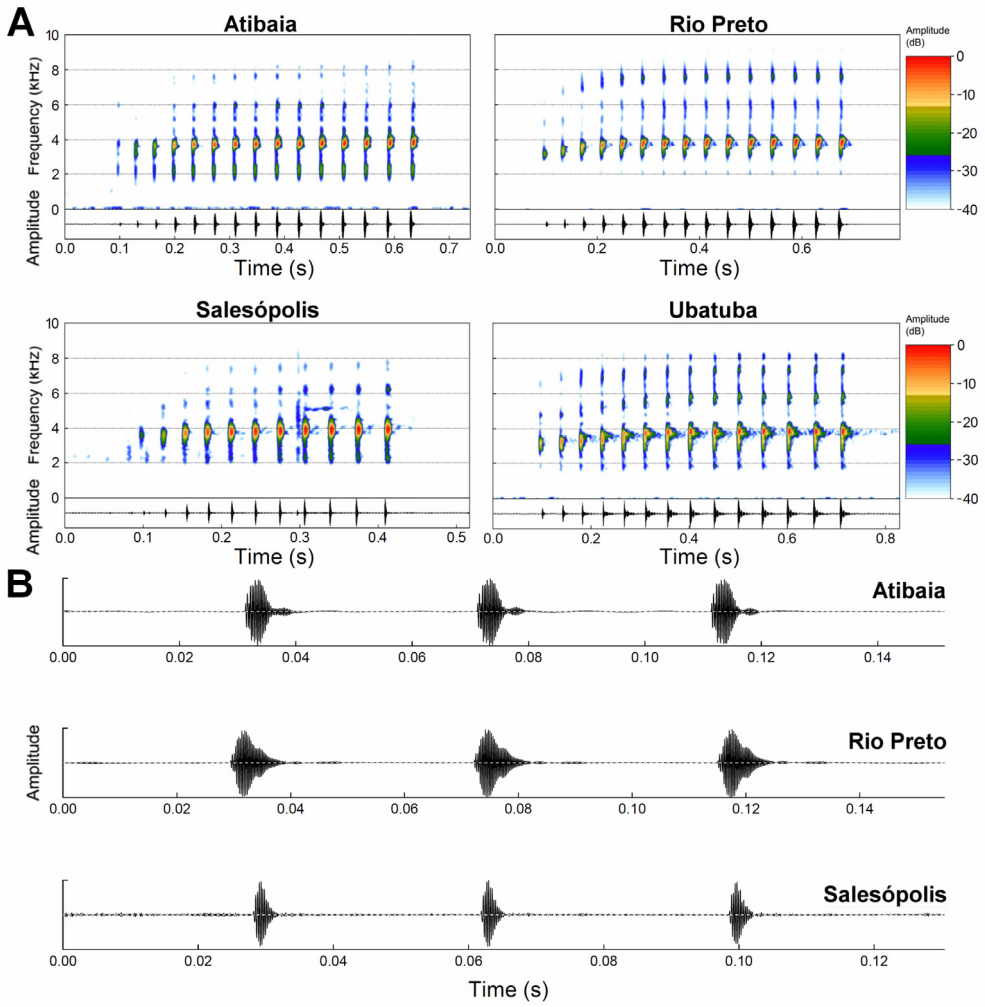

Figure 3. (A) Spectrograms (top) and corresponding oscillograms (bottom) of advertisement calls of *Ischnocnema parva* from four sampled localities: municipality of Atibaia, state of São Paulo; sound file: Ischnoc_parvaAtibaiaSP4bIAH_AAGm670; 19:59 h, 15 October 2013; air 18.6ºC; unvouchered; Rio Preto (MG); file: Ischnoc_parvaRioPretoMG3bFSA_AAGmt; 18:43 h, 06 November 2015; air 19.2ºC; Voucher (ZUEC 23214); Salesópolis (SP); file: Ischnoc_parvaSalesopolisSP3bFSA_AAGm671; 18:51 h, 28 December 2015; air 21.5ºC; unvouchered; Ubatuba (SP); file: Ischnoc_parvaUbatubaSP2aAAGm; 21:00 h, 29 October 2010; air 19.0ºC; unvouchered. (B) Oscillograms of three pulses of the same calls above. Excerpts of these original analyzed calls (in wav format) are available at AmphibiaWeb (http://amphibiaweb.org/).

call are detailed in Table 1; spectrograms and oscillograms are depicted in Figure 3A; its pulse structure is in Figure 3B. As a general pattern, the advertisement call consists of a single pulsed note emitted sporadically and with a slightly continuous increase in amplitude over time; the amplitude peak is reached near the midpoint of the call, thenceforth the amplitude reaches a plateau that goes until the end of the call; there is also a slight and quick increase in frequency until the fourth or fifth pulse (in the first third of the call), thereafter the frequency also reaches a plateau that lasts throughout the duration of the signal. The Fundamental frequency peaks at around 2000 Hz and the dominant (=second harmonic) at around 4000 Hz (Fig. 3A).

The call has a duration of 0.183–1.077 s and is emitted at a rate of 1.8–5.5 calls/minute. It is com-

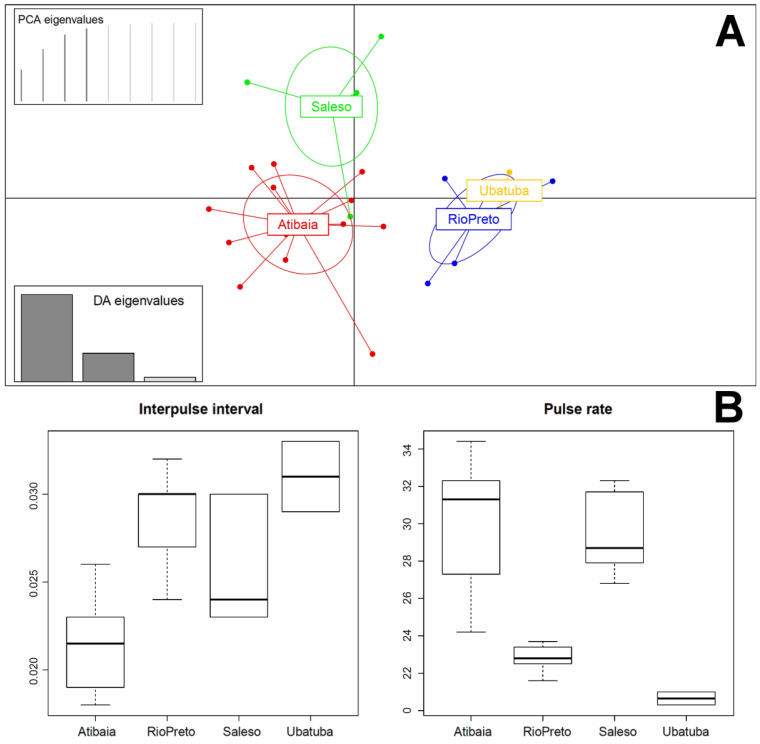

Figure 4. (A) Scatterplot with the two first axes of the Discriminant Analysis on the first four Principal Components on the acoustic dataset of *Ischnocnema parva*, variance explained by the axes: LD1 (x-axis) = 72.5 % (F-statistic = 19.2) and LD2 (y-axis) = 23.7 % (F-statistic = 6.3); and (B) boxplots of the two major variables promoting variation between localities. Abbreviation: saleso = municipality of Salesópolis.

posed of 5 to 35 pulses, which last from 3–27 ms separated by intervals of 3–38 ms and released at rate from 20–33 pulses/second (Fig. 3B). Fundamental frequency peaks at 1734–2412 Hz. While Dominant frequency (=second harmonic) peaks at 3359–4350 Hz, minimum frequency at 2297–3919 Hz and maximum at 3747–4828 Hz. In addition to these first two bands (fundamental and dominant frequencies), up to two other harmonically-related emphasized frequency bands may be present (Fig. 3A).

Statistical comparisons

In Random Forest multivariate approach we found 0% of classification error to Atibaia males; males from other localities had considerable classification error (Rio Preto = 40%, Salesópolis = 60%, and Ubatuba = 100%), most of them classified as belonging to Atibaia. Accordingly, the dapc resulted only in a partial discrimination of sampled populations, with a slight separation along axis 1 (LD1=72.5%), whereas variation along LD2 (23.7%) did not contribute to separation. Pulse rate (40%) and interpulse interval (30%) mainly accounted for population separation along LD1 (Fig. 4A). In dapc, the two southwesternmost localities (Atibaia and Salesópolis; Fig. 1), which have the calls with highest pulse rate (Fig. 4B), were closer to one another than to the other two localities.

Population differences were noticed in temporal traits. Salesópolis differed from Atibaia by having a shorter pulse duration (p = 0.02) and longer interpulse interval (p = 0.05). From Rio Preto, Atibaia differed by having longer pulse duration (p = 0.02) and interpulse interval (p < 0.01) and a lower pulse rate (p < 0.01). Calls from Rio Preto had a longer pulse duration (p = 0.02) and lower pulse rate (p = 0.02), compared to Salesópolis calls (Table 1).

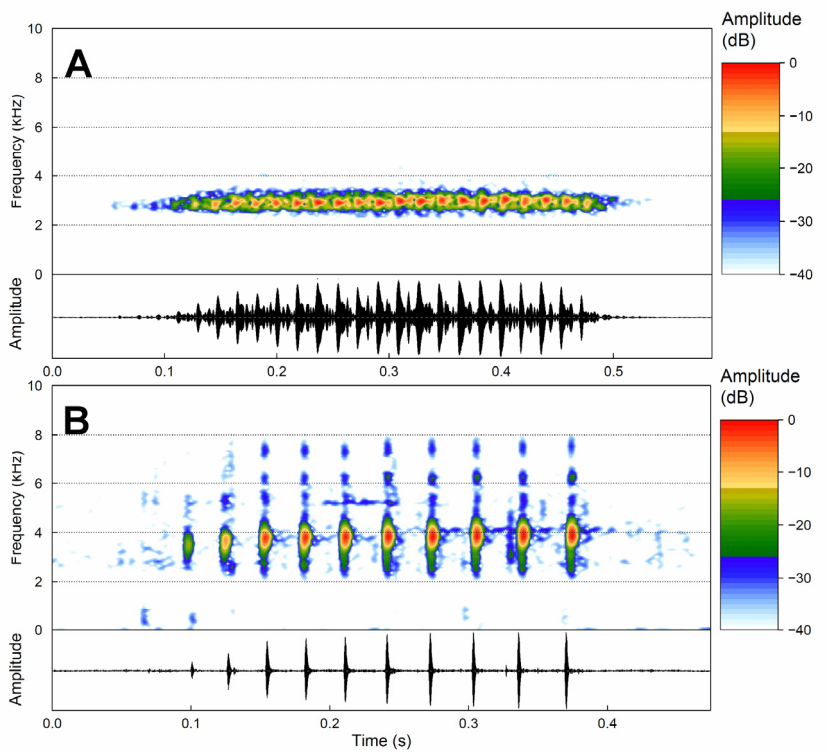

Figure 5. (A) Spectrogram (top) and corresponding oscillogram (bottom) of advertisement call of *Ischnocnema parva* from Boracéia, municipality of Salesópolis, state of São Paulo, described by Heyer et al. (1990); (B) Spectrogram (top) and corresponding oscillogram (bottom) of one of our recordings from the same locality; file: Ischnoc_parvaSalesopolisSP3cFSA_AAGm671; 18:53 h, 28 December 2015; air 21.5ºC; unvouchered.

Discussion

Our samples from Salesópolis are especially important because they are from a site where the call has been previously described (Heyer et al. 1990) and the original description is in disagreement with our newly gathered data. Heyer et al. (1990) provided notes on the advertisement call of *Ischnocnema parva* from Estação Biológica da Boracéia, Salesópolis (SP) based on an undetermined number of males. According to them, the advertisement call consists of 20–25 spaced notes with two major pulses each. We did not find different pulse types. We think that the call of *I. parva* is better defined as composed of a single note with ca. 15 pulses (one note [= call] refers to a unit of sound of this signal; see Fig. 3A). Compared to our data, the call described by Heyer et al. (1990) has a higher pulse (called note there) rate (54–60 pulses per second vs 20–33 in this study) and lower dominant frequency (2400–3500 Hz; 3359–4091 Hz in this study). Furthermore, those authors interpreted the call as having no harmonic structure, and with an ascending amplitude modulation up to the middle of the call, followed by a descendant modulation that goes until its end. Given these remarkable differences, it is possible that the call described by Heyer et al. (1990) may not be of *I. parva* (see these temporal and spectral differences in Figure 5).

Cruz et al. (2009) reported the occurrence of *I. parva* in Parque Estadual do Ibitipoca, municipality of Lima Duarte (MG), which is about 30 km north from our sample from Rio Preto (MG). This species also was already reported for Atibaia and Ubatuba by Giaretta & Facure (2008) and Siqueira-Jr et al. (2004), respectively.

Despite the lack of acoustic data for topotypic *I. parva* (municipality of Rio de Janeiro, RJ), this is the first acoustic study within *I. parva* species se-

ries which includes recordings of several populations. Molecular evidence pointed that this widely distributed species may correspond to more than one taxon (Brusquetti et al. 2013). Air temperature at the time of the recordings in our fieldworks overlapped in their ranges, thus we do not attribute the acoustic differences found to temperature influence. Since we did not access molecular data of our sampled localities and because of the small sample size of some localities, we prefer to attribute the found (e.g. pulse rate) differences of the temporal traits to an intraspecific variation.

Due to lack of acoustic data for *I. pusilla* and *I. nanahallux*, we cannot make an interspecific comparison to assess if there is an acoustic diagnosis for *I. parva* among the *I. parva* species series. Finally, further acoustic studies are needed in order to significantly improve species delimitation within *Ischnocnema*.

Acknowledgements. We thank Fabio Hepp and the two anonymous reviewers for discussions and comments during the review process. We also thank William P. Costa for fieldwork help and Luís Felipe Toledo for providing laboratory facilities. Coordination for the Improvement of Higher Education Personnel (CAPES) provided MSc scholarship to FSA and IAH, and São Paulo Research Foundation (FAPESP) provide PhD scholarship to FSA (Process #2015/10728-7). National Counsel of Technological and Scientific Development (CNPq) provided grants to AAG (#446935/2014-0 and #300903/2015-4). CNPq and Minas Gerais Research Foundation (FAPEMIG) financially support A. A. Giaretta's research. FSA thanks The Cornell Lab of Ornithology (Bioacoustics Research Program) for the license for Raven Pro. Collection permit: SISBIO/ICMBio #30059-1 and #30059-7.

References

Bioacoustics Research Program (2014): Raven Pro: Interactive Sound Analysis Software, version 1.5. The Cornell Lab of Ornithology, Ithaca, New York. <http://www.birds.cornell.edu/raven, accessed> at: 2015.02. 05

Bokermann, W.C.A. (1967): Una nueva especie de *Eleutherodactylus* del sudeste brasileño (Amphibia, Leptodactylidae). Neotropica 13: 1–3.

Breiman, L. (2001): Random Forests. Machine Learning 45: 5–32.

Brusquetti, F., Thomé, M.T.C., Canedo, C., Condez, T.H., Haddad, C.F.B. (2013): A new species of *Ischnocnema parva* species series (Anura, Brachycephalidae) from northern state of Rio de Janeiro, Brazil. Herpetologica 69(2): 175–185.

Canedo, C., Haddad, C.F.B. (2012): Phylogenetic relationships within anuran clade Terrarana, with emphasis on the placement of Brazilian Atlantic rainforest frog genus *Ischnocnema* (Anura: Brachycephalidae). Molecular Phylogenetics and Evolution 65(2): 610–620.

Crocoft, R.B., Ryan, M.J. (1995): Patterns of advertisement call evolution in toads and chorus frogs. Animal Behaviour 49(2): 283–303.

Cruz, C.A.G., Feio, R.N., Caramaschi, U. (2009): Anfíbios do Ibitipoca. Bicho do Mato, Belo Horizonte.

Frost, D.R. (2015): Amphibian Species of the World: an Online Reference. Version 6.0. American Museum of Natural History, New York, USA. <http://research.amnh.org/herpetology/amphibia/index.html>, accessed at: 2015.11.13.

Gerhardt, H.C., Huber, F. (2002): Acoustic communication in insects and anurans: common problems and diverse solutions. The University of Chicago Press, Chicago, USA.

Giaretta, A.A., Facure, K.G. (2008): Reproduction and habitat of ten Brazilian frogs (Anura). Contemporary Herpetology 3: 1–4.

Heyer, W.R., Rand, A.S., Cruz, C.A.G., Peixoto, O.L., Nelson, C.E. (1990): Frogs of Boracéia. Arquivos de Zoologia, São Paulo 31(4): 231–410.

Hothorn, T., Hornik, K., Van de Wiel, M.A., Zeileis, A. (2008): Implementing a class of permutation tests: the coin package. Journal of Statistical Software 28(8): 1–23.

Jombart, T. (2008): adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24(11): 1403–1405.

Jombart, T., Devillard, S., Balloux, F. (2010): Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11(94): 1–15.

Korkmaz, S., Goksuluk, D., Zararsiz, G. (2014): MVN: An R Package for Assessing Multivariate Normality. The R Journal 6(2): 151–163.

Liaw, A., Wiener, M. (2002): Classification and Regression by randomForest. R News 2/3: 18–22.

Martins, A.C.J.S., Klefer, M.C., Siqueira, C.C., Van Sluys, M., Menezes, V.A., Rocha, C.F.D. (2010): Ecology of *Ischnocnema parva* (Anura: Brachycephalidae) at the Atlantic Rainforest of Serra da Concórdia, state of Rio de Janeiro, Brazil. Zoologia 27(2): 201–208.

McLister, J.D., Stevens, E.D., Bogart, J.P. (1995): Comparative contractile dynamics of calling and locomotor muscles in three hylid frogs. The Journal of Experimental Biology 198(7): 1527–1538.

Padial, J.M., Grant, T., Frost, D.R. (2014): Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. Zootaxa 3825(1): 1–132.

Pohar, M., Blas, M., Turk, S. (2004): Comparison of Logistic Regression and Linear Discriminant Analysis: A Simulation Study. Metodološki Zvezki 1(1): 143–161.

R Core Team (2015): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/> accessed, at: 2015.12.15.

Siqueira-Jr, S., Ananias, F., Recco-Pimentel, S.M. (2004): Cytogenetics of three Brazilian species of *Eleutherodactylus* (Anura, Leptodactylidae) with 22 chromosomes and re-analysis of multiple translocations in *E. binotatus*. Genetics and Molecular Biology 27(3): 363–372.

Sueur, J., Aubin T., Simonis C. (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.