REPRODUCTION, SEXUAL DIMORPHISM, AND DIET OF *LEPTODACTYLUS CHAQUENSIS* (ANURA, LEPTODACTYLIDAE) IN NORTHEASTERN BRAZIL

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Abstract.—Descriptive natural history parameters are important for determining if and how traits vary in related species/populations in response to environmental characteristics. Additionally, filling information gaps from less-explored regions, such as northeastern Brazil, for widely distributed species complexes can help us better understand the evolution of life histories. Here, we studied reproduction, sexual dimorphism, and diet of Cei's White-lipped Frog (*Leptodactylus chaquensis*) from an ecotone area between the Caatinga and Atlantic Forest biomes in northeastern Brazil, Rio Grande do Norte State. We collected reproductive individuals during both rainy and dry seasons, but observed calling males, foam nests, and tadpole schools only during the rainy season. There was no sexual dimorphism in body size, but sexes differed in shape variables: females had longer tibias than males, whereas males had longer forearms. We identified three distinct calls emitted by males: growl, grunt, and trill, as well as a less common call combining both growl and trill elements. The species is a dietary generalist and preyed on invertebrates, mainly beetles (Coleoptera) and ants (Formicidae). Finally, the population studied showed similar reproductive and call characteristics to *L. chaquensis* from Chaco and Cerrado biomes.

Key Words.—bioacoustics; Caatinga; natural history; reproductive behavior; shape variables

INTRODUCTION

The Neotropical frog genus *Leptodactylus* includes 74 recognized species that exhibit a diversity of body sizes and reproductive behaviors (Heyer 1969; Frost 2016). Species often show sexual dimorphism, complex acoustic repertoires, tadpole schooling behavior, and parental care (de Sá et al. 2014). Despite such diversity in reproductive strategies (mostly regarding calling and oviposition sites), almost all species of *Leptodactylus* lay eggs in foam nests (Heyer 1969; Haddad and Prado 2005). Moreover, even closely related species can exhibit differences in reproductive strategies in response to ecological constraints. For example, gametogenesis is interrupted during the coldest and hottest temperatures in Cei's White-lipped Frog (*L. chaquensis*), but it is continuous in *L.* aff. *latrans* (Cei 1980).

The *L. latrans* group comprises eight morphologically similar species broadly distributed in South America (de Sá et al. 2014). Many studies on the natural history of species in the *L. latrans* group have been published, addressing aspects of their reproductive biology (e.g., vocalizations, sexual dimorphism, and reproductive period; Barrio 1966; Cei 1980; Heyer and Giaretta 2009) and diet (Teixeira and Vrcibradic 2003; Schaefer et al. 2006). These studies were conducted in different biomes across South America, mainly open formations, but such information is scarce for species of the *L. latrans* group in northeastern Brazil, where the semi-arid Caatinga biome meets the wet Atlantic Forest biome (Cei 1962; Magalhães et al. 2013). The biodiverse Atlantic Forest in Brazil is distributed from Rio Grande do Sul to Rio Grande do Norte states, ranging 29° in latitude and covering over 150 million ha (Ribeiro et al. 2009). At its northern limit in northeastern Brazil, the climate is substantially different compared to the southern portion and communities are highly influenced by the adjacent Caatinga. For example, during an intensive survey of

498

frogs in more than 20 forest fragments in southern Rio Grande do Norte State, no forest-exclusive species were captured (Lion et al. 2014). In fact, several frogs and lizards typically characteristic of the Caatinga were captured, such as Muller's Termite Frog (*Dermatonotus muelleri*), Kluge's Dwarf Gecko (*Lygodactylus klugei*), and Peters' Four-eyed Frog (*Pleurodema diplolister*; Lion et al. 2016).

The lack of natural history information on species of the *L. latrans* group from less-explored regions, such as northeastern Brazil, precludes a better understanding of the ecology, evolution, and taxonomy of species (Angeloni et al. 2008; Vitt 2013; Nali et al. 2014). Because of the cryptic diversity of species in the *L. latrans* group (de Sá et al. 2014), the recognition of geographic variation in such traits could be especially important for species conservation and management (Morrison and Hero 2003). Here, we present data on reproduction (reproductive period, behavior, and calls), sexual dimorphism, and diet of *L. chaquensis* in an ecotone area between Caatinga and Atlantic Forest in northeastern Brazil.

Materials and Methods

Study organism.—A recent phylogenetic study corroborated the monophyly of the eight species comprising the *L. latrans* group (de Sá et al. 2014), which includes *L. chaquensis*. These species are morphologically similar and hence difficult to identify using external morphology alone, as is the case of *L. macrosternum* and *L. chaquensis* (Heyer and Giaretta 2009; de Sá et al. 2014). Currently, *L. chaquensis* is restricted to arid ecosystems in northern Argentina and adjacent Bolivia, Brazil, Paraguay, and northern Uruguay, whereas *L. macrosternum* is restricted to its type locality in Bahia State, Brazil (de Sá et al. 2014). Specimens belonging to the *L. latrans* group distributed in northeastern Brazil, especially within the Caatinga biome, were not assigned to any species and their taxonomic statuses remain uncertain (de Sá et al. 2014). Advertisement calls of frogs from these regions in northeastern Brazil (the populations studied herein included) unequivocally associate them to *L. chaquensis* (see Results).

Study area.—We conducted field work in the Escola Agrícola de Jundiaí (05°53'06.68"S; 35°22'01.28"W), Macaíba Municipality, Rio Grande do Norte State, in northeastern Brazil. The region represents a transition zone between Caatinga and Atlantic Forest biomes, and harbors frog and plant species that are typical of both domains, although the vegetation is predominantly Caatinga (Cestaro and Soares 2004; Magalhães et al. 2013). We collected individuals of *L. chaquensis* during an extensive anuran inventory in this area (see Magalhães et al. 2013), which occurred during the 2009 rainy (February to July: accumulated precipitation 1,556 mm) and dry seasons (October, November, and December: accumulated precipitation 14 mm), and the 2010 rainy season (February to June: accumulated precipitation 607 mm). During the rainy season, we actively searched for frogs in a 33-ha flooded area with extensive aquatic vegetation and small bushes on the perimeter, whereas during the dry season, we found frogs along the margins of a narrow sandy bottom river (Jundiaí River) that crosses the area. We conducted all fieldwork from 1800 to 0000 and collected all individuals by hand. We killed all collected individuals with lidocaine (5%) and fixed them in formalin (10%), usually immediately after collection but a few on the following morning. We preserved specimens in ethanol (70%) and deposited them at Coleção de Anfíbios e Répteis (CLAR-AAGARDA) at the Universidade Federal do Rio Grande do Norte. One additional male (AAGARDA8967) recorded and collected in the area after the survey period was included in the bioacoustic analysis only (voucher recording ASUFRN 229).

Reproductive biology.—We determined the sex of 50 males and 50 females by dissection and direct observation of gonads and through the presence of secondary sexual characteristics in males (dark vocal sacs, hypertrophied arms, and nuptial spines). To determine the reproductive period of our study population, we classified individuals as reproductive or non-reproductive and examined variation in frequencies among months and between wet and dry seasons. We considered males reproductive when they exhibited secondary sexual characteristics and large quantities of spermatozoa in seminiferous tubules with compacted chromatin (Báo et al. 1991; Maragno and Cechin 2009; Vitt and Caldwell 2014). We used a qualitative scale based on the overall density of spermatocytes surrounded by germinate cells with highly compacted chromatin as observed in histological slides of the testis (Báo et al. 1991; Ferreira and Mehanna 2012). We considered females reproductive if they exhibited visible and developed oocytes with a compact oviduct of similar volume to the ovaries in macroscopic analysis following Vitt and Caldwell (2014).

Sexual dimorphism.—To investigate sexual dimorphism in size and shape, we measured only sexually mature individuals. Individuals with snout-vent length (SVL) equal to or larger than the smallest reproductive individual for each sex were considered sexually mature, which yielded 28 males and 26 females for analysis. We measured morphometric variables following Duellman and Trueb (1986) using

TABLE 1. Mean and standard deviation of morphometric variables (in mm) for sexually mature Cei's White-lipped Frog (*Leptodactylus chaquensis*). Mean and standard deviation of size-adjusted shape variables are presented in parentheses.

Variable	Males (n = 28)	Females (n = 26)
Snout-vent length	74.02 ± 5.64 (0.55 ± 0.01)	74.13 ± 5.5 (0.56 ± 0.01)
Head width	26.17 ± 2.45 (0.11 ± 0.01)	26.1 ± 1.98 (0.10 ± 0.01)
Head length	25.36 ± 1.89 (0.09 ± 0.02)	25.36 ± 1.99 (0.09 ± 0.02)
Diameter of tympanum	5.68 ± 0.58 (-0.56 ± 0.02)	5.62 ± 0.49 (-0.56 ± 0.02)
Eye diameter	7.52 ± 0.74 (-0.44 ± 0.04)	7.66 ± 0.81 (-0.42 ± 0.04)
Forearm length	30.3 ± 2.56 (0.17 ± 0.02)	29.01 ± 2.15 (0.16 ± 0.01)
Hand length	16.93 ± 1.31 (-0.09 ± 0.01)	16.61 ± 1.2 (-0.09 ± 0.02)
Tibia length	36.93 ± 2.7 (0.25 ± 0.01)	37.74 ± 2.47 (0.27 ± 0.01)
Foot length	54.53 ± 3.7 (0.42 ± 0.02)	54.19 ± 3.25 (0.43 ± 0.01)
Arm diameter	6.61 ± 1.24 (-0.50 ± 0.06)	5.72 ± 0.5 (-0.55 ± 0.03)

digital calipers (nearest 0.01 mm): SVL, head width (HW), head length (HL), diameter of tympanum (DT), eye diameter (ED), forearm length (FaL), hand length (HaL), tibia length (TL), foot length (FL), and arm diameter (AD). We log-transformed (base 10) all morphometric variables and screened the data for multivariate outliers using adaptive outlier detection in the R package mvoutlier (Filzmoser et al. 2005). We considered three females (11.5% of all females) outliers, and dropped them from further analyses.

To analyze morphometric variation in size, we defined body size as an isometric size variable (Rohlf and Bookstein 1987) following the procedure described by Somers (1986). We calculated an isometric eigenvector, defined a priori with values equal to $p^{-0.5}$, where p is the number of variables (Jolicoeur 1963), and obtained scores from this eigenvector, hereafter called Body Size, by post-multiplying the n × p matrix of log10-transformed data, where n is the number of observations, by the p × 1 isometric eigenvector. To analyze morphometric variation in shape, we removed the effect of size from the log10-transformed variables using Burnaby's method (Burnaby 1966). We post-multiplied the n × p matrix of the log10-transformed data by a p × p symmetric matrix, L, defined as: $L = I_p - V(V^T V)^{-1}V^T$, where I_p is a p × p identity matrix, V is the isometric size eigenvector defined above, and V^T is the transpose of matrix V (Rohlf and Bookstein 1987). Hereafter, we refer to the resulting size-adjusted

variables as Shape Variables (Table 1). We tested for differences in Body Size between sexes using an analysis of variance (ANOVA) and tested for differences between sexes in Shape Variables using a Bayesian logistic regression in the Arm package for R (Gelman and Hill 2007). For the latter, we compared the full model against a constant-only (null) model using a chi-square test of the scaled deviance to evaluate the statistical significance of the full model based on Shape Variables (Chambers and Hastie 1992; Faraway 2006). We also calculated the significance of the logistic regression model with a chi-square test on the value of the difference between the residual deviations from the full model and the null model (Tabachnick and Fidell 2001). We then assessed the importance of each variable in discriminating the two sexes by model selection via single term addition (Chambers and Hastie 1992): 1) the full model was tested against a constant-only model; 2) the significant term with the lowest Akaike's Information Criterion (AIC) value was added to the null model; 3) step 2 was repeated; 4) any non-significant terms were dropped from the model; and 5) steps 3 and 4 were repeated until no significant terms could be added or no non-significant terms could be dropped from the model. After the model selection analysis, we assessed the misclassification error of the reduced model using 1,000 bootstrap replications of a linear discriminant analysis with R package ipred (Peters, A., T. Hothorn, B. Ripley, T. Therneau, and B. Atkinson. 2013. ipred: Improved Predictors, 2013. R package version 0.9-3. Available at http://cran.r-project.org/web/packages/ipred/index.html [Accessed 08 November 2016].). We further assessed variable importance using model averaging, retaining only models with $\Delta AIC_c < 4$ (Crawley 2007). We conducted model averaging in the MuMIn package in R (Burnham and Anderson 2002). We performed all analyses in R v3.0.2 (R Development Core Team 2013). We used an $\alpha = 0.05$.

Vocalizations.—We describe calls based on recordings from five individuals. We recorded frogs using a Marantz PMD 661 (Marantz Professional, Kawasaki, Kanagawa, Japan) coupled with a Sennheiser ME66 (Sennheiser, Wedemark, Niedersachsen, Germany) directional microphone at 50–100 cm distance. We analyzed advertisement calls in Raven Pro 1.4 (Bioacoustics Research Program, Cornell Lab of Ornithology, Ithaca, New York, USA) and constructed audio spectrograms with the seewave package (Sueur et al. 2008) in R under the following parameters: FFT window width = 256 (except for the grunt call, which was 450), Frame = 100, Overlap = 80, and flat top filter. We follow the terminology for call descriptions used by Duellman and Trueb (1986), McLister et al. (1995), and Heyer and Giaretta (2009), who consider a
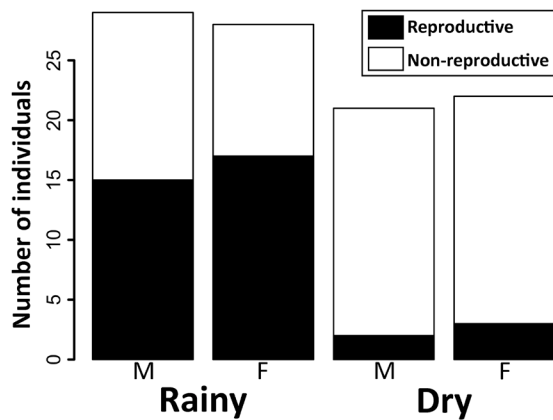
FIGURE 1. Total number of Cei's White-lipped Frog (*Leptodactylus chaquensis*) collected during the rainy and dry seasons in an Atlantic Forest-Caatinga ecotone area in Rio Grande do Norte State, northeastern Brazil. Abbreviations are M = male and F = Female.

note as a single unit of sound consisting of one or more pulses produced during a single airflow cycle. Variables quantified include note duration, pulse duration, pulses per note, pulse rate, and dominant frequency. We deposited recording files in the collection Arquivos Sonoros da Universidade Federal do Rio Grande do Norte (ASUFRN).

Diet.—To characterize the diet of *L. chaquensis*, we dissected and removed the stomachs from 90 ethanol-preserved specimens that were collected in both rainy and dry seasons. We identified prey to the lowest taxonomic level possible, generally order. We measured the length (l) and width (w) of intact prey and calculated their volumes (V) using the ellipsoid formula (Colli et al. 2003):

$$V = \frac{4}{3}\pi\left(\frac{w}{2}\right)^2\left(\frac{l}{2}\right)$$

We calculated numerical and volumetric percentages of each prey type for each individual. We determined the relative contribution of each prey category by calculating

an index of relative importance for individuals and pooled stomachs using the average of percentage of prey occurrence (F%), numeric percentage (N%), and volumetric percentage (V%), according to the equation (Pinkas et al. 1971):

$$IRI = F\% \times (N\% + V\%)$$

RESULTS

Reproductive biology.—We collected 57 individuals during the rainy season and 43 during the dry season. Of the 50 individuals of each sex, 34% of males and 40% of females were reproductive (Fig. 1). The SVL of the smallest reproductive male and female measured was 64.60 mm and 66.43 mm, respectively. Reproductive individuals occurred in a higher proportion and frequency during the rainy season ($\chi^2 = 18.97$, df = 1, $P < 0.001$; Fig. 1). Based on observations of calling males, foam nests, and tadpole schools, mostly after heavy rains, reproduction occurred from February to June in 2009 and 2010. Although we collected few reproductive individuals of both sexes during the dry season (with male sex hibiting secondary sexual characters), there is no evidence that reproduction occurred (e.g., no calling males nor tadpole schools observed). On 24 June 2010, we observed a female sitting still beside a tadpole school for approximately 30 min (at about time 2000) while tadpoles swam around her (Fig. 2). When we approached the tadpole school, the female made no attempt to escape, nor did she attempt to defend the tadpole school.

Sexual dimorphism.—Body Size score of reproductive males averaged 4.15 ± 0.10 (SD; range = 3.92–4.33) and reproductive females averaged 4.12 ± 0.10 (range = 3.94–4.29). There was no sexual dimorphism in adult Body Size ($F_{1,43} = 0.67$, $P = 0.406$), but sexes differed in Body Shape ($\chi^2 = 47.56$, df = 1, $P < 0.001$). Tibia length and forearm length largely explained shape differences between sexes (Table 2). Females had longer tibias than males whereas males had longer forearms than females (Table 1). Misclassification error

TABLE 2. Results of model selection of shape variables differing between female and male Cei's White-lipped Frog (*Leptodactylus chaquensis*). The best model includes only the shape variables manually selected using the Akaike's Information Criterion (AIC). Model results are for Full Model = Full, Best Model = Best, Model-averaged Coefficient = MAC, and Relative Variable Importance = RVI. Abbreviations are I = Intercept, SVL = snout vent-length, HW = head width, HL = head length, DT = diameter of tympanum, ED = eye diameter, FaL = forearm length, HaL = hand length, TL = tibia length, FL = foot length, and AD = arm diameter. *P*-value model indicated by asterisks: * < 0.05, ** < 0.01.

Model	I	SVL	HW	HL	DT	ED	FaL	HaL	TL	FL	AD	AIC	χ^2	P
Full	13.89	-25.58	-60.78	21.90	-11.72	-14.50	113.52**	-41.56	-139.41**	33.34	13.93	43.74	48.47	0.001
Best	21.7	–	40.65*	34.72	–	38.05	44.43**	–	62.23**	–	–	38.54	43.67	0.001
MAC	17.91	-36.13	-72.52	29.77	-14.11	-24.57	96.41*	-34.60	-130.72**	37.25	22.56	–	–	–
RVI	–	0.14	0.78	0.52	0.19	0.53	0.99	0.26	1.00	0.47	0.55	–		

FIGURE 2. Female Cei's White-lipped Frog (*Leptodactylus chaquensis*) attending a tadpole school in an Atlantic Forest-Caatinga ecotone area in Rio Grande do Norte State, northeastern Brazil. (Photographed by Diego J. Santana).

based on the five selected variables in the Best Model was 0.18.

Vocalizations.—We observed male *L. chaquensis* calling only during the rainy season, mostly at night, in a large flooded pond (about 5–15 cm depth), usually hidden within dense vegetation with the body partially submerged in water. We occasionally observed males calling while embedded in foam nests containing developing embryos. Less frequently, we observed males calling during twilight and some during the daytime when the weather was cloudy. Males mostly called after heavy rains and did not actively call for long periods, even when reproductive sites were available.

We identified three distinct calls emitted by male *L. chaquensis* (growl, grunt, and trill; *sensu* Heyer and Giaretta 2009). The growl was the most common call emitted by all individuals and we consider this call to

be the advertisement call (Table 3; Fig. 3A). The trill (a high-pitched call in comparison to the growl and grunt; Fig. 3B) and grunt (a low intensity and short sound similar to a throat scratch; Fig. 3C) calls were less frequently emitted by recorded males.

We also detected a fourth type of call containing both growl and trill elements (within the same note), which we refer to as the growl/trill call (Fig. 3D). Based on 14 calls from one individual, the growl/trill call had an average note duration of 0.49 ± (SD) 0.06 s (range = 0.34–0.59), had a dominant frequency of 554 ± 165 Hz (range = 345–689), and was composed of 19 ± 3 pulses/note (range = 13–26). The average rate of emission was 38.1 ± 5.1 pulses/s (range = 27.1–46.3). In the growl/trill call, the male started with a growl call (ranging from 5–15 pulses at low frequency) and finished with a trill call (ranging from 5–12 pulses at higher frequency; see Fig. 3D).

Diet.—Of the 90 specimens examined, 61 had stomach contents (68%). The diet of *L. chaquensis* was composed of nine prey categories: seven invertebrate orders, unidentified larvae, and an unidentified anuran (Table 4). Among these, ants, larvae, and beetles were the most abundant (N%), frequent (F%), and important (IRI) prey items, whereas homopterans and beetles accounted for most of the relative volume (23.89% and 26.27%, respectively; Table 4).

DISCUSSION

Variation in environmental conditions can affect reproductive strategies and morphological traits in anurans (Prado and Haddad 2005). For instance, in seasonally dry biomes (such as the Caatinga) precipitation is crucial for anuran reproduction because many temporary reproductive sites are created (Arzabe

TABLE 3. Advertisement call (growl call) parameters of Cei's White-lipped Frog (*Leptodactylus chaquensis*) recorded at Macaíba Municipality, Rio Grande do Norte State, Brazil. Values are presented as mean ± SD with ranges below in parentheses. Abbreviations are n = number of notes analysed, SVL = snout-vent length in mm, ND = note duration, PD = pulse duration, P/N = pulse/note, PR = pulse rate, DF = dominant frequency and At = air temperature.

| Recording No.	ND (s)	PD (ms)	P/N	PR (pulses/s)	DF (Hz)	At [° C]
ASUFRN 143 (n = 13, SVL = 79.8)	0.462 ± 0.049 (0.328–0.510)	8 ± 2 (5–13)	24 ± 2 (18–27)	51 ± 3 (45–55)	373 ± 67 (345–517)	27.3
ASUFRN 159 (n = 15, SVL = 80.6)	0.327 ± 0.019 (0.302–0.356)	9 ± 1 (5–12)	19 ± 1 (18–22)	60 ± 2 (58–66)	345 ± 0 (345–345)	29.7
ASUFRN 160 (n = 15, SVL = 78.8)	0.463 ± 0.031 (0.422–0.523)	11 ± 2 (4–17)	23 ± 1 (22–26)	50 ± 2 (46–53)	459 ± 84 (345–517)	28.7
ASUFRN 161 (n = 14, unvouchered)	0.453 ± 0.017 (0.418–0.472)	9 ± 1 (6–13)	23 ± 1 (21–24)	51 ± 1 (48–53)	455 ± 86 (345–517)	28.7
ASUFRN 229 (n = 6, SVL = 87.0)	0.459 ± 0.067 (0.378–0.566)	16 ± 2 (12–23)	19 ± 2 (16–22)	42 ± 2 (39–45)	345 ± 0 (345–345)	23.4
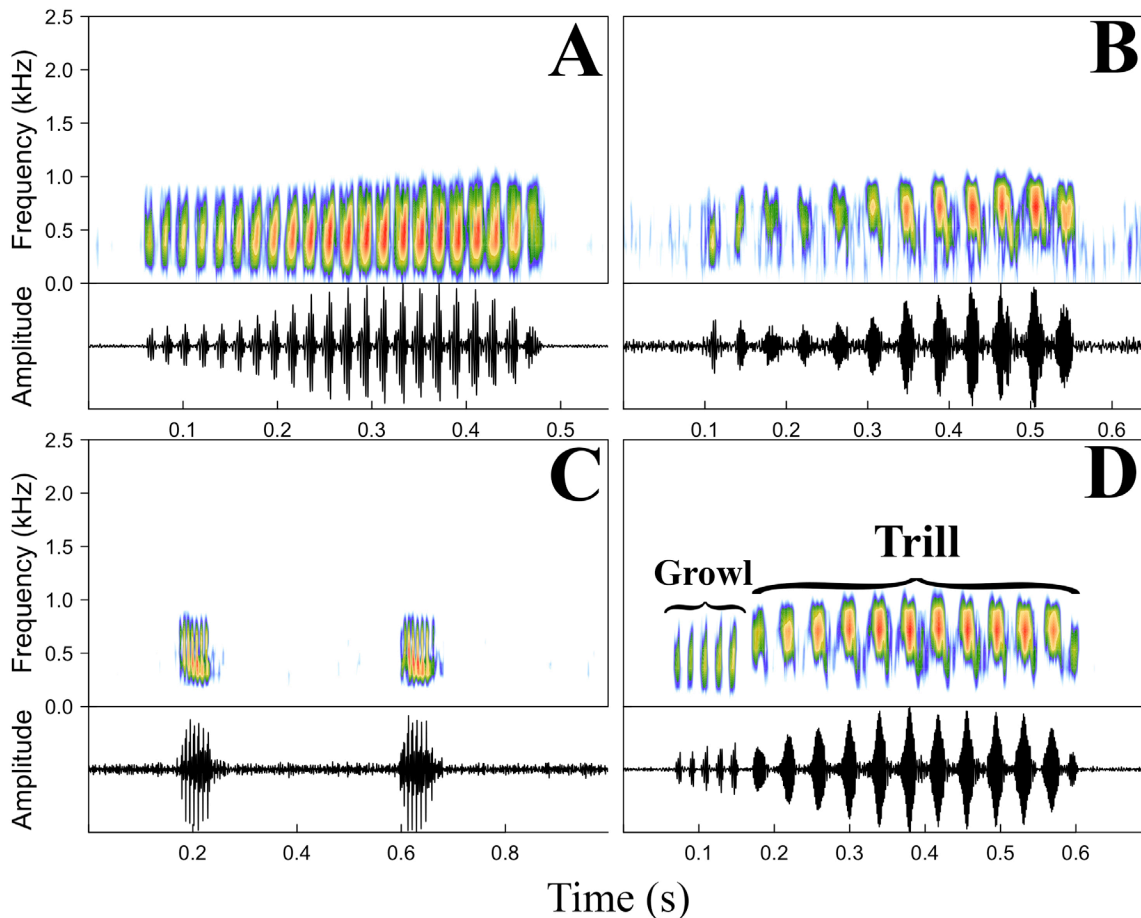
FIGURE 3. Oscillogram (above) and spectrogram (below) of calls emitted by Cei's White-lipped Frog (*Leptodactylus chaquensis*) from an Atlantic Forest-Caatinga ecotone area in Rio Grande do Norte State, northeastern Brazil. For calls, Growl is panel A, Trill is panel B, Grunt is panel C, and Growl/Trillis panel D (recording ASUFRN 143). For each, air temperature was 27.3°C.

1999; Prado and Haddad 2005; Wells 2007). Because precipitation is largely restricted to the wet season in our study region, most reproductive individuals in our population were observed during this period. Nevertheless, we also found a small number of reproductive individuals of both sexes during the dry season. Similarly, continuous reproduction was also reported for species in the *L. latrans* group from both Chaco (Cei 1980) and Cerrado (Heyer and Giaretta 2009) populations, which are biomes also characterized by highly seasonal precipitation. Hence, although environmental seasonality plays an important role in anuran reproduction (Gallardo 1964; Prado et al. 2005), it does not seem to be the only factor determining the presence of reproductive individuals in this species.

Female-biased sexual size dimorphism (SSD) is the norm for most anurans because larger females are usually selected due to a fecundity advantage (Shine 1979; Nali et al. 2014). Nevertheless, there is no consensus on which evolutionary forces drive the lack of SSD or make males larger in some species (Han

and Fu 2013; Nali et al. 2014). In the large species of *Leptodactylus* (e.g., *L. chaquensis*, *L. insularum*, *L. latrans*, and *L. pentadactylus*), there is either no SSD or males are larger than females (Cei 1980; Prado et al. 2000; de Sá et al. 2014). Some alternative anuran reproductive behaviors such as multi-male spawning and satellite males, all reported for *Leptodactylus* (Prado et al. 2000; Prado and Haddad 2003), may allow smaller males to reproduce, thereby weakening selection for large male size (Nali et al. 2014). Conversely, selection could favor traits other than size that are important in male contests and maintenance of amplexus (Han and Fu 2013; Nali et al. 2014).

Indeed, male *Leptodactylus* commonly exhibit dimorphic traits (e.g., enlarged forelimbs with prepollical spines) related to intrasexual competition (Wells 1977; Navas and James 2007). Because many male anurans compete for clasping females in amplexus, as reported for *L. chaquensis* (Prado and Haddad 2003), some authors have suggested that robust forelimb muscles have evolved, especially because amplexus

TABLE 4. Prey consumed by Cei's White-lipped Frog (*Leptodactylus chaquensis*; n = 61) in a transition area between Caatinga and Atlantic Forest biomes in Rio Grande do Norte State, Brazil. Abbreviations are n = number of prey; N% = numerical percentage; F = frequency of occurrence; F% = relative frequency of occurrence; V = volume in mm³; V% = relative volume; IRI = index of relative importance.

Prey category	n	N%	F	F%	V	V%	IRI
Formicidae	38.0	29.46	26.0	26.80	1,787.35	10.62	1,074.13
Unidentified larvae	33.0	25.58	20.0	20.62	2,184.24	12.97	794.94
Lepidoptera	7.0	5.43	5.0	5.15	0.00	0.00	27.97
Coleoptera	26.0	20.17	22.0	22.68	4,423.60	26.27	1,053.03
Homoptera	6.0	4.65	6.0	6.19	4,022.00	23.89	176.54
Aranae	13.0	10.08	13.0	13.40	1,406.10	8.35	146.99
Diptera	2.0	1.55	2.0	2.06	0.00	0.00	3.20
Blattaria	3.0	2.33	2.0	2.06	0.00	0.00	4.80
Anura	1.0	0.78	1.0	1.03	3,013.00	17.90	19.25

may last several hours before spawning (Mao et al. 2014). In such cases, male forelimbs are larger, heavier, and more resistant to fatigue compared to females (Navas and James 2007; Mao et al. 2014). The longer forelimbs of male *L. chaquensis* seem to support these observations. Although arm diameter was not identified as a dimorphic trait in model selection, arms of males are visually thicker than those of females but only in reproductive individuals, thus exhibiting temporary dimorphism (Wells 2007). Because we used all mature males in our analysis (reproductive and non-reproductive individuals), dimorphism in this variable may have been masked in the model. Indeed, when we included only reproductive individuals in the analysis, arm diameter

was the most important variable explaining shape dimorphism (results not shown).

The longer tibia of females in comparison to males has also been reported for other anuran species (Zhi-hua and Xiang 2005; Mao et al. 2014; Neves et al. 2014). However, females were larger than males in these previous studies and such differences were possibly related to gravid females needing stronger propulsion. Parental care behavior by females may explain the difference in tibia length between sexes in *L. chaquensis*. For example, pumping, channel-digging, and defensive tadpole school behavior have been reported for other species in the *L. latrans* group (Wells and Bard 1988; Heyer and Giaretta 2009; Rodrigues

TABLE 5. Call parameters of Cei's White-lipped Frog (*Leptodactylus chaquensis*), Macaíba Municipality, Rio Grande do Norte State, Brazil, in comparison to *Leptodactylus chaquensis* (*sensu* de Sá et al. 2014) from Argentina (de Sá et al. 2014), and Minas Gerais, Brazil (Heyer and Giaretta 2009). Abbreviations are n = number of notes analysed, ND = note duration, P/N = pulse/note, PD = pulse duration, PR = pulse rate, and DF = dominant frequency.

Location	Call	ND (s)	P/N	PD (s)	PR (pulses/s)	DF (Hz)
Macaíba	Growl (n = 63)	0.43 ± 0.07 (0.3–0.57)	22 ± 2.4 (16–27)	0.015 ± 0.003 (0.01–0.02)	52 ± 5.4 (39–66)	402 ± 82 (345–517)
	Grunt (n = 37)	0.074 ± 0.010 (0.05–0.09)	7.6 ± 1.0 (5–9)	0.003 ± 0.0009 (0.003–0.007)	103 ± 7.2 (90.9–118)	393 ± 43 (344–431)
	Trill (n = 11)	0.46 ± 0.14 (0.3–0.68)	12 ± 4.0 (6–17)	0.02 ± 0.06 (0.07–0.03)	25.3 ± 3 (22–31)	611 ± 84 (517–689)
Argentina	Growl	0.41–0.66	16–30	–	46–49	343–515
	Grunt	0.10–0.12	8–10	–	71–100	263–343
	Trill	0.48–0.81	11–16	–	–	424–520
Brazil	Growl	0.41–0.66	25.8 ± 3.3 (21–30)	0.016 ± 0.003 (0.01–0.02)	47.5 ± 1.6 (46–49)	345 ± 2 (343–348)
	Grunt	0.11 ± 0.01 (0.10–0.12)	9.2 ± 0.8 (8–10)	0.011 ± 0.002 (0.005–0.014)	82.8 ± 10.7 (71–100)	291 ± 40 (263–343)
	Trill	0.62 ± 0.03 (0.60–0.66)	15 ± 0.9 (14–16)	0.029 ± 0.007 (0.011–0.042)	23.5 ± 1.9 (21–26)	460 ± 41 (428–514)

et al. 2011). Because there is no previous evidence of male parental care in this species group, a longer tibia may enhance female success in defending the shoal (stronger propulsion to combat predators or for digging channels faster). However, research focusing on sexual dimorphism of hind limb muscles and performance are needed to test this hypothesis.

Despite the complex acoustic repertoire emitted by male *L. chaquensis*, the importance of each of these calls in intrasexual and intersexual communication is unknown. Playback and choice tests in species of the *L. latrans* species group are needed to clarify their roles (Heyer and Giaretta 2009). Growl and grunt calls had similar dominant frequencies, while the trill call was about 100 Hz higher. This same pattern was observed for populations of *L. chaquensis* from Minas Gerais, Brazil (Heyer and Giaretta 2009) and Argentina (de Sá et al. 2014). Moreover, the acoustic parameters of the three call types were similar between populations of *L. chaquensis* from Minas Gerais and Argentina and the population of *L. chaquensis* studied here (Table 5). The advertisement call has been used as an important taxonomic tool to diagnose morphologically similar species because it is considered a strong premating isolating mechanism (Duellman and Trueb 1986; Gerhardt 1994; Wells 2007). Based solely on acoustic parameters measured here, our population of uncertain taxonomic status is clearly associated with *L. chaquensis*. Nunes and Juncá (2006) described the call of a population of frogs belonging to the *L. latrans* group from Feira de Santana municipality, Bahia State, Brazil. Nevertheless, the shorter note duration (range = 0.28–0.39s) and lower number of pulses/note (range = 9–12) distinguishes this call from the previously mentioned populations of *L. chaquensis* (Table 5) and our study population, indicating that at least two distinct species of the *L. latrans* group occur in northeastern Brazil.

The diets of *L. latrans* and *L. chaquensis* are well documented and are relatively similar to the diet we report (e.g., Strüssmann et al. 1984; Teixeira and Vrcibradic 2003; Maneyro et al. 2004); however, the relative importance of prey types may vary regionally (see Solé et al. 2009; Cossovich et al. 2011). The diet of *L. chaquensis* was composed of many invertebrate groups (mainly Formicidae and Coleoptera) and one frog species, characterizing it as a dietary generalist. Although frogs (adult and tadpoles) can constitute the diet of large *Leptodactylus* species, their frequency is usually low (see França et al. 2004; Sanabria et al. 2005; Solé et al. 2009; Mendes et al. 2012).

We found no appreciable differences in natural history traits (e.g., calls, SSD, and diet) in *L. chaquensis* compared to other species of the *L. latrans* group distributed along the open formations in South America (e.g., Chaco, Cerrado, and Caatinga). However, more studies are necessary to determine if and how these traits vary among species and populations. The sharp climatic differences among the open biomes of South America have been implicated in driving differentiation of the local herpetofauna (Oliveira et al. 2015). Ecological, physiological, and behavioral traits likely respond to the marked differences in rainfall and seasonality along the Chaco-Cerrado-Caatinga open diagonal. Long-term studies that address other characters, such as larval development, ecological specialization, growth rates, and demographic characters are needed to better describe and compare populations across the region.

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