A new account for the endangered Cerrado Rocket Frog *Allobates* goianus (Bokermann, 1975) (Anura: Aromobatidae), with comments on taxonomy and conservation

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Submitted on 2015, 11th November; revised on 2016, 23rd March; accepted on 2016, 26th March Editor: Paolo Casale

Abstract. We report a new distributional record of *Allobates goianus* in Niquelândia (Goiás State, Central Brazil), and its rediscovery in Minaçu since the creation of the Serra da Mesa dam lake in 1997. Moreover, we describe the species advertisement call from Niquelândia, reporting on a new calling emission pattern for the species, plus we provide the first comprehensive definition, diagnosis, and interspecific comparisons of *A. goianus* based on our two newly collected additional specimens since its original description in the mid-1970s. *Allobates goianus* is compared with the 22 *Allobates* species known to occur in Brazil due to geographic proximity. Morphological, color, and acoustic traits diagnose *A. goianus* from all analyzed congeners, except *A. brunneus* and *A. olfersioides*. Our study draws attention to the importance of rediscovering *A. goianus* at the type locality (Chapada dos Veadeiros) aiming to properly address the species limits of the three Brazilian *Allobates* species (*A. brunneus*, *A. goianus*, and *A. olfersioides*) that are indistinguishable with regard to phenotypic traits. The conservation status of *A. goianus*, endemic to the Cerrado of Central Brazil, is discussed as its natural habitats are under increased pressure due to habitat fragmentation in the Cerrado.

Keywords. Advertisement call, Allobates brunneus, Allobates olfersioides, habitat fragmentation, threatened species, type locality.

INTRODUCTION

The genus *Allobates* currently comprises 51 species distributed along South America on both sides of the Andes, and Central America (Grant et al., 2006; Frost, 2015). *Allobates goianus* (Bokermann, 1975) was described from Central Brazil (Alto Paraíso de Goiás, Chapada dos Veadeiros, Goiás State) and is regarded as a Cerrado endemic species. The species was recently reported as endangered according to the latest Brazilian list of threatened species (ICMBio, 2014), due to habitat loss, the increasing fragmentation of habitats, and restricted extent of occurrence. Besides the type locality, *A. goianus* has been reported from only two additional localities, both in Goiás State: i) Floresta Nacional de Silvânia, a federal protected area (Bastos et al., 2003), from where its advertisement call was recently described by Bastos et al. (2011), and ii) Minaçu (Serra da Mesa region), where it was recorded in 1996 by Brandão and Araújo (2008), before the creation of the Serra da Mesa dam lake, supposedly flooding its habitats according to these authors.

Recent faunal inventories failed to locate *A. goianus*, including in the areas reported here (see Oda et al., 2009;

Morais et al., 2012; Santoro and Brandão, 2014). Furthermore, this species has not been reported from its type locality either since its original description in the mid 1970's (Santoro and Brandão, 2014).

The purposes of this study were to report a new distributional record of *A. goianus* in Central Brazil, based on two male specimens and its rediscovery in Minaçu since the creation of the Serra da Mesa dam lake in 1997, based on a call recording. Furthermore, we describe the advertisement call of *A. goianus* from Niquelândia based on a larger sample size, and report on a pattern of calling emission other than the previously described by Bastos et al. (2011); Also, we provide the first comprehensive definition, diagnosis, and interspecific comparisons of *A. goi anus* based on our two newly collected specimens since its original description by Bokermann (1975).

MATERIALS AND METHODS

Fieldwork was conducted from November to December 2014 in the Serra da Mesa region, municipalities of Niquelândia (14°21'50"S, 48°30'17"W; ca. 710 m a.s.l.) and Minacu (13°45'35"S, 48°18'31"W; ca. 900 m a.s.l.), Goiás State, Central Brazil (Fig. 1). These localities lie 110 km southwest and 95 km northwest from the type locality of A. goianus, respectively. Given that A. goianus is a Brazilian Cerrado endemic species, interspecific comparisons were restricted to Allobates species occurring in Brazil due to geographic proximity, *i.e.* the 18 species listed in the latest Brazilian amphibian list (Segalla et al., 2014): A. brunneus (Cope, 1887), A. caeruleodactylus (Lima and Caldwell, 2001), A. conspicuus (Morales, 2002), A. crombiei (Morales, 2002), A. femoralis (Boulenger, 1884), A. flaviventris Melo-Sampaio, Sousa and Peloso, 2013, A. fuscellus (Morales, 2002), A. gasconi (Morales, 2002), A. grilissimilis Simões, Sturaro, Peloso and Lima, 2013, A. hodli Simões, Lima and Farias, 2010, A. marchesianus (Melin, 1941), A. masniger (Morales, 2002), A. nidicola (Caldwell and Lima, 2003), A. olfersioides (Lutz, 1925), A. paleovarzensis Lima, Caldwell, Biavati and Montanarin, 2010, A. subfolionidificans (Lima, Sanchez and Souza, 2007), A. sumtuosus (Morales, 2002), A. vanzolinius (Morales, 2002), with the addition of A. bacurau Simões, 2016, A. magnussoni Lima, Simões and Kaefer, 2014, A. myersi (Pyburn, 1981), and A. tapajos Lima, Simões and Kaefer, 2015, which were not included in this species list. New specimens are deposited in the Collection of Amphibians of the Museu de Biodiversidade do Cerrado at the Universidade Federal de Uberlândia (AAG-UFU) under the following accession numbers: AAG-UFU 5064-5065. The type series of A. goianus is deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP) under the following accession numbers: MZUSP 76652 (holotype), and MZUSP 73706, 76651 (paratypes).

Morphological and coloration characters follow the diagnostic traits listed in Kok et al. (2013). Definitions and terminology are according to Grant et al. (2006). Snout-vent length (SVL) was measured with digital calipers of 0.01 mm precision and follows Duellman (1970); relative finger lengths follow Grant et al. (2006). Morphological and color traits were assessed under a stereomicroscope; finger lengths were measured with a micrometric piece coupled to a stereomicroscope.

Calls were recorded using a Sennheiser K6/ME66 directional microphone and a M-Audio Microtrack-II digital recorder, and K6/ME67 directional microphones and Marantz PMD 670 or PMD 671 digital recorders. Both recorders were set at a sampling rate of 48 kHz and a sample size of 16 bits. Calls were analyzed with Raven Pro 32-bit 1.5 beta version (Bioacoustics Research Program, 2012) with the following settings: window size = 256 samples; window type = Hann; 3dB filter bandwidth = 270 Hz; overlap = 85%; hop size = 0.79 ms; DFT size = 1024 samples; grid spacing = 46.9 Hz. Temporal traits were measured from the oscillogram and spectral traits from the spectrogram. Dominant frequency was determined from the entire call using the "Peak Frequency" measurement function. Sound figures were generated with Seewave package version 1.7.3 (Sueur et al., 2008) on R platform version 3.1.0 (R Core Team, 2014) with the following settings: window type = Hanning; window length = 512 samples (FFT); overlap = 85%. Definitions of call traits follow those of Cocroft and Ryan (1995) in most cases with terminological modifications in Carvalho et al. (2015). Note rate is expressed in notes/sec, call rate in calls/min. See Appendix 1 for information about sound recordings and voucher specimens.

RESULTS

New distributional record

Our new distributional record of *A. goianus* in Niquelândia is located approximately 110 km southwestward from its type locality, whereas the site where we rediscovered the species in Minaçu is located approximately 95 km northwestward from the type locality (Fig. 1).

Morphology and color patterns

The type series of *A. goianus* is composed of three specimens: the holotype (MZUSP 76652, an adult female with SVL 17.4 mm); and two paratypes (MZUSP 73706, a juvenile with SVL 12.8 mm; and MZUSP 76651, probably a sub-adult with SVL 14.7 mm). Due to poor preservation conditions, we decided not to seek for further evidence for sexing these specimens (vocal slits or internal morphology). Color of the holotype was fainted so that coloration comparisons were restricted to general coloration of dorsum. Dorsal blotch was barely discernible and ill-outlined, but even so this color trait could be associated with a lanceolate or hourglass-shaped blotch.

Morphological features and size of our newly two collected adult male specimens from Niquelândia essentially matched the holotype description of *A. goianus* (Boker-

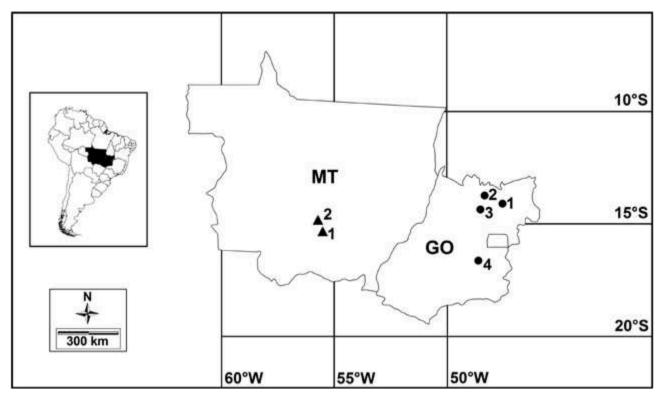


Fig. 1. Distribution map of *Allobates goianus* (circles) and *A. brunneus* (triangles) in the Brazilian Cerrado. <u>Circles</u>: (1) Alto Paraíso de Goiás, Chapada dos Veadeiros (type locality); (2) Minaçu (Brandão and Araújo, 2008; present study); (3) Niquelândia (present study); (4) Silvânia (Bastos et al., 2003); Triangles: (1) Chapada dos Guimarães [collection sites 1–3 in Lima et al. (2009)], presumably the type locality [a site that lies approximately 30 miles northeast from Cuiabá according to the original description: Cope (1887)]; (2) Other record sites in Chapada dos Guimarães [collection sites 4–9 in Lima et al. (2009)]. Brazilian state abbreviations: GO (Goiás), MT (Mato Grosso).

mann, 1975), so we assigned the newly collected specimens to *A. goianus* after the re-examination of the species holotype. Given that holotype's color traits could not be properly assessed, and also taking into account that five out of six diagnostic traits of *A. goianus* are color traits (see in the next paragraph), species definition, diagnosis, and interspecific comparisons were based on the two newly collected adult males from Niquelândia, for which color traits were assessed both in life and in preservative.

Allobates goianus (Figs 2-3) definition and diagnosis: the specimens are assigned to Allobates by the absence of median lingual process, throat collar, and dark dermal collar, and by a diurnal calling activity. The specimens of *A. goianus* can be defined by the following phenotypic traits: (1) SVL, 16.8 mm and 17.0 mm; (2) in life skin on dorsum shagreened to faintly granular posteriorly; (3) annulus tympanicus indistinct posterodorsally; (4) vocal sac distinct, subgular; (5) maxillary teeth absent; (6) distal tubercle on finger IV present; (7) tip of finger IV not reaching distal subarticular tubercle of finger III when fingers are pressed together; (8) relative lengths of fingers I (3.5 mm and 3.5 mm) and II (3.4 mm and 3.4 mm) subequal (finger I 3% longer than finger II, n = 2) in length; (9) finger discs weakly expanded; (10) lateral fringes on fingers absent; (11) metacarpal ridge absent; (12) finger III not swollen; (13) carpal pad absent; (14) thenar tubercle conspicuous, weakly protuberant; (15) tarsal keel tubercle-like and strongly curved at proximal end, extending from metatarsal tubercle; (16) tarsal fringe absent; (17) toe IV disc moderately expanded; (18) basal webbing only between toes II-III, and between toes III-IV; (19) metatarsal fold absent; (20) weak pre- and postaxial fringes on toes II-IV (preaxial ones more conspicuous); (21) dorsal coloration sorrel with a distinctive dark brown hourglass-shaped blotch; (22) dorsolateral stripe absent; (23) oblique lateral stripe as a diffuse broad area, extending from groin to about mid-body length, including light-colored, small spots, arranged irregularly; (24) ventrolateral stripe present, whitish, with no distinctive dark blotches ventrolaterally; (25) paracloacal marks present, light yellow; (26) throat coloration yellow in life, cream in preservation; (27) belly coloration cream to yellow in life, and cream in preservation; (28) iris metallic gold; (29) dorsal thigh color pattern brown with dark



Fig. 2. Two adult males of *Allobates goianus* from Niquelândia (Goiás State, Central Brazil). Above: AAG-UFU 5064 (SVL 17.0 mm); below: AAG-UFU 5065 (SVL 16.8 mm).

brown bands/blotches. *Allobates goianus* is diagnosable from all compared species by the combination of character states 12, 21, 22, 24, 26, 27.

Comparison with Brazilian congeners

Allobates goianus (character states of comparative species are between parentheses) can be distinguished from *A. bacurau* by having an hourglass-shaped blotch (absent; Simões et al., 2016); from *A. caeruleodactylus* by having fingers and toe discs dark-colored with white speckles (blue-colored; Lima and Caldwell, 2001); from *A. conspicuus*, *A. masniger*, *A. paleovarzensis*, and *A. tapajos* by lacking dorsolateral stripe (present; Morales, 2002; Lima et al., 2010; Lima et al., 2015); from *A. subfolionidificans* by having ventrolateral stripe (absent; Lima

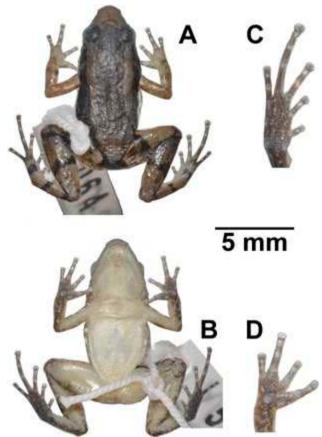


Fig. 3. Dorsal (A) and ventral (B) views, sole of foot (C) and palm of hand (D) of an adult male of *Allobates goianus* from Niquelândia, Central Brazil: AAG-UFU 5064 (SVL 17.0 mm).

et al., 2007); males of A. goianus can be distinguished from those of A. crombiei (transparent whitish throat; Lima et al., 2012), A. flaviventris (gray or violet gray throat; Melo-Sampaio et al., 2013), A. grillisimilis (white to translucent throat; Simões et al., 2013b), A. magnussoni (grayish violet throat; Lima et al., 2014), A. marchesianus (gray throat; Caldwell et al., 2002), A. nidicola (black to light gray throat; Caldwell and Lima, 2003), and A. sumtuosus (white to light gray throat; Simões et al., 2013a) by having a yellow throat; from A. fuscellus, A. gasconi, A. sumtuosus and A. vanzolinius by having finger III not swollen (finger III swollen; Morales, 2002); from A. femoralis (two morphotypes with reddish or black and white ventral coloration; Simões et al., 2008) and A. hodli (black, white, and reddish-orange ventral coloration; Simões et al., 2010) by having a cream to yellow ventral coloration. No morphological and color trait can unambiguously distinguish A. goianus from A. olfersioides (Verdade and Rodrigues, 2007) and A. brunneus (Lima et al., 2009).

	Allobates goianus		
	Niquelândia n = 4 (81/82)	Minaçu n = 1 (3/9)	
Call duration (sec)	3.9±0.8 (0.3-11.8)	2.6±0.4 (2.1-2.9)	
Call interval (sec)	2.8±1.7 (0.4-15.3)	7.7±2.5 (5.9-9.4)	
Calls/min	8.9±1.8 (7.3-11.9)	5.9	
Notes per call	14.4±2.7 (2-41)	8.0±1.0 (7-9)	
Notes/sec	3.5±0.1 (3.1-3.9)	2.8±0.2 (2.5-2.9)	
Note duration (msec)	40.0±2.8 (30-51)	38.4±2.4 (36-43)	
Note interval (msec)	261.0±8.7 (203-483)	308.5±118.6 (239-599)	
Fundamental frequency (Hz)	2630.1±56.1 (2531-2766)	2554.7±25.1 (2531-2578)	
Dominant frequency (HZ)	5230.7±184.7 (4922-5578)	5085.9±100.2 (4922-5156)	
Air temperature (°C)	23.0-26.7	25.0	

 Table 1. Advertisement call traits of Allobates goianus from the municipalities of Niquelândia and Minaçu, Goiás State, Central Brazil. n = number of recorded males (analyzed calls / analyzed notes). mean ± SD (min-max).

Advertisement call of Allobates goianus from Niquelândia.

Vocalization of A. goianus (n = 4 males; Table 1)consists of a multi-note call (Fig. 4A-B) composed of 2-41 notes (mean = 14.4, SD = 2.7), with duration varying from 0.3-11.8 sec (mean = 3.9, SD = 0.8), separated by intervals of 0.4-15.3 sec (mean = 2.8, SD = 1.7), and emitted at rates of 7.3-11.9 calls per minute (mean = 8.9, SD = 1.8). A few isolated notes (n = 4 in our sample) are emitted between typical multi-note calls. Notes consist of one type of short, non-pulsed signal with ascendant frequency modulation, and may also have irregular amplitude modulations (Fig. 4C). Notes last from 30-51 msec (mean = 40.0, SD = 2.8), separated by intervals of 203-483 msec (mean = 261.0, SD = 8.7), and emitted at rates of 3.1-3.9 notes per second (mean = 3.5, SD = 0.1). The dominant frequency is always within the second harmonic, varying from 4922-5578 Hz (mean = 5230.7, SD = 184.7). Fundamental frequency (first harmonic) peaks from 2531-2766 Hz (mean = 2630.1, SD = 56.1). See Table 1 and Fig. 5 for comparative acoustic traits for the male recorded in Minacu.

Comparison with advertisement calls of Brazilian congeners

The call of *A. goianus* can be distinguished from those of *A. caeruleodactylus* (x = 62 msec; Lima and Caldwell, 2001) and *A. magnussoni* (65-104 msec; Lima et al., 2014) by a shorter note duration (30-51 msec); from those of *A. crombiei* (45-69 msec; Lima et al., 2012) and *A. marchesianus* (119-212 msec; Caldwell et al., 2002) by a longer note interval (203-483 msec), and from that of *A. subfo*-

lionidificans (550-860 msec; Lima et al., 2007) by a shorter note interval (203-483 msec); from those of A. femoralis (2-note or 4-note call patterns; Simões et al., 2008), A. hodli (calls formed by two whistle-like notes; Simões et al., 2010), and A. tapajos (2-note call pattern; Lima et al., 2015) by having a multi-note call (mean = 14 notes/call, mode = 16); from that of A. *flaviventris* (up to 10-note calls; Melo-Sampaio et al., 2013) by a greater number of notes per call (up to 41-note calls); from those of A. bacurau (5890-6340 Hz; Simões, 2016), A. grillisimilis (5868-6651 Hz; Simões et al., 2013 b), and A. sumtuosus (5609-6549 Hz; Simões et al., 2013 a) by a lower dominant frequency (4922-5578 Hz), and from those of A. masniger (4363-4694 Hz; Tsuji-Nishikido et al., 2012), A. myersi (< 3000 Hz; Simões and Lima, 2011), A. nidicola (3896-4413 Hz; Tsuji-Nishikido et al., 2012), and A. paleovarzensis (4045–5093 Hz, mean = 4534.2; Kaefer and Lima, 2012) by a higher dominant frequency (4922-5578 Hz, mean = 5230.7). No acoustic trait can distinguish A. goianus (Table 1) from A. brunneus (Lima et al., 2009).

Habitat and natural history

Males were observed calling in rainy days on, or under moist leaf litter, or on twigs close to the ground inside riverine forests. In Niquelândia, specimens were found from the border towards the inner gallery forest of approximately 50 m wide, whose abutting areas were already completely altered for pasture. In Minaçu, they were found in a rocky grassland area, calling from inside a forest patch alongside a seasonal streamlet. At both sites, specimens were found calling in mid-afternoon (ca. 16:00 h on), stopping calling activity at sunset.

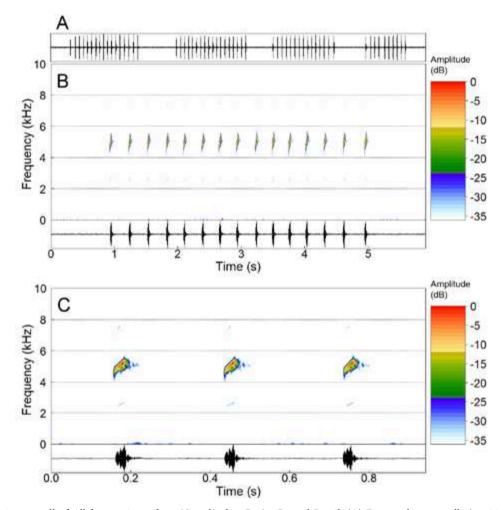


Fig. 4. Advertisement call of *Allobates goianus* from Niquelândia, Goiás, Central Brazil. (A) Four multi-note calls (ca. 24.5 sec); (B) spectrogram and respective oscillogram of the third multi-note call from A; and (C) spectrogram and respective oscillogram of three mid-notes from B. Sound file: Allobates_goianusNiquelandiaGO1cTRC_AAGm (see Appendix 1 for additional information).

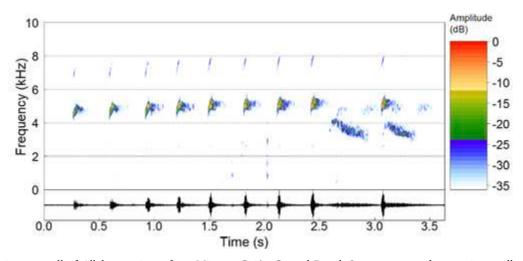


Fig. 5. Advertisement call of *Allobates goianus* from Minaçu, Goiás, Central Brazil. Spectrogram and respective oscillogram of one multi-note call. Sound file: Allobates_goianusMinaçuGO1LBM_AAGm671 (see Appendix 1 for additional information).

DISCUSSION

Our new distributional record of *A. goianus* in Niquelândia was expected by Oda et al. (2009), due to the geographic proximity and similar anuran species compositions between this locality and Minaçu in northern Goiás (Serra da Mesa region), the latter being the locality from where *A. goianus* was recorded before 2001 (Brandão and Araújo, 2008). Our sound recording of the species in Minaçu confirms that the species is still present at that locality.

The first description of the call of A. goianus was based on one male from Silvânia (Bastos et al., 2011) and did not provide a detailed description of the emission pattern, nor any sound figure with a wide time frame (see Fig. 2 in Bastos et al., 2011). Therefore, we could not infer if the emission pattern could be assigned to a multi-note call or a continuous call. In Silvânia, it was rare to find males of A. goianus in calling activity, and the calls had a continuous emission pattern (A. R. Morais pers. comm.). By contrast, the advertisement call of A. goianus from both Niquelândia and Minaçu consists of a multi-note call, and we thus could not compare the call rates among populations. Spectral call traits (fundamental and dominant frequencies, i.e. second harmonic) for the individual from Silvânia had relatively lower values than those from Niquelândia and Minaçu (see Table 1; Bastos et al., 2011), whereas values for note duration (= call in Bastos et al., 2011) from Silvânia completely diverged (65-79 ms) from those from Niquelândia and Minaçu populations (combined value ranges: 30-51 ms). Because of small sample sizes (N < 5 males recorded for each population), we could not investigate whether the between-population differences observed for note duration could be better explained by individual variability or the expected temperature influence on temporal traits of call (see Gerhardt and Huber, 2002).

If *A. goianus* emits its calls in two different emission patterns, it falls under the same pattern that was previously described for *A. brunneus* by Lima et al. (2009), in which conspecific males had both multi-note (trilled) and continuous emission patterns. Additionally, Caldwell et al. (2002) reported that two males of *A. marchesianus* switched from series of notes (= multi-note call) to a continuous call pattern. Variation in note emission patterns has been described for other species of *Allobates* as well (Simões et al., 2008, 2013 a; Simões and Lima, 2011; Lima et al., 2009, 2014). The behavior of switching note emission patterns might convey different messages to conspecific and heterospecific individuals (Amézquita et al., 2006), and ought to be addressed within an experimental framework.

We did not find any straightforward character (size, morphology, color pattern, advertisement call) that could distinguish *A. goianus* from *A. brunneus* (*sensu* Lima et al., 2009) and *A. olfersioides* (*sensu* Verdade and Rodrigues, 2007). *Allobates goianus* (hourglass-shaped blotch) might possibly be differentiated from *A. olfersioides* (single or multiple intercrossing Xs; Verdade and Rodrigues, 2007) by different character states for the dorsal marks. On the other hand, these color patterns may sometimes be so variable in shape, outline, and contrasting background color to the extent that these character states could be hardly discriminated from each other in some cases. So we assume that this color trait might not represent a reliable feature for species discrimination between *A. goianus* and *A. olfersioides*.

It is noteworthy that there is a large gap (ca. 750 km horizontal distance) between the distributional records for the two species (A. brunneus and A. goianus) that occur in the Cerrado of Central and Western Brazil (Fig. 1), and both Cerrado species are completely isolated from A. olfersioides (at least 800 km between the distributional records for A. goianus and A. olfersioides), which is restricted to the Atlantic Forest (see Fig. 1 in Verdade and Rodrigues, 2007). These results shed light on the importance of rediscovering A. goianus at the type locality (Chapada dos Veadeiros), which would allow a formal re-description of the species, as well as a comparative analysis of the three Brazilian species of Allobates without a clear species-level discrimination through phenotypic traits, including a reappraisal of their species limits supplemented by genetic information.

Further studies are still required to assess additional population-level information about *A. goianus*, improving our knowledge on the conservation threats and risks of extinction of its isolated populations in Central Brazil. For instance, the population recorded by us in Niquelândia occurs in an almost completely human-altered area, and such a forest remnant is probably maintained owing to the course of a streamlet inside the forest. Nevertheless, government monitoring and oversight are limited and these areas are not free of threats. Furthermore, despite being in protected areas, populations from Chapada dos Veadeiros and Silvânia also seem to be under threat (Morais et al., 2012; ICMBio, 2014; Santoro and Brandão, 2014) due to habitat fragmentation (ICMBio, 2014).

Therefore, this unfavorable scenario requires immediate attention, especially conservation strategies aiming to the maintenance of the few remaining forested areas and establishment of connections between them in the Brazilian Cerrado, which might help not only this endangered and poorly known *Allobates* species, but also several other forest dwellers inhabiting this region, which are currently under increased pressure mostly due to habitat fragmentation.

ACKNOWLEDGEMENTS

Financial support was provided by CNPq, FAPEMIG, and FAPESP. A research grant conceded by CNPq to AAG. Doctoral fellowships by FAPESP to TRC and CNPq to LBM. Special thanks to Christine Strussmann for making available a comparative recording of *A. brunneus*, Taran Grant for making available type material under his care at Museu de Zoologia da Universidade São Paulo, Alessandro R. Morais for sharing with us relevant field information on *A. goianus*, Leandro Drummond for providing *Allobates* comparative pictures, Philippe Kok and anonymous reviewers for valuable comments on earlier versions of this study. Collection permit was granted by Instituto Chico Mendes through the online platform Sistema de Autorização e Informação em Biodiversidade (ICMBio/SISBIO 30059-5).

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APPENDIX 1

List of sound recordings of *Allobates goianus* from Serra da Mesa Region (Niquelândia and Minaçu, Goiás State: GO) with the respective voucher males (AAG-UFU accession numbers), and additional information.

Locality	Sound recording	Voucher	Time; Date
Niquelândia (GO)	Allobates_goianusNiquelandiaGO1aTRC_AAGm	5064	17:49 h; 19 Dec 2014
-	Allobates_goianusNiquelandiaGO1bTRC_AAGm	5064	17:52 h; 19 Dec 2014
	Allobates_goianusNiquelandiaGO1cTRC_AAGm	5064	17:53 h; 19 Dec 2014
	Allobates_goianusNiquelandiaGO2TRC_AAGm	Uncollected	18:16 h; 19 Dec 2014
	Allobates_goianusNiquelandiaGO3LBM_AAGm671	Uncollected	17:20 h; 20 Dec 2014
	Allobates_goianusNiquelandiaGO4aLBM_AAGm671	5065	17:40 h; 20 Dec 2014
	Allobates_goianusNiquelandiaGO4bLBM_AAGm671	5065	17:41 h; 20 Dec 2014
Minaçu (GO)	Allobates_goianusMinacuGO1LBM_AAGm671	Uncollected	17:15 h; 22 Nov 2014