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Complex acoustic signals in *Crossodactylodes* (Leptodactylidae, Paratelmatobiinae): a frog genus historically regarded as voiceless

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ABSTRACT

Vocal sounds occur in most anurans and are often emitted as simple and stereotyped acoustic signals. Some frog groups, however, have complex signals and others can produce distinctive acoustic structures, such as purely ultrasonic calls. *Crossodactylodes* is a genus of bromeligenous frogs that is understudied in many aspects. This genus has been historically regarded as voiceless, but recent studies reported briefly on vocal sounds in two species. Here, we provide the first quantitative description of vocalisations of *Crossodactylodes* frogs and describe the vocal repertoires of three species. Vocalisations are formed of up to three call types, reported herein as creaking, chirp and squeak calls. We discuss the major call patterns and the repertoire of *Crossodactylodes*. We also discuss the evolutionary and functional implications of the low-intensity calls produced at the water–air interface inside bromeliads. The absence of some morphological structures normally involved in sound reception (elements of the middle ear) in *Crossodactylodes* frogs indicates that extratympanic pathways might be the main auditory route in these highly specialised leptodactylids.

Introduction

Acoustic communication is widespread across vertebrates (Bradbury and Vehrencamp 1998). In anurans, acoustic signals are crucial for mate choice and play a prominent role in coordinating distinct behaviours such as mating, maintenance of territories, and anti-predator mechanisms (Ryan 2001; Gerhardt and Huber 2002; Ferreira et al. 2019a). Although anurans can have complex repertoires, most species generally produce stereotyped and simple advertisement calls attributed to a dual role in reproduction and aggression (Wells 2007). Other species, however, are regarded as voiceless and intraspecific communication can be entirely mediated by, for example, visual and chemical cues

(Hödl and Amézquita 2001; Lee et al. 2002; Byrne and Keogh 2007). Although less common, reduced vocal behaviour also occurs across major anuran groups. Tailed frogs (*Ascaphus*) were reported to sometimes produce faint sounds (Stephenson and Verrell 2003). New Zealand frogs (*Leiopelma*) and toads of the Californian desert (*Anaxyrus exsul* and *A. nelsoni*) are able to produce distress, alarm, or release calls, but advertisement calls have never been recorded (Stephenson and Stephenson 1957; Bell 1978; Green 1988; Elliott et al. 2009).

For Neotropical frogs, vocal repertoires have been described in most species of the frog family Leptodactylidae (e.g. De Sá et al. 2014; Carvalho et al. 2020; Hepp and Pombal 2020). Nonetheless, vocal records remain undocumented for paratelmatobiine frogs of the genus *Crossodactylodes*. The genus has been previously reported as voiceless (Peixoto 1983), but acoustic signalling was briefly reported recently for *C. itambe* and *C. izecksohni* in two natural history studies (Barata et al. 2018a; Ferreira et al. 2019b). This frog genus has a series of morphological modifications in its hearing structures, i.e. the loss of the elements constituting the tympanic middle ear (Pereyra et al. 2016; Santos et al. 2020a), as well as the absence of vocal slits (except for *C. bokermanni*; Santos et al. 2020a), which are present in adult males of most vocal frogs in other groups.

The genus *Crossodactylodes* comprises bromeligenous species, with their life cycle completely dependent on the aquatic ecosystem formed by rainwater accumulated between the leaves of the plants (Ferreira et al. 2019b). This genus contains five species (plus three putative new species; sensu Santos et al. 2020b) endemic to the montane Atlantic Forest and *campo rupestre* (i.e. Brazilian rupestrian grasslands; sensu Silveira et al. 2016) ecosystems of eastern Brazil (Santos et al. 2020a). During fieldwork in the Espinhaço Mountain Range and Atlantic Forest of south-eastern Brazil, vocalisations of two species (*C. itambe* and *C. izecksohni*) and a third unnamed species (*Crossodactylodes* sp.) were recorded. Here we provide the first quantitative analysis of *Crossodactylodes* vocalisations. We also comment on the different levels of variability in their vocal repertoires and the structural complexity and distinctiveness of their sounds. Lastly, we discuss the functional and evolutionary implications of such unique vocalisations and calling behaviour in this rarely vocal genus of leptodactylid frogs.

Materials and methods

Study sites and field data collection

Fieldwork was carried out at three study sites in south-eastern Brazil: two sites in the Espinhaço Mountain Range in the state of Minas Gerais and one site in the Atlantic Forest in the state of Espírito Santo. In Minas Gerais, we visited (1) Parque Estadual do Pico do Itambé, the type locality of *C. itambe* 18°23′52.00"S, 43°20′39.00"W; 1,929 m elevation; in all cases datum = WGS84 on 6 March 2018, an open environment with a high density of rupicolous bromeliads; and (2) Parque Estadual da Serra Negra (18°00′39.19"S, 42°44′30.34"W; 1,556 m elevation) from 28 February to 4 March 2018, a forest patch formed by low trees sparsely distributed and the presence of many ground and epiphytic bromeliads. Both sites are located within a unique mountaintop ecosystem (*campo rupestre*) characterised by a grassy-shrubby vegetation mosaic associated with rocky outcrops and sandy, stony, and waterlogged grasslands (Silveira et al. 2016). In

Espírito Santo, we visited a private property in the municipality of Santa Teresa (19° 51′54.98"S, 40°34′41.26"W; 922 m elevation), near the Reserva Biológica Augusto Ruschi, and on the outskirts of the type locality of *C. izecksohni*, from 22 November 2016 to 19 December 2016. This site has a high density of ground and epiphytic bromeliads on a forested mountaintop.

Specimens were euthanised in 5% lidocaine solution, fixed in 10% commercial grade formalin, and preserved in 70% ethanol. Voucher specimens were deposited in the Célio F. B. Haddad Amphibian Collection (CFBH), at São Paulo State University (UNESP), in Rio Claro, São Paulo, Brazil, under the following accession numbers: *C. itambe* (CFBH 44651, 45186–7), *Crossodactylodes* sp. (CFBH 44889–90). The two recorded males of *C. izecksohni* could not be collected because this population was being monitored in a study based on capture/recapture data (Ferreira et al. 2019b). Collected specimens match the diagnoses of the two species (Peixoto 1983; Barata et al. 2013; Santos et al. 2020a). A third species, referred herein to as *Crossodactylodes* sp., was not identified at species level and a recent phylogenetic study reported it as a putative new species equivalent to *Crossodactylodes* sp. 3 of Santos et al. (2020b).

Acoustic analysis

The high variability and complexity observed in the vocalisations and the insufficient behavioural observations prevented us from identifying the roles of the multiple call types composing the repertoire of *Crossodactylodes* (e.g. advertisement, courtship, aggressive; see Wells 2007). For this reason, we named call types using onomatopoeic expressions based on acoustic features that helped researchers to discriminate the distinct calls of *Crossodactylodes* in the field. For this group of frogs, we propose the following distinctive call types: creaking call (a two-component and continuous wood creaking sound; Figures 1 and 2), chirp call (a short, non-pulsed signal; Figure 3(a,b)), and squeak call (a multi-pulsed signal much longer relative to chirps; Figure 3(d,f)). Chirps and squeaks were considered introductory calls because they commonly preceded the main acoustic signal consisting of long emissions of creaking calls. Temporal and frequency patterns of each of these calls are described in the results.

Vocalisations of *C. itambe* and *Crossodactylodes* sp. were recorded with a Marantz PMD 661 digital recorder using a sampling rate of 44.1 kHz and a sample size of 16 bits, and a Sennheiser ME66/K6 unidirectional microphone. The recording input gain was kept unaltered throughout each recording. Recordings were stored as uncompressed wave files. Calls of *C. izecksohni* were recorded using a Tascam DR-40 digital recorder equipped with in-built microphones. Recordings were originally stored in MP3 format. For these, the original files were converted into wave format (same sampling rate and sample size indicated earlier). Call recordings of the three *Crossodactylodes* species were deposited in the new repository of sound files from the CFBH collection (CFBH-voc) under the following accession numbers: CFBH-voc 1–3 (*C. itambe*), CFBH-voc 4–5 (*Crossodactylodes* sp.), and CFBH-voc 6–7 (*C. izecksohni*). Calls were analysed using a customised version of Soundruler 0.9.6.1 (Gridi-Papp 2007) written in Matlab 6.5.2 (Mathworks, Inc.). The fundamental frequency of the analysed call types of *Crossodactylodes* was assessed through the fundamental frequency track tool (function 'fund') using seewave 2.1.0 (Sueur et al. 2008) in R 3.5.0 (R Core Team 2018). Then,
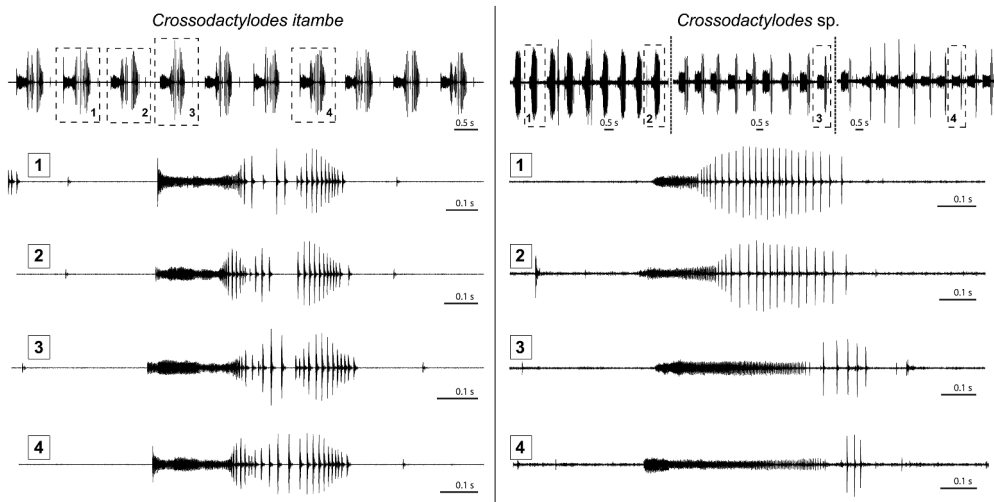
Figure 1. Variation in the temporal structure of creaking calls of *Crossodactylodes itambe* and *Crossodactylodes* sp. produced in sustained calling (over 1-minute continuous calling). A 10-second section of continuous calling of *C. itambe* and three distinct 10-second sections during the sustained calling of *Crossodactylodes* sp. are presented.

a 200-Hz high-pass filter was applied prior to conducting the analysis to reduce the background noise introduced by wind. Acoustic traits were quantified through automated analysis in the time domain from the oscillogram window and in the frequency domain from the spectrogram window. Call repetition rate was quantified manually in Audacity 2.1.1 (Audacity Team 2017). The frequencies of pulse trains were assessed through the instantaneous frequency function (function 'ifreq') using seewave. The frequency of individual pulses was determined by averaging the instantaneous frequency values for each sample within a pulse. The frequency modulation of pulse trains was obtained by subtracting the frequency of the last pulse from that of the first pulse. Quantified acoustic traits essentially followed the definitions and terminology of Carvalho et al. (2019). In addition to these, we estimated the quality factor of the main frequency band of the two components of creaking calls using the 'tuning' ($Q_{20dB-SPL}$) measurement by dividing the peak frequency by the bandwidth at 10% peak amplitude. Data are presented as mean and corresponding standard deviation, and range. In the case of *C. itambe*, for which we recorded creaking calls from more than one male, averaged means and corresponding standard deviations were calculated from individual means of each of the three recorded males. Ranges include the span of values from the raw dataset. The general spectrogram settings were used as follows: fast Fourier transform (FFT) size = 1024 points, FFT overlap = 90%, window type = Hanning, and contrast = 70%. The specific settings for the automated recognition of acoustic signals in Soundruler are provided as part of the Supplementary Material. Sound figures were produced using Soundruler or seewave 2.1.0 (Sueur et al. 2008) and tuneR 1.3.2. (Ligges et al. 2017) in R 3.5.0 (R Core Team 2018) with the following parameters: window type = Hanning, FFT size = 256 or 512 points, and FFT overlap = 90%; the strength of frequency components were indicated by a relative 36-dB colour scale (red = maximum amplitude).
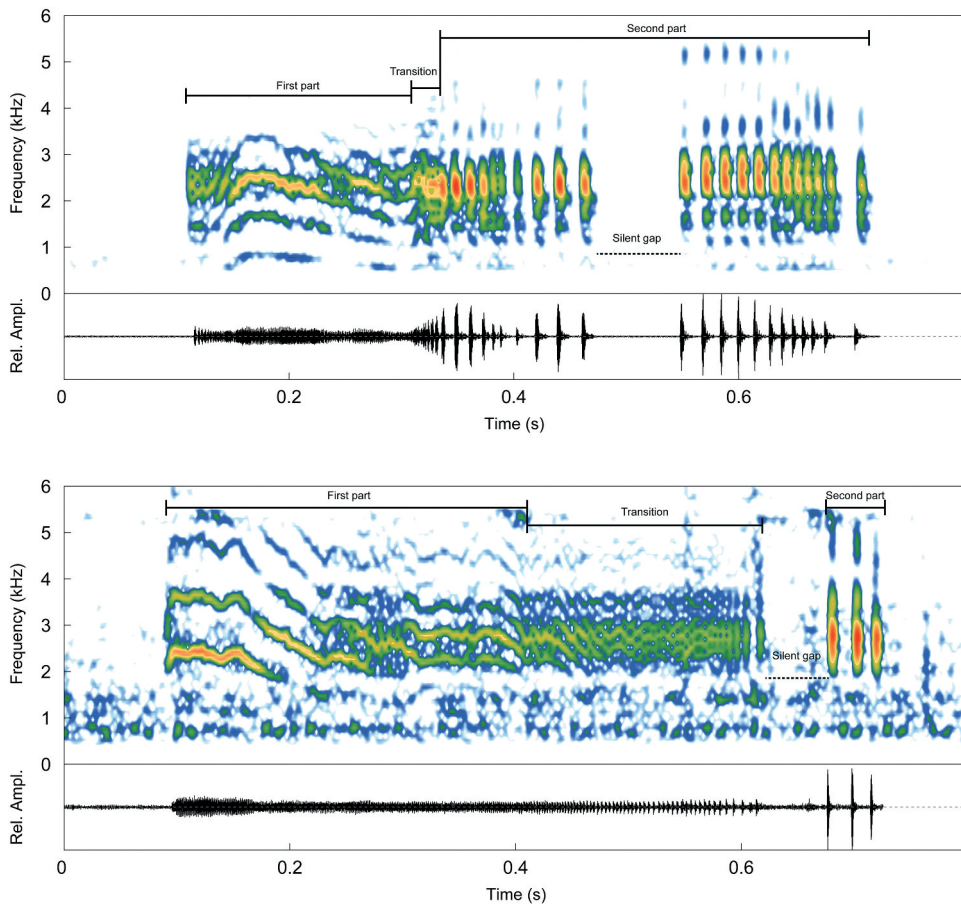
Figure 2. Detail on the spectral and temporal structure of one creaking call of *Crossodactylodes itambe* (above) and *Crossodactylodes* sp. (below) derived from the sustained calling in Figure 1. Spectrograms produced using a FFT size = 512 points.

Results

Vocalisations of the three studied species of *Crossodactylodes* were emitted from inside bromeliads with accumulated rainwater (Figure 4). Direct observations of calling males were rare for the three species. Based on a video recording (see Supplementary Material) and direct observations reported by Ferreira et al. (2019b), calling males of *C. izecksohni* were positioned (1) either on or underneath bromeliad leaves with the body partially underwater and the head above the water surface, (2) completely out of water (but always close to it) sitting on leaves, or (3) completely submerged. For *Crossodactylodes* sp. (present study), calling males had half their bodies in the water with the head held low at water level or, less often, completely out of water. Males of *C. itambe* (three observations reported by Barata et al. 2018a, plus those described in the present study) were only observed calling with the body partially underwater and the head above the water surface.

Crossodactylodes itambe called from inside a single species of rupicolous bromeliad (*Vriesea medusa*) in an open outcrop at Pico do Itambé (Figure 4(a)). *Crossodactylodes*
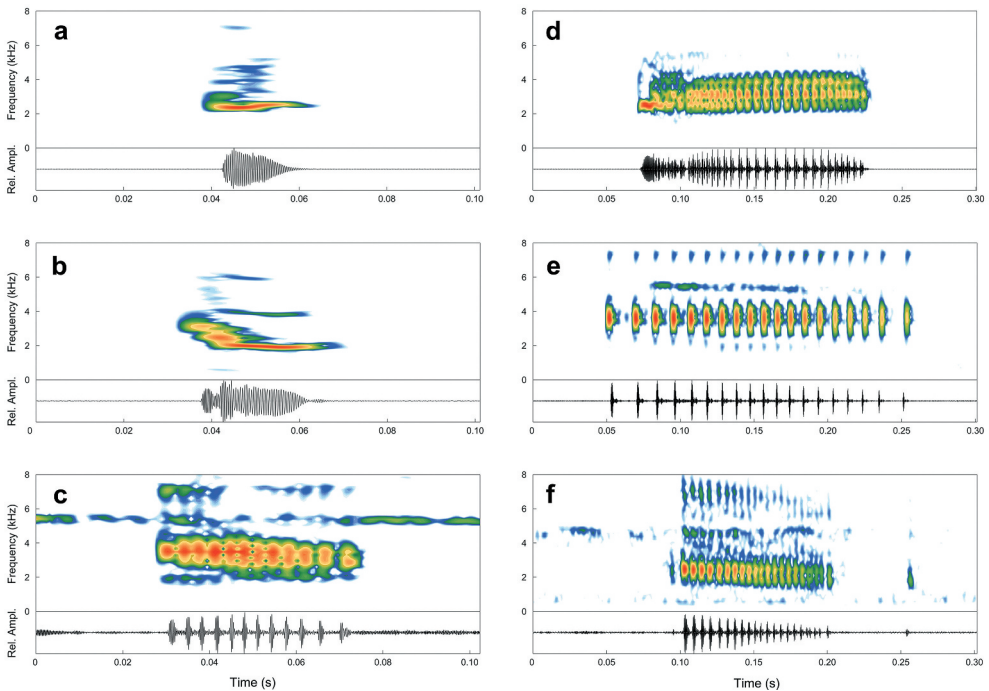
Figure 3. Spectral and temporal structure of chirp calls and squeak calls of the three studied species of *Crossodactylodes*. (a, b) Chirp calls of *C. itambe*, barely frequency modulated and downward frequency modulated, respectively. (c) Short squeak call of *Crossodactylodes* sp. (d, e, f) Squeak calls of *C. itambe*, *Crossodactylodes* sp., and *C. izecksohni*, respectively. Spectrograms produced using a FFT size = 256 points.

izecksohni called from several different bromeliad species (mainly *Aechmea capixabae*, *Neoregelia macrosepala*, *N. pauciflora*, *Nidularium cariacicaensis*, and *Vriesea vagans*) on the ground in a forest patch in Santa Teresa (Figure 4(b)). *Crossodactylodes* sp. called from two bromeliad species (*Vriesea longicaulis* and *V. schwackeana*) on the ground or attached to trees in a forest patch at Serra Negra (Figure 4(c)). When compared to *C. itambe* (three individuals calling on one night in a surveyed area of <0.5 km^2), calling activity was higher at Serra Negra, where *Crossodactylodes* sp. was heard during the evening on a few consecutive days, although calls were given sporadically each evening. Calling activity in *C. izecksohni* has been rarely observed despite the continued monitoring of the Santa Teresa population (Ferreira et al. 2019b). On all occasions, we recorded only one male calling from each plant for the three species.

Among the three studied species, *Crossodactylodes itambe* produced the most complex vocal repertoire, formed by three call types: creaking, chirp, and squeak calls (see Tables 1 and Tables 2 for a quantitative characterisation). The main signal broadcast by the three recorded males of *C. itambe* was the creaking call, which has two components with distinctive temporal and frequency structures that are merged into a single vocal emission (Figure 2). This call is made up of a frequency-modulated and narrowly tuned component (first component; Table 1), followed by a pulsed broad-bandwidth component with higher amplitude that approximates the sound amplitude of squeak calls (second component; Table 1). The dominant frequency of the first component

coincides with that of the third and fourth harmonics, whereas that of the second component coincides with the fundamental harmonic. There might be inharmonic structures on either side of the principal frequency band of the second component (Figure 2(a)).

Unlike chirps and squeaks that were emitted sporadically, creaking calls were emitted in a long-standing series (over 6 minutes of sustained calling) at a relatively fast repetition rate (approximately 60 calls per minute). One male of *C. itambe* emitted introductory calls (preceding the main call type, i.e. creaking calls) which were composed of two main types of signals: chirp calls, consisting of non-pulsed signals with a downward frequency modulation (FM) or without FM (Figure 3(a,b)); and squeak calls, which were pulsed signals about five times longer in duration than the chirps (Figure 3(d)).

The two recorded males of *C. izecksohni* emitted acoustic signals at irregular intervals as isolated calls or in short series of two or three calls. Based on the general patterns described earlier, we assigned all recorded calls to the squeak call type (Table 1, Figure 3(f)). Based on a video file of a third male (Supplementary Material), we also observed a long series of shorter calls with similar structure to regular squeak calls but given at a higher rate – previously assigned to courtship calls by Ferreira et al. (2019b). These vocalisations were elicited by the presence of a female and were emitted shortly before and during amplexus (see Supplementary Material for the video showing the courtship calling and male-female interactions).

Crossodactylodes sp. produced two call types (creaking and squeak calls). One of the two recorded males produced a long series of creaking calls (Figure 1). The other male was only recorded after a conspecific female had been experimentally placed inside the same bromeliad. This male emitted pulsed-only calls that were similar to squeak calls but had varying durations (Table 1, Figure 3(c,e)). A short series of creaking calls was also recorded from this second male, but it could not be analysed quantitatively because of low signal-to-noise ratio.

Overall, similar temporal and frequency patterns were documented in the three species, but some differences in quantitative traits were detected (Table 1). In general, creaking calls did not differ much between *C. itambe* and *Crossodactylodes* sp. (Figure 1). This call type was not recorded for *C. izecksohni*. The main sources of variation in creaking calls are the relative duration of each of the two parts of the calls, as well as the pulse rate and the presence of a silent gap in the second part of the calls (Table 1, Figures 1 and 2). High variation is also observed in the temporal structure of creaking calls, especially in the transition between the first and second parts of calls, which can be abrupt or gradual (Figure 2). In a few cases, creaking calls of *C. itambe* have an amplitude peak at the very onset (rise time around 2% of call duration), as opposed to most calls which have an amplitude peak coinciding with the pulsed part of the call (i.e. second component). Additionally, the temporal organisation of pulses greatly varied throughout sustained calling, in some cases forming pulse groups separated by noticeable silent gaps or being homogeneously produced throughout the pulsed part of call. This was not observed in calls of *Crossodactylodes* sp., whose creaking calls exhibited a continuously varying temporal structure during the sustained calling. For the latter species, the relative duration of the first and second parts of calls varied from a predominantly pulsed component, intermediate non-pulsed and pulsed components with similar durations, to a predominantly non-pulsed component ending with a few pulses (see Figure 1).

Figure 4. Calling and breeding habitat of the bromeligenous frogs of the genus *Crossodactylodes*. In-situ photograph of a male of (a) *C. itambe* in a *Vriesea medusa* bromeliad at Pico do Itambé, its type locality, (b) *C. izecksohni* in an *Aechmea capixabae* bromeliad in the municipality of Santa Teresa, and (c) Detail of the vegetation at Parque Estadual da Serra Negra, the only area where *Crossodactylodes* sp. is known to occur. Credits of Figure 4(a): Leandro Drummond.

Squeak calls were the only call type analysed for all three species (Figure 3(d–f)) and varied in almost all acoustic variables among species (Table 2). A particular type of variation was observed in the spectral structure of squeak calls. The onset of some calls had a tonal component (Figure 3(d)). Despite having the longest squeak calls, one of the two recorded males of *Crossodactylodes* sp. emitted unusually short calls twice (referred as 'atypical squeaks' in Table 2). The atypical squeak calls were interspersed between regular squeak calls and had a higher pulse rate (Table 2; Figure 3(c)).

Discussion

Multi-component acoustic signalling

Other paratelmatobiine frogs have vocal repertoires composed of two or more call types (e.g. Juncá and Lugli 2009; Giaretta and Magrini 2013; Santos et al. 2019), but

Table 1. Quantitative description of the creaking call of two *Crossodactylodes* species. Data are reported as mean ± standard deviation (range). N = recorded males/quantified calls.

Call type/traits	*C. itambe* N = 3/54	*Crossodactylodes* sp. N = 1/22
Creaking call (two-component call)		
Duration (ms)	577 ± 47 (469–662)	554 ± 50 (480–640)
Relative rise time (%)	52 ± 40 (2–93)	64 ± 12 (44–86)
Call rate (calls per minute)	60 ± 4 (56–63)	54
Series duration (s)	186 ± 153 (88–362)	83
Dominant frequency (Hz)	2415 ± 166 (2261–2649)	2705 ± 80 (2476–2778)
Creaking calls (1st component)		
Duration (ms)	210 ± 33 (173–235)	202 ± 89 (118–390)
Dominant frequency (Hz)	2193 ± 207 (1954–2317)	2573 ± 204 (2218–2907)
Frequency modulation (Hz)	−40 ± 121 (−156–86)	−189 ± 283 (−603–258)
Tuning (unitless)	3.93 ± 1.44 (2.68–5.51)	10.54 ± 5.00 (3.01–17.52)
Creaking calls (2st component)		
Duration (ms)	317 ± 39 (273–347)	263 ± 119 (91–395)
Pulse number	15 ± 2 (13–18)	14 ± 9 (4–25)
Pulse rate (pulses per second)	46 ± 7 (38–50)	45 ± 14 (31–66)
Pulse duration (ms)	7 ± 1 (6–8)	4 ± 1 (3–4)
Pulse interval (ms)	16 ± 3 (14–20)	21 ± 8 (11–29)
Pulse dominant frequency (Hz)	2409 ± 127 (2325–2556)	2735 ± 48 (2660–2793)
Frequency modulation (Hz)	39 ± 115 (−65–163)	43 ± 46 (0–129)
Tuning (unitless)	2.42 ± 0.39 (1.98–2.68)	1.92 ± 0.25 (1.60–2.24)

Table 2. Quantitative description of the squeak and chirp calls of three *Crossodactylodes* species. Data are reported as mean ± standard deviation (range). N = recorded males/quantified calls. *In many cases, squeak calls have incomplete pulses (i.e. pulse interval absent).

Call type/traits	*C. itambe*	*C. izecksohni*	*Crossodactylodes* sp.
Squeak call	N = 1/2	N = 2/8	N = 1/12
Duration (ms)	143 ± 4 (140–145)	109 ± 10 (61–163)	239 ± 43 (185–331)
Relative rise time (%)	69 ± 3 (67–71)	45 ± 2 (7–65)	19 ± 5 (7–35)
Pulse number	31 ± 1 (30–32)	15 ± 1 (10–23)	24 ± 5 (17–32)
Pulse rate (pulses per second)	229 ± 7 (224–234)	141 ± 9 (95–224)	100 ± 10 (88–115)
Pulse duration (ms)	4 ± 1 (3–4)	7 ± 1 (3–9)	3 ± 1 (2–3)
Pulse interval (ms)*	2 ± 1 (1–2)*	2 ± 1 (1–4)*	7 ± 1 (5–8)
Pulse dominant frequency (Hz)	3019 ± 80 (2800–3092)	2536 ± 112 (2387–2638)	3641 ± 54 (3580–3685)
Frequency modulation (Hz)	288	−473 ± 91 (−584 to −387)	92 ± 40 (47–122)
Chirp call	N = 1/7	Not recorded	Not recorded
Duration (ms)	27 ± 10 (16–41)	–	–
Relative rise time (%)	33 ± 17 (16–63)	–	–
Dominant frequency (Hz)	2267 ± 180 (1960–2433)	–	–
Frequency modulation (Hz)	−246 ± 331 (−603–129)	–	–
Atypical (short squeak) call	Not recorded	Not recorded	N = 1/2
Duration (ms)	–	–	37 ± 9 (31–43)
Relative ise time (%)	–	–	37 ± 6 (33–41)
Pulse number	–	–	12 ± 1 (11–12)
Pulse rate (pulses per second)	–	–	326 ± 64 (281–372)
Pulse duration (ms)	–	–	3 ± 1 (2–3)
Pulse interval (ms)*	–	–	1 ± 1 (1–2)*
Pulse dominant frequency (Hz)	–	–	3449 ± 10 (3442–3456)
Frequency modulation (Hz)	–	–	−376 ± 282 (−575 to −176)

Crossodactylodes is unique in having particularly complex acoustic signals with high variation in their temporal and spectral structures. The main call type broadcast by *Crossodactylodes* species (i.e. creaking call) is a compound signal made up of two distinct acoustic components, even though they are able to produce simpler, one-component

calls. The integration of these components into creaking calls may serve two-fold roles of signalling to male and female conspecifics.

Two-component calls have been shown to play contrasting behavioural roles in the coquí frog, *Eleutherodactylus coqui* (Narins and Capranica 1978) and in the tree frog *Dendrosophus minutus* (Toledo et al. 2015). In male túngara frogs (*Engystomops pustulosus*), however, the facultative addition of a second call type (chuck call) to whine-only calls increases their attractiveness to conspecific females (Ryan and Rand 1990). In addition to compound signals formed by qualitatively distinct components, quantitative variation in acoustic features of calls has also been coupled to behavioural context. Species of various frog families have been shown to produce graded calls by combining advertisement and aggressive signals together with a single acoustic emission (Wells and Schwartz 1984; Toledo et al. 2015). The ability of the North American green tree frog (*Dryophytes cinereus*) to discriminate temporal call traits varying continuously was experimentally demonstrated by Gerhardt (1978). The distinctive vocal repertoire of *Crossodactylodes* species is noteworthy for exhibiting both qualitative (call types with distinct temporal structure) and quantitative (continuously varying components of a compound signal, i.e. creaking calls) complexity. Behavioural experiments could elucidate the roles of each of the signals composing the complex acoustic signals among *Crossodactylodes* frogs.

Rarity of calling activity

Regardless of the extensive monitoring effort applied to two of the three *Crossodactylodes* species in this study (Barata et al. 2017, 2018b; Ferreira et al. 2019b), insufficient records of calling activity hindered our quantitative analysis of some call types and detailed assessment of intraspecific variation. In addition to *Crossodactylodes*, reduced vocal activity has been suggested for additional frog groups with prolonged breeding, such as sooglossid frogs (Nussbaum et al. 1982). In other paratelmatobiine genera, however, some species are explosive breeders and call for a few days throughout the year (e.g. *Paratelmatobius* spp.; Garcia et al. 2009; Santos et al. 2019). Additionally, vocalisations among paratelmatobiine frogs generally have noticeably lower intensity than in other anurans (M.T.T. Santos, personal observation). The genus *Crossodactylodes* represents the most extreme case in Paratelmatobiinae, with vocal activity being observed to date only a couple of times, reinforcing that the species in this genus are rarely vocal (Barata et al. 2018a; Ferreira et al. 2019b; present study).

Vocalisations remain unknown for three other *Crossodactylodes* species (*C. septentrionalis, C. pintoi*, and *C. bokermanni*), which inhabit remote locations that are difficult to survey. *Crossodactylodes bokermanni* is more commonly found in nature than the other two species. Only three adult specimens of *C. septentrionalis* have been collected to date and *C. pintoi* was last observed in 1909 (Santos et al. 2020a). Calling behaviour is therefore rare or difficult to observe for the entire genus. The behavioural and ecological drivers for male frogs of *Crossodactylodes* to vocalise remain unclear, although the presence of females appears to have contributed to male calling bursts in the three studied species (Ferreira et al. 2019b; present study).

A reduction in vocal signalling in frogs has been linked to noise, such as that generated in a crowded acoustic space or by flowing water (review in Schwartz and Bee 2013). These

examples are unlikely to apply, however, to the peculiar mountaintop habitats that *Crossodactylodes* frogs inhabit. The genus is composed exclusively of bromeligenous species and, as such, vocal activity has always been observed from inside bromeliads in open areas on outcrops or inside montane forest patches. Few other anuran species co-occur with *Crossodactylodes* in these peculiar habitats and interference of heterospecific calls should be low or absent, given that their call dominant frequencies differ from those of *Crossodactylodes* and the absence of noisy choruses. In addition, there are almost no sources of abiotic noise that could result in acoustic interference (e.g. wide-band river noise). However, calls of *Crossodactylodes* might be subject to interference from wind, mainly in open-area habitats, even though the three call types analysed here for *Crossodactylodes* are relatively high-pitched (emphasised frequencies higher than 2 kHz). All three call types recorded from *Crossodactylodes* frogs are low-intensity calls and hard to detect even from a close range (<1 m away from calling males; authors' personal observation). This might suggest a constraint related to vocal communication in *Crossodactylodes*, whose species produce particularly weak acoustic signals and could be limited to short-range interactions (Boistel et al. 2011; Womack et al. 2017).

It is possible that *Crossodactylodes* species use visual or chemical signals for intraspecific communication. One of the recorded males of *Crossodactylodes* sp. began to call shortly after a conspecific female was placed inside that male's bromeliad, indicating that visual cues can trigger vocal behaviour in male frogs of this genus. Regarding chemical cues, these frogs are strictly associated with water accumulated inside bromeliads through which chemical signalling may take place. Despite being understudied, there is evidence that the biochemical composition in the skin of other leptodactylids can mediate intraspecific interactions (Cei et al. 1967; King et al. 2005).

Functional and evolutionary implications

The acquisition of acoustic data for the remaining *Crossodactylodes* species may have further evolutionary implications, especially for *C. bokermanni*. This is the only species in the genus with vocal slits and internal vocal sac (Santos et al. 2020a), which could imply that the vocalisations of *C. bokermanni* have a higher sound intensity and different signal envelopes. Data on this species could help close the current gap in our understanding of acoustic communication in the genus, as *C. bokermanni* represents an intermediary between its congeners (i.e. bromeligenous species without vocal slits and vocal sac) and members of the other Paratelmatobiinae genera (i.e. non-phytotelm species, with or without vocal slits and vocal sac). According to morphological studies of Paratelmatobiinae (Pombal and Haddad 1999; Santos et al. 2019, 2020a), vocal slits are absent in three of the seven species of *Paratelmatobius* (*P. lutzii, P. gaigeae* and *P. poecilogaster*). Due to the uncertain phylogenetic position of *P. lutzii*, the evolution of this character remains to be addressed. The low intensity of sound output and radiation of sound frequencies in paratelmatobiine frogs, notably in *Crossodactylodes*, seems to be related to the loss of vocal slits and vocal sac. The lack of these structures, directly related to sound production and radiation in the vast majority of anurans, does not prevent the production of vocal sounds (e.g. Nunes-de-almeida et al. 2016; Preininger et al. 2016; this study). However, the low intensity of acoustic signals, especially in earless species, suggests a limited range over which acoustic communication could occur (Boistel et al. 2011; Womack et al. 2017). Future research on the evolution of vocal

communication in Paratelmatobiinae should test for correlations between their call structure (e.g. sound intensity) and morphology (e.g. development of vocal sac) with the recurrent loss of vocal slits in the two genera (*Crossodactylodes* and *Paratelmatobius*).

The low-intensity sounds produced by *Crossodactylodes* species may be explained by their use of the environment for signal transmission. There are many examples of frog groups using calling sites other than bromeliads as amplifiers and resonators. Frogs of the genera *Neobatrachus* (former *Heleioporus*) in Australia and *Eupsophus* in Chile benefit from sound amplification by calling from burrows (Bailey and Roberts 1981; Penna and Solís 1996). In fact, the amplification by burrows favours signal detection by nearby conspecifics (Muñoz and Penna 2016) and influences the choice of specific calling sites that can be recognised by females (Cui et al. 2012). Calling males of the Bornean tree-hole frog (*Metaphrynella sundana*) explore tree cavities by adjusting their call frequency to the resonant frequency of the hole, which varies with the amount of water retained in it (Lardner and Bin Lakim 2002).

Acoustic signals of *Crossodactylodes* frogs are possibly radiated both into aerial and aquatic media because they can call either when completely submerged, with their bodies partially underwater but their heads above the water surface, or completely out of the water. Furthermore, vibrational signals travelling through a bromeliad leaf were demonstrated to modulate calling behaviour in the golden rocket frog (Narins et al. 2018) and could also serve as a venue for signal transmission in *Crossodactylodes*. The 3D structure of the bromeliad may influence airborne sound in ways that the frogs could potentially exploit. Females may therefore receive the male signals from the air, the water or the leaves of the bromeliad. Interestingly, this frog genus lacks all elements of the tympanic middle ear (Pereyra et al. 2016; Santos et al. 2020a). These structures play a fundamental role in transmitting acoustic signals from the environment to the inner ear in anurans that hear through their eardrums (Smotherman and Narins 2004), but they would not be necessary if the sounds produced by *Crossodactylodes* could be detected through extratympanic pathways (Hetherington 2001). In this scenario, underwater sounds could cross the head tissues when the animal is sub-merged and reach the inner ear even in the absence of the elements of the middle ear (see Gridi-Papp and Narins 2008 for a review of sensory ecology of hearing in vertebrates). Various other mechanisms of sound reception have been identified in amphibians, such as the detection of ground vibrations through the opercularis system, and airborne sound through the body wall in the lungs or via bone-based pathway (Hetherington 2001; Mason 2007; Boistel et al. 2013). Some of these could have a role in sound transmission in *Crossodactylodes* frogs, which transit between the aerial and aquatic media supported by bromeliad leaves while vocalising. The various potential routes of signal transmission available to *Crossodactylodes* frogs and their hearing specialisations indicate that, although soft and rare, their calls may enhance their breeding success and promote adaptations.

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Ethical statement

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