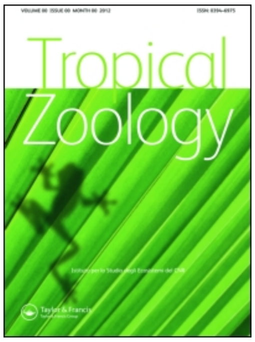

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Ecological niche modeling and new distribution records of the central dwarf frog *Physalaemus centralis* Bokermann, 1962 (Anura, Leptodactylidae, Leiuperinae) with comments on its taxonomic status

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Physalaemus centralis is distributed over open areas of eastern South America. Herein, we perform an extensive review of occurrence records and propose a potential distribution based on ecological niche modeling. In order to confirm species identification, as well taxonomic status along its distribution, we provide morphological and molecular data from analysed specimens. To reach our goals, we compiled data available in the literature and from scientific collections, as well as unpublished data. We identified records of *P. centralis* in 123 localities, 86 of those records remain unpublished. Consequently, it is possible to extend its range of distribution to the state of Rondônia, Brazil, and also to the Caatinga formation. The Maxent algorithm provided a model of potential distribution consistent with the distribution proposed here. Results indicate that *P. centralis* has a large distribution associated to open areas of South America (Cerrado, Caatinga and Pantanal), with no evidence that this is a case of species complex. The multiple approach proposed herein, that combines occurrence data from different sources and molecular data to confirm species identification, provided consistent results. Therefore, we recommend that this framework be used as a protocol to assess species distribution in future studies, especially in the case of widely distributed species.

Keywords: Cerrado domain; distribution; modeling; *Physalaemus cuvieri* group; species complex

Introduction

Brazil shelters the richest amphibian fauna worldwide and over 1000 species have been regarded as valid taxa in the country (Frost 2016), which bears around 15% of the amphibian diversity recorded worldwide so far. Several species have been described in the past years (e.g. Brcko et al. 2013; Magalhães et al. 2014; Peloso et al. 2014). Nonetheless, the number of undescribed species remains high (see Loebmann and Haddad 2010; Fouquet et al. 2012). Keeping record of this surprisingly great amphibian biodiversity while their natural environments suffer with rapid destruction has become a great challenge for taxonomists, who have gathered efforts to discover and describe new species before they disappear. Additionally, the recognition of morphologically cryptic species has pushed

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taxonomy to a more complex level, demanding other sources of evidence beyond morphology. In this sense, molecular techniques have been broadly used to solve taxonomic confusion in many animal groups, including amphibians (e.g. Coloma et al. 2012; Teixeira et al. 2012).

Considering that amphibian biodiversity is not yet completely understood, one could expect that current knowledge about the geographic distribution of many amphibians is also greatly deficient (Vrcibradic et al. 2008), especially in countries with great biodiversity, such as Brazil. In fact, the subject remains so poorly understood that some scientific journals have maintained special sections to address the issue (see *Check List*, *Herpetological Review*, and *Herpetology Notes*).

Geographic distribution maps are often produced through expert-driven criteria (Giovanelli et al. 2010). These maps, also known as “range maps”, are created using locations points from currently known distributions of the target species and aim to provide the limits of distribution (IUCN 2016). In amphibians, the Global Amphibian Assessment range maps are useful to showcase the known distribution of most species. However, points of occurrence of amphibians have been found far from the edges of such range maps in South America (Ficetola et al. 2014).

The use of statistical modeling methods to produce predictive distribution maps from species occurrence and environmental data (Guisan and Thuiller 2005) has increased considerably over the past years. These models are applied to meet several distinct scientific interests, including predicting potential invasion by alien species (e.g. Peterson et al. 2003; Papes and Peterson 2003; Ficetola et al. 2007; Giovanelli, Haddad, et al. 2008), assessing potential geographic distribution of species (e.g. Guisan and Thuiller 2005; Elith et al. 2006; Giovanelli, Araújo, et al. 2008), indicating geographical distribution of species in the past (e.g. Hugall et al. 2002) and providing additional information to plan conservation strategies and select protection areas (e.g. Jeganathan et al. 2004; Young 2007), among other interests.

The neotropical frog genus *Physalaemus* Fitzinger, 1826 is distributed from southern Mexico to northern Argentina (Nascimento et al. 2005), containing 47 recognized species (see Frost 2016). Most species in the *Physalaemus cuvieri* group (*sensu* Lourenço et al. 2015) are widely distributed, except for *P. cuqui*, that has a restricted distribution in northern Argentina and southern Bolivia (Lobo 1993; De La Riva et al. 2000), and *P. erikae*, which occurs only in Atlantic rainforest areas from southern Bahia (Cruz and Pimenta 2004). Four species from this group are distributed in the “dry diagonal” of the neotropics (Werneck 2011): *Physalaemus albifrons* and *P. kroyeri* are both associated to the Brazilian xeric formation called Caatinga, and *P. albonotatus* and *P. centralis* occur in open formations of Cerrado, Pantanal and Chaco ((see distribution details for these species in species in Frost 2016). *Physalaemus cuvieri* is the species with the largest distribution, occurring both in open areas such forest formations in eastern South America, although it has been recognized as a species complex (Lourenço et al. 2015). Herein, we provide information on the taxonomic status of *P. centralis* based on morphological and molecular data, and present an updated distribution map, including a distribution based on ecological niche modeling. The study was motivated by fact that this species’ identification in northern Brazil is unclear, considering that *P. centralis* is morphologically similar to other species from the *Physalaemus cuvieri* group. Therefore, it is possible that *P. centralis* could be a species complex throughout its distribution.

Material and methods

Species identification

To confirm identification of *P. centralis* throughout its distribution, we examined and compared specimens from 21 localities based on external morphological characters. All characteristics matched the original description (Bokermann 1962). Snout–vent length (SVL) was measured in other specimens from the *Physalaemus cuvieri* group once this parameter is useful to distinguish some species in the group. All analysed specimens were preserved and housed in the Célio F. B. Haddad amphibian collection (CFBH) located at UNESP (Departamento de Zoologia, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil), in a museum at UNICAMP (Museu de Zoologia Adão José Cardoso: ZUEC, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil) and in a museum at USP (Museu de Zoologia da Universidade de São Paulo: MUZUSP, São Paulo, Brazil) (Appendix 1).

Additionally, we obtained partial sequences of the cytochrome-c oxidase subunit 1 (COI) gene and the 16S ribosomal RNA (16S) gene from specimens of *P. centralis* collected at 12 localities of six Brazilian states (Appendix 2). In order to quantify inter-species divergence, we included sequences available in GenBank for *P. centralis* and out-group sequences of other closely related *Physalaemus* species in all analyses of COI and 16S (Appendix 2).

Genomic DNA was extracted from ethanol-preserved muscle from each individual using QIAquick DNEasy kits (Qiagen, Hilden, Germany), following the manufacturer's protocol. Fragments were obtained via PCR using primers and conditions described in Lyra et al. (2016). PCR products were submitted to enzymatic purification and sequenced on an automated DNA sequencer (Applied Biosystems ABI 3130XL) at Macrogen Inc. (Seoul, South Korea) and at the Southern China DNA Barcode Center, Kunming Institute of Zoology, Chinese Academy of Science. GenBank accession numbers for each gene fragment are listed in Appendix 2. Gene sequences and metadata were also deposited at the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007) under project code "PCENT".

Sequences were visualized and assembled into contigs using Geneious v6.0 (Biomatters). Sequence alignment was performed with Muscle (Edgar 2004) and verified by eye. Sequence distances were quantified using the uncorrected p-distance and complete deletion of gaps and missing data, and displayed in a neighbour-joining (NJ) tree, using MEGA6 software (Tamura et al. 2013). Node supports were assessed using 1000 bootstrap replicates.

Data from species records and ecological niche modeling development

In order to provide an updated distribution map for *P. centralis,* we compiled data available in the literature and in Brazilian scientific collections, in addition to new records of occurrence presented in this work. Localities of occurrence were obtained from the following scientific collections: CFBH; Coleção de Herpetologia, Universidade de Brasília (CHUNB), Brasília, Brazil; and Coleção de Vertebrados, Universidade Federal do Mato Grosso (UFMT), Cuiabá, Brazil. In addition, we accessed the online database of the species's Link project (2014) and obtained additional records of *P. centralis* from a collection at UNESP (Coleção do Departamento de Zoologia de São José do Rio Preto (DZSJRP), Universidade Estadual Paulista, São José do Rio Preto, Brazil) and from a collection at UNICAMP (Coleção de Anfíbios do Museu de História Natural "Prof. Dr. Adão José Cardoso" (ZUEC-AMP), Universidade Estadual de Campinas, Campinas, Brazil).

Table 1. Mean and standard deviation of snout–vent length for species of *Physalaemus cuvieri* group.

Species	Males	Females	Source
Physalaemus albifrons	28.6 ± 2.0 (*N* = 7)	29.7 ± 4.9 (*N* = 41)	Loebmann (unpub. data)
Physalaemus albonotatus	24.9 ± 1.9 (*N* = 4)	23.5 ± 2.3 (*N* = 41)	Brasileiro and Haddad (2015) and Prado and Haddad (2005)
Physalaemus centralis	36.3 ± 2.4 (*N* = 27)	34.5 ± 2.7 (*N* = 9)	Brasileiro and Martins (2006)
Physalaemus cuqui	30.0 ± 1.2 (*N* = 25)		Lobo (1993)
Physalaemus cuvieri	27.6 ± 3.0 (*N* = 525)	29.5 ± 2.7 (*N* = 680)	Brasileiro et al. (2005)
Physalaemus ephippifer	24.7 ± 1.0 (*N* = 6)	27.5 ± 2.1 (*N* = 2)	Brasileiro and Haddad (2015)
Physalaemus erikae	23.7 ± 2.1 (*N* = 5)	23.1 ± 2.3 (*N* = 13)	Cruz and Pimenta (2004)
Physalaemus fischeri	25.5 ± 2.4 (*N* = 5)		Brasileiro and Haddad (2015)
Physalaemus kroyeri	26.3 ± 2.3 (*N* = 3)		Brasileiro and Haddad (2015)

Species records were used to perform the ecological niche modeling. To check data consistency for the geographical positioning and to construct a map of species distribution, all records of occurrence were computed to DIVA-GIS software, version 7.5.0.0. Ecological niche modeling was performed using Maxent, version 3.3.3k (Phillips et al. 2006, available at http://www.cs.princeton.edu/~schapire/maxent/). The Maxent algorithm has been ranked among the most effective methods for species distribution modeling using presence-only data (Elith et al. 2006; Giovanelli et al. 2010). To run the model, we used 19 bioclimatic interpolated climate surfaces for global land areas (1950–2000) and a Derived Hydrological Digital Elevation Model (DEM-H). This data set is based on weather conditions recorded between 1950 and 2000, with 2.5 min resolution (Hijmans et al. 2005). The resulting model was evaluated with the receiver operating characteristics curve, calculating the area under the curve (AUC) (Fielding and Bell 1997).

We used the minimum presence threshold that equals the minimum model prediction value for any of the training occurrence point data, thus avoiding inflating commission errors (see Giovanelli et al. 2010). We generated a binary map depicting the predicted area for the model using a threshold value.

Results and discussion

Species identification

A total of 39 specimens of *P. centralis* were checked considering external morphology. All specimens were in accordance with the variation proposed in the original description (Bokermann 1962). One relevant aspect that is not mentioned in the species description refers to the colouration of the axillary and inguinal regions in live specimens, which are both orange in *P. centralis*. This is an important characteristic because it distinguishes *P. centralis* from other species of the *P. cuvieri* group. In *P. cuvieri,* this colouration is predominately reddish, while in *P. albonotatus*, *P. cuqui* and *P. kroyeri*, these regions are whitish (Lobo 1993). SVL comparisons among species show that *P. centralis* is larger than other species in the *Physalaemus cuvieri* group, reaching up to 43 mm of SVL (Bokermann 1962) (see SVL for other species of the group in Table 1).

Molecular analyses corroborate assumptions based on morphological data, showing that *P. centralis* has a large distribution associated to open areas of South America and there is no evidence that this species is a species complex throughout its geographical distribution. Mean intraspecific genetic distances found for COI and 16S were 0.01 (range 0.0–0.034) and 0.019 (range 0.0–0.027), respectively. These values are within the range expected for intraspecific distances in amphibians, in general less than 0.03–0.05 for 16S and 0.1 for COI (Fouquet et al. 2007). Distances between *P. centralis* and other *Physalaemus* species ranged from 0.118 (*P. centralis* vs. *P. cuvieri*) to 0.209 (*P. centralis* vs. *P. nattereri*) for COI and from 0.098 (*P. centralis* vs. *P. cuvieri*) to 0.175 (*P. centralis* vs. *P. nattereri*) for 16S. Although Jansen et al. (2011) suggested the existence of cryptic species in *P. centralis* from Bolivia based on molecular data (0.034 of divergence), our results suggest that it is a case of intraspecific variation with a weak geographic structure (see Figure 1).

Distribution records and ecological niche modeling of **Physalaemus centralis**

Compiled data provided 123 georeferenced records for *P. centralis*, 37 recovered from the literature, 74 coming from scientific collections and 12 being new occurrence records (Table 2). Those records are distributed between the countries of Bolivia (1 record), Brazil (115 records) and Paraguay (4 records). The edges of the geographic distribution are marked by coordinates 63.7097°– 41.3920° W and 2.4878°–23.8156° S and altitude ranges from sea level to 1265 m above sea level (Figure 2). Our findings include the first record for the state of Rondônia (CFBH 20424), extending the species' distribution in Brazil in about 490 km in a straight-line westward of the Lucas do Rio Verde municipality, in the state of Mato Grosso (UFMT 2454, 2462, 2476, 2482, 2485, 2487). In addition, our records for the states of Bahia and Piauí are the first to mark this specie's occurrence in the Caatinga.

In Brazil, *P. centralis* was previously reported in the states of Bahia, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, São Paulo and Tocantins (Bokermann 1962; Strüssmann et al. 2000; Toledo et al. 2003; Kokubum and Menin 2002; Gordo and Campos 2003; Nascimento et al. 2005; Vasconcelos and Rossa-Feres 2005; Brasileiro and Martins 2006; Silveira 2006; Van-Silva et al. 2007; Brasileiro et al. 2008; Valdujo et al. 2009; Roberto et al. 2013). Data compilation shows that *P. centralis* is widely distributed in eastern South America, especially in open areas of Cerrado. However, we now show that the species also occurs in contact areas between the Cerrado and forested areas from closed formations of the Atlantic and Amazon rainforests. Admittedly, records from the Atlantic and Amazon forests are strongly associated with disturbed areas, i.e. areas deforested by anthropic activities. These results indicate that *P. centralis* is an opportunistic species and may extend its range of distribution with the advancement of deforestation. On the other hand, the species does not seem to tolerate extreme climatic conditions regarding temperature and precipitation in the Caatinga, keeping in mind that records presented here are limited to ecotonal areas of Cerrado and Caatinga.

The Maxent modeling of 123 presence records yielded an AUC of 0.94. Figure 3 shows the results of the jackknife test of variable importance generated by Maxent. The environmental variable with the highest gain when used in isolation is Temperature Annual Range (BIO7). The environmental variable that decreases in gain the most when omitted is Annual Precipitation (BIO12), which therefore appears to provide more information compared to other variables.

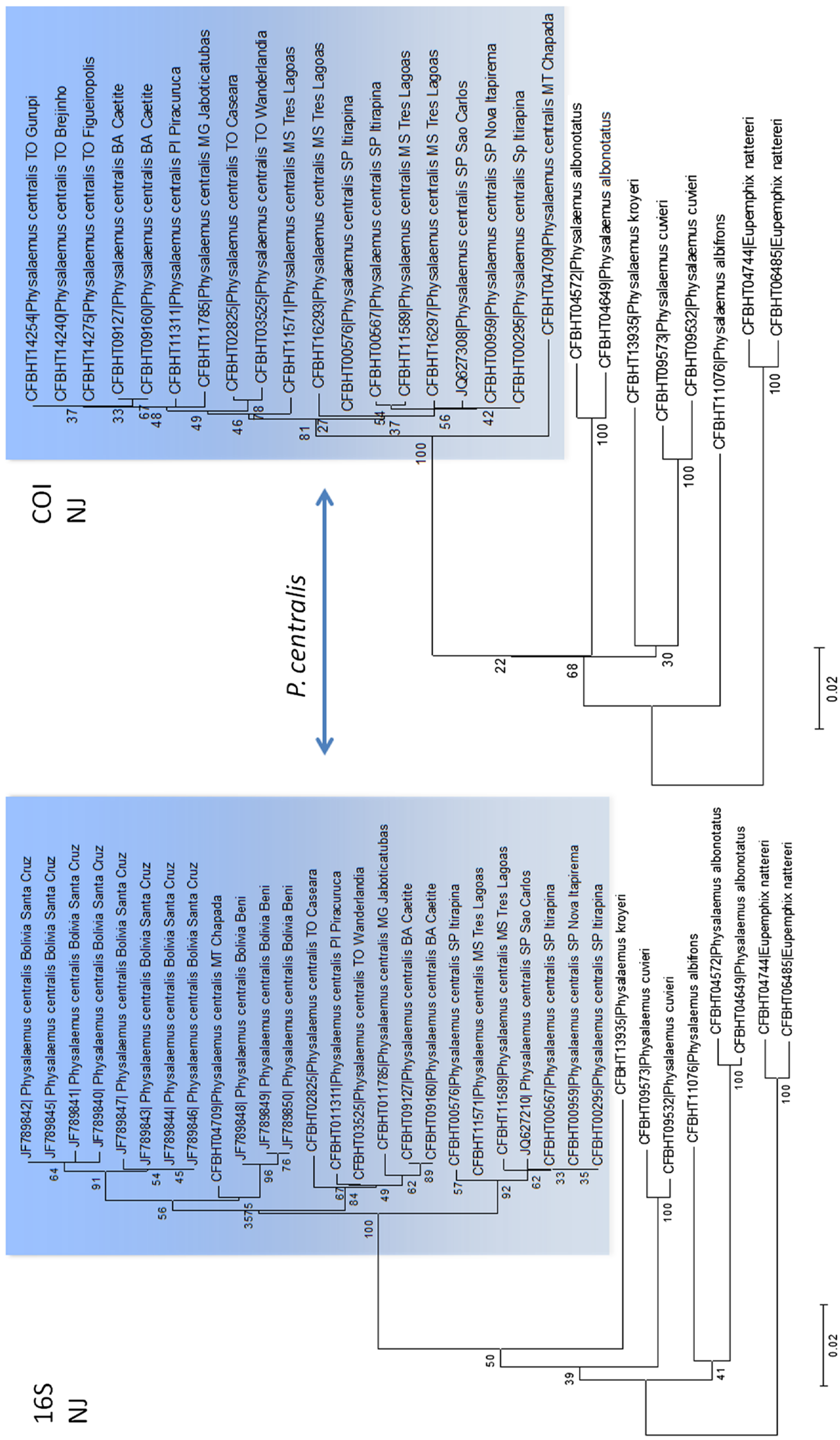

Figure 1. Neighbour-joining trees for 16S and COI of analysed *Physalaemus* species. All samples of *Physalaemus centralis* are grouped into a single clade. Out-groups are represented by other *Physalaemus* species.

Table 2. Records of *Physalaemus centralis* in Brazil based on field work (present study), bibliographical data, and voucher specimens in Brazilian collections.

Municipality	State/Province	Country	Longitude	Latitude	Source
Capitán Bado	Amambay	Paraguay	−55.5554	−23.2661	Brusquetti and Lavilla (2006)
Pedro Juan Caballero	Amambay	Paraguay	−56.0546	−22.6218	Brusquetti and Lavilla (2006)
Santa Rosa del Aguaray	San Pedro	Paraguay	−56.2952	−23.8126	Smith et al. (2012)
Caetité	Bahia	Brazil	−42.4833	−14.0667	Present study (CFBH 21079, 21080)
Cocos	Bahia	Brazil	−44.5344	−14.1839	CHUNB
Correntina	Bahia	Brazil	−44.6367	−13.3433	CHUNB
Jaborandi	Bahia	Brazil	−44.4328	−13.6194	CHUNB
Lençóis	Bahia	Brazil	−41.3920	−12.5574	CFBH
São Desidério	Bahia	Brazil	−45.0833	−12.7000	Valdujo et al. (2009)
São Desidério	Bahia	Brazil	−44.6000	−13.0167	Valdujo et al. (2009)
São Desidério	Bahia	Brazil	−44.9667	−12.3667	CHUNB
Iténez	Beni	Bolivia	−63.7097	−13.2648	Padial and Köhler (2001)
Itanará	Canindeyú	Paraguay	−55.7801	−23.8156	Brusquetti and Lavilla (2006)
Acreúna	Goiás	Brazil	−50.3883	−17.3867	CFBH
Alvorada do Norte	Goiás	Brazil	−46.4922	−14.4808	CHUNB
Anápolis	Goiás	Brazil	−48.9257	−16.3112	CFBH
Aporé	Goiás	Brazil	−51.8806	−18.6739	Van-Silva et al. (2007)
Baliza	Goiás	Brazil	−52.545	−16.1961	CFBH
Britânia	Goiás	Brazil	−51.1500	−15.2333	CHUNB
Catalão	Goiás	Brazil	−47.9500	−18.1667	CHUNB
Corumbá de Goiás	Goiás	Brazil	−48.8000	−15.9167	CHUNB
Indiara	Goiás	Brazil	−49.9809	−17.1273	CFBH
Mineiros	Goiás	Brazil	−52.5667	−17.5667	CHUNB
Palmeiras de Goiás	Goiás	Brazil	−49.9384	−16.7897	CFBH
Rio Verde	Goiás	Brazil	−50.9446	−17.7718	CFBH
São Domingos	Goiás	Brazil	−46.3167	−13.4000	CHUNB
São Simão	Goiás	Brazil	−50.5332	−19.0056	CFBH
Silvânia	Goiás	Brazil	−48.6081	−16.6589	Nascimento et al. (2005)
Carolina	Maranhão	Brazil	−47.4694	−7.3328	Present study

Table 2. (*Continued*).

Municipality	State/Province	Country	Longitude	Latitude	Source
Estreito	Maranhão	Brazil	−47.4286	−6.0106	Brasileiro et al. (2008)
Estreito	Maranhão	Brazil	−47.3614	−6.2733	Brasileiro et al. (2008)
Porto Franco	Maranhão	Brazil	−47.4000	−6.3333	Present study (CFBH 25953)
Santo Amaro do Maranhão	Maranhão	Brazil	−43.2928	−2.4878	CFBH
Chapada dos Guimarães	Mato Grosso	Brazil	−55.7497	−15.4606	CFBH
Chapada dos Guimarães	Mato Grosso	Brazil	−55.7500	−15.4333	CHUNB
Cuiabá	Mato Grosso	Brazil	−56.0833	−15.5833	CHUNB
Lucas do Rio Verde	Mato Grosso	Brazil	−55.8899	−13.1001	UFMT
Nossa Senhora do Livramento	Mato Grosso	Brazil	−53.3323	−15.7882	UFMT
Paranatinga	Mato Grosso	Brazil	−54.0557	−14.4723	UFMT
Porto Alegre do Norte	Mato Grosso	Brazil	−51.6325	−10.8769	CHUNB
Ribeirão Cascalheira	Mato Grosso	Brazil	−51.8241	−12.9419	ZUEC-AMP
São Félix do Araguaia	Mato Grosso	Brazil	−50.6691	−11.6172	ZUEC-AMP
Xingu	Mato Grosso	Brazil	−52.4031	−12.0056	Nascimento et al. 2005;
Bonito	Mato Grosso do Sul	Brazil	−56.5056	−20.6508	Gordo and Campos (2003)
Dourados	Mato Grosso do Sul	Brazil	−54.8056	−22.2211	Tombini and Béda (2002)
Três Lagoas	Mato Grosso do Sul	Brazil	−51.7718	−20.9762	Present study (CFBH CFBH 13595, 18482, 18483, 22631, 24016, 24020, 24040)
Belo Horizonte	Minas Gerais	Brazil	−43.9378	−19.9208	CFBH
Buritis	Minas Gerais	Brazil	−46.4233	−15.6178	Nascimento et al. (2005)
Buritizeiro	Minas Gerais	Brazil	−44.9622	−17.3511	Nascimento et al. (2005)
Chapada Gaúcha	Minas Gerais	Brazil	−45.6183	−15.3056	CHUNB
Conquista	Minas Gerais	Brazil	−47.5417	−19.9372	Nascimento et al. (2005)
Diamantina	Minas Gerais	Brazil	−43.6003	−18.2494	Nascimento et al. (2005)

Table 2. (*Continued*).

Municipality	State/Province	Country	Longitude	Latitude	Source
Esmeraldas	Minas Gerais	Brazil	−44.3138	−19.7628	UNICAMP
Formoso	Minas Gerais	Brazil	−46.2319	−14.9467	CHUNB
Jaboticatubas	Minas Gerais	Brazil	−43.7450	−19.5136	ZUEC-AMP
João Pinheiro	Minas Gerais	Brazil	−46.1667	−17.7333	Silveira (2006)
Lagoa Santa	Minas Gerais	Brazil	−43.8897	−19.6272	Nascimento et al. (2005)
Manga	Minas Gerais	Brazil	−43.9322	−14.7558	Nascimento et al. (2005)
Pirapora	Minas Gerais	Brazil	−44.9419	−17.345	Nascimento et al. (2005)
Porteirinha	Minas Gerais	Brazil	−43.0283	−15.7433	Nascimento et al. (2005)
Riacho dos Machados	Minas Gerais	Brazil	−43.0494	−16.0061	Nascimento et al. (2005)
Sacramento	Minas Gerais	Brazil	−47.4400	−19.8653	Nascimento et al. (2005)
Santana do Riacho	Minas Gerais	Brazil	−43.7144	−19.1689	Nascimento et al. (2005)
Uberlandia	Minas Gerais	Brazil	−48.3000	−18.9833	Kokubum and Menin (2002)
União de Minas	Minas Gerais	Brazil	−50.3416	−19.5359	CFBH
Várzea da Palma	Minas Gerais	Brazil	−44.7308	−17.5981	Nascimento et al. (2005)
Piracuruca	Piauí	Brazil	−41.6789	−4.1933	Present study (CFBH 19396, 19397)
Colorado do Oeste	Rondônia	Brazil	−60.3937	−13.0385	Present study/ New state record (CFBH 20424)
Angatuba	São Paulo	Brazil	−48.3844	−23.5009	CFBH
Araraquara	São Paulo	Brazil	−48.1697	−21.7903	ZUEC-AMP
Bauru	São Paulo	Brazil	−49.0600	−22.3100	DZSJRP
Botucatu	São Paulo	Brazil	−48.445	−22.8858	Nascimento et al. (2005)
Brotas	São Paulo	Brazil	−48.1267	−22.2842	CFBH
Corumbataí	São Paulo	Brazil	−47.6258	−22.2200	Nascimento et al. (2005)
Guaíra	São Paulo	Brazil	−48.3106	−20.3183	DZSJRP
Ibirá	São Paulo	Brazil	−49.2408	−21.0803	DZSJRP
Icem	São Paulo	Brazil	−49.195	−20.3417	UNESPSJRP
Itirapina	São Paulo	Brazil	−47.8228	−22.2528	CFBH
Jaguariuna	São Paulo	Brazil	−46.9797	−22.7003	ZUEC-AMP
José Bonifácio	São Paulo	Brazil	−49.6883	−21.0528	UNESPSJRP

Table 2. (*Continued*).

Municipality	State/Province	Country	Longitude	Latitude	Source
Luiz Antônio	São Paulo	Brazil	−47.7987	−21.6057	Prado et al. (2009)
Magda	São Paulo	Brazil	−50.2261	−20.6439	UNESPSJRP
Nova Aliança	São Paulo	Brazil	−49.5431	−21.0761	UNESPSJRP
Nova Granada	São Paulo	Brazil	−49.3142	−20.5339	DZSJRP
Nova Itapirema	São Paulo	Brazil	−49.5333	−21.0667	Vasconcelos and Rossa-Feres (2005)
Novo Horizonte	São Paulo	Brazil	−49.2208	−21.4681	DZSJRP
Palestina	São Paulo	Brazil	−49.4300	−20.3900	ZUEC-AMP
Paulínia	São Paulo	Brazil	−47.1500	−22.7603	ZUEC-AMP
Pirassununga	São Paulo	Brazil	−47.4244	−21.9978	Brasileiro and Martins (2006)
Planalto	São Paulo	Brazil	−49.9739	−21.0014	FAPESP
Rio Claro	São Paulo	Brazil	−47.5500	−22.4167	Toledo et al. (2003)
Santa Fé do Sul	São Paulo	Brazil	−50.9258	−20.2111	UNESPSJRP
Santo Antônio do Aracanguá	São Paulo	Brazil	−50.3490	−20.9260	DZSJRP
São José do Rio Preto	São Paulo	Brazil	−49.3794	−20.8197	UNESPSJRP
Tanabi	São Paulo	Brazil	−49.6492	−20.6264	UNESPSJRP
Turmalina	São Paulo	Brazil	−50.4655	−20.0521	CFBH
União Paulista	São Paulo	Brazil	−49.9261	−20.9211	FAPESP
Vitoria Brasil	São Paulo	Brazil	−50.4844	−20.1967	UNESPSJRP
Votuporanga	São Paulo	Brazil	−49.9728	−20.4228	UNESPSJRP
Araguacema	Tocantins	Brazil	−49.5564	−8.8036	Present study (CFBH 10313)
Araguaina	Tocantins	Brazil	−48.2072	−7.1911	Present study
Babaçulândia	Tocantins	Brazil	−48.0153	−7.0842	Brasileiro et al. (2008)
Cazeara	Tocantins	Brazil	−49.9556	−9.2783	CFBH
Colinas do To-cantins	Tocantins	Brazil	−48.475	−8.0592	CHUNB
Darcinópoles	Tocantins	Brazil	−47.7608	−6.7147	Brasileiro et al. (2008)
Dois Irmãos	Tocantins	Brazil	−48.0878	−6.7808	Present study
Formoso do Araguaia	Tocantins	Brazil	−49.5289	−11.7967	ZUEC-AMP
Guaraí	Tocantins	Brazil	−48.5103	−8.8342	Present study
Lizarda	Tocantins	Brazil	−46.6731	−9.5942	CFBH
Mateiros	Tocantins	Brazil	−46.4211	−10.5475	CHUNB

Table 2. (*Continued*).

Municipality	State/Province	Country	Longitude	Latitude	Source
Palmas	Tocantins	Brazil	−48.4361	−10.4208	CHUNB
Palmeirante	Tocantins	Brazil	−47.9258	−7.8600	Present study
Palmeirópolis	Tocantins	Brazil	−48.4022	−13.0439	CHUNB
Paraíso do Tocantins	Tocantins	Brazil	−48.8667	−10.1761	CHUNB
Paranã	Tocantins	Brazil	−47.8831	−12.6153	CHUNB
Pedro Afonso	Tocantins	Brazil	−48.1747	−8.9675	CHUNB
Porto Nacional	Tocantins	Brazil	−48.4172	−10.7081	CHUNB
São Félix	Tocantins	Brazil	−50.6703	−11.5636	Present study
Wanderlândia	Tocantins	Brazil	−47.9631	−6.8492	CFBH

Abbreviations: CFBH – Colecão de Anfíbios Célio F. B. Haddad; DZSJRP – Coleção do Departamento de Zoologia de São José do Rio Preto; ZUEC-AMP – Coleção de Anfíbios do Museu de História Natural “Prof. Dr. Adão José Cardoso”; CHUNB – Coleção Herpetológica da Universidade de Brasília; UFMT – Coleção de Vertebrados da Universidade Federal do Mato Grosso.

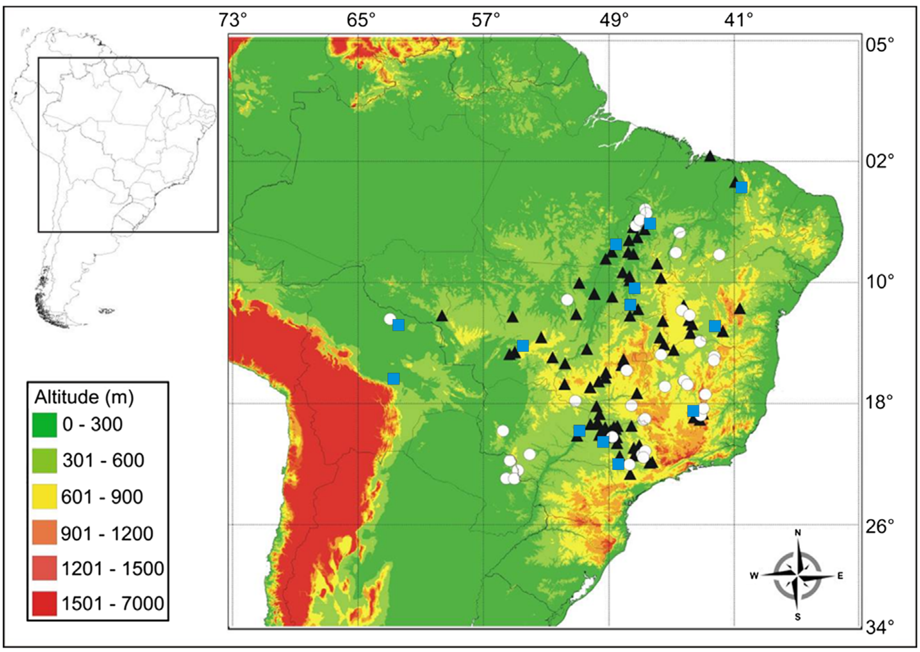

Figure 2. Map showing current known distribution of *Physalaemus centralis*. White circles represent records available in the literature. Black triangles are unpublished records (records obtained from scientific collections or presented for the first time in the present study). Blue squares represent specimens with molecular data accessed.

The ecological niche modeling suggests that *P. centralis* has potential to occur in open areas eastward of South America, including the Caatinga, Chaco (in Paraguay) and mainly the Cerrado (Figure 4). The model also points out that the Atlantic rainforest from south-eastern Brazil, as well as transitional zones between the Cerrado and the Amazon

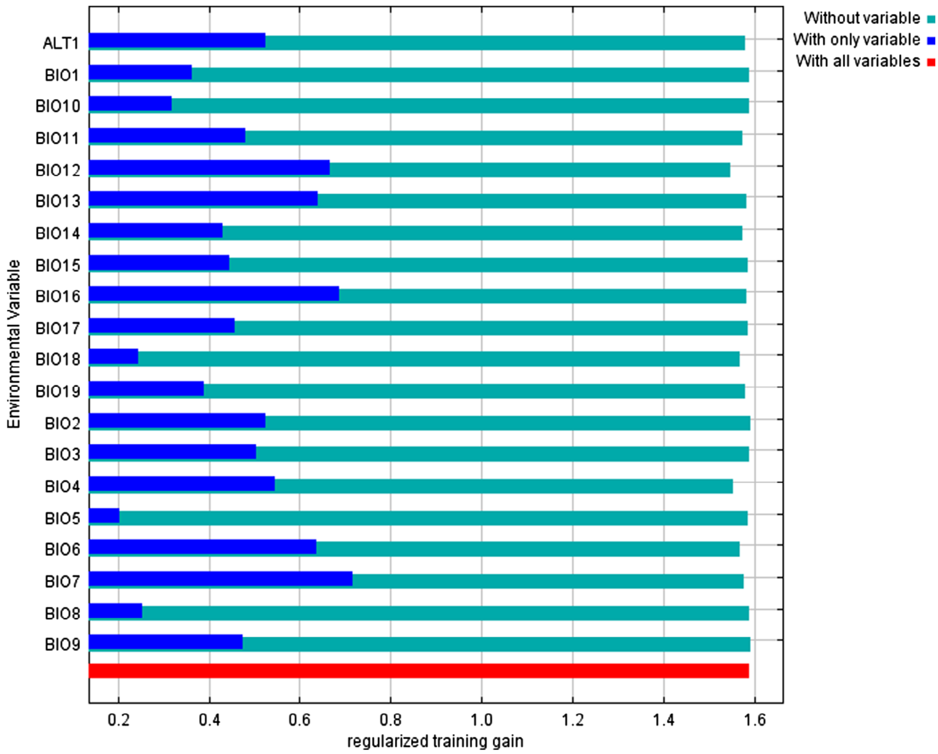

Figure 3. Results of the jackknife test of variable importance generating by Maxent. The environmental variables Temperature Annual Range (BIO7) and Annual Precipitation (BIO12) were the most important variables.

rainforest, are potential areas for species occurrence. It is possible that these regions have become suitable after the fragmentation of the Atlantic rainforest and the formation of the "arc of deforestation" in Amazonia, mainly in the states of Rondônia and Pará, which caused the species to invade recently opened areas.

However, some areas of occurrence predicted in the model should be discarded. For example, the occurrence prediction for the Plateau of Ibiapaba, in the state of Ceará, Brazil (04° S; 40° W) is probably a bias of the model, given that this region was exhaustively sampled in the years 2007–2009 and no *P. centralis* specimen was found (see Loebmann and Haddad 2010; Roberto and Loebmann 2016). The Plateau of Ibiapaba has a relatively high altitude (ca. 900 m above sea level) and extends throughout the frontier of the states of Piauí and Ceará. Although we have confirmed the presence of *Physalaemus* species in the state of Piauí, municipality of Piracuruca (ca. 40 km from the base of the Plateau of Ibiapaba), the high altitude from of the plateau may act as a barrier for the species. Therefore, the predicted occurrence of *P. centralis* in Ibiapaba, in other areas of the state of Ceará, as well as in the states of Pernambuco and Rio Grande do Norte, should be considered possible biases of the model. In conclusion, the model is relatively consistent with records of occurrence presented herein, with these few exceptions.

The present work used an inductive framework to develop maps of geographic distribution integrating occurrence data obtained from museums, a genetic approach

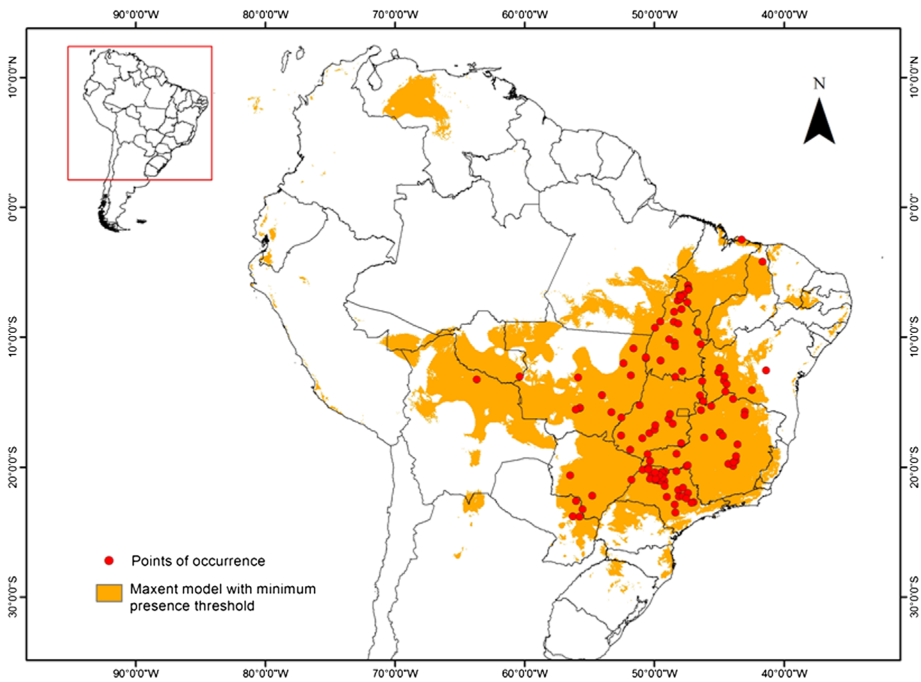

Figure 4. Prediction of the potential distribution of *Physalaemus centralis* based on the Maxent algorithm.

and ecological niche modeling. This multiple approach revealed that *P. centralis* is in fact widely distributed and strongly related to the Cerrado biome. We also conclude that *P. centralis* should not be considered a species complex. Therefore, we recommend that future studies involving widely distributed species should consider an integrative approach similar to what was developed here.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix 1.

Specimens of *Physalaemus cuvieri* group morphologically analysed.

Physalaemus centralis. BRAZIL. State of Goiás, municipality of Baliza (CFBH 688), state of Bahia, municipality of Lençóis (CFBH 6659, 6660), municipality of São Desidério (CFBH 20539), municipality of Jaborandi (CFBH 20551), municipality of Bahianópolis (CFBH 21007), municipality of Caetité (CFBH 21079, 21080); state of Minas Gerais, municipality of Belo Horizonte (CFBH 1479, 1480); state of Mato Grosso do Sul, municipality of Três Lagoas (CFBH 13595, 18482, 18483, 22631, 24016, 24020, 24040); state of Mato Grosso, municipality of Chapada dos Guimarães (CFBH 14392); state of Piauí, municipality of Piracuruca (CFBH 14392, 19396); state of Rondônia, municipality of Colorado do Oeste (CFBH 20424); state of São Paulo, municipality of Corumbataí (CFBH 1340, 2057), municipality of Rio Claro (CFBH 2368, 4580), municipality of Itirapina (CFBH 5830), municipality of Brotas (CFBH 9805, 9830, 9831), municipality of Angatuba (CFBH 23118, 23138, 23149); state of Tocantins, municipality of Caseara (CFBH 10282), municipality of Araguacema (CFBH 10313), municipality of Wanderlândia (CFBH 11452), municipality of Lizarda (CFBH 13221), municipality of Gurupi (CFBH 20962, 20963, 22035).

Physalaemus albifrons (Spix, 1824): BRAZIL. State of Ceará, municipality of Caucaia (UFCA 4093), municipality of Jati (MNRJ 55529), municipality of Caucaia (UFCA 4093), São Gonçalo do Amarante (URCA-H 5603, 6401, 6405), municipality of Trairi (URCA-H 5666), municipality of Viçosa do Ceará (CFBH 16137-16141).

Physalaemus albonotatus (Steindachner, 1864): BRAZIL. State of Mato Grosso, municipality of Cáceres, ZUEC 7067-7-78 (topotypes), municipality of Acorizal, CFBH 14240, 14142-14144, municipality of Dom Aquino, CFBH14331-14332; State of Mato Grosso do Sul, municipality of Bonito, CFBH 14253-14254; municipality of Corumbá, CFBH 8987; state of Tocantins, municipality of Mateiros, MZUSP 133262-133267.

Physalaemus cuvieri Fitzinger, 1826: BRAZIL. State of Tocantins, municipality of Babaçulândia, CFBH 11408; municipality of Araguacema, CFBH 10291; state of São Paulo, municipality of Assis, CFBH 20015; municipality of Itirapina, CFBH 5712, 6026, 9828, 9838, 21934; municipality of Mauá, CFBH 9908; municipality of São Paulo, CFBH 9140; municipality of São José do Rio Preto, CFBH 9139; CFBH 11316. municipality of Rio Claro, CFBH 5587; state os Santa Catarina, municipality of Campos Novos, CFBH 13639.

Physalaemus ephippifer (Steidachner, 1864): BRAZIL. State of Pará, municipality of Belém, MNRJ 32276-80, MNRJ 69637-69644 (topotypes); ZUEC 13724-13741.

Physalaemus erikae Cruz and Pimenta, 2004*:* BRAZIL. State of Bahia, minicipality of Graratinga, MNRJ 35401 (topotype); municipality of Ilhéus, MNRJ 48647-50.

Physalaemus fischeri (Boulenger, 1890): VENEZUELA. Province of Apure, municipality of Mantecal, ZUEC 10475-10476, 9369, 9362.

Physalaemus kroyeri (Reinhardt and Lutken, 1862): BRAZIL. State of Bahia, municipality of Maracás, MZUSP 96472-96547.

Appendix 2. Localities, voucher information, and GenBank accession numbers for specimens included in the molecular analysis.

			GenBank num.			
Species	Tissue number	Voucher	16S	COI	Country, Region, Locality	Reference
Physalaemus centralis	CFBHT00567		–	–	Brazil, São Paulo, Itirapina	This work
Physalaemus centralis	CFBHT00576		–	–	Brazil, São Paulo, Itirapina	This work
Physalaemus centralis	CFBHT00295		–	–	Brazil, São Paulo, Itirapina	This work
Physalaemus centralis	CFBHT00959		–	–	Brazil, São Paulo, Nova Itapirema	This work
Physalaemus centralis	CFBHT16293	CFBH 32335	–	–	Brazil, Mato Grosso do Sul, Três Lagoas	This work
Physalaemus centralis	CFBHT11589	CFBH 24040	–	–	Brazil, Mato Grosso do Sul, Três Lagoas	This work
Physalaemus centralis	CFBHT16297	CFBH 32334	–	–	Brazil, Mato Grosso do Sul, Três Lagoas	This work
Physalaemus centralis	CFBHT11571	CFBH 24020	–	–	Brazil, Mato Grosso do Sul, Três Lagoas	This work
Physalaemus centralis	CFBHT11785	CFBH 24366	–	–	Brazil, Minas Gerais, Jaboticatubas	This work
Physalaemus centralis	CFBHT04709	CFBH 14392	–	–	Brazil, Mato Grosso, Chapada dos Guimarães	This work
Physalaemus centralis	CFBHT09127	CFBH 21080	–	–	Brazil, Bahia, Caetite	This work
Physalaemus centralis	CFBHT09160	CFBH 21079	–	–	Brazil, Bahia, Caetite	This work
Physalaemus centralis	CFBHT14275	CFBH 28947	–	–	Brazil, Tocantins, Figueirópolis	This work
Physalaemus centralis	CFBHT14254	CFBH 28925	–	–	Brazil, Tocantins, Gurupi	This work
Physalaemus centralis	CFBHT14240	CFBH 28915	–	–	Brazil, Tocantins, Brejinho do Nazareth	This work
Physalaemus centralis	CFBHT02825	CFBH 10282	–	–	Brazil, Tocantins, Caseara	This work
Physalaemus centralis	CFBHT03525	CFBH 11452	–	–	Brazil, Tocantins, Wanderlândia	This work
Physalaemus centralis	CFBHT11311		–	–	Brazil, Piauí, Piracuruca	This work
Physalaemus centralis	Isolate 117		JQ627210	JQ627308	Brazil, São Paulo, São Carlos	Unpublished
Physalaemus centralis		SMF 88240	JF789840		Bolivia Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
Physalaemus centralis		MNKA 9400	JF789841		Bolivia Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
Physalaemus centralis		MNKA 9412	JF789842		Bolivia Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)

Physalaemus centralis		MNKA 9437	JF789843		Bolivia Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
Physalaemus centralis		MNKA 9769	JF789844		Bolivia Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
Physalaemus centralis		MNKA 9514	JF789845		Bolivia Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
Physalaemus centralis		MNKA 9789	JF789847		Bolivia Santa Cruz, Ñuflo de Chavez, San Sebastián	Jansen et al. (2011)
Physalaemus centralis		MNKA 9580	JF789846		Bolivia Santa Cruz, Velasco, Caparu	Jansen et al. (2011)
Physalaemus centralis		MNKA 9324	JF789848		Bolivia Beni, Yucuma, Los Lagos	Jansen et al. (2011)
Physalaemus centralis		MNKA 9376	JF789849		Bolivia Beni, Yucuma, Los Lagos	Jansen et al. (2011)
Physalaemus centralis		MNKA 9371	JF789850		Bolivia Beni, Yucuma, Los Lagos	Jansen et al. (2011)
Physalaemus albifrons	CFBHT11076	CFBH 22688	–	–	Brazil, Bahia, Livramento de Nossa Senhora	This work
Physalaemus albonotatus	CFBHT04572	CFBH 14253	–	–	Brazil, Mato Grosso do Sul, Bonito	This work
Physalaemus albonotatus	CFBHT04649	CFBH 14332	–	–	Brazil, Mato Grosso, Dom Aquino	This work
Physalaemus cuvieri	CFBHT09573		–	–	Brazil, Rio Grande do Sul, São Sepe	This work
Physalaemus cuvieri	CFBHT09532	CFBH 18687	–	–	Brazil, São Paulo, Teodoro Sampaio	This work
Physalaemus kroyeri	CFBHT13935	CFBH 28647	–	–	Brazil, Bahia, Jequié	This work
Physalaemus nattereri	CFBHT06485	CFBH 8021	–	–	Brazil, São Paulo, Rio Claro	This work
Physalaemus nattereri	CFBHT04744	CFBH 14427	–	–	Brazil, Mato Grosso, Acorizal	This work