



**Delimitação de espécies no complexo *Physalaemus ephippifer* (Anura:
Leptodactylidae)**

Izadora Emanuelle Costa Silva

Diadema, São Paulo, Brasil
Novembro – 2021



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Izadora Emanuelle Costa Silva

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ORIENTADOR: Prof. Dr. Marcelo José Sturaro

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Resumo

Embora ameaçada, a região Neotropical abriga uma riqueza ainda subestimada, pois diversas espécies novas vêm sendo descritas para a região. Nesta região é muito comum a ocorrência de espécies morfologicamente crípticas e nestes casos, o uso de várias fontes de evidências e análises de delimitação têm sido recomendados para investigar a diversidade de táxons, concedendo estabilidade taxonômica aos grupos estudados. *Physalaemus ephippifer* está amplamente distribuída na região norte do Neotrópico (Amazônia e nordeste brasileiro) e recentemente foi indicada como um possível complexo de espécies. Portanto, empregando uma abordagem integrativa utilizando dados morfológicos e moleculares em análises de delimitação baseadas em locus único (ABGD, mPTP e GMYC) e nos modelos coalescente multiespécies (BPP e iBPP), busquei inferir as possíveis linhagens e suas relações dentro do complexo *P. ephippifer* e testar hipóteses de delimitação de espécies. A análise filogenética recuperou cinco linhagens distintas dentro do complexo *P. ephippifer*, estruturadas geograficamente por rios e/ou biomas. Os métodos de delimitação baseados num locus único divergiram em relação ao número e configuração das espécies candidatas (1–3 possíveis espécies). Entretanto, através de testes de hipóteses de delimitação com métodos coalescentes de multiespécies (dados moleculares e/ou morfológicos) foram recuperadas cinco possíveis espécies candidatas em *P. ephippifer*.

Palavras-Chave: Diversidade; Amazônia; Conservação; Taxonomia Integrativa; Anuros.

Abstract

The Neotropics harbors underestimated fauna diversity due to the ongoing description of novel species, albeit current threats to its conservation. Morphologically cryptic species are frequent in the Neotropics, while such a scenario implies using multiple data types and species delimitation analyses to determine taxon diversity and stability. The widespread distribution of *Physalaemus ephippifer* in northern Neotropics (*i.e.*, Amazon and northeastern Brazil) is suggested as a potential species complex. Herein, I infer the relationships of different lineages in the *P. ephippifer* species complex and further test multiple species delimitation hypotheses. Moreover, this hypothesis testing relied on employing an integrative approach using morphological and molecular data under single-locus delimitation analyses (*e.g.*, ABGD, mPTP, GMYC) and on the multispecies coalescent model (*e.g.*, BPP and iBPP). The phylogenetic analysis recovered five distinct lineages within the *P. ephippifer* species complex, which are geographically structured by rivers and/or biomes. Delimitation methods based on a single locus diverged in the number and configuration of candidate species (from one to three potentially novel species). However, testing delimitation hypotheses with multi-species coalescent methods (molecular and/or morphological data) revealed five potentially novel species.

Keywords: Diversity; Amazonia; Conservation; Integrative Taxonomy; Anuran.

Introdução Geral

Delimitar e descrever espécies apropriadamente são importantes ferramentas para a compreensão de diversas questões biológicas, além auxiliar na pavimentação de estratégias de conservação de qualidade, especialmente num contexto em que 80% a 90% das espécies do planeta permanecem desconhecidas (Cracraft, 2002; Bickford et al., 2007; Mace, 2004; Rannala and Yang, 2020). Além disso, parte das espécies já descritas está ameaçada, sendo os anfíbios o grupo de vertebrados em maior risco, com 41% das espécies com algum grau de ameaça (IUCN, 2021). A crescente disponibilidade de bancos de dados baseados em multilocus e a integração com outras fontes de evidências (por exemplo: morfologia e aspectos ecológicos) têm auxiliado os pesquisadores no acesso a esta diversidade, pois favorece o descobrimento de linhagens crípticas anteriormente classificadas sob o mesmo nome (Beheregaray and Caccone, 2007; Fujita et al., 2012; Solís-Lemus et al., 2015; Sturaro et al., 2018; Domingos et al., 2017).

Nos últimos anos, o modelo coalescente de multiespécies tem sido bastante utilizado nas análises de delimitação de espécies, por fazer uso de dados multilocus para identificar linhagens evolutivas independentes através do teste hipóteses levando em consideração parâmetros populacionais como taxa evolutiva e tamanho populacional (Fujita 2012; Knowles and Carstens, 2007; Solís-Lemus et al. 2015). Porém, o uso desta abordagem, e isto configura uma limitação, por vezes pode levar a uma superestimação de linhagens classificadas como espécies (Sukumaran and Knowles, 2017; Hillis, 2019), portanto, a integração de diferentes fontes de dados é recomendada para analisar cuidadosamente os limites entre as espécies (Padial et al., 2010; Hillis, 2007; Hillis 2019; Solís-Lemus et al. 2015).

Os estudos de delimitação que utilizam multi-dados (e.g. morfologia, acústica, DNA) são uma importante peça da taxonomia integrativa, que parte da premissa de que a investigação dos processos de especiação deve contar com diferentes fontes de evidências (Padial 2010; Fujita, 2012; Angulo and Reichle, 2008; Padial and De La Riva, 2009, Peloso et al., 2014). Nos últimos anos, diversos estudos têm usufruído dessa integração para desvendar a diversidade críptica em anuros (e.g. Rojas et al., 2018; Ortega-Andrade et al., 2015; Caminer et al., 2017; Rivadeneira et al., 2018; Vacher et al., 2017; Simões et al., 2013, Sturaro et al., 2020). Espécies com ampla distribuição são fortes candidatas a serem crípticas e/ou apresentarem um alto grau de estruturação (Angulo & Icochea 2010; Funk et al., 2012), como é o caso da rã neotropical *Physalaemus*

ephippifer (Steindachner, 1864), que ocorre em grande parte da região neotropical, incluindo Amazônia e no nordeste brasileiro (Frost 2021), uma região com diversidade ainda subestimada e que possui uma rica fauna de anfíbios (Giam et al 2012; Duellman 1999; Vacher *et al.* 2020, Araújo *et al.* 2020).

Physalaemus ephippifer foi descrito por Steindachner (1864) como *Leiuperus ephippifer*, baseado em espécimes (depositados na Herpetological Collection of the Naturhistorisches Museum, Zoologische Abteilung, Viena, Áustria – NHMW) coletados por Jon Natterer em uma localidade apontada como “Parà und Caiçara”. Infelizmente, os síntipos dessa espécie não foram catalogados no NHMW e esses espécimes nunca foram encontrados (Häupl et al., 1994; Häupl & Tiedemann 1978; Gemel et al., 2019). Posteriormente, Gunther (1865) transferiu *Leiuperus ephippifer* para o gênero *Gomphobates* e finalmente, Bokermann (1966) restringe a localidade original para “Belém, Pará, Brazil” e transfere *Gomphobates ephippifer* para o gênero *Physalaemus*. Nascimento et al. (2005) realizou a revisão taxonômica do gênero *Physalaemus*, e alocou *P. ephippifer* no grupo *Physalaemus cuvieri* juntamente com outras oito espécies. Posteriormente, Lourenço et al. (2015) apresentou a filogenia do gênero, na qual recuperou amostras de *P. ephippifer* da localidade tipo como grupo irmão de um clado de *P. cuvieri* do nordeste brasileiro.

Em um estudo utilizando análise filogenética baseada em marcadores moleculares e análise citogenética do complexo *P. cuvieri* – *P. ephippifer*, Nascimento et al. (2019) amostraram *P. ephippifer* de duas regiões da Amazônia (Escudo das Guiana e leste da Amazônia) além de amostras de *P. cuvieri* de diversas localidades. Os autores constataram que a diversidade dentro do grupo é maior do que era estimada previamente, demonstrando a ocorrência de pelo menos duas linhagens distintas dentro de *P. ephippifer*. Ademais, a linhagem de *P. cuvieri* do nordeste foi recuperada dentro de *P. ephippifer*, corroborando o estudo de Lourenço *et al.* (2015). Segundo os autores, as amostras do norte do Rio Amazonas formam um clado irmão do grupo de *P. ephippifer* da região metropolitana de Belém (Pará, Brasil) e de *P. cuvieri* de localidades da região norte do nordeste (Maranhão, Ceará, Pernambuco, Paraíba e Bahia). Devido à baixa amostragem de espécimes da região Amazônica, os autores indicam a necessidade de um estudo mais amplo sobre a diversidade escondida sob o táxon. Além disso, a integração de outras fontes de evidências como a morfologia é essencial para auxiliar na busca pelos limites dessas possíveis espécies.

Um bom conhecimento em taxonomia e sistemática do grupo de estudo é essencial para uma análise precisa dos padrões e processos de especiação (Vieites et al., 2009). Devido a ampla disponibilidade de material (espécimes e amostras de tecidos depositados em coleções científicas) de *P. ephippifer* e *P. cuvieri* do nordeste brasileiro (aqui tratado como complexo *P. ephippifer*), foi possível realizar uma análise integrativa com marcadores mitocondriais e nucleares, e dados morfológicos para estimar a diversidade de espécie desse grupo.

Objetivo geral

Investigar e estudar a diversidade de espécies dentro do complexo *Physalaemus ephippifer* integrando dados morfológicos e moleculares.

Objetivos Específicos

- Inferir as relações dentro do complexo *Physalaemus ephippifer*;
- Inferir a diversidade de espécies utilizando DNA barcoding;
- Testar hipóteses de delimitação de espécies utilizando análises coalescentes com dados morfológicos e/ou moleculares;
- Apontar possíveis espécies candidatas dentro do grupo.

Resultados

A diversidade em *Physalaemus ephippifer* foi analisada integrando dados moleculares (fragmentos de genes mitocondriais e nucleares) e morfológicos (morfometria). Primeiramente foi realizada uma análise filogenética utilizando os dados moleculares concatenados (mitocondriais e nucleares). Nessa análise foram recuperados cinco grupos que estão estruturados geograficamente, sendo limitados por alguns rios amazônicos e fitofisionomias.

Além disso, foram utilizados três métodos de delimitação de espécies utilizando locus único (DNA barcoding, gene ribossomal *16s*), que se baseiam em diferentes parâmetros evolutivos. O primeiro método, o General Mixed Yule Coalescent (GMYC) faz uso de uma árvore ultramétrica datada para encontrar a melhor delimitação de espécies que combina a diversificação e ramificações genealógicas entre as espécies (Fujisawa and Barraclough, 2013). O segundo método, o multi-rate Poisson Tree Process (mPTP) modela o processo de ramificação baseado no número de substituições esperadas acumuladas entre os eventos de especiação subsequentes (Kapli et al., 2017) para este, foi utilizada uma árvore inferida não ultramétrica. Por último, o Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012) particiona as amostras em espécies candidatas baseado nas lacunas do código de barras (*barcode gap*), ou seja, estima as menores distâncias intraespécificas e as maiores distâncias interespécificas.

Utilizando a proposta menos conservativa das análises anteriores, foram definidas *a priori* as possíveis espécies para as análises no Bayesian Phylogenetics and Phylogeography (BPP) e no Integration of genes and traits for Bayesian Phylogenetics and Phylogeography (iBPP) (Rannala and Yang, 2003; Yang and Rannala, 2010; Solis-Lemus et al., 2015), com a finalidade de testar a melhor hipótese de delimitação de espécies nesse complexo. As hipóteses de delimitação foram testadas no BPP utilizando os dados moleculares, e no iBPP utilizando os dados morfológicos e ambos os bancos de dados de forma integrada. Para checar a consistência dos resultados e possíveis efeitos populacionais e demográficos no complexo *P. ephippifer* foram testados quatro cenários: (1) tamanho populacional ancestral elevado e divergência profunda; (2) tamanho populacional ancestral baixo e divergência profunda; (3) tamanho populacional ancestral elevado e divergência recente; e (4) tamanho populacional ancestral baixo e divergência recente.

As análises de delimitação de espécies com dados moleculares, morfológicos e de forma integrada apontam pelo menos cinco espécies no complexo *P. ephippifer*, as quais estão

estruturadas geograficamente: (1) no médio-alto interflúvio Xingu-Tocantins, (2) nordeste do Brasil, (3) Ilha do Marajó e localidades adjacentes do baixo interflúvio Xingu-Tocantins, limitadas ao norte pelo rio Amazonas, oeste pelo rio Xingu e leste pelo rio Pará, (4) ao norte do Rio Amazonas (Escudo das Guianas) e oeste do rio Xingu (sul do rio Amazonas), e (5) e a leste do rio Tocantins e extremo leste da Amazônia. Essas novas evidências suportam e ampliam os estudos anteriores (Nascimento et al. (2019) sobre a possibilidade de diversidade críptica em *P. ephippifer*. Até então eram apontadas apenas duas possíveis espécies sob este nome, entretanto, com a ampliação das amostragens geográfica, morfológica e molecular, o presente estudo amplia este número, estimando cinco possíveis espécies.

Os métodos e resultados detalhados do presente estudo estão organizados no Capítulo 1, o qual está escrito na forma de manuscrito científico que será submetido para um periódico da área de Biodiversidade.

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Integrative species delimitation in the *Physalaemus ephippifer* complex (Anura: Leptodactylidae)

Izadora Emanuelle Costa Silva^{1,2*}, Thiago Ribeiro de Carvalho³, Ariovaldo Antonio Giaretta⁴ and Marcelo José Sturaro¹

¹ Universidade Federal de São Paulo, Departamento de Ecologia e Biologia Evolutiva, Av. Professor Artur Riedel, 275, Jardim Eldorado, Diadema, CEP 09972-270, São Paulo, Brazil

² Programa de Pós-Graduação em Ecologia e Evolução, Universidade Federal de São Paulo

³ Laboratório de Herpetologia, Departamento de Zoologia e Centro de Aquicultura, Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil

⁴ Laboratório de Taxonomia, Sistemática e Evolução de Anuros Neotropicais, Universidade Federal de Uberlândia, Faculdade de Ciências Integradas do Pontal, Rua 20, 1600, Bairro Tupã, 38304-402. Ituiutaba, MG, Brazil

* Corresponding author: cs.izadora@gmail.com

Abstract

The Neotropics harbors the largest amphibian fauna in the world. To investigate this diversity, methods for species delimitation allow highlighting cryptic lineages and enable more extensive taxonomic studies. Estimations suggest at least three distinct lineages in the *Physalaemus ephippifer* complex, which resides in central and eastern Amazon, alongside northeastern Brazil. However, its distribution remains undersampled. We used morphological and molecular data of this complex, thus including samples from almost the entire distribution to infer the relationships and species diversity. To estimate the initial hypotheses of species boundaries, we used single-locus (i.e., *16s* DNA barcode) delimitation methods and the lineages inferred in a multi-locus tree and their geographic distribution. The widest species delimitation hypothesis relied on using coalescent-based methods with combined molecular, morphological, and combined datasets under different population scenarios. The phylogenetic reconstruction recovered five geographically structured lineages (less conservative species delimitation), while single-locus delimitation methods recovered one to three

species in this complex. The coalescent based validation recovered five species in all scenarios within all types of evidence (*i.e.*, morphological, molecular, and combined datasets). Our integrated approach and large-scale sampling approach allowed us to discover higher species diversity in the *P. ephippifer* complex than previous estimations. Future studies integrating other evidence (such as coloration patterns and bioacoustics) are needed to support our findings and describe these species.

Keywords: Diversity; Amazonia; Conservation; Integrative Taxonomy; Anuran.

1. Introduction

The Neotropics correspond to one of the most species-rich areas in the world, thus encompassing different biomes and habitats, which are the result of a complex geological history (Antonelli et al., 2018; Ulloa Ulloa et al., 2017). The intricate interaction between biotic and abiotic (geological and topographic) factors in this region favored the diversification of several taxa groups (Antonelli and Sanmartín, 2011; Hughes et al., 2013). The most diverse set of amphibian in the world lives in the neotropical region, and despite being threatened, the number of species described in this region continues to grow (De Carli Monteiro et al., 2018; De La Riva et al., 2018; Duellman, 1988; Frost 2021; Mângia et al., 2018; Menéndez-Guerrero et al., 2020; Pinheiro et al., 2018; Sturaro and Peloso, 2014). Methods of species delimitation contribute to fauna diversity estimations and rely on genetic distances (Hebert et al., 2003), in multispecies coalescent models using multi-locus and multi-trait data (Knowles and Carstens 2007, Solís-Lemus et al., 2015).

The most widely used delimitation methods differ in many technical aspects. Among single-locus-based methods, the Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012) is based on the genetic distance to estimate species boundaries, whereas General Mixed Yule-Coalescent (GMYC) and Multi-rate Poisson tree processes (mPTP) require a gene tree. The latter depends upon the number of accumulated substitutions and tends to perform better than GMYC when interspecific distances are smaller (Kapli et al. 2017; Pons et al., 2006; Zhang et al., 2013).

Compared to other methods based on the coalescent model, Bayesian Phylogenetics and Phylogeography (BPP) and Integration of genes and traits for Bayesian Phylogenetics and Phylogeography (iBPP) (Solís-Lemus et al., 2015; Yang and Rannala, 2010; Yang, 2015) perform better on both simulated and empirical data, despite being sensitive to the guide tree and the choice of prior parameters (Camargo et al., 2012; Eberle et al., 2016; Ence and Carstens, 2011; Hime et al., 2016). These methods are responsible for highlighting cryptic lineages and enabling deepened studies in areas that lack meticulous taxonomic studies in an integrated approach (Hajibabaei et al., 2007; Padial et al., 2009, 2010). Biodiversity underestimation reflects morphologically indistinguishable cryptic species, challenging to diagnose, misclassified under an existing taxon, and the current lack of studies focusing on such issues (Angulo and Icochea 2010; Bickford et al., 2007; Vacher et al., 2017). Amphibians have a propensity for cryptic diversity due to the presence of non-visual reproductive signals, which are

responsible for a conservative trend in morphological evolution (Bickford et al. 2007; Cherry et al., 2014; Stuart et al., 2006). Furthermore, widely distributed species are more likely to be cryptic, being an opportunity and also a challenge to study species delimitation, as is the case of the *Physalaemus ephippifer* complex.

This species complex, recovered as a monophyletic group, comprises *P. ephippifer* (Steindachner, 1864), that occurs in eastern and central Amazonia, including the mouth of the Amazon River, in the Guyana Shield and few localities in Venezuela (state of Bolívar) (Frost 2021), and the *Physalaemus cuvieri* clade from northeastern Brazil, from Maranhão to southern Bahia states (Lourenço et al., 2015; Miranda et al., 2019; Nascimento et al., 2019). Based on molecular markers and cytogenetics data, one previous study inferred a possible cryptic diversity in the *P. ephippifer* complex, with the occurrence of at least two distinct lineages (Nascimento et al., 2019). Therefore, the authors reinforced the need for a broader study on the diversity hidden under this group. It is paramount to consider reports that used sampling that did not contemplate the entire complex distribution, especially in the Amazonia, which could have underestimated species diversity.

To identify independent evolutionary lineages and to properly delineate species boundaries, it is advisable to use multiple lines of evidence (Hillis 2007; Hillis 2019). Following the assumptions of integrative taxonomy (Padial et al., 2010), several studies have taken advantage of multi-locus/multi-trait datasets and species delimitation methods to unveil cryptic lineages previously classified under the same name (Beheregaray and Caccone 2007; Domingos et al., 2017; Fujita et al 2012; Ortega-Andrade, 2015; Ramos et al., 2019; Sturaro et al., 2018; Trevisan et al., 2020).

In this study, we assess the species diversity within *Physalaemus ephippifer* complex (1) sampling almost its entire geographic distribution, including localities in Amazonia not sampled previously (Lourenço et al., 2015; Miranda et al., 2019; Nascimento et al., 2019); and (2) employing an integrated species delimitation approach (Figure 1), using both molecular markers (mitochondrial and nuclear) and morphological data.

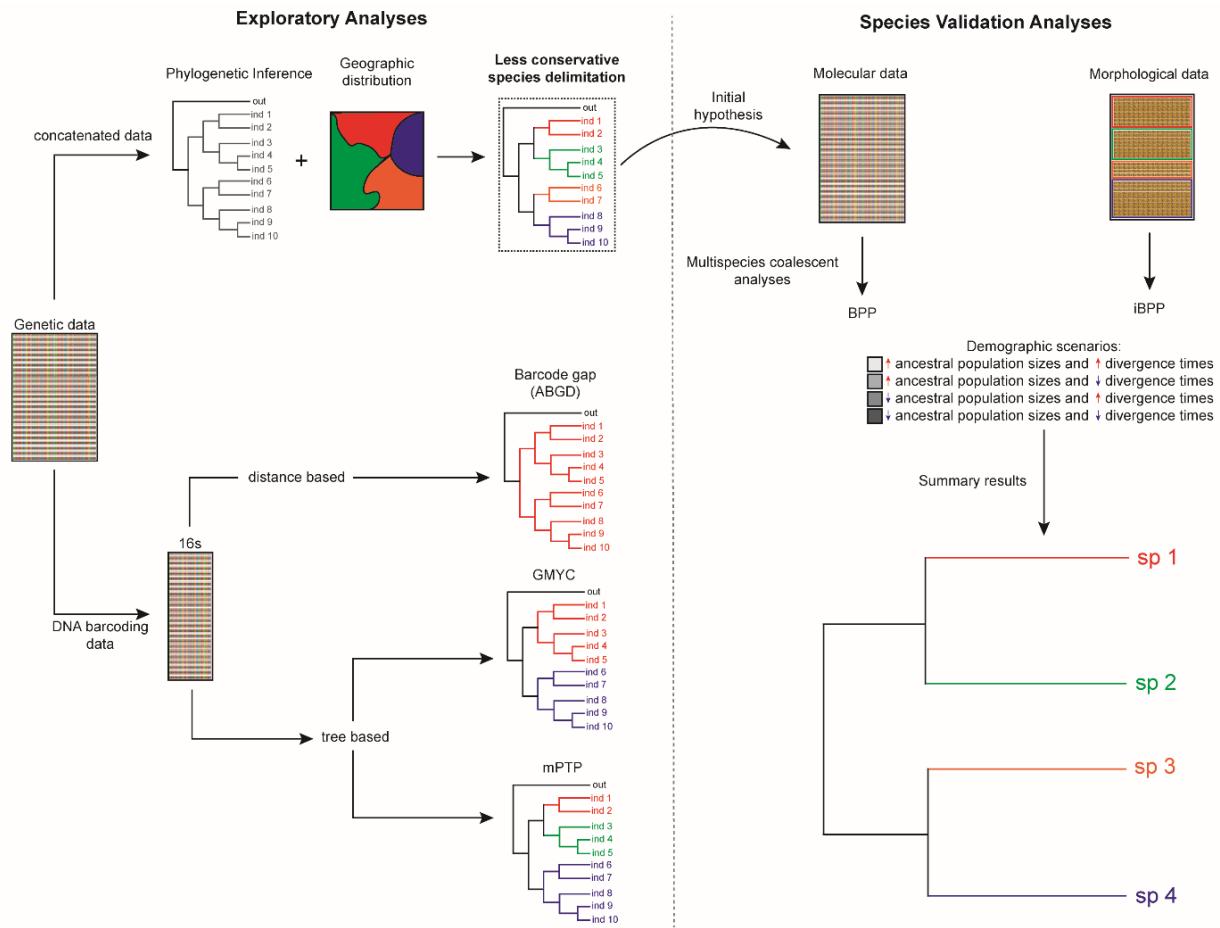


Figure 1. Workflow for species delimitation in the *Physalaemus ephippifer* complex.

Exploratory analyses: Phylogenetic tree and geographic distribution using all loci (mtDNA and nuDNA), and ABGD, GMYC and mPTP analyses including 16s dataset (DNA barcode). Species validation analyses: BPP including all loci and iBPP using morphological and combined dataset (molecular and morphology) to test the initial hypothesis (less conservative species delimitation of exploratory analyses).

2. Methods

2.1. Sampling and laboratory methods

2.1.1. DNA data set

Our molecular data set comprised 86 samples of the species complex *P. ephippifer*, an increase of 46 new samples from the Amazonia, further sampling almost the entire known geographic distribution. The new samples are deposited in the Coleção Herpetológica Osvaldo Rodrigues da Cunha, Museu Paraense Emílio Goeldi, Belém, Pará, Brazil (MPEG) and Coleção Herpetológica da Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil (AAG-UFG). We also included in our

dataset of 41 other species of *Physalaemus* and *Engystomops* available in the Genbank (Benson et al. 2013) from published studies (Appendix B).

DNA extractions were performed with the Extraction Kit Promega. Two mitochondrial genes (mtDNA: *16s* large subunit RNA and Cytochrome Oxidase subunit 1) and two nuclear genes (nuDNA: Recombination Activating 1 - RAG-1 and C-X-C Motif Chemokine Receptor 4 - CXCR4) were amplified following Faivovich et al., (2005) and Funk et al. (2012) (Table 1). PCR products were purified by precipitation using polyethylene glycol solution (20% PEG 800 and 2.5 M NaCl) and sequenced in both fragment directions using Big Dye Terminators (Perkin Elmer, Norwalk, Connecticut) following the manufacturer's guidelines, and purifying by precipitation with sodium acetate. The sequences were generated by the automated DNA MegaBase sequencer (GE Healthcare), and later revised and edited in Geneious, version 9 (Kearse et al., 2012).

The sequences were aligned independently for each gene with MAFFT online program, version 7, using the strategy Q-ins-i for *16s*, G-ins-i for COI and L-ins-i for RAG-1 and CXCR4, under default parameters (Katoh et al., 2005; Katoh and Toh, 2008; Katoh and Standley, 2013). We concatenated the dataset with the software Sequence Matrix, version 1.8 (Vaidya et al., 2011).

2.1.2. Morphological data set

We measured 460 specimens of *Physalaemus ephippifer* complex deposited in MPEG, the Herpetological Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil (INPA); and the Célio Fernando Batista Haddad Collection (CFBH), Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil (Appendix A). The sex of adults was determined based on the observation of the presence (males) or absence (females) of secondary characters (vocal sac and/or nuptial pad).

The sampling sought to cover the entire known geographic distribution of this complex. These specimens allowed quantitative morphological analyses, which consisted of the measurement of the following characters: SVL (snout-vent length), HL (head length), HW (head width), END (eye-naris distance), EL (eye length), EW (eyelid width), TL (tympanum length), IOD (interorbital distance), IND (internarial distance), ESD (eye-snout distance), NSD (naris-snout distance), HAL (hand length), UAL (upper arm length), FAL (forearm length), FD3 (finger disc 3 width), TBL (tibia length), THL (thigh length) and FOL (foot length). The measurements were taken using a digital

caliper (accuracy of 0.1 mm) under a stereomicroscope, following (Peloso et al., 2014, 2016) and Watters et al. (2016)

2.2. Phylogenetic analysis

Outgroup definition was according to Lourenço et al. (2015) and further selected for the analysis sequences of other species of *Physalaemus* and *Engystomops*. Tree was rooted using *Engystomops pustulosus*.

The best partitioning scheme and evolutionary models relied on PartitionFinder version 2.1.1 (Lanfear et al., 2017), thus using corrected Akaike Information Criterion (AICc), following the author's instructions. We tested all substitution models available for "MrBayes" under the search algorithm "greedy" and set the ribosomal gene and codon positions (for coding genes) as possible partitions.

Bayesian inference contributed to inferring the phylogenetic relationships using the best partition scheme and substitution models obtained from PartitionFinder. The analysis was conducted on MrBayes version 3.2.7 (Huelsenbeck & Ronquist, 2001), with two independent searches with four chains, each with 10 million generations and sampling every 1,000 generations, in the CIPRES portal, version 3.3. (Miller et al., 2011). The effective sample size (ESS) and search stability were performed on Tracer, version 1.7.2 (Rambaut et al., 2018), thus considering the minimum ESS > 200, and a burn-in value of 25%.

2.3. Exploratory species delimitation

We assessed the initial hypotheses of species boundaries by applying three different methods of species delimitation using a single locus (*i.e.*, 16s DNA barcoding). These single locus methods are based on different elements of molecular evolution. We inferred species delimitation in Automatic Barcode Gap Discovery (ABGD) web server (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>) (Puillandre et al. 2012), using a prior intraspecific divergence limit of P = 0.01 as recommended by Puillandre et al. (2012), testing different values for relative gap width (X) (1.5 and 1.0), with simple distance under default parameters. This method partitions samples within species based on DNA barcoding gaps that delimit both the smallest intraspecific and longer interspecific distances. We used the 16s alignment dataset in FASTA format without outgroup to calculate the genetic distance between samples.

For the tree-based species delimitation analysis with mPTP, we generated a non-ultrametric likelihood tree in Raxml, version 8.2.10 (Stamatakis, 2014) with 16s dataset including the outgroups, with GTRGAMMA substitution model, under default parameters. We ran a mPTP analysis in web server (<http://mptp.h-its.org/>) (Kapli et al., 2017), using this non-ultrametric tree and setting all other *Physalaemus* species and *Engystomops pustulosus* as outgroups. This method models the branching process based on the number of expected substitutions accumulated between subsequent speciation events (Kapli et al. 2017). For the other tree-based method (GMYC), we ran a time-calibrated ultrametric tree with full 16s dataset in BEAST, version 1.8 (Drummond et al., 2012), using relaxed clock lognormal model with a substitution rate of 0.00249 substitutions per site per million years (SD = 0.00029), following Evans et al. (2004). Stationarity, convergence and ESS (considering the minimum > 200) were visualized in software Tracer, version 1.7.2 (Rambaut et al., 2014). This tree was used as input for a single threshold analysis in GMYC (Pons 2006) web interface (<https://species.h-its.org/gmyc/>). This analysis finds the maximum likelihood solution for a model that links species diversification and genealogical branching (Fujisawa and Barraclough 2013).

2.4. Multi-trait species validation

We used Bayesian Phylogenetics and Phylogeography (BPP) to test species boundaries in *Physalaemus ephippifer* complex (Yang and Rannala, 2010; Yang 2015) and iBPP (Solís-Lemus et al., 2015) with three datasets: (1) molecular (mtDNA and nuDNA), (2) morphological, and (3) combined (morphological and molecular). Both methods use the multispecies coalescent to compare hypotheses of species boundaries in a Bayesian framework, accounting for incomplete lineage sorting due to discordance between a gene tree and ancestral polymorphism (Yang and Rannala, 2010; Rannala and Yang, 2013; Solís-Lemus et al., 2015). For all methods, we ran the ‘A10’ analysis setting a guide species tree based on our less conservative exploratory delimitation hypothesis for the *P. ephippifer* complex.

We performed species validation analyses with the molecular dataset in BPP, version 3.2.4 (Yang and Rannala, 2010; Yang 2015) under four scenarios combining inverse-gamma priors for theta (Θ) and tau (τ) parameters following Ramos et al. (2019): (1) large ancestral population sizes and deep divergence ($\Theta \sim \text{IG}(3, 0.04 - \text{mean } 0.02)$, $\tau \sim$

$\text{IG}(3, 0.02 - \text{mean } 0.01)$), (2) small ancestral population size and deep divergence ($\Theta \sim \text{IG}(3, 0.004 - \text{mean } 0.002)$, $\tau \sim \text{IG}(3, 0.02 - \text{mean } 0.01)$), (3) large ancestral population sizes and shallow divergences ($\Theta \sim \text{IG}(3, 0.04 - \text{mean } 0.02)$, $\tau \sim \text{IG}(3, 0.002 - \text{mean } 0.001)$), and (4) small ancestral population sizes and shallow divergences ($\Theta \sim \text{IG}(3, 0.004 - \text{mean } 0.002)$, $\tau \sim \text{IG}(3, 0.002 - \text{mean } 0.001)$). Each scenario was run twice to check consistency between analyses with 10 million rjMCMC generations, sampling every 100 generations, automatic finetune adjustments, and a burn-in period of 100,000 generations.

We performed species validation tests in iBPP version. 2.1 (Solís-Lemus et al., 2015) using morphological and combined datasets. We removed any possible size effects in the morphological data following Strauss (1985), Dos Reis et al. (1990) and Sturaro et al. (2018): (1) we log-transformed (base 10) all measurements and removed the outliers using R packages “mvoutlier” (Filzmoser and Gschwandtner 2015); (2) employed a multivariate normality test with R package “MVN” (Korkmaz et al., 2014) – the dataset following normal multivariate distribution; (3) ran a Principal component analysis, regressing all variables with the first component and using the residuals of that in the in iBPP. With morphological and combined datasets, we performed combinations of gamma priors for theta (Θ) and tau (τ) parameters under four scenarios: (1) large ancestral population sizes and deep divergence ($\Theta \sim \text{G}(2, 100 - \text{mean } 0.02)$, $\tau \sim \text{G}(2, 200 - \text{mean } 0.01)$), (2) small ancestral population size and deep divergence ($\Theta \sim \text{G}(2, 1000 - \text{mean } 0.002)$, $\tau \sim \text{G}(2, 200 - \text{mean } 0.01)$), (3) large ancestral population sizes and shallow divergences ($\Theta \sim \text{G}(2, 100 - \text{mean } 0.02)$, $\tau \sim \text{G}(2, 2000 - \text{mean } 0.001)$), and (4) small ancestral population sizes and shallow divergences ($\Theta \sim \text{G}(2, 1000 - \text{mean } 0.002)$, $\tau \sim \text{G}(2, 2000 - \text{mean } 0.001)$).

To maintain the same scenarios independently of dataset type or analysis, we thus standardized mean values for small and large ancestral population sizes while deep and shallow divergence times.

Genetic distances between species recovered in the validation analyses were estimated using uncorrected p-distances and pairwise-deletion options in MEGA X, version 10.2.6 (Kumar et al., 2018).

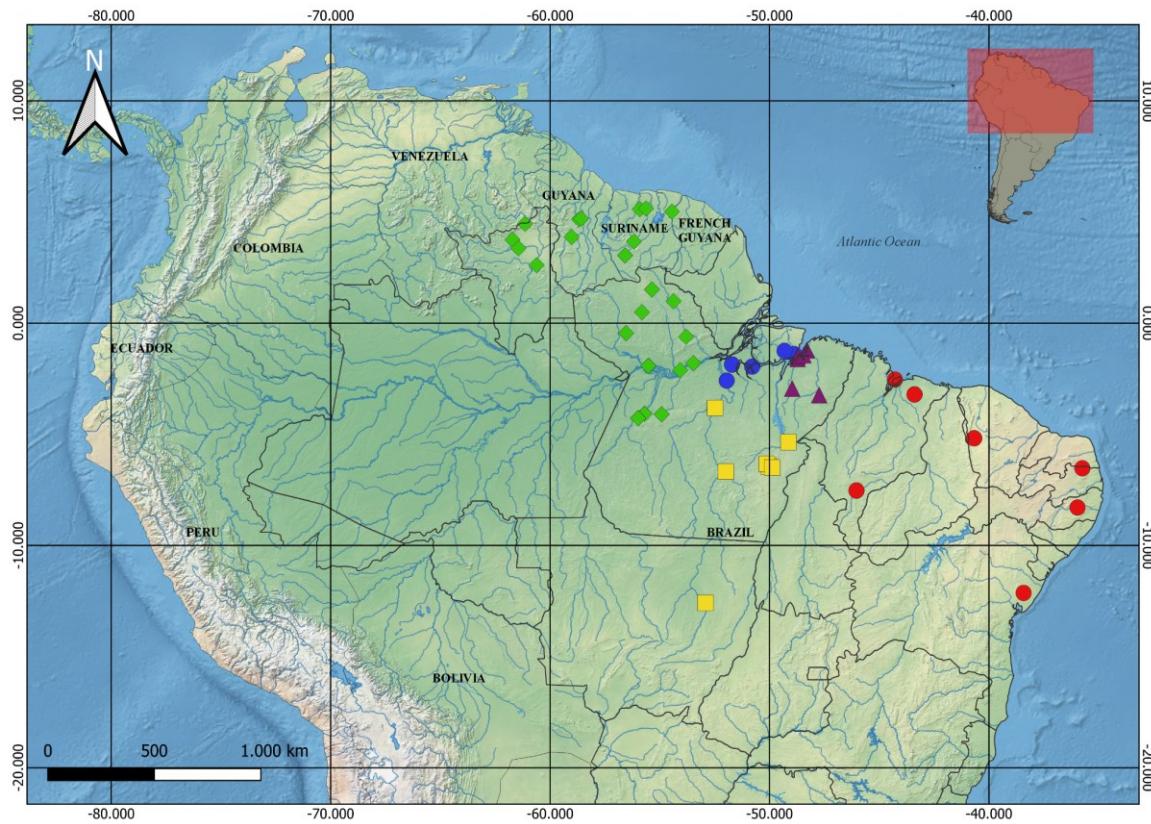


Figure 2. Distribution of the *Physalaemus ephippifer* complex DNA samples used in the present study. The colors represent species recovered in validation analyses: green diamond = NOE; Blue circle = MAR; Yellow square: AEX; Purple triangle = AEB; Red Circle = NOD.

Table 1. Primers used in this study.

Primers	Direction	Sequence (5'-3')	References
16s AF	Forward	5'- CGC CTG TTT ATC AAA AAC AT -3'	Palumbi <i>et al.</i> (1991)
16s AR	Reverse	5'- CCG GTC TGA ACT CAG ATC ACG T -3'	Palumbi <i>et al.</i> (1991)
COI-PFD	Forward	5' - TTT CAA CHA AYC AYA AAG AYA TYG G -3'	Peloso <i>et al.</i> (2014)
COI-PRD	Reverse	5'- TAN ACT TCN GGG TGD CCA AAR AAT CA -3'	Peloso <i>et al.</i> (2014)
R1-GFF	Forward	5' - GAGAAGTCTACAAAAAVGGCAAAG – 3'	Faivovich <i>et al.</i> (2005)
R1-GFR	Reverse	5' - GAAGCGCCTGAACAGTTATTAC – 3'	Faivovich <i>et al.</i> (2005)
CXCR4-F10	Forward	5'- TGA CTG ATA AGT ACC GGC TGC -3'	Funk <i>et al.</i> (2012)
CXCR4-R10	Reverse	5'- CCA AGG CTT CTG TGA TGG AG -3'	Funk <i>et al.</i> (2012)

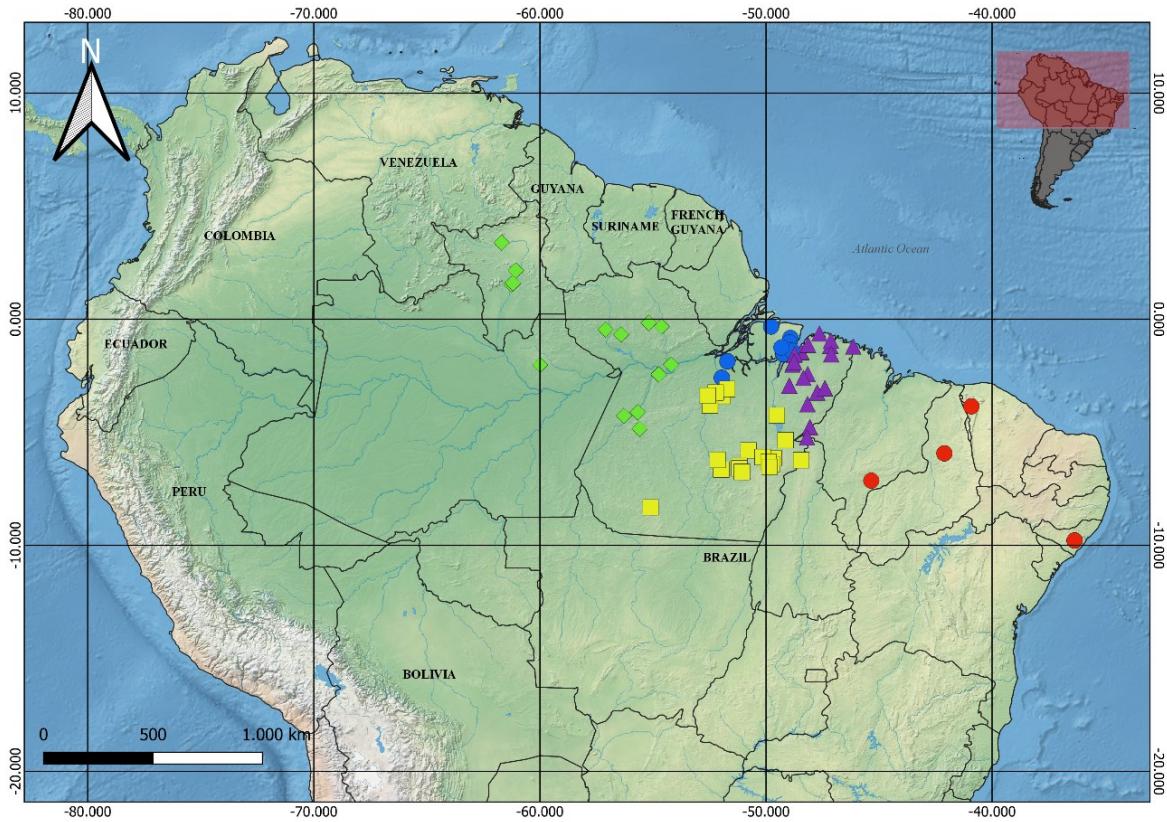


Figure 3. Distribution of the *Physalaemus ephippifer* complex specimens used in morphological analyses of this study. Colors represent species recovered in the validations analyses: Green Circle = NOE; Blue Circle = MAR; Yellow Square: AEX; Purple Triangle = AEB; Red Circle = NOD.

3. Results

3.1. Phylogenetic analysis

The concatenated molecular dataset included 127 terminals and 3,230 aligned characters (16s: 492 bp, COI: 718 bp, CXCR4: 598, RAG1: 1422). The Bayesian inference found strong support for the monophyly of the *Physalaemus ephippifer* complex and recovered it as a sister clade of *P. cuvieri* from central Brazil (Tocantins state) (Figure 4). In the *P. ephippifer* complex (Figure 5), five monophyletic groups were recovered: (1) one composed by samples from south of the Amazon River and east of the Tocantins River (including the type locality of *P. ephippifer* Belém, Pará, Brazil) (AEB, pp = 0.60 Figure 5), which is sister of two others, thus comprising (2) samples from localities between middle-high Xingu and Tocantins rivers (AEX, pp = 1) and (3) samples from northeastern Brazil, which are distributed from the municipality of São Luís (Maranhão state, Brazil) to the north of the state of Bahia (Brazil), in the municipality of

Alagoinhas (NOD, pp = 0.33), supported by posterior probability of 0.81. These three groups were recovered as sister of two clades (4) with individuals from Marajó Island and localities of lower interfluve Xingu-Tocantins rivers (MAR, pp = 1), and (5) samples from the north region of Amazon River, including Pará and Roraima states in Brazil, Guyanas, and Suriname, and located to the west of the Xingu River (south of the Amazon River) (NOE, pp < 0.5).

3.2. Exploratory Species delimitation

The number of delimited species differs between the methods utilized. The barcode gap analysis (ABGD), despite the different parameters ($X = 1.5$ and 1.0), yielded identical results with only one species for the entire *P. ephippifer* complex. The single threshold GMYC analysis delimited two species, one composed by samples from MAR and NOE and other from AEB, AEX and NOD. The mPTP approach delimited three species: (1) one with samples from eastern Amazonia and northeastern Brazil (AEB, AEX and NOD), (2) a second from Marajó and adjacent localities lower Xingu-Tocantins (MAR), and (3) a third from north of Amazon river and western Xingu (NOE). A summary of these different species delimitation analyses was shown in Figure 5.

3.3. Multi trait species validation

For species validation tests, we defined the species tree guide while considering the monophyletic clades, and respective geographic recovered in our multi-locus phylogenetic inference, as an initial proposal for species delimitation of the *P. ephippifer* complex in BPP and iBPP (Figure 5). For the iBPP analyses using morphological data, our initial dataset (Appendix A) diminished to 418 individuals after outlier removals.

All the scenarios in both BPP and iBPP (morphological and combined datasets) recovered five putative species with high support (pp = 1.0) (Figure 5), validating the initial hypothesis. These results indicate that inside *P. ephippifer* complex at least 4 species remain undescribed: one from the north of Amazon River to west of Xingu River (NOE), another from Marajó island and localities between lower Xingu-Tocantins (MAR), the clade from the east side of Xingu river to the extreme east of Amazon (AEX), and finally the clade in northeastern Brazil, which extends from the state of Maranhão to the north of Bahia (NOD). Furthermore, a fifth species came from samples from the type locality (AEB).

The mean uncorrected pairwise genetic distance in 16s rDNA between the five recovered species of the *Physalaemus ephippifer* complex in our validation analyses varied from 1.3% (between AEB and AEX) to 4.4% (between MAR and NOD) (Table 2).

Table 2. Uncorrected p-distances between and within (in bold) putative *Physalaemus ephippifer* complex species from validation analyses based on 16s rDNA.

	MAR	NOD	AEX	AEB	NOE
MAR	0.01				
NOD	0.044	0.01			
AEX	0.042	0.014	0.00		
AEB	0.037	0.014	0.013	0.00	
NOE	0.042	0.033	0.032	0.027	0.01

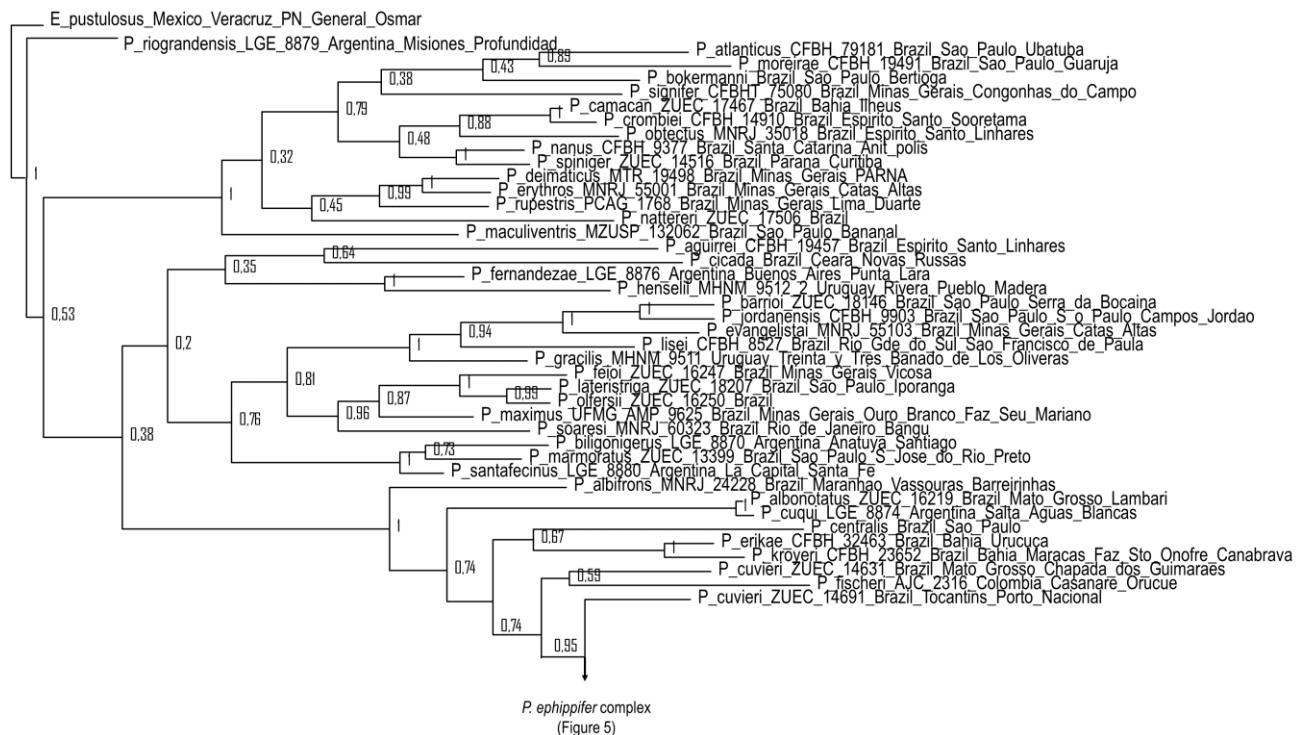


Figure 4. Bayesian phylogenetic inference using the concatenated dataset (*i.e.*, mtDNA and nuDNA) of genus *Physalaemus*. Values at nodes = posterior probability.

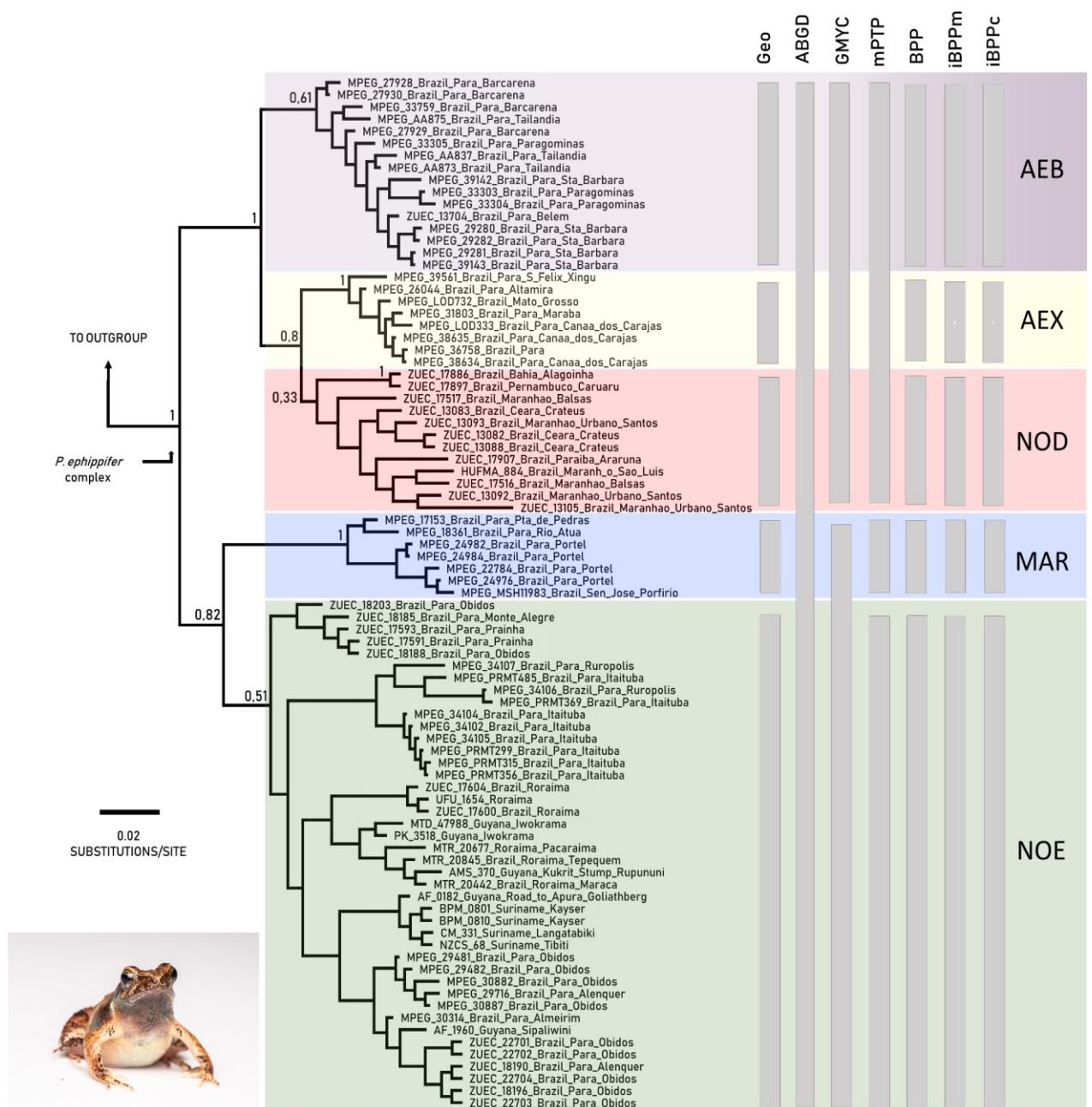


Figure 5. Summary including Bayesian phylogenetic inference using the concatenated dataset (mtDNA and nuDNA) of the *Physalaemus ephippifer* complex. Gray bars represent phylogenetic and geographic (GEO), single-locus (ABGD, GMYC and mPTP), multilocus (BPP), morphological (iBPPm), and combined (molecular and morphology) (iBPPc) species delimitation results. Values at nodes = posterior probability. Colors represent the clades and respective geographical distribution: Purple = AEB; Yellow = AEX; Red = NOD; Blue = MAR and Green = NOE. Values at nodes = posterior probability.

4. Discussion

4.1. Phylogenetic analysis

Our Bayesian inference recovered the monophyly of the *Physalaemus ephippifer* complex with relatively strong support and as a sister group of a clade of *P. cuvieri* from central Brazil (Porto Nacional, Tocantins). The monophyly of this complex has been found by other studies (Lourenço et al., 2015; Miranda et al., 2019; Nascimento et al., 2019). However, our results on the sister group of this complex differ from those studies, which recovered samples of *P. cuvieri* of Central to Southern Brazil and Argentina as the sister group of *P. ephippifer* complex.

Within the *P. ephippifer* complex, our results recovered five clades which differ in number from previous studies that inferred one to three clades. The results of Lourenço et al. (2015) support only two main clades in this group, one with samples from Belém and other with samples of *P. cuvieri* from northeastern Brazil. Miranda et al. (2019) carrying out a phylogeographic study, added more samples from eastern Amazonia and northeastern Brazil, that were recovered as a monophyletic clade treated as a lineage "D" of *P. cuvieri*. We tried to include such samples in this study. However, these samples remain inaccessible in Genbank or other repositories. In the last study about this species complex, Nascimento et al. (2019) increased the sampling of *P. ephippifer* with individuals from the vicinity of Belém and north of the Amazon River in Brazil (Parque Nacional do Viruá, Roraima, and northern Pará). These authors recovered three main clades in the *P. ephippifer* complex, one including samples from northern Amazonia that is sister of two others from eastern Amazonia and northeastern Brazil. These differences between this study and previous ones are due to their limited geographic sampling, particularly in the Amazon region. We included samples from well-represented regions (e.g., northern and eastern Amazonia), albeit with improved geographic coverage. Further, we provide new samples from novel localities, such as the western Tocantins river and Marajó Island.

4.2. Exploratory Species delimitation

Our results show that the tree-based (mPTP and GMYC) and distance-based (ABGD) methods of single-locus species delimitation were not fully congruent (Figure 5), with the barcode gap analysis being the most conservative delimiting for a single species. This difference between methods is congruent with other comparative studies of

delimitation methods in amphibians (Guarnizo et al., 2015; Paz and Crawford, 2012) and other taxa (Blair and Bryson, 2017; Boissin et al., 2017; Pentinsaari et al., 2017; Song et al., 2018). This fact shows that ABGD tends to estimate less species than other methods. The ABGD method is known for over lump and perform poorly on more speciose datasets (Dellicour and Flot, 2015, 2018). Its performance also depends on user-selected parameters, such as the distance model and the prior upper limit to intraspecific divergence (P) (Kekkonen and Hebert, 2014). Under the recommended P values (0.01 – Puillandre et al., 2012), we tested different X values to avoid species underestimation, albeit it retrieved only one group only in all partitions.

In contrast to ABGD, GMYC is known for over-splitting (Fujisawa & Barraclough 2013; Hamilton et al., 2014; Paz and Crawford 2012; Talavera et al., 2013). Similar to results by Blair and Bryson (2017), this method seemed conservative and did not show the over-split pattern with our dataset, thus recovering just two putative species: one comprising samples from AEB, AEX and NOD, and other including MAR e NOE. The mPTP approach recovered the eastern (AEB+ AEX+ NOD), the Marajó (MAR), and the Guiana Shield and west of Xingu river (NOE) clades as candidate species, thus being the single-locus method with more similarity to the geographic distribution of the clades recovered in our phylogenetic analysis. Furthermore, this analysis was less conservative than other single-locus methods since contrasting to previous comparisons provided by other studies (Kapli et al., 2017; Blair and Bryson 2017; Young et al., 2021; Becchimanzi et al., 2021), which found this approach more conservative among species delimitation methods.

Single-locus delimitation methods help us testing the initial hypothesis of species boundaries, although we cannot rely only on these tools to make assumptions in species boundaries in the *P. ephippifer* complex, as previously recommended by other authors (Esselstyn et al., 2012; Lohse, 2009; Puillandre et al., 2012; Talavera et al., 2013; Zhang et al., 2013). Thence, putative species accessed in our study were part of an integrative investigation using multi-locus and traits coalescent-based approaches (Fujita et al. 2012; Solís-Lemus et al., 2015; Yang and Rannala, 2010).

4.3. Multi trait species validation

Despite our prior parameters testing, BPP and iBPP analyses recovered five putative species in the *Physalaemus ephippifer* complex (Figure 5), with high posterior probabilities (pp = 1). Although these analyses are very sensible to prior parameter

(Eberle et al., 2016), the similar results of all combinations corroborated, at least in part, to the conclusions from Nascimento et al., (2019), who pointed out that inside the *Physalaemus ephippifer* complex were at least three unnamed species. Although these five putative species are highly supported by all validation analyses and geography, the putative species AEB, NOD and NOE have low support in concatenate phylogeny, and the latter was also recovered in the analysis of mPTP. The other two delimited species (AEX and MAR) have high support in the phylogenetic inference and also geographic correspondence, and the species MAR was also retrieved in the mPTP results (Figure 5).

The Bayesian coalescent species delimitation approach solely based upon molecular data received criticism because this method may recover population structure instead of species features (Sukumaran and Knowles, 2017). In our study, we also incorporated analyses with both morphological and integrative data (molecular and morphology), thus aiming to circumvent this issue and further increase accuracy in the species delimitation (Bauer et al., 2010; Edwards and Knowles, 2014; Pavón-Vázquez et al., 2018; Ramos et al., 2019; Solís-Lemus et al., 2015).

4.4. Species boundaries and future directions

The AEB delimited species corresponds to *Physalaemus ephippifer*, because this lineage groups samples from the type-locality (Belém, Pará, Brazil) and nearby areas, and also localities from eastern Tocantins River, limited western by Mearim river (Maranhão state, Brazil). This species was also recovered by Nascimento et al. (2019), albeit their molecular sampling was limited by few samples from surrounding areas of Belém without morphological data. The putative species from northeastern Brazil (NOD) was also identified by Lourenço et al. (2015), in their phylogeny (as a lineage 1 of *P. cuvieri*), and Nascimento et al. (2019), in almost all species delimitation analyses. The latter report suggests that it may indicate population structuring rather than true species boundaries (Sukumaran and Knowles, 2017). Furthermore, Miranda et al. (2019) did not find eastern Amazonia and northeastern Brazil clades as monophyletic groups in their molecular phylogenetic tree. Based on this fact, *P. ephippifer* could occur from eastern Amazonia to northeastern Brazil. Notwithstanding, cytogenetics data support the distinction between AEB and NOD putative species, because in *Physalaemus ephippifer* was observed heteromorphic sex chromosomes (Nascimento et al., 2010), but not in the samples from northeastern Brazil (Quinderé et al., 2009), thus

suggesting a possible incipient speciation process. Our results likewise recover these lineages and support them as distinct species. This potentially new species from northeastern Brazil is currently considered as *P. cuvieri*. Nonetheless, this species was not recovered as monophyletic clade in our and previous studies (Lourenço et al., 2015; Nascimento et al., 2019). Any taxonomic decision solely based on that fact would be premature, because the type of *Physalaemus cuvieri* has not been found in the recent list of the Herpetological Collection of Museum of Natural History of Vienna, Austria, and the type-locality is ambiguous ("America, Brasilia"). Therefore, it is not advisable to attribute any name for this putative species.

Our extensive sampling in northern and western Amazonia corroborated the candidate species of the *P. ephippifer* complex in this region as suggested by Nascimento et al., (2019). However, this species is not restricted to the Guianas Shield (including Guyana, Suriname, French Guyana and in the state of Roraima, Brazil) but also the region between the Tapajós and Xingu rivers, south of Amazon river. Nascimento et al. (2019) found geographic structuring between samples from Roraima and western Pará (municipalities of Óbidos, Prainha and Monte Alegre) in their phylogenetic analyses, retrieving them as reciprocally monophyletic groups, as well in a species delimitation analysis. The genetic distance between the two groups and cytogenetic data also pointed out differences between these lineages (called as "Western Pará Clade" and "Viruá Clade"), although authors indicate that these differences may indicate intra or interpopulation variation. In our study, we did not find geographic structure in the multi-locus tree, thus corroborating these findings as intraspecific variation. In NOE species delimited in our study, samples from south and north of Amazon river were not recovered as reciprocal monophyletic groups, although this river is known to impose barrier to the dispersal of amphibians (Fouquet et al., 2015; Gascon et al., 1998, 2000; Godinho and Da Silva, 2018; Moraes et al., 2016; Vacher et al., 2020). Despite not yet being tested, this absence of geographic structure might be explained by the floating meadows dispersion mechanism, indicating that this river does not represent a relevant barrier to the dispersal of NOE species (Fonte et al., 2021). There is no available name of this possible species because it is currently recognized as *P. ephippifer* sensu stricto (Amazonian lineages), which does not have any synonyms.

MAR species delimited in this study includes samples from Marajó Island and lower interfluvial Xingu-Tocantins, which were never sampled (Lourenço et al., 2015; Nascimento et al., 2019). The identification of novel species should continue (as

described here), as expected in other areas of the Amazonia (Funk et al., 2012). Nonetheless, species delimitation studies, thus including such samples are scarce from this region (Vacher et al., 2020), still require an extensive geographic representation. The Marajó Island and lower interfluve Xingu-Tocantins have a complex hydrological and geological history that could explain this speciation event (Rossetti and Valeriano, 2007). Therefore, studies focusing on the historical biogeography of the *P. ephippifer* complex are necessary to better understand this pattern.

Putative species from middle and high Xingu-Tocantins interfluve (AEX) were never proposed before due to sampling gaps (Lourenço et al., 2015; Miranda et al., 2019; Nascimento et al., 2019). New species of vertebrates have been described from this region (De Carvalho et al., 2019), and our findings support the need for new efforts to elucidate this underestimation of species diversity.

Pairwise distance analysis combined with species delimitations resulted in an integrative framework (Padial et al., 2010), which was based on molecular and morphological datasets, thus allowing us to infer five species inside the *P. ephippifer* complex. All species delimited in our validation analyses have at least 1.3% of uncorrected pairwise genetic divergences in 16s rRNA (Table 2), a value between the threshold stipulated by Vieites et al. (2009) for confirmed candidate species. We did not explore qualitative morphological or bioacoustic characters, albeit the iBPP analyses using morphometric data supported all five putative species in the *P. ephippifer* complex.

5. Conclusion

Our integrative approach demonstrates that there are five candidate species within the *Physalaemus ephippifer* complex. These findings stem from employing morphological and molecular characters in different species delimitation methods, in addition to pairwise genetic distance. This study expands the knowledge on frog species diversity in the Amazon region and northeastern Brazil, although further studies on this complex should describe these new species using other approaches (e.g., larval morphology, bioacoustics, cytogenetics).

Acknowledgements

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Appendix A. Specimens of *Physalaemus ephippifer* analyzed in our study. Individuals from Museu Paraense Emilio Goeldi (MPEG), Belém, Brazil; Instituto Nacional de Pesquisas da Amazônia Herpetological Collection (INPA), Manaus, Brazil; Celio F.B. Haddad Collection (CFBH), São Paulo, Brazil.

Município	Voucher	Lat	Long
Belém	MPEG_861	-1.4439840	-48.4430060
Belém	MPEG_862	-1.4439840	-48.4430060
Belém	MPEG_865	-1.4439840	-48.4430060
Belém	MPEG_866	-1.4439840	-48.4430060
Belém	MPEG_870	-1.4439840	-48.4430060
Belém	MPEG_871	-1.4439840	-48.4430060
Belém	MPEG_873	-1.4439840	-48.4430060
Belém	MPEG_877	-1.4439840	-48.4430060
Belém	MPEG_879	-1.4439840	-48.4430060
Belém	MPEG_881	-1.4439840	-48.4430060
Belém	MPEG_882	-1.4439840	-48.4430060
Belém	MPEG_883	-1.4439840	-48.4430060
Belém	MPEG_884	-1.4439840	-48.4430060
Belém	MPEG_892	-1.4439840	-48.4430060
Belém	MPEG_893	-1.4439840	-48.4430060
Belém	MPEG_894	-1.4439840	-48.4430060
Belém	MPEG_900	-1.4439840	-48.4430060
Marapanim	MPEG_949	-0.6315290	-47.6369480
Alto Alegre, Roraima	MPEG_1080	3.2617531	-61.0708919
Belém	MPEG_1439	-1.4439840	-48.4430060
Belém	MPEG_1441	-1.4439840	-48.4430060
Belém	MPEG_1460	-1.4439840	-48.4430060
Belém	MPEG_3127	-1.4439840	-48.4430060
Belém	MPEG_3128	-1.4439840	-48.4430060
Belém	MPEG_3129	-1.4439840	-48.4430060
Belém	MPEG_3130	-1.4439840	-48.4430060
Belém	MPEG_3133	-1.4439840	-48.4430060
Belém	MPEG_3136	-1.4439840	-48.4430060
Belém	MPEG_3137	-1.4439840	-48.4430060
Belém	MPEG_3138	-1.4439840	-48.4430060
Tucuruí	MPEG_3730	-3.7792481	-49.7082619

Tucuruí	MPEG_3735	-3.7792481	-49.7082619
Capanema	MPEG_3886	-1.1789930	-47.1589560
Capanema	MPEG_3889	-1.1789930	-47.1589560
Capanema	MPEG_3890	-1.1789930	-47.1589560
Capanema	MPEG_3891	-1.1789930	-47.1589560
Capanema	MPEG_3892	-1.1789930	-47.1589560
Capanema	MPEG_3894	-1.1789930	-47.1589560
Capanema	MPEG_3895	-1.1789930	-47.1589560
Belém	MPEG_3917	-1.4439840	-48.4430060
Cachoeira do Arari	MPEG_4643	-1.0325000	-48.9719444
Cachoeira do Arari	MPEG_4648	-1.0325	-48.97194444
Cachoeira do Arari	MPEG_4649	-1.0325	-48.97194444
Cachoeira do Arari	MPEG_4650	-1.0325	-48.97194444
Cachoeira do Arari	MPEG_4651	-1.0325	-48.97194444
Cachoeira do Arari	MPEG_4652	-1.0325000	-48.9719444
Cachoeira do Arari	MPEG_4653	-1.0325	-48.97194444
Cachoeira do Arari	MPEG_4662	-1.0325000	-48.9719444
Cachoeira do Arari	MPEG_4663	-0.8261500	-48.9202950
Cachoeira do Arari	MPEG_4664	-1.0325	-48.97194444
Cachoeira do Arari	MPEG_4668	-1.0325	-48.97194444
Cachoeira do Arari	MPEG_4690	-1.0325	-48.97194444
Cachoeira do Arari	MPEG_4697	-1.0325000	-48.9719444
Breves	MPEG_4708	-1.7127778	-50.4577778
Breves	MPEG_4709	-1.7127778	-50.4577778
Breves	MPEG_4710	-1.7127778	-50.4577778
Breves	MPEG_4711	-1.7127778	-50.4577778

Breves	MPEG_4712	-1.7127778	-50.4577778
Breves	MPEG_4727	-1.7127778	-50.4577778
Breves	MPEG_4734	-1.7127778	-50.4577778
Breves	MPEG_4743	-1.7127778	-50.4577778
Belém	MPEG_6125	-1.4439840	-48.4430060
Santarém	MPEG_6364	-2.4420631	-54.7470081
Santarém	MPEG_6368	-2.4420631	-54.7470081
Santarém	MPEG_6369	-2.4420631	-54.7470081
Santarém	MPEG_6374	-2.4420631	-54.7470081
Santarém	MPEG_6375	-2.4420631	-54.7470081
Santarém	MPEG_6376	-2.4420631	-54.7470081
Santarém	MPEG_6377	-2.4420631	-54.7470081
Paragominas	MPEG_7130	-3.0525000	-47.3952778
Paragominas	MPEG_7142	-3.0525000	-47.3952778
Paragominas	MPEG_7148	-3.0525000	-47.3952778
Paragominas	MPEG_7149	-3.0525000	-47.3952778
Paragominas	MPEG_7156	-3.0525000	-47.3952778
Paragominas	MPEG_7171	-3.0525000	-47.3952778
Paragominas	MPEG_7175	-3.0525000	-47.3952778
Paragominas	MPEG_7177	-3.0525000	-47.3952778
Paragominas	MPEG_7178	-3.0525000	-47.3952778
Paragominas	MPEG_7186	-3.0525000	-47.3952778
Paragominas	MPEG_7189	-3.0525000	-47.3952778
Paragominas	MPEG_7190	-3.0525000	-47.3952778
Roraima	MPEG_7640	1.5496000	-61.2661000
São Félix do Xingu	MPEG_9352	-6.2202778	-52.1372222

Muaná	MPEG_9584	-1.5044950	-49.2580710
Muaná	MPEG_9585	-1.5044950	-49.2580710
Muaná	MPEG_9586	-1.5044950	-49.2580710
Muaná	MPEG_9587	-1.5044950	-49.2580710
Muaná	MPEG_9594	-1.5044950	-49.2580710
Muaná	MPEG_9600	-1.5044950	-49.2580710
Santo Antônio do Tauá	MPEG_9637	-1.1293750	-48.1389990
Vitória do Xingú	MPEG_10339	-3.3908333	-51.9250000
Vitória do Xingú	MPEG_10352	-3.3908333	-51.9250000
Vitória do Xingú	MPEG_10384	-3.3908333	-51.9250000
Vitória do Xingú	MPEG_10706	-3.3908333	-51.9250000
Paragominas	MPEG_14642	-3.7562010	-48.1644290
Paragominas	MPEG_14682	-3.7562010	-48.1644290
Muaná	MPEG_16413	-1.5343930	-49.2233710
Muaná	MPEG_16416	-1.5044950	-49.2580710
Muaná	MPEG_16417	-1.5343930	-49.2233710
Muaná	MPEG_16418	-1.5343930	-49.2233710
Muaná	MPEG_16419	-1.5343930	-49.2233710
Muaná	MPEG_16420	-1.5343930	-49.2233710
Muaná	MPEG_16423	-1.5343930	-49.2233710
Muaná	MPEG_16425	-1.5343930	-49.2233710
Muaná	MPEG_16426	-1.5343930	-49.2233710
Muaná	MPEG_16427	-1.5343930	-49.2233710
Curionópolis	MPEG_16862	-6.4141667	-49.7461111
Curionópolis	MPEG_16877	-6.4141667	-49.7461111
Ourilândia do Norte	MPEG_17068	-6.7482631	-51.0683669

Ponta de Pedras	MPEG_17155	-1.3900000	-48.8708333
Ponta de Pedras	MPEG_17157	-1.3900000	-48.8708333
Muaná	MPEG_18360	-1.2466667	-49.3150000
Muaná	MPEG_18362	-1.2466667	-49.3150000
Barcarena	MPEG_20277	-1.5870420	-48.7552640
Barcarena	MPEG_20280	-1.5870420	-48.7552640
Barcarena	MPEG_20281	-1.5870420	-48.7552640
Barcarena	MPEG_20283	-1.5870420	-48.7552640
Barcarena	MPEG_20285	-1.5870420	-48.7552640
Barcarena	MPEG_20286	-1.5870420	-48.7552640
Barcarena	MPEG_20287	-1.5870420	-48.7552640
Barcarena	MPEG_20288	-1.5870420	-48.7552640
Barcarena	MPEG_20292	-1.5870420	-48.7552640
Paragominas	MPEG_20889	-3.2366194	-47.7330556
Portel	MPEG_22784	-1.956472222	-50.78985556
Curionópolis	MPEG_23445	-6.4141667	-49.7461111
Curionópolis	MPEG_23447	-6.4141667	-49.7461111
Curionópolis	MPEG_23448	-6.4141667	-49.7461111
Curionópolis	MPEG_23449	-6.4141667	-49.7461111
Curionópolis	MPEG_23450	-6.4141667	-49.7461111
Curionópolis	MPEG_23451	-6.4141667	-49.7461111
Curionópolis	MPEG_23461	-6.4141667	-49.7461111
Curionópolis	MPEG_23464	-6.4141667	-49.7461111
Curionópolis	MPEG_23465	-6.4141667	-49.7461111
Curionópolis	MPEG_23467	-6.4141667	-49.7461111
Curionópolis	MPEG_23803	-6.1511050	-49.6526650

Portel	MPEG_24973	-1.8626056	-51.7072889
Portel	MPEG_24977	-1.8626056	-51.7072889
Portel	MPEG_24981	-1.8626056	-51.7072889
Portel	MPEG_24985	-1.8626056	-51.7072889
Portel	MPEG_24987	-1.8626056	-51.7072889
Portel	MPEG_24990	-1.8626056	-51.7072889
Portel	MPEG_24991	-1.8626056	-51.7072889
Altamira	MPEG_25603	-3.8144444	-52.4802222
Altamira	MPEG_26026	-3.8144444	-52.4802222
Altamira	MPEG_26028	-3.8144444	-52.4802222
Marabá	MPEG_28259	-5.3509831	-49.1340931
Marabá	MPEG_28260	-5.3509831	-49.1340931
Marabá	MPEG_28261	-5.3509831	-49.1340931
Marabá	MPEG_28262	-5.3509831	-49.1340931
Marabá	MPEG_28263	-5.3509831	-49.1340931
Marabá	MPEG_28264	-5.3509831	-49.1340931
Marabá	MPEG_28265	-5.3509831	-49.1340931
Marabá	MPEG_28266	-5.3509831	-49.1340931
Tomé-Açu	MPEG_29014	-2.4236560	-48.1547310
Vila Nova	MPEG_29017	-5.2273080	-48.1870305
Vila Nova	MPEG_29018	-5.2273080	-48.1870305
Óbidos	MPEG_29480	-0.4584083	-57.0983222
Óbidos	MPEG_29481	-0.4584083	-57.0983222
Óbidos	MPEG_29483	-0.4584083	-57.0983222
Óbidos	MPEG_29484	-0.4584083	-57.0983222
Óbidos	MPEG_29485	-0.4584083	-57.0983222

Alenquer	MPEG_29486	-0.6766667	-56.4041667
Alenquer	MPEG_29487	-0.6766667	-56.4041667
Alenquer	MPEG_29488	-0.6766667	-56.4041667
Alenquer	MPEG_29489	-0.6766667	-56.4041667
Alenquer	MPEG_29490	-0.6766667	-56.4041667
Alenquer	MPEG_29491	-0.6766667	-56.4041667
Alenquer	MPEG_29492	-0.6766667	-56.4041667
Alenquer	MPEG_29493	-0.6766667	-56.4041667
Alenquer	MPEG_29494	-0.6766667	-56.4041667
Alenquer	MPEG_29716	-0.1654889	-55.1864000
Alenquer	MPEG_29722	-0.1654889	-55.1864000
Alenquer	MPEG_29723	-0.1654889	-55.1864000
Alenquer	MPEG_29724	-0.1654889	-55.1864000
Ourilândia do Norte	MPEG_29899	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29900	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29901	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29902	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29903	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29904	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29905	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29906	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29907	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29908	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_29909	-6.5927631	-51.1781319
Almeirim	MPEG_30315	-0.3197222	-54.6144444
Altamira	MPEG_31487	-8.3179069	-55.1007181

Altamira	MPEG_31489	-8.3179069	-55.1007181
Altamira	MPEG_31490	-8.3179069	-55.1007181
Altamira	MPEG_31493	-8.3179069	-55.1007181
Altamira	MPEG_31494	-8.3179069	-55.1007181
Itaituba	MPEG_31585	-4.2753056	-56.2840000
Itaituba	MPEG_31586	-4.2753056	-56.2840000
Itaituba	MPEG_31587	-4.2753056	-56.2840000
Itaituba	MPEG_31588	-4.2753056	-56.2840000
Itaituba	MPEG_31590	-4.2753056	-56.2840000
Itaituba	MPEG_31592	-4.2753056	-56.2840000
Primavera	MPEG_33255	-0.9449800	-47.1291520
Paragominas	MPEG_33303	-3.2366194	-47.7330556
Paragominas	MPEG_33304	-3.2366194	-47.7330556
Itaituba	MPEG_34102	-4.2753056	-56.2840000
Itaituba	MPEG_34103	-4.2753056	-56.2840000
Itaituba	MPEG_34104	-4.2753056	-56.2840000
Itaituba	MPEG_34105	-4.2753056	-56.2840000
Rurópolis	MPEG_34106	-4.1144444	-55.6769444
Rurópolis	MPEG_34109	-4.1144444	-55.6769444
Brasil Novo	MPEG_34566	-3.3766610	-52.5744444
Brasil Novo	MPEG_34567	-3.3766610	-52.5744444
Marabá	MPEG_34793	-5.6563210	-49.416231
Marabá	MPEG_34794	-5.6563210	-49.416231
Marabá	MPEG_34795	-5.6563210	-49.416231
Marabá	MPEG_34796	-5.6563210	-49.416231
Marabá	MPEG_34797	-5.6563210	-49.416231

Marabá	MPEG_34798	-5.6563210	-49.416231
Marabá	MPEG_34799	-5.6563210	-49.416231
Marabá	MPEG_34800	-5.6563210	-49.416231
Marabá	MPEG_34801	-5.6563210	-49.416231
Marabá	MPEG_34802	-5.6563210	-49.416231
São Geraldo de Araguaia	MPEG_36390	-6.2280556	-48.4438889
São Geraldo de Araguaia	MPEG_36393	-6.2280556	-48.4438889
São Geraldo de Araguaia	MPEG_36397	-6.2280556	-48.4438889
Chaves	MPEG_37087	-0.3242430	-49.7745340
Chaves	MPEG_37088	-0.3242430	-49.7745340
Chaves	MPEG_37089	-0.3242430	-49.7745340
Senador José Porfírio	MPEG_38472	-3.8518950	-51.8526280
Vitória do Xingú	MPEG_38998	-3.3908333	-51.9250000
Vitória do Xingú	MPEG_38999	-3.3908333	-51.9250000
São Félix do Xingu	MPEG_39388	-6.6453680	-51.9821510
São Félix do Xingu	MPEG_39389	-6.6453680	-51.9821510
São Félix do Xingu	MPEG_39390	-6.6453680	-51.9821510
São Félix do Xingu	MPEG_39391	-6.6453680	-51.9821510
São Félix do Xingu	MPEG_39392	-6.6453680	-51.9821510
São Félix do Xingu	MPEG_39393	-6.6453680	-51.9821510
São Félix do Xingu	MPEG_39394	-6.6453680	-51.9821510
São Félix do Xingu	MPEG_39561	-6.6453680	-51.9821510
São Félix do Xingu	MPEG_39562	-6.6453680	-51.9821510
Marabá	MPEG_40215	-5.7987831	-50.7749531
Marabá	MPEG_40216	-5.7987831	-50.7749531
Marabá	MPEG_40217	-5.7987831	-50.7749531

Marabá	MPEG_40259	-5.7987831	-50.7749531
Parauapebas	MPEG_40971	-6.0983170	-50.1422776
Parauapebas	MPEG_40972	-6.0983170	-50.1422776
Parauapebas	MPEG_40975	-6.0983170	-50.1422776
Parauapebas	MPEG_40976	-6.0983170	-50.1422776
Parauapebas	MPEG_40977	-6.0983170	-50.1422776
Parauapebas	MPEG_40979	-6.0983170	-50.1422776
Parauapebas	MPEG_40980	-6.0983170	-50.1422776
Rondon do Pará	MPEG_41021	-4.7790600	-48.0589831
Rondon do Pará	MPEG_41024	-4.7790600	-48.0589831
Rondon do Pará	MPEG_41025	-4.7790600	-48.0589831
Rondon do Pará	MPEG_41026	-4.7790600	-48.0589831
Rondon do Pará	MPEG_41027	-4.7790600	-48.0589831
Rondon do Pará	MPEG_41028	-4.7790600	-48.0589831
Rondon do Pará	MPEG_41029	-4.7790600	-48.0589831
Rondon do Pará	MPEG_41030	-4.7790600	-48.0589831
Ourém	MPEG_41177	-1.5523790	-47.1167010
Ourém	MPEG_41178	-1.5523790	-47.1167010
Vitória do Xingú	MPEG_10280	-3.3908333	-51.9250000
Vitória do Xingú	MPEG_10459	-3.3908333	-51.9250000
Vitória do Xingú	MPEG_10460	-3.3908333	-51.9250000
Vitória do Xingú	MPEG_10631	-3.3908333	-51.9250000
Senador José Porfírio	MPEG_11513	-2.5836100	-51.9502650
Senador José Porfírio	MPEG_14116	-2.5836100	-51.9502650
Senador José Porfírio	MPEG_14117	-2.5836100	-51.9502650
Senador José Porfírio	MPEG_14127	-2.5836100	-51.9502650

Senador José Porfírio	MPEG_14209	-2.5836100	-51.9502650
Senador José Porfírio	MPEG_14381	-2.5836100	-51.9502650
Ourilândia do Norte	MPEG_16457	-6.4866050	-51.1757761
Ourilândia do Norte	MPEG_16458	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_16459	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_16460	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_16461	-6.5927631	-51.1781319
Ourilândia do Norte	MPEG_16462	-6.5927631	-51.1781319
Junco do Maranhão	MPEG_1671	-1.8274369	-46.1173389
Curionópolis	MPEG_16880	-6.4141667	-49.7461111
Ponta de Pedras	MPEG_17151	-1.3900000	-48.8708333
Ponta de Pedras	MPEG_17152	-1.3900000	-48.8708333
Ponta de Pedras	MPEG_17153	-1.3900000	-48.8708333
Ponta de Pedras	MPEG_17154	-1.3900000	-48.8708333
Ponta de Pedras	MPEG_17156	-1.3900000	-48.8708333
Ponta de Pedras	MPEG_17158	-1.3900000	-48.8708333
Ponta de Pedras	MPEG_17159	-1.3900000	-48.8708333
Muaná	MPEG_18361	-1.2466667	-49.3150000
Tucuruí	MPEG_19540	-4.2469167	-49.5252778
Tucuruí	MPEG_19541	-4.2469167	-49.5252778
Tucuruí	MPEG_19542	-4.2469167	-49.5252778
Tomé-Açu	MPEG_19580	-2.5956111	-48.3491389
Tomé-Açu	MPEG_19581	-2.5956111	-48.3491389
Tomé-Açu	MPEG_19582	-2.5956111	-48.3491389
Tomé-Açu	MPEG_19583	-2.5956111	-48.3491389
Monte Alegre	MPEG_19759	-2.0074800	-54.1970855

Monte Alegre	MPEG_19760	-2.0074800	-54.1970855
Paragominas	MPEG_20880	-3.2366194	-47.7330556
Paragominas	MPEG_20885	-3.2366194	-47.7330556
Paragominas	MPEG_20886	-3.2366194	-47.7330556
Paragominas	MPEG_20887	-3.2366194	-47.7330556
Paragominas	MPEG_20888	-3.2366194	-47.7330556
Paragominas	MPEG_20891	-3.2366194	-47.7330556
Altamira	MPEG_21957	-3.2413339	-52.2234750
Portel	MPEG_24974	-1.8626056	-51.7072889
Portel	MPEG_24975	-1.8626056	-51.7072889
Portel	MPEG_24976	-1.8626056	-51.7072889
Portel	MPEG_24980	-1.8626056	-51.7072889
Portel	MPEG_24982	-1.8626056	-51.7072889
Portel	MPEG_24983	-1.8626056	-51.7072889
Portel	MPEG_24984	-1.8626056	-51.7072889
Portel	MPEG_24986	-1.8626056	-51.7072889
Portel	MPEG_24988	-1.8626056	-51.7072889
Portel	MPEG_24989	-1.8626056	-51.7072889
Anapú	MPEG_25969	-3.0886819	-51.7189281
Vitória do Xingú	MPEG_25973	-3.3908333	-51.9250000
Anapú	MPEG_25975	-3.0886819	-51.7189281
Anapú	MPEG_25978	-3.0886819	-51.7189281
Anapú	MPEG_25983	-3.0886819	-51.7189281
Anapú	MPEG_25989	-3.0886819	-51.7189281
Anapú	MPEG_25996	-3.0886819	-51.7189281
Vitória do Xingú	MPEG_26005	-3.3908333	-51.9250000

Anapú	MPEG_26018	-3.0886819	-51.7189281
Anapú	MPEG_26019	-3.0886819	-51.7189281
Vitória do Xingú	MPEG_26021	-3.3908333	-51.9250000
Altamira	MPEG_26024	-3.8144444	-52.4802222
Altamira	MPEG_26025	-3.2413339	-52.2234750
Altamira	MPEG_26027	-3.8144444	-52.4802222
Altamira	MPEG_26029	-3.2413339	-52.2234750
Anapú	MPEG_26031	-3.0886819	-51.7189281
Anapú	MPEG_26033	-3.0886819	-51.7189281
Anapú	MPEG_26034	-3.0886819	-51.7189281
Altamira	MPEG_26044	-3.8144444	-52.4802222
Altamira	MPEG_26045	-3.2413339	-52.2234750
Parauapebas	MPEG_28511	-6.0983170	-50.1422776
Vila Nova	MPEG_29015	-5.2273080	-48.1870305
Vila Nova	MPEG_29016	-5.2273080	-48.1870305
Santa Bárbara do Pará	MPEG_29281	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29286	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29287	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29289	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29290	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29292	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29293	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29294	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29296	-1.9925000	-48.8275000
Santa Bárbara do Pará	MPEG_29297	-1.9925000	-48.8275000
Óbidos	MPEG_29482	-0.4584083	-57.0983222

Almeirim	MPEG_30314	-0.3197222	-54.6144444
Óbidos	MPEG_30882	-1.9175000	-55.5180556
Óbidos	MPEG_30883	-1.9175000	-55.5180556
Óbidos	MPEG_30884	-1.9175000	-55.5180556
Óbidos	MPEG_30885	-1.9175000	-55.5180556
Óbidos	MPEG_30886	-1.9175000	-55.5180556
Óbidos	MPEG_30887	-1.9175000	-55.5180556
Altamira	MPEG_31493	-8.3179069	-55.1007181
Itaituba	MPEG_31589	-4.2753056	-56.2840000
Itaituba	MPEG_31591	-4.2753056	-56.2840000
Itaituba	MPEG_31593	-4.2753056	-56.2840000
Itaituba	MPEG_31594	-4.2753056	-56.2840000
Itaituba	MPEG_31595	-4.2753056	-56.2840000
Itaituba	MPEG_31596	-4.2753056	-56.2840000
Itaituba	MPEG_31597	-4.2753056	-56.2840000
Parauapebas	MPEG_31749	-6.3043970	-49.8898170
Marabá	MPEG_31803	-5.3509831	-49.1340931
Barcarena	MPEG_31831	-1.5468431	-48.7337100
Barcarena	MPEG_31832	-1.5468431	-48.7337100
Paragominas	MPEG_33305	-3.2366194	-47.7330556
Rurópolis	MPEG_34107	-4.1144444	-55.6769444
Rurópolis	MPEG_34108	-4.1144444	-55.6769444
Ourilândia do Norte	MPEG_34491	-6.7482631	-51.0683669
Ourilândia do Norte	MPEG_34492	-6.7482631	-51.0683669
Ourilândia do Norte	MPEG_34493	-6.7482631	-51.0683669
Brasil Novo	MPEG_34568	-3.3766610	-52.5744444

Brasil Novo	MPEG_34572	-3.3766610	-52.5744444
Tailândia	MPEG_34750	-2.9399131	-48.9702831
Tailândia	MPEG_34752	-2.9399131	-48.9702831
Tailândia	MPEG_34753	-2.9399131	-48.9702831
Moju	MPEG_34754	-1.8819519	-48.7625461
Tailândia	MPEG_34755	-2.9399131	-48.9702831
Moju	MPEG_34756	-1.8819519	-48.7625461
Moju	MPEG_34758	-1.8819519	-48.7625461
Moju	MPEG_34759	-1.8819519	-48.7625461
Moju	MPEG_34761	-1.8819519	-48.7625461
Moju	MPEG_34765	-1.8819519	-48.7625461
São Geraldo de Araguaia	MPEG_36388	-6.2280556	-48.4438889
São Geraldo de Araguaia	MPEG_36389	-6.2280556	-48.4438889
São Geraldo de Araguaia	MPEG_36391	-6.2280556	-48.4438889
São Geraldo de Araguaia	MPEG_36392	-6.2280556	-48.4438889
São Geraldo de Araguaia	MPEG_36395	-6.2280556	-48.4438889
São Geraldo de Araguaia	MPEG_36396	-6.2280556	-48.4438889
Tucuruí	MPEG_3745	-3.7792481	-49.7082619
Viseu	MPEG_6038	-1.2032950	-46.1439889
Viseu	MPEG_6039	-1.2032950	-46.1439889
Viseu	MPEG_6052	-1.2032950	-46.1439889
Santarém	MPEG_6371	-2.4420631	-54.7470081
Santarém	MPEG_6372	-2.4420631	-54.7470081
Santarém	MPEG_6373	-2.4420631	-54.7470081
Santarém	MPEG_6378	-2.4420631	-54.7470081
Santarém	MPEG_6379	-2.4420631	-54.7470081

Paragominas	MPEG_7145	-3.0525000	-47.3952778
São Félix do Xingu	MPEG_9289	-6.2202778	-52.1372222
São Félix do Xingu	MPEG_9355	-6.2202778	-52.1372222
São Félix do Xingu	MPEG_9356	-6.2202778	-52.1372222
São Félix do Xingu	MPEG_9357	-6.2202778	-52.1372222
São Félix do Xingu	MPEG_9358	-6.2202778	-52.1372222
Amajari	INPA_26503	3.38405030	-61.6965058
Caracaraí	INPA_19103	1.58939323	-61.1853409
Amajari	INPA_19128	3.38405030	-61.6965058
Amajari	INPA_26505	3.38405030	-61.6965058
Amajari	INPA_19127	3.38405030	-61.6965058
Caracaraí	INPA_27545	1.58939323	-61.1853409
Caracaraí	INPA_27563	1.58939323	-61.1853409
Trairão	INPA_27690	-4.84876149	-55.5873283
Iracema	INPA_31813	2.15551040	-61.0583844
Trairão	INPA_27689	-4.84876149	-55.5873283
Santarém	INPA_10855	-2.50157447	-54.9504146
Caracaraí	INPA_36274	1.60000000	-61.90000000
Caracaraí	INPA_19105	1.58939323	-61.1853409
Caracaraí	INPA_19104	1.58939323	-61.1853409
Presidente Figueiredo	INPA_34307	-2.0186926	-59.9915341
Amajari	INPA_19129	3.38405030	-61.6965058
Amajari	INPA_19106	3.38405030	-61.6965058
Iracema	INPA_31812	2.15551040	-61.0583844
Presidente Figueiredo	INPA_34309	-2.0186926	-59.9915341
Amajari	INPA_26502	3.38405030	-61.6965058

Amajari	INPA_19126	1.58939323	-61.1853409
Presidente Figueiredo	INPA_29558	-2.0186926	-59.9915341
Presidente Figueiredo	INPA_29556	-2.0186926	-59.9915341
Caracaraí	INPA_28477	1.58939323	-61.1853409
Caracaraí	INPA_28478	1.58939323	-61.1853409
Amajari	INPA_26504	3.38405030	-61.6965058
Presidente Figueiredo	INPA_29557	-2.0186926	-59.9915341
Caracaraí	INPA_36266	1.60000000	-61.90000000
Presidente Figueiredo	INPA_34319	-2.0186926	-59.9915341
Iracema	INPA_31811	2.15551040	-61.0583844
Campo Alegre	CFBH_16396	-9.782554242	-3.635071314
Campo Alegre	CFBH_16389	-9.782554242	-3.635071314
Campo Alegre	CFBH_16421	-9.782554242	-3.635071314
Campo Alegre	CFBH_16408	-9.782554242	-3.635071314
Campo Alegre	CFBH_16387	-9.782554242	-3.635071314
Campo Alegre	CFBH_16441	-9.782554242	-3.635071314
Campo Alegre	CFBH_16411	-9.782554242	-3.635071314
Canaã dos Carajás	CFBH_3670	-6.530055865	-49.85179942
São Félix do Piauí	CFBH_31512	-5.931527164	-42.11213774
Campo Alegre	CFBH_16435	-9.782554242	-3.635071314
Campo Alegre	CFBH_16367	-9.782554242	-3.635071314
Campo Alegre	CFBH_16417	-9.782554242	-3.635071314
Sambaíba	CFBH_32695	-7.138599533	-45.34656441
Canaã dos Carajás	CFBH_3668	-6.530055865	-49.85179942
Campo Alegre	CFBH_161418	-9.782554242	-3.635071314
Ubajara	CFBH_16171	-3.8514645	-40.91959482

Campo Alegre	CFBH_16372	-9.782554242	-3.635071314
São Félix do Piauí	CFBH_31511	-5.931527164	-42.11213774
São Félix do Piauí	CFBH_31510	-5.931527164	-42.11213774
Campo Alegre	CFBH_16405	-9.782554242	-3.635071314
Campo Alegre	CFBH_16420	-9.782554242	-3.635071314
Campo Alegre	CFBH_16433	-9.782554242	-3.635071314
Ubajara	CFBH_16170	-3.8514645	-40.91959482
Campo Alegre	CFBH_16388	-9.782554242	-3.635071314
Campo Alegre	CFBH_16414	-9.782554242	-3.635071314
Ubajara	CFBH_16172	-3.8514645	-40.91959482
Campo Alegre	CFBH_18578	-9.782554242	-3.635071314
Campo Alegre	CFBH_16368	-9.782554242	-3.635071314
Campo Alegre	CFBH_16398	-9.782554242	-3.635071314

Appendix B. Sequences of genus *Physalaemus* and *Engystomops* used in our phylogenetic inference and species delimitation analyses and their respective references.

Espécies	Número de Tombo	Número do Tecido	Localidade	16S	RAG1	COI	CXC R4	Referências
<i>Engystomops pustulosus</i>	LW101A ¹ /TNHC-GDC 19498 ² /USNM 572717 ³		Laguna Verde, Veracruz, Mexico ¹ ; Parque Nacional General Omar Torrijos H., El Copé, Panamá ³	DQ3372 49 ¹	KX20 8655 ²	FJ766 700 ³	—	Ron et al. (2006) ¹ ; Feng et al. (2017) ² ; Crawford et al. (2010) ³
<i>Physalaemus aguirrei</i>	CFBH 19457	CFBH-T 9041	Linhares, Espírito Santo, Brasil	KP1460 65	KP145 999	—	—	Lourenço et al. (2015)
<i>Physalaemus albifrons</i>	MNRJ 24228	SMRP 74.21	Vassouras, Barreirinhas, Maranhão, Brasil	KP1460 09	KP145 977	—	—	Lourenço et al. (2015)
<i>Physalaemus albonotatus</i>	ZUEC 16219	SMRP 263.15	Lambari D'Oeste, Mato Grosso, Brasil	KP1460 50	KP145 983	—	—	Lourenço et al. (2015)
<i>Physalaemus atlanticus</i>	CFBH 7918 ¹	CFBH-T 1398 ¹ /CFBH-T 1397 ²	Ubatuba, São Paulo, Brasil1,2	KP1459 40 ¹	—	KU49 4635 ²	—	Lourenço et al. (2015) ¹ ; Lyra et al. (2016) ²
<i>Physalaemus barrioi</i>	ZUEC 18146 ¹	SMRP 303.1 ¹ /CFBHT10 353 ²	Serra da Bocaina, São Paulo, Brasil1,2	KP1460 67 ¹	KP145 950 ¹	KU49 4636 ²	—	Lourenço et al. (2015) ¹ ; Lyra et al. (2016) ²
<i>Physalaemus biligonigerus</i>	LGE 8870	LGE 8870	Añatuya, Santiago del Estero, Argentina	KP1460 84	KP145 964	—	—	Lourenço et al. (2015)
<i>Physalaemus bokermanni</i>	ZUEC 17448 ¹	SMRP 375.1 ¹ /TG 417 ²	Bertioga, São Paulo, Brasil1,2	KP1459 36 ¹	KP145 962 ¹	KU49 4639 ²	—	Lourenço et al. (2015) ¹ ; Lyra et al. (2016) ²
<i>Physalaemus camacan</i>	ZUEC 17467	SMRP 353.2	Ilhéus, Bahia, Brasil	KP1460 94	KP145 976	—	—	Lourenço et al. (2015)
<i>Physalaemus centralis</i>	ZUEC 13697 ¹	SMRP 96.11 ¹ /117 ²	Vitória Brasil, São Paulo, Brasil1; São Carlos, São Paulo, Brasil2	KP1460 61 ¹	KP145 968 ¹	JQ627 308 ²	—	Lourenço et al. (2015) ¹ ; Genbank unpublished data ²
<i>Physalaemus cicada</i>	CFBH 19395 ¹	CFBH-T 11307 ¹ /CFBH-T 11312 ²	Novas Russas, Ceará, Brasil1,2	KP1460 64 ¹	KP146 000 ¹	KU49 4641 ²	—	Lourenço et al. (2015) ¹ ; Lyra et al. (2016) ²
<i>Physalaemus crombiei</i>	CFBH 14910	CFBH-T 5899	Sooretama, Espírito Santo, Brasil	KP1460 95	—	—	—	Lourenço et al. (2015)
<i>Physalaemus cuvieri</i>	ZUEC 14631	SMRP 92.180	Chapada dos Guimarães, Mato Grosso, Brasil	KP1460 25	KP145 960	—	—	Lourenço et al. (2015)

<i>Physalaemus</i>									
<i>cuvieri</i>	ZUEC 14691	SMRP 92.200	Porto Nacional, Tocantins, Brasil	KP1460 47	KP145 970	–	–	Lourenço et al. (2015)	
<i>Physalaemus</i>	LGE 8874	LGE 8874	2.5 Km SE from Aguas Blancas, Salta, Argentina	KP1460 71	–	–	–	Lourenço et al. (2015)	
<i>cuqui</i>									
<i>Physalaemus</i>	MTR 19498	–	Minas Gerais, Brasil	KP1459 35.1	–	–	–	Lourenço et al. (2015)	
<i>deimaticus</i>									
<i>Physalaemus</i>	HUFMA 884	SMRP 92.247	São Luís, Maranhão, Brasil	KP1460 21	KP145 945	–	–	Lourenço et al. (2015)	
<i>ephippifer</i>	ZUEC 13082	SMRP 97.5	Crateús, Ceará, Brasil	KP1460 15	KP145 951	–	–	Lourenço et al. (2015)	
<i>Physalaemus</i>	ZUEC 13083	SMRP 97.8	Crateús, Ceará, Brasil	KP1460 13	–	–	–	Lourenço et al. (2015)	
<i>ephippifer</i>	ZUEC 13088	SMRP 97.13	Crateús, Ceará, Brasil	KP1460 14	–	–	–	Lourenço et al. (2015)	
<i>Physalaemus</i>	ZUEC 13092	SMRP 92.17	Urbano Santos, Maranhão, Brasil	KP1460 20	KP146 002	–	–	Lourenço et al. (2015)	
<i>ephippifer</i>	ZUEC 13093	SMRP 92.18	Urbano Santos, Maranhão, Brasil	KP1460 18	KP146 001	–	–	Lourenço et al. (2015)	
<i>Physalaemus</i>	ZUEC 13105	SMRP 92.30	Urbano Santos, Maranhão, Brasil	KP1460 19	KP145 995	–	–	Lourenço et al. (2015)	
<i>ephippifer</i>	ZUEC 17516	SMRP 92.226	Balsas, Maranhão, Brasil	KP1460 22	KP145 956	–	–	Lourenço et al. (2015)	
<i>Physalaemus</i>	ZUEC 17517	SMRP 92.227	Balsas, Maranhão, Brasil	KP1460 16	KP145 955	–	–	Lourenço et al. (2015)	
<i>ephippifer</i>	ZUEC 17886	SMRP 92.273	Alagoinha, Bahia, Brasil	KP1460 11	–	–	–	Lourenço et al. (2015)	
<i>Physalaemus</i>	ZUEC 17897	SMRP 92.284	Caruaru, Pernambuco, Brasil	KP1460 12	–	–	–	Lourenço et al. (2015)	
<i>ephippifer</i>	ZUEC 17907	SMRP 419.6	Araruna, Paraíba, Brasil	KP1460 17	–	–	–	Lourenço et al. (2015)	
<i>Physalaemus</i>	MPEG 17153	–	Sítio Chapéu Virado, Rio Quiáparaná, Ponta de Pedras, Ilha do Marajó, Pará, Brasil	Sequenciado	–	Sequenciado	Sequenciado	Presente estudo	
<i>ephippifer</i>	MPEG 18361	–	Fazenda Socorro (Berta Grande). Rio Atuá. Ilha do Marajó, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo	
<i>Physalaemus</i>	MPEG 22784	–	Fazenda Riacho Monte Verde. Precious Woods, Portel, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo	
<i>ephippifer</i>	MPEG 24976	–	FLONA Caxiuanã. Plote PPBIO, Km 750, Portel, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo	
<i>Physalaemus</i>	MPEG 24982	–	FLONA Caxiuanã. Plote PPBIO, Km 1400, Portel, Pará, Brasil	Sequenciado	–	–	Sequenciado	Presente estudo	
<i>ephippifer</i>									

<i>Physalaemus ephippifer</i>	MPEG 24984	—	FLONA Caxiuanã. Plote PPBIO, Km 600, Portel, Pará, Brasil	Sequenciado	—	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 26044	—	Tapuama. UHE de Belo Monte. Margem direita do Rio Xingu, Altamira, Pará, Brasil	Sequenciado	—	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 27928	—	Área da Futura refinaria ABC, Barcarena, Pará, Brasil	Sequenciado	—	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 27929	—	Área da Futura refinaria ABC, Barcarena, Pará, Brasil	Sequenciado	—	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 27930	—	Área da Futura refinaria ABC, Barcarena, Pará, Brasil	Sequenciado	—	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 29280	—	Parque Ecológico de GUNMA, Santa Bárbara do Pará, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 29281	—	Parque Ecológico de GUNMA, Santa Bárbara do Pará, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	—	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 29282	—	Parque Ecológico de GUNMA, Santa Bárbara do Pará, Pará, Brasil	Sequenciado	—	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 29481	—	FLOTA Trombetas, Óbidos, Pará, Brasil	Sequenciado	—	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 29482	—	FLOTA Trombetas, Óbidos, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 29716	—	ESEC Grão Pará, Alenquer, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	—	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 30314	—	REBIO Maicurú, Almeirim, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 30882	—	ESEC Grão Pará, Centro, Óbidos, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 30887	—	ESEC Grão Pará, Centro, Óbidos, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 31803	—	Marabá, Pará, Brasil	Sequenciado	—	Sequeciado	—	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 33303	—	Área de mineração de bauxita da VALE, Paragominas, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 33304	—	Área de mineração de bauxita da VALE, Paragominas, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 33305	—	Área de mineração de bauxita da VALE, Paragominas, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 33759	—	Trilha Ecológica do Hotel Samauma, Barcarena, Pará, Brasil	Sequenciado	Sequeciado	—	Sequeciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 34102	—	Barreiras, Itaituba, Pará, Brasil	Sequenciado	Sequeciado	Sequeciado	Sequeciado	Presente estudo

<i>Physalaemus ephippifer</i>	MPEG 34104	—	Barreiras, Itaituba, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 34105	—	Barreiras, Itaituba, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	—	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 34106	—	Rurópolis, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 34107	—	Rurópolis, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 36758	—	Mina do Sossego, Canaã dos Carajás, Pará, Brasil	Sequenciado	—	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 38634	—	Serra do Tarzan, Flona dos Carajás, Canaa dos Carajás, Brasil	Sequenciado	—	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 38635	—	Serra do Tarzan, Flona dos Carajás, Canaa dos Carajás, Brasil	Sequenciado	—	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 39142	—	Santa Bárbara do Pará, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 39143	—	Santa Bárbara do Pará, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MPEG 39561	—	Sao Félix do Xingu, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	AA 837	—	Tailândia, Pará, Brasil	Sequenciado	—	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	AA 873	—	Tailândia, Pará, Brasil	Sequenciado	—	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	AA 875	—	Tailândia, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	LOD 333	—	Bichos Vale, Canaã dos Carajás, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	LOD 732	—	Tanguro, Mato Grosso, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	MSH 11983	—	Senador José Porfirio, Pará, Brasil	Sequenciado	—	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	PRMT 299	—	Itaituba, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	PRMT 315	—	Itaituba, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	PRMT 356	—	Itaituba, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	PRMT 369	—	Itaituba, Pará, Brasil	Sequenciado	—	Sequenciado	Sequenciado	Presente estudo

<i>Physalaemus ephippifer</i>	PRMT 485	—	Itaituba, Pará, Brasil	Sequenciado	Sequenciado	Sequenciado	Sequenciado	Presente estudo
<i>Physalaemus ephippifer</i>	ZUEC 13704	SMRP 252.6	Belém, Pará, Brasil	KP146003	—	—	—	Lourenço et al. (2015)
<i>Physalaemus ephippifer</i>	ZUEC 17604	—	Parque Nacional do Viruá, Roraima, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 17600	—	Parque Nacional do Viruá, Roraima, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 17591	—	Prainha, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 17593	—	Prainha, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 18188	—	Óbidos, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 18203	—	Óbidos, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 22704	—	Óbidos, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 22703	—	Óbidos, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 22702	—	Óbidos, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 22701	—	Óbidos, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 18196	—	Óbidos, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 18185	—	Monte Alegre, Pará, Brasil	MK2415	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	ZUEC 18190	—	Alenquer, Pará, Brasil	KDQF01004009	—	—	—	Nascimento et al. (2019)
<i>Physalaemus ephippifer</i>	PK 3518	—	Turtle Camp, Iwokrama, Guyana	KDQF01003781	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	NZCS 68	—	Tibiti, Suriname	KDQF01003738	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	MTR 33890	—	Fazenda Monte Verde, Peixe-Boi, Pará, Brasil	KDQF01003522	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	MTR 20845	—	Riacho alojamento, Tepequem, Roraima, Brasil	KDQF01003490	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	MTR 20677	—	Pacaraima, Roraima, Brasil	—	—	—	—	Vacher et al. (2020)

<i>Physalaemus ephippifer</i>	MTR 20442	—	E.E. Maraca, Roraima, Brasil	KDQF0 1003461	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	MTD 47988	—	Iwokrama, Guyana	KDQF0 1003076	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	CM 331	—	Langatabiki, Suriname	KDQF0 1002296	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	BPM 0810	—	Kayser, Suriname	KDQF0 1001924	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	BPM 0801	—	Kayser, Suriname	KDQF0 1001920	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	AMS 370	—	Oeste de Rupununi R Swamp, Kukrit Stump, Guyana	KDQF0 1001725	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	AF 1960	—	Sipaliwini, Guyana	KDQF0 1000766	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	AF 0182	—	Pista Goliathberg PK7 avant, Rua para Apura, Suriname	KDQF0 1000078	—	—	—	Vacher et al. (2020)
<i>Physalaemus ephippifer</i>	UFU 1654	—	Cantá, Roraima	Sequenciado KP1460	—	—	—	de Thiago de Carvalho
<i>Physalaemus erikae</i>	CFBH 32463	CFBH-T 16267	Uruçuca, Bahia, Brasil	08 KP1459 38	—	—	—	Lourenço et al. (2015)
<i>Physalaemus erythros</i>	MNRJ 55001	MNRJ 55001	Catas Altas, Minas Gerais, Brasil	KP1460 69	—	—	—	Lourenço et al. (2015)
<i>Physalaemus evangelistai</i>	MNRJ 55103	MNRJ 55103	Catas Altas, Minas Gerais, Brasil	KP1460 80	—	—	—	Lourenço et al. (2015)
<i>Physalaemus feioi</i>	ZUEC 16247	SMRP 247.1	Viçosa, Minas Gerais, Brasil	KP1460 68	—	—	—	Lourenço et al. (2015)
<i>Physalaemus fernandezae</i>	LGE 8876	LGE 8876	Punta Lara, Buenos Aires, Argentina	KP1460 33	—	—	—	Lourenço et al. (2015)
<i>Physalaemus fischeri</i>	AJC 2316	—	Reserva Wisirare, Orucue, Casanare, Colombia	KP1494 73	KP145 979	KP149 227	—	Guarnizo et al. (2015)
<i>Physalaemus gracilis</i>	MHNM 9511	MHNM 9511	Bañado de los Oliveras, Treinta y Tres, Uruguay	KP1460 24/34	—	—	—	Lourenço et al. (2015)
<i>Physalaemus henselii</i>	MHNM 9512	MHNM 9512	Ruta 5, km 492, Pueblo Madera, Rivera, Uruguay	KP1459 70	—	—	—	Lourenço et al. (2015)
<i>Physalaemus jordanensis</i>	CFBH 9903	CFBH-T 2291	Campos do Jordão, São Paulo, Brasil	KP1460 997	KP145 —	—	—	Lourenço et al. (2015)
<i>Physalaemus kroyeri</i>	CFBH 23652	CFBH-T 11432	Faz. Santo Onofre e Canabrava, Maracás, Bahia, Brasil	KP1460 05	KP145 941	—	—	Lourenço et al. (2015)
<i>Physalaemus lateristriga</i>	ZUEC 18207	SMRP 365.2	Iporanga, São Paulo, Brasil	KP1460 77	KP145 946	—	—	Lourenço et al. (2015)

<i>Physalaemus lisei</i>	CFBH 8527	CFBH-T 1790	São Francisco de Paula, Rio Grande do Sul, Brasil	KP1460 74	KP145 961	–	–	Lourenço et al. (2015)
<i>Physalaemus maculiventris</i>	MZUSP 132062	CTMZ 01093	Bananal, São Paulo, Brasil	KP1459 37	KP145 978	–	–	Lourenço et al. (2015)
<i>Physalaemus marmoratus</i>	ZUEC 13399	SMRP 43.48	São José do Rio Preto, São Paulo, Brasil	KP1460 83	KP145 957	–	–	Lourenço et al. (2015)
<i>Physalaemus maximus</i>	UFMG-AMP 9625	UFMG-T 1818	Fazenda Seu Mariano, Ouro Branco, Minas Gerais, Brasil	KP1460 79	–	–	–	Lourenço et al. (2015)
<i>Physalaemus moreirae</i>	CFBH 19491	CFBH-T 8341	Guarujá, São Paulo, Brasil	KP1460 89 ¹	KP145 949 ¹	KU49 4647 ²	–	Lourenço et al. (2015) ¹ ; Lyra et al. (2016) ²
<i>Physalaemus nanus</i>	CFBH 9377	CFBH-T 2096	Anitápolis, Santa Catarina, Brasil	KP1460 96	–	–	–	Lourenço et al. (2015)
<i>Physalaemus nattereri</i>	ZUEC 17506 ¹	–	Três Lagoas, Mato Grosso do Sul, Brasil1;UHE Lajeado, Tocantins, Brasil2	KP1461 03 ¹	–	–	–	Lourenço et al. (2015) ¹ ; Fouquet et al. (2013) ²
<i>Physalaemus obtectus</i>	MNRJ 35018	MNRJ 35018	Linhares, Espírito Santo, Brasil	KP1460 93	–	–	–	Lourenço et al. (2015)
<i>Physalaemus olfersii</i>	ZUEC 16250 ¹	SMRP 247.4 ¹ /CFBH-T 10357 ²	Linhares, Espírito Santo, Brasil	KP1460 78 ¹	KP145 993 ¹	KU49 4651 ²	–	Lourenço et al. (2015) ¹ ; Lyra et al. (2016) ²
<i>Physalaemus riograndensis</i>	LGE 8879	LGE 8879	5 Km N from Profundidad, Misiones, Argentina	KP1460 82	–	–	–	Lourenço et al. (2015)
<i>Physalaemus rupestris</i>	PCAG 1768	–	P.E. Ibitipoca, Lima Duarte, Minas Gerais, Brasil	KP1460 99	–	–	–	Lourenço et al. (2015)
<i>Physalaemus santafecinus</i>	LGE 8880	LGE 8880	Santa Fé, La Capital, Argentina	KP1460 81	KP145 994	–	–	Lourenço et al. (2015)
<i>Physalaemus signifer</i>	CFBH-T 75080	–	Congonhas do Campo, Minas Gerais. Brasil1	KP1460 90	KP145 944	KU23 4712 ²	–	Lourenço et al. (2015) ¹ ; Genbank unpublished data ²
<i>Physalaemus soaresi</i>	MNRJ 60323	MNRJ 60323	Bangu, Rio de Janeiro, Brasil	KP1460 76	–	–	–	Lourenço et al. (2015)
<i>Physalaemus spiniger</i>	ZUEC 14516	SMRP 294.12	Curitiba, Paraná, Brasil	KP1460 97	–	–	–	Lourenço et al. (2015)

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