PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

An integrative analysis of the taxonomic identity of *Hypsiboas* (Anura, Hylidae) related to *H. joaquini* (B. Lutz, 1968) and *H. semiguttatus* (A. Lutz, 1925)

Autor: Ronaldo Libardi Widholzer
Orientador: Santiago Castroviejo-Fisher

DISSERTAÇÃO DE MESTRADO

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1. SUMÁRIO

2. RESUMO:

Hypsiboas joaquini e *H. semiguttatus* são duas espécies fenotipicamente semelhantes do grupo de espécies *H. pulchellus,* com uma história taxonômica complexa e inter-relacionada devido a problemas tais como escassas descrições originais, descrições de vários sinonimias, redescrições com base em exemplares de outras espécies, e de grande variação populacional. Várias espécies estreitamente relacionadas têm sido descritas nos últimos anos e, atualmente, estamos diante de um panorama complexo, com cinco espécies relativamente bem caracterizados em termos de morfologia externa em suas respectivas localidades tipo (*H. curupi, H. caipora, H. joaquini, H. semiguttatus* e *H. stellae*), mas para os quais a variação geográfica é mal compreendida e com sobreposições. Assim, o objetivo do nosso estudo é avaliar o status taxonômico de espécimes anteriormente determinadas e estreitamente ligadas, como *H. joaquini* ou *H. semiguttatus* usando adulto e morfologia larva, sequências de DNA, e chamados de advertência. Nós comparamos a variação entre esses quatro conjuntos de caracteres em nove unidades taxonômicas, as cinco espécies reconhecidas atualmente mencionadas acima e quatro conjuntos adicionais de espécimes agrupadas segundo seus locais de reprodução e de coleta (ou seja, *H.* aff. *curupi*, *H.* cf. *joaquini*, *H* . aff. *joaquini* e *H.* aff. 2 *joaquini*). Os principais resultados são: (i) extensa sobreposição e sem caracteres claros de diagnósticos morfométricos de machos adultos, larvas e caracteres quantitativos de adultos do sexo masculino; (ii) extensa variação na morfologia diagnóstica do disco oral dos girinos *H.* aff. 2 *joaquini*; (iii) sobreposição extensiva e sem claros caracteres diagnósticos acústicos, exceto para *H. stellae*; (iv) um clado fortemente sustentado que inclui *H. joaquini* sensu stricto e todos os espécimes pertencentes às quatro unidades comparativas aqui definidos e que apresenta ramos curtos, baixas distâncias genéticas (<1%), e nenhuma estrutura geográfica. De acordo com nossos resultados podemos considerar espécimes de *H.* aff. *curupi*, *H.* cf. *joaquini*, *H.* aff. *joaquini* e *H.* aff. 2 *joaquini* como parte de *H. joaquini*, que se torna uma espécie amplamente distribuída com grande variação fenotípica. A variação excepcional da morfologia do disco oral dos girinos de *H. joaquini* sensu, como adaptações locais e de plasticidade fenotípica, é discutida neste trabalho.

3. ABSTRACT:

An integrative analysis of the taxonomic identity of *Hypsiboas* (Anura, Hylidae) related to *H. joaquini* (B. Lutz, 1968) and *H. semiguttatus* (A. Lutz, 1925)

Hypsiboas joaquini and *H. semiguttatus* are two phenotypically similar species of the *H. pulchellus* species group with a complex and interrelated taxonomic history due to problems such as poor original descriptions, descriptions of several synonyms, redescritpions based on specimens of other species, and large population variation. Several closely related species have been described in recent years and currently, we face a complex panorama, with five species relatively well characterized in terms of external morphology in their respective type localities (*H. curupi, H. caipora, H. joaquini, H. semiguttatus,* and *H. stellae*) but for which geographic variation is poorly understood and overlapping. Thus, the goal of our study is to evaluate the taxonomic status of specimens formerly identified as closely related to either *H. joaquini* or to *H. semiguttatus* using adult and larva morphology, DNA sequences, and advertisement calls. We compared variation among these four sets of characters across nine taxonomic units, the five currently recognized species mentioned above and four additional sets of specimens grouped according to breeding and collection sites (i.e., *H.* aff. *curupi*, *H.* cf. *joaquini*, *H.* aff. *joaquini*, and *H.* aff. 2 *joaquini*). Our main results are: (i) extensive overlap and no clear diagnostic characters of morphometric characters of adult males and larvae, and quantitative characters of adult males; (ii) extensive variation on tadpole oral disc morphology diagnosing *H.* aff. 2 *joaquini*; (iii) extensive overlap and no clear diagnostic characters of acoustic characters except for *H. stellae*; (iv) a strongly supported clade that includes *H. joaquini* sensu stricto and all specimens belonging to the four comparative units defined herein and that shows short branches, low genetic distances (< 1%), and no geographical structure. According to our results we consider specimens of *H.* aff. *curupi*, *H.* cf. *joaquini*, *H.* aff. *joaquini*, and *H.* aff. 2 *joaquini* as part of *H. joaquini*, which becomes a widely distributed species with large phenotipic variation. We further discuss the outstanding variation of oral disc morphology of tadpoles of *H. joaquini* sensu this work, with local adaptation and phenotypic plasticity as possible causes.

APRESENTAÇÃO

Com 951 espécies conhecidas (Frost 2016), a família Hylidae (Laurenti 1768) é a mais diversa dentre os anuros. Trata-se de um grupo monofilético que distribui-se amplamente, mas com maior diversidade concentrada na região Neotropical (Faivovich *et al*. 2005; Frost, 2016). A família é composta por três sub-famílias também monofiléticas sendo Hylinae (Rafinesque 1815) a mais diversa, com 683 espécies descritas das quais a maioria tem distribuição restrita à região Neotropical. Em seguida temos Pelodryadinae (Günther 1858) com 208 espécies e distribuição pela Austrália e Papua Nova Guiné e Phyllomedusinae (Günther 1858) com 60 espécies e distribuição também neotropical (Frost 2016).

O gênero *Hypsiboas* (Wagler 1830) contém 90 espécies reconhecidas de pererecas arborícolas (Frost 2016). A postura de ovos pode ser na água, no solo ou na vegetação e seus girinos habitam desde córregos com água abundante até pequenos acúmulos de águas em plantas. Este gênero distribui-se da América Central até as regiões subtropicais da América do Sul e tem representantes em diferentes biomas como florestas úmidas e áreas secas e abertas, bem como em diversas elevações, desde o nível do mar, até 2.200 m de elevação (Faivovich *et al.* 2005). *Hypsiboas* é diagnosticado por 33 transformações em nucleotídeos dos genomas nucleares e mitocondrial (Faivovich *et al*. 2005). As espécies deste gênero estão organizadas em sete grupos descritos por Faivovich *et al*. (2005), sendo eles *H. albopunctatus, H. benitezi, H. faber, H. pellucens, H. pulchellus, H. punctatus* e *H. semilineatus*.

As 33 espécies reconhecidas do grupo *Hypsiboas pulchellus* são conhecidas comumente como pererecas gladiadoras, pois os machos possuem um espinho saliente nos membros superiores que são usados nas brigas por territórios (Köhler *et al.* 2010). Este grupo possui características típicas, tais como: uma listra verde irregular que se estende desde o olho até a narina, uma mancha verde pode ser observada entre os olhos e o tímpano; sua cabeça é na mesma proporção do corpo; os membros posteriores são moderadamente magros, com uma dobra tarsal interior discreta (Duellman *et al*. 1997; Amphibiaweb 2014). Porém uma característica marcante é a região que rodeia o tímpano, com cor castanho escuro, enquanto a região posterior do lábio superior possui uma cor creme (Duellman *et al*. 1997; Amphibiaweb 2014).

O grupo *Hypsiboas pulchellus* se originou na Mata Atlântica com uma posterior radiação para outras regiões, como foi o caso da formação do clado Andino, sendo que sua maioria está presente na América do Sul (Faivovich *et al*. 2004; 2005). Do grupo Hypsiboas pulchellus, seis ocorrentes na região sul (IUCN, 2013). Este grupo é diagnosticado por 55 transformações em nucleotídeos dos genomas nuclear e mitocondrial. A ausência do comprovante do *m. depressor mandibulae* que se origina na face dorsal no nível do *m. dorsalis scapularis* é uma provável sinapomorfia do grupo (Faivovich *et al*. 2005).

Embora alguns trabalhos tenham contribuído no avanço do conhecimento da filogenética e diversidade de espécies do grupo, a taxonomia do grupo *Hypsiboas pulchellus* fica longe de ser estável (Duellman *et al.* 1997; Faivovich *et al*. 2004, 2005; Köhler *et al.* 2010). Isto se mostra pela descrição de 19 espécies novas apenas nos últimos 10 anos (Frost, 2016). O aumento no número de espécies de anfíbios descritos recentemente , têm acentuado na escala global (Köhler *et al*. 2005; Vieites *et al*. 2009). As razões para isso são: (i) maior exploração de áreas pouco pesquisadas e (ii) utilização de uma combinação de conjuntos de caracteres nas avaliações taxonômicas (p.e., Padial & De la Riva 2010).

Uma nova abordagem conhecida como taxonomia integrativa (Dayrat, 2005; para revisões ver Padial & De la Riva, 2010; Padial *et al*., 2010) é promissora, uma vez que o uso combinado das linhas de evidência independentes, tais como filogenética molecular, bioacústica, ecologia, e morfologia têm revelado espécies crípticas e resolvido problemas taxonômicos em muitos grupos de anfíbios (Vences & Wake 2007), além de acelerar a descoberta de espécies (p.e., Vieites *et al*. 2009). A abordagem de taxonomia integrativa para o grupo *H. pulchellus* é necessária devido à variação marcante em caracteres morfológicos e padrão de cor intra e interpopulacional, muitas vezes sobrepondo variações interespecíficas (Duellman *et al.* 1997; Köhler *et al.* 2010).

No nordeste do Rio Grande do Sul, foi detectada algumas populações que poderiam corresponder a uma espécie nova do grupo *H. pulchellus* (Kwet 2001; Kwet *et al*. 2010). Esta população, inicialmente identificada como *Hypsiboas* aff. *semigutatus*, habita riachos pequenos e grandes no planalto das araucárias, possui hábito de vida e canto diferenciados, além de menor tamanho (Kwet 2001). Trabalhos mais recentes identificaram esta população como *Hypsiboas* aff. *joaquini* (Garcia *et al.* 2007; Kwet 2008; Kwet *et al*, 2010) para a região de São Francisco de Paula e *Hypsiboas* cf. *joaquini* para a região de Bom Jesus, Cambará e São José dos Ausentes. Algumas características desta população são: tamanho do corpo 30–40 mm; coloração dorsal castanho claro a castanho escuro; faixa lateral distinta, branca ou amarela; flancos castanhos escuro com manchas amarelas arredondadas; atividade reprodutiva ao longo do ano (exceto nos meses mais frios); desova de 150-300 ovos com superfície superior preta e grandes cápsulas gelatinosas, grudada na vegetação suspensa sobre a água; girino cinza, de padrão corporal com manchas escuras, alimentando-se sobre a superfície das pedras (Kwet & Di Bernardo 2010). No entanto, o status taxonômico dessas populações permanece incerto, e as comparações morfológicas externas não parecem fornecer provas decisivas para se tomar uma decisão taxonômica.

A identificação correta dos indivíduos desta população ampliaria o conhecimento quanto à diversidade de espécies de anfíbios no estado, podendo contribuir ou não para existência de uma nova espécie. Qualquer tipo de evidência pode ser usado para iniciar uma nova hipótese delimitando uma nova espécie, porém, se uma espécie é descrita baseada em uma evidência morfológica, com adição de filogenética do DNA e barreiras reprodutivas, podemos dar uma maior confiança nos nossos resultados, e é nesta linha que a taxonomia integrativa trabalha, utilizando linhas de evidências diferenciadas para delimitar as espécies (Padial *et al.* 2009; Padial & Riva 2010).

Os fragmentos de DNA 16S e 12S são uma importante ferramenta para o estudo da biodiversidade, pois são conhecidos como o código de barras de DNA para as espécies de anuros (Raharivololoniaina *et al.* 2006; Haas & Das 2011 Além disso, sequências de códigos de barra de DNA podem, em alguns casos, ajudar a responder questões filogenéticas, filogeográficas e ecológicas (Crawford *et al.* 2010; Strauß *et al*. 2010).

Com objetivo de avaliar o status taxonômico destas populações determinadas como *H.* aff *joaquini*, este estudo propõe-se a analisar diferentes fontes de evidência independente (*i.e.*, morfologia e morfometria de girinos e adultos, vocalização e fragmentos de DNA) acerca dos indivíduos desta população.

Esta dissertação está estruturada no formato básico de artigo científico, formato recomendado pelo Programa de Pós-graduação em Biociências da PUCRS. O artigo está apresentado como um capítulo e redigidos em inglês. O artigo foi preparado de acordo às normas para submissão à **Zoologica Scripta**. As instruções para submissão de artigos a este jornal foram fornecidas aos membros da banca de avaliação como documentos separados à tese.

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An integrative analysis of the taxonomic identity of *Hypsiboas* (Anura, Hylidae) related to *Hypsiboas joaquini* (B. Lutz, 1968) and *H. semiguttatus* (A. Lutz, 1925)

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Abstract

Hypsiboas joaquini and *H. semiguttatus* are two phenotypically similar species of the *H. pulchellus* species group with a complex and interrelated taxonomic history due to problems such as poor original descriptions, descriptions of several synonyms, redescritpions based on specimens of other species, and large population variation. Several closely related species have been described in recent years and currently, we face a complex panorama, with five species relatively well characterized in terms of external morphology in their respective type localities (*H. curupi*, *H. caipora*, *H. joaquini*, *H. semiguttatus,* and *H. stellae*) but for which geographic variation is poorly understood and overlapping. Thus, the goal of our study is to evaluate the taxonomic status of specimens formerly identified as closely related to either *H. joaquini* or to *H. semiguttatus* using adult and larva morphology, DNA sequences, and advertisement calls. We compared variation among these four sets of characters across nine taxonomic units, the five currently recognized species mentioned above and four additional sets of specimens grouped according to breeding and collection sites (i.e., *H.* aff. *curupi*, *H.* cf. *joaquini*, *H.* aff. *joaquini*, and *H.* aff. 2 *joaquini*). Our main results are: (i) extensive overlap and no clear diagnostic characters of morphometric characters of adult males and larvae, and quantitative characters of adult males; (ii) extensive variation on tadpole oral disc morphology diagnosing *H.* aff. 2 *joaquini*; (iii) extensive overlap and no clear diagnostic characters of acoustic characters except for *H. stellae*; (iii) a strongly supported clade that includes *H. joaquini* sensu stricto and all specimens belonging to the four comparative units defined herein and that shows short branches, low genetic distances (< 1%), and no geographical structure. According to our results we consider specimens of *H.* aff. *curupi*, *H.* cf. *joaquini*, *H.* aff. *joaquini*, and *H.* aff. 2 *joaquini* as part of *H. joaquini*, which becomes a widely distributed species with large phenotipic variation. We further discuss the outstanding variation of oral disc morphology of tadpoles of *H. joaquini* sensu this work, with local adaptation and phenotypic plasticity as possible causes.

Keywords: Argentina, Brazil, *H. pulchellus* species group, morphology, phylogenetics, phenotypic plasticity, tadpole

Introduction

Hypsiboas is part of Hylidae and was resurrected from its synonymy with *Hyla* by Faivovich *et al*. (2005) on the basis of a phylogenetic analysis of the family using molecular and morphological data. Currently, the genus contains 91 species distributed through tropical Central and South America, from Nicaragua to Argentina and the islands of Trinidad and Tobago (Faivovich *et al.* 2005; Duellman *et al*. 2016; Frost 2016). Duellman *et al*. (2016) reallocated *Ecnomiohyla tuberculosa* to *Hypsiboas* without evidence but arguing that *Hypsiboas* is a "catch-all genus". We do not follow their proposal because there is no evidence substaining that *E. tuberculosa* is part of *Hypsiboas* or that *Hypsiboas* is a non-monophyletic genus (although it may become with the inclusion of *E. tuberculosa*). There are representatives of the genus in different biomes, from lowland rainforests and dry shrublands to high elevation species occurring above 2,000 m a.s.l. (Faivovich *et al*. 2005; Duellman *et al*. 2016).

Hypsiboas includes small to large tree frogs related to snout-to-vent length (2.6–12.5 cm). Their eggs are protected by a transparent capsule and are laid on soil, water, rocks or vegetation (Kwet 2008). Tadpoles normally hatch at early stages (circa state 25 of Gosner 1960) and develop in lotic and lentic waters as well as in water contained in phytotelmata. *Hypsiboas* is diagnosed by 33 molecular transformations of nuclear and mitochondrial DNA sequences (Faivovich *et al.* 2005). The diversity of this genus is organized into seven species groups (Faivovich *et al*. 2005): *H. albopunctatus, H. benitezi, H. faber, H. pellucens, H. pulchellus, H. punctatus*, and *H. semilineatus*. Additionally, two species, *H. fuentei* (Goin & Goin, 1968) and *H. varelae* (Carrizo, 1992) remain unassigned to a species group.

The first described species that are now part of the *Hypsiboas pulchellus* group sensu Faivovich *et al.* (2005) have a convoluted taxonomic and nomenclatural history. For example, according to Bokermann (1965), Fitzinger (1826) corrected the mistake made by Raddi (1823), who used the name *Hyla lateralis* [currently *Dryophytes cinereus* (Schneider, 1799), from north America] for a specimen reported by Daudin (1800) from near the city of Rio de Janeiro, Brazil. The name *Hyla raddiana* was proposed for this specimen by Fitzinger (1826). Duméril & Bibron (1841) described *Hyla leucomelas* and *H. pulchella* from Montevideo, Uruguay. Steindachner (1864) considered both species described by Duméril & Bibron (1841) as synonyms of *Hyla raddiana*. However, *Hyla* *raddiana* was later considered a synonym of *Hyla albomarginata* by Bokermann (1965), while *H. pulchella* was considered a different and valid species.

Alpha taxonomic nomenclature problems aside, the *Hypsiboas pulchellus* group has its origins on the work of Bokermann (1963). Barrio (1965) considered it to be a single polytypic species, with 5 subspecies. B. Lutz (1973), based on Bokermann (1963), proposed the formal recognition of the "cycle of *Hyla pulchella*". She included five subspecies [*H. pulchella pulchella*, *H. p. cordobae* Barrio, 1965 currently *Hypsiboas cordobae*, *H. p. riojana* Koslowski, 1895 currently *Hypsiboas riojanus*, *H. p. andina* Müller, 1924 now a junior synonym of *H. riojanus*, and *H. p. joaquini* Lutz, 1968, currently *Hypsiboas joaquini*], plus *Hyla prasina* Burmeister, 1856, *H. marginata* Boulenger, 1887, *H. semiguttata* Lutz, 1925, and *H. cymbalum* Bokermann, 1963. B. Lutz (1973) considered *H. guentheri* Boulenger, 1886 and *H. bischoffi* Boulenger, 1887 as members of the *H. polytaenia* group, despite recognizing their similarity with *H. pulchellus*.

Kluge (1979) recognized an assembly of species of Neotropical *Hyla*, called "gladiator frogs", in which males have a greatly enlarged prepollex spine and hypertrophied forearms and that included members of the *Hyla pulchella* group of B. Lutz (1973). Faivovich (1996) included *Hyla caingua* Carrizo, 1991 in the group, without further comment. This action was corroborated by Cruz & Caramaschi (1998). Langone (1997) considered *H. p. joaquini* and *H. semiguttata* as synonyms of *H. marginata*. Furthermore, Langone (1997) suggested that *H. guentheri* could be related to the *H. pulchella* group. Duellman *et al.* (1997) proposed the fusion of *H. circumdata* and *H. pulchella* groups based in males having a greatly enlarged prepollical spine and hypertrophied forearms. Several subsequent studies ignored Duellman *et al*'s. (1997) taxonomy (*e.g.*, Cruz & Caramaschi 1998, 2000; Eterovick & Brandão 2001). Later on, Garcia *et al*. (2001, 2003) and Faivovich *et al*. (2004) provided critical evidence against the fusion of the *H. circumdata* and *H. pulchella* groups. Namely, Garcia *et al*. (2001, 2003) and Faivovich *et al.* (2004) defended that morphology of the distal prepollical element is different when compared among members of both groups. Species of the *H. pulchella* and *H. boans* groups (Da Silva 1998; Kluge 1981) have a posterior projection in the distal element, not observed in species of the *H. circumdata* group (Napoli 2000).

The hypertrophy of forearms is observed in other hylids, such as several members of *Bokermannohyla* and *Hypsiboas* [*B. alvarengai* (Bokermann, 1956), *B. martinsi* (Bokermann, 1964), and *B. langei* (Bokermann, 1965) of the *B. martinsi* group (Napoli

2000; Caramaschi *et al*. 2001), *B. pseudopseudis* (Miranda-Ribeiro, 1937), *B. saxicola* (Bokermann, 1964), and *B. ibitiguara* (Cardoso, 1983) of the *B. pseudopseudis* group (Napoli 2000; Caramaschi *et al*. 2001), *H. biobeba* (Bokermann & Sazima, 1974) of the *H. boans* group (Martins & Haddad 1988)]. These characters are more frequent in montane species that reproduce in currents of water, suggesting that these structures may have an adaptive role and the possibility of convergence among unrelated phylogenetic groups.

Faivovich *et al.* (2005) assessed the relationships of Hylidae and, among other taxonomic changes, redefined the *Hypsiboas pulchelus* group as a natural group on the basis of 55 nucleotide transformations of nuclear and mitochondrial genes. Faivovich *et al.* (2005) also suggested as synapomorphy of the group the absence of the slip of the *m. depressor mandibulae* that originates on the *dorsal fascia* at the level of the *m. dorsalis scapularis*. Within the *H. pulchelus* group, Faivovich *et al.* (2005) also recognized the taxa *Hypsiboas polytaenus* clade. More recent phylogenetic studies including a broad taxon sampling of the group (*e.g.* Caminer & Ron 2014; Pyron & Wiens 2011; Duellman *et al*. 2016) have not challenged the monophyly of the *H. pulchellus* group.

Since the proposal of Faivovich *et al*. (2005), the content of the group has changed due to the synonym of *Hypsiboas andinus* with *H. riojanus* (Köhler *et al*. 2010) and the addition of nine new species. Currently, with 38 recognized species (Appendix 1), this is the most species rich group within *Hypsiboas*. This clade, despite originating in the Atlantic Forest, has radiated into several regions of South America (Faivovich *et al*. 2004, 2005).

The taxonomic identity of various members of *Hypsiboas pulchellus* group have been the subject of several studies due to difficulties associated to poor original descriptions and problematic diagnostic characters (*e.g.*, Garcia *et al.* 2003, 2007; Faivovich *et al.* 2004, 2005; Antunes *et al*. 2008; Köhler *et al*. 2010). One of the most notable cases involves *H. semiguttatus* and *H. joaquini*, two species from Southern Brazil and northeastern Argentina—a hotspot for medium-sized hylids of *H. pulchellus* group (Kwet 2008)—with a complicated and intermixed taxonomic history that, as described below, is still not solved.

The original description of *Hyla semiguttata* was based on specimens collected in São Bento do Sul, state of Santa Catarina (SC), Brazil. The taxon was later considered a synonym of *Hyla polytaenia* (currently *Hypsiboas polytaenus*) by Cochran (1955). Cei & Roig (1961) revalidated *Hyla semiguttata*, but used specimens that proceeded from

Misiones, Argentina (currently *Hypsiboas curupi* Garcia, Faivovich & Haddad, 2007) for their description. B. Lutz (1968) described *Hyla raddiana joaquini* based on two samples from São Joaquim, SC, and considered *Hyla semiguttata* sensu Cei & Roig (1961) as *H. r. raddiana*.

B. Lutz (1973) identified the samples from Misiones and São Bento do Sul as *Hyla semiguttata* and accepted the proposals made by Bokermann (1965), such as changing the name *H. raddiana* to *H. pulchella*. B. Lutz (1973) changed the name *H. r. joaquini* to *H. p. joaquini*, and removed *Hyla prasina* from the synonym with *H. pulchella* when proposed the "cycle of *Hyla pulchella*". She also extended the known distribution of *H. semiguttata* southward to Canela, state of Rio Grande do Sul (RS), Brazil. Later, Langone (1993) extended the geographic range of *H. semiguttata* into the state of Paraná (PR), Brazil and recommended *H. semiguttata* and *H. p. joaquini* as junior synonyms of *H. marginata*. Garcia *et al.* (2001, 2003) rejected the proposal of Langone (1993) providing evidence for the validity of both taxa.

Garcia *et al.* (2007) redescribed *Hypsiboas semiguttatus,* which allowed for the discovery of a morphologically similar new species, and described the populations from Misiones—originally identified by Cei & Roig (1961) as *H. semiguttata*—as a new species, *H. curupi*. Kwet (2008) discovered another new species, presumably from this clade, from Sinimbú, RS, *H. stellae,* which is morphologically most similar to *H. curupi* but differs in advertisement call and external characteristics, such as body size and colouration. Finally, Antunes *et al*. (2008) described *H. caipora* from the Atlantic Forest of southeastern São Paulo (SP), Brazil. This species was inferred as sister of *H. semiguttatus* and most similar to it and to *H. curupi*. Despite these important advances in the taxonomy of *Hypsiboas,* other problems remain unsolved on the *Hypsiboas semiguttatus* clade, which includes *H. caipora, H. curupi*, *H. joaquini*, *H. semiguttatus*, and *H. stellae*.

The populations from northeast RS, used by B. Lutz (1973) as representatives of *Hyla p. joaquini*, when compared to *Hyla p. semiguttata* are larger and have white blotches in the dorsal surface of legs (blotches absent in *H. semigutatta*). Another character present in *Hyla p. joaquini* but absent in *H. semiguttata* is a metalic coloration of dorsal surfaces (evidence of iridophores). This population from northeast RS was referred to as "*Hyla semiguttatus* large form" by Kwet & Di Bernardo (1999) and Kwet (2001). Garcia *et al.* (2007) considered this population more similar to *Hypsiboas joaquini* than to *H. semiguttatus* and called it *Hypsiboas* cf. *joaquini,* a nomenclature subsequentelly followed by Kwet *et al*. (2010). Kwet (2008) proposed that *Hypsiboas joaquini* and *H.* cf. *joaquini* are coespecific without further investigating the issue.

Kwet & Di Bernardo (1999) used the name "*Hyla semiguttata* small form" for specimens from the surroundings of São Francisco de Paula, RS. Kwet & Di Bernardo (1999) reported that these specimens, besides being smaller than *H. semiguttatus,* have a different advertisment call and dorsal coloration—brown and, sometimes, with green hues. Kwet (2001) followed Kwet & Di Bernardo (1999), and Garcia *et al.* (2007) and Kwet (2008) used the name *Hypsiboas* sp. (aff. *joaquini*) for these populations, a nomenclature subsequentelly followed by Kwet *et al*. (2010) and Kwet & Marquez (2010). Talles & Martins (2011) used the name *H.* aff. *pulchellus*.

Another population of *Hypsiboas* apparently associated with *H. semiguttatus* and *H. joaquini*, which identity remains unsolved, was called *Hypsiboas* sp. aff. *semiguttatus* by Kwet (2008) and *Hypsiboas* cf. *semiguttatus* Kwet & Marquez (2010). These specimens are from the municipalities of Ponta Grossa and Palmeira, PR. Specimens from this population are smaller than *H. semiguttatus* and have a polymorphic dorsal pattern, mixing dark brown blotches fragmented in several small blotches. Their vocalization has a slightly lower dominant frequency than that of *Hypsiboas semiguttatus*.

Kwet (2008) reported one population of uncertain taxonomie identity from Serra do Tabuleiro, SC and was considered more similar to *H. semiguttatus* and *H. marginatus* by Kwet (2008). It was named *Hypsiboas* sp. in Kwet & Marquez (2010).

As things stand, we face a complex panorama, with five species relatively well characterized in terms of external morphology in their respective type localities (*Hypsiboas curupi*, *H. caipora*, *H. joaquini*, *H. semiguttatus*, and *H. stellae*) but for which geographic variation is poorly understood and overlapping. In other words, there is a strong intra and interpopulational variability in morphometric characters and color patterns, in addition to overlapping interspecific variation (Duellman *et al*. 1997). This situation indicates that one needs to not only compare specimens from different localities but also use integrative approaches (Dayrat 2005) in the taxonomy of these frogs.

The use of the combined independent lines of evidence, such as molecular phylogenetics, bioacoustics, ecology, morphometry, and morphology has already uncovered many cryptic species and solved problems in many taxonomic groups of amphibians (Vences & Wake 2007; Padial *et al*. 2009; Padial & De la Riva 2010), thereby accelerating the rate of new species discoveries (*e.g.* Vieites *et al*. 2009).

Our theoretical framework coincides with the cumulative approach described by Padial *et al.* (2010), where differences in any of the organismal attributes—which may have independent rates of evolution—are taxonomic characters that can provide evidence supporting the existence of a species. Under this approach, the congruence of characters is desired, but not required.

The goal of our study is to evaluate the taxonomic status of specimens belonging to the *Hypsiboas pulchellus* species group, formerly identified as closely related to either *H. joaquini* or to *H. semiguttatus* using independent sources of evidence.

Material and Methods

Species concept

We consider species to be separately evolving metapopulation lineages (Simpson 1951; Wiley 1978, 1981), operationally discoverable to the extent that isolation from its putative sister lineage can be inferred from character data (Wiley 1978; Frost & Kluge 1994). Reciprocal monophyly, fixed morphological differences, and fixed differences in reproductive behaviors (*e.g.*, advertisement calls) are examples of the kinds of evidence that can be used to infer species (see Padial *et al.* 2010 for a review).

Comparative units

Taxonomic studies such as this one aim to find evidences, or their absence, of lineage divergence and independence among populations. Although characters are observed on specific individuals, fixation of characters states on a sampled population is interpreted as indicative of the existence of independently evolving evolutionary lineages above the population level unless the observed variation can be explained by other phenomena such as isolation by distance, phenotypic plasticity or plesiomorphy. In this study, we work with five initial taxonomic hypotheses at the species level (*Hypsiboas caipora*, *H. curupi*, *H. joaquini*, *H. semiguttatus*, and *H. stellae*) and a number of specimens, from different localities, of ambiguous taxonomic identity according to available diagnosis and data. Our strategy was to use different collecting sites as comparative units. This was done under the premise that collecting sites for anurans normally correspond to aggregated breading sites along bodies of water, which is the case in species of *H. pulchellus* group.

Nonetheless, we did not discard a priori the possibility of syntopic species inasmuch as several of our analyses (*e.g.*, phylogenetic trees of DNA sequences, principal component analysis) compare the data of each specimen in the context of all the data and allow for the identification of coexisting divergent lineages. Following the literature (Garcia *et al.* 2003, 2007; Faivovich *et al.* 2004; Kwet 2008) and our own field and laboratory observations, we defined the following four taxonomic comparative units besides the five recognized species.

Hypsibas aff. *curupi* for specimens coming from the region of Xanxere and Campos Novos, SC. The association with *H. curupi* is based on proximity to type locality and similarity in external morphology.

Hypsiboas cf. *joaquini* (Fig. 1) for specimens coming from the region of the border between RS and SC (*e.g.* São José dos Ausentes, Cambará do Sul, Bom Jesus). Specimens from this region were mentioned in B. Lutz (1973), Braun & Braun (1980), Kwet & Di-Bernardo (1999), Kwet (2001, 2008), Garcia *et al.* (2007) and Pinheiros (2013).

Hypsiboas aff. *joaquini* (Fig. 2) for specimens from the region of São Francisco de Paula, RS. Mentioned by Kwet & Di Bernardo (1999) as "*Hyla semiguttata* small form" and by Garcia *et al.* (2007) as *Hypsiboas* sp. (aff. *joaquini*).

Hypsiboas aff. 2 *joaquini* (Fig. 3) for specimens from Caraá, RS. This correspond to a new locality found by collaborators and us during fieldwork.

Data acquisition in the field

Fieldwork was conducted from November 2014 to January 2016 in four localities in the Brazilian states of SC and RS (Fig. 4), from where *Hypsiboas* with uncertain taxonomic affinities have been reported (*H.* aff. *joaquini*, *H.* cf. *joaquini*, and *H.* aff. 2. *joaquini*). In these localities, we conducted nocturnal acoustic and visual searches along streams for adults. The same streams were searched during the day with dip nets for tadpoles.

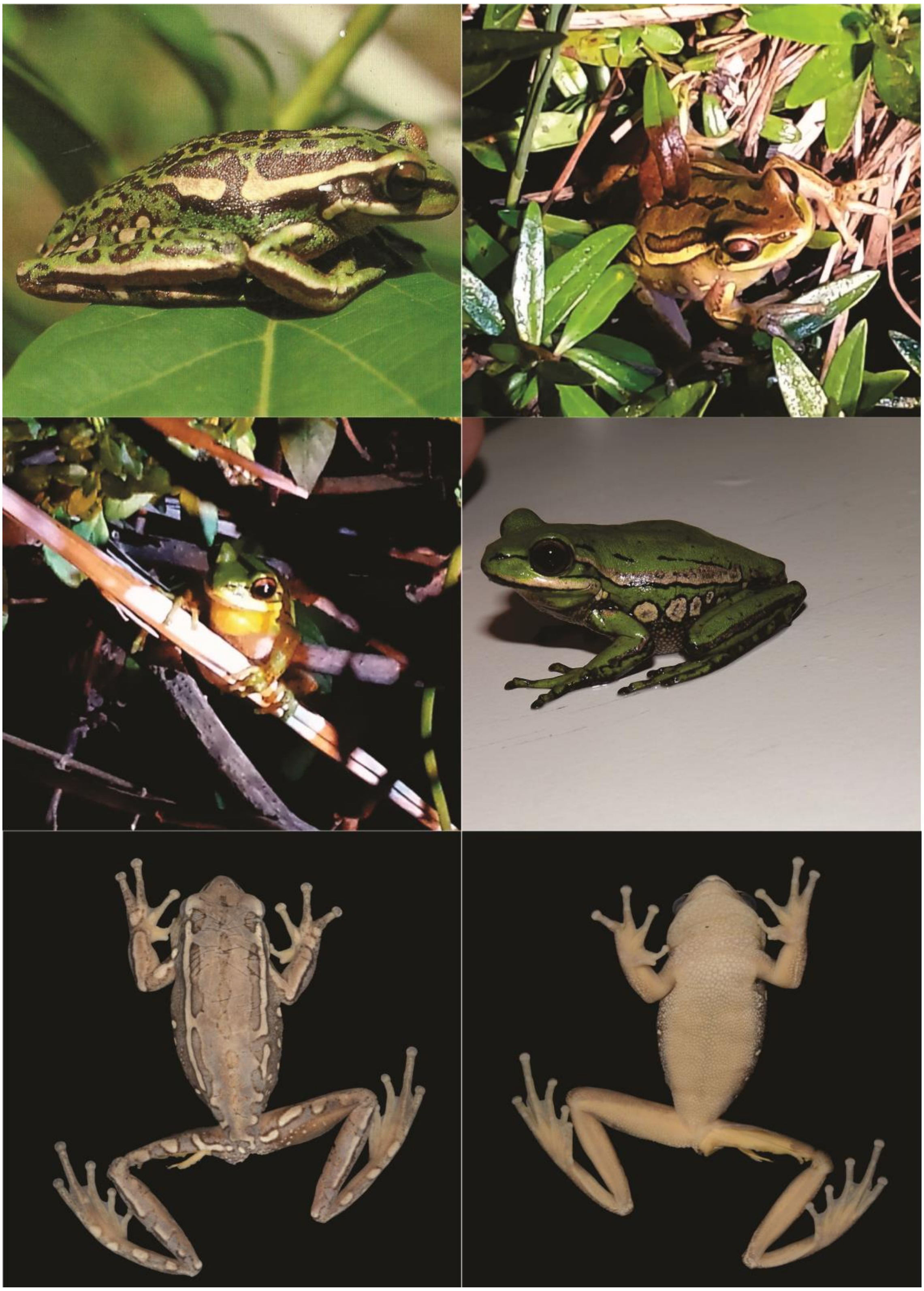

Figure 1. Adult specimens of *Hypsiboas* cf. *joaquini*.

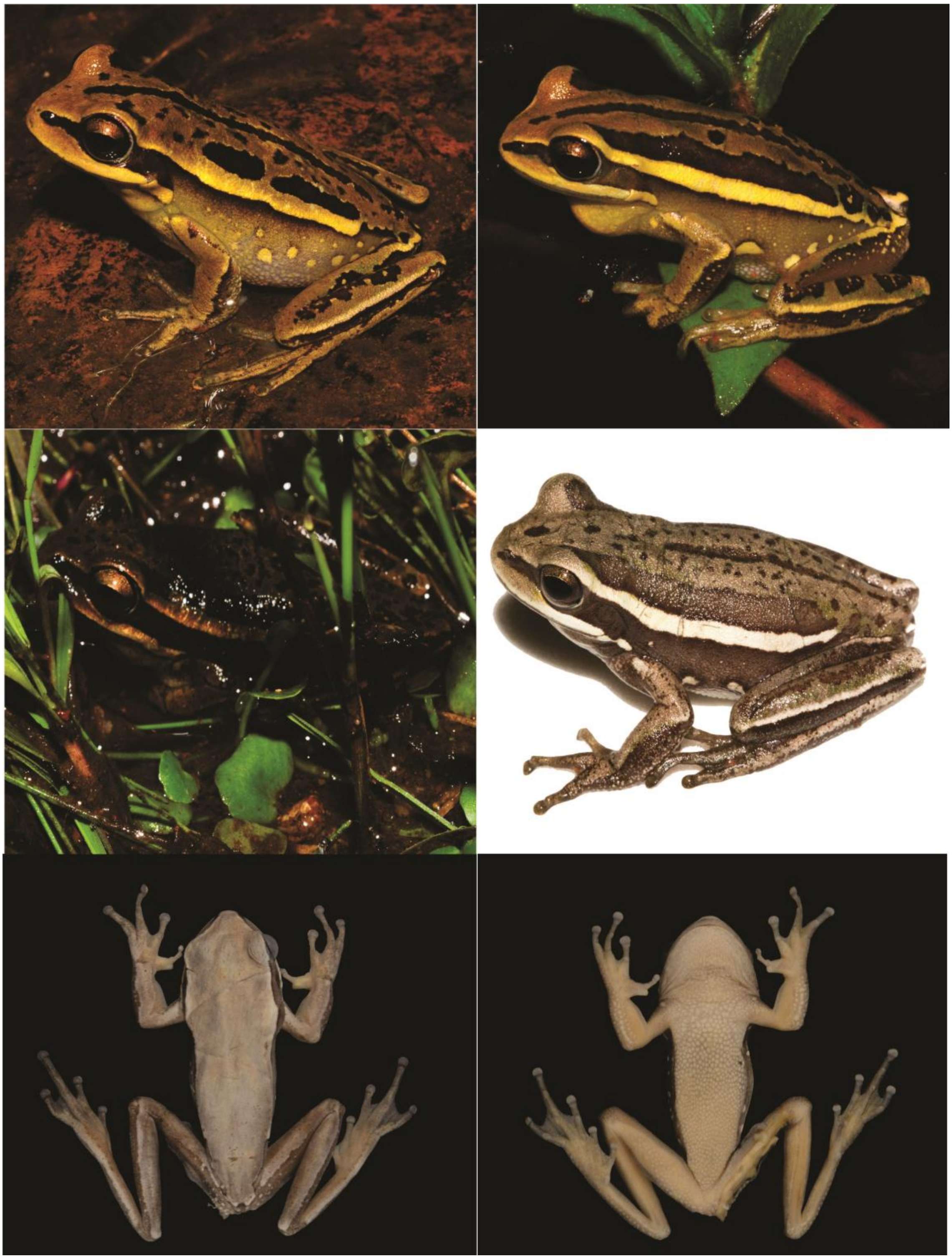

Figure 2. Adult specimens of *Hypsiboas* aff. *joaquini*.

Figure 3. Adult specimens of *Hypsiboas* aff. 2 *joaquini*.

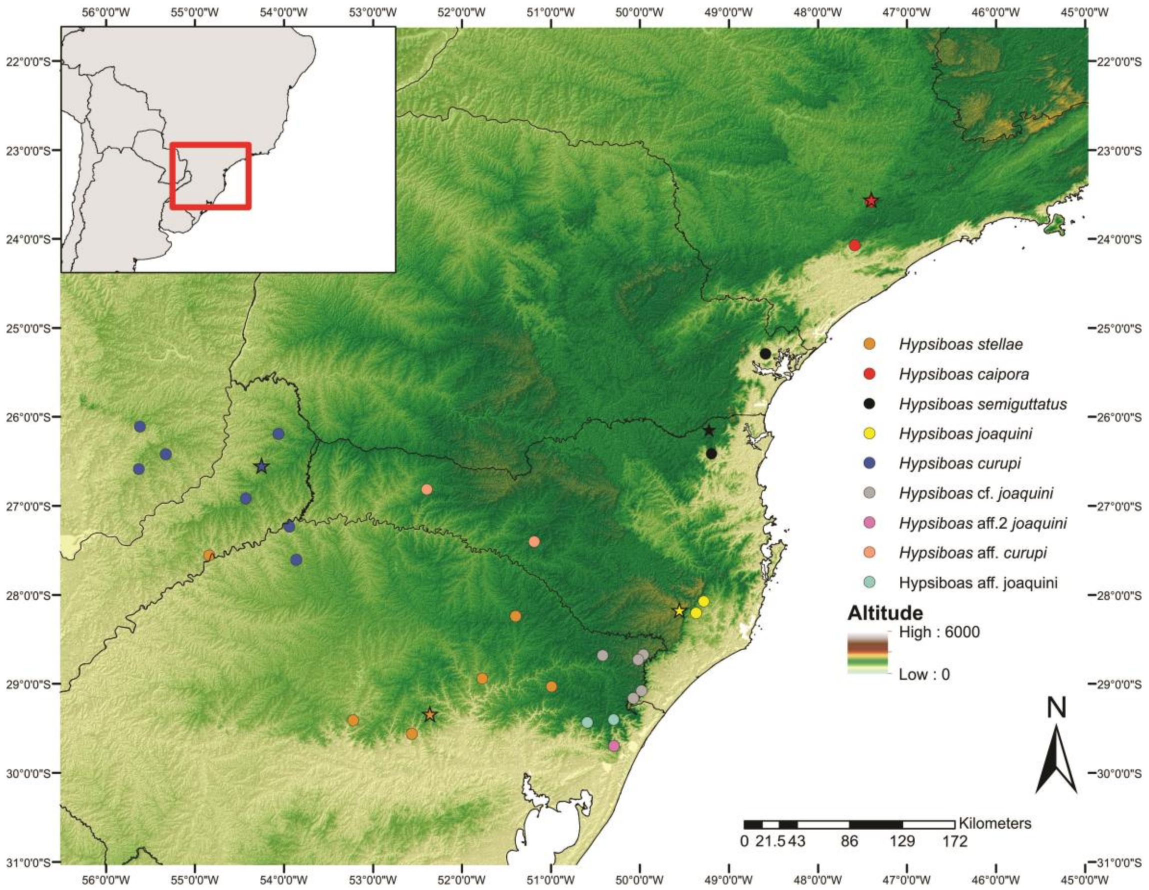

Figure 4. Map showing the localities of specimens studied according to the comparative units used in this study. Stars indicate type localities of currently recognized species.

Advertisement calls were recorded with a Marantz PMD670 digital recorder and a directional microphone positioned about 2 m from each recorded male, at sampling rate of 48 kHz in Waveform Audio (.WAV). Recordings are listed in Appendix 2. Adult specimens were collected manually, euthanized with an overdose of topical anesthetic (lidocaine 50mg/g) following approved protocols (Heyer *et al*. 1994), fixed in 5% formalin, and preserved in 70% ethanol. Tadpoles were euthanized with lidocaine diluted in water and fixed in 10% formalin. A sample of muscle tissue for molecular studies was preserved in 95% ethanol from each collected specimen before fixation. Voucher specimens were deposited in the amphibian collection of PUCRS (MCP-anfíbios), in Porto Alegre, Brazil.

Data acquisition from scientific collections

In addition to the newly 32 collected specimens, we examined 205 vouchers (including adults male, female and tadpoles) of ten species of *Hypsiboas pulchellus* group. Specimens were accessed through collection loans from or visits to Laboratorio de Genética Evolutiva, Instituto de Biología Subtropical, Universidad Nacional de Misiones (CONICET-UNaM), Universidade Estadual Paulista (CFBH); Universidade Federal de Minas Gerais (UFMG-AMP), Coleção do Laboratório de Herpetologia da UFRGS (UFRGS), Museu de Ciências Naturais Fundação Zoobotânica do Rio Grande do Sul (MCN-FZB), Museu de Ciência e Tecnologia of PUCRS (MCP–anfíbios), Universidade Federal de Santa Maria (ZUFSM), Museu de Zoologia "Adão José Cardoso" of Universidade Estadual de Campinas (ZUEC), Museu Nacional do Rio de Janeiro (MNRJ), and Universidade Federal de Minas Gerais (UFMG-AMP). These specimens corresponded to 24% of the species currently included in the *Hypsiboas. pulchellus* group. More importantly, type and topotypic material of the four focus species (*H. curupi*, *H. joaquini*, *H. semiguttatus* and *H. stellae*) was included as well as four specimens of ambiguous taxonomic identity from six localities from southern Brazil. We did not examine specimens of *H. caipora* and data from this species were taken from Antunes *et al*. (2008). All specimens studied are listed in Appendix 3.

Advertisement call samples of topotypic males of *Hypsiboas joaquini* were obtained from recordings deposited at Fonoteca Neotropical Jacques Vielliard (FNJV). Additional recordings were provided by Axel Kwet, which also included call samples of topotypical of *H. joaquini*. Two additonal call samples of *H.* cf. *joaquini* from Cambará do Sul, RS, were recorded by Paulo Pinheiros (UFMG). FNJV also provided call samples of *Hypsiboas semiguttatus* recorded in Ponta Grossa, PR. Recordings tentatively identified as *H. semiguttatus* (Lebon Régis, SC) were provided by Axel Kwet and Diego Baldo. Recordings of *H. curupi* are provided by Diego Baldo. All recordings of *H. stellae* were provided by Axel Kwet from Sinimbú, RS.

Tissue samples for molecular analyses were obtained from the following collections: CFBH, CONICET-UNaM, MCN-FZB, MCP–anfíbios PUCRS, and UFRGS. These included samples from 48 individuals of three species of the *Hypsiboas pulchellus* group and the four additional comparative units described above, originating from 14 localities in southern Brazil and Argentina, including topotypic samples of *H. joaquini*, *H. stellae* and a sample near 50 Km from *H. curupi* type locality. Additional DNA sequences were downloaded from GenBank. Material used for molecular analyses is listed in Appendix 4.

Morphological analyses

Sex was determined by secondary sexual characters: vocal slits and prepolical spines. The combined effort of the study of material housed at collections and newly collected material from fieldwork rendered only 22 female specimens. Thus, although morphological data was taken from males and females, statistical analyses described below were based only on males. A total 146 adult males and 90 tadpole voucher specimens were grouped according to the comparative units described above. We evaluated the presence and state of 24 discrete morphological characters commonly used in *Hypsiboas* taxonomy (*e.g.*, Garcia *et al.* 2007; Kwet 2008).

We measured 19 morphometric variables from preserved adult specimens using a digital caliper or a stereo microscope Zeis SV11 with graduated lenses (precision 0.1 mm). Variables were: snout-to-vent length (SVL); head length (HL), head width (HW), interorbital distance (IOD), internasal distance (ID), nostril-tip of snout distance (NS), eye-naris distance (EN), eye diameter (ED), distance between the anterior margins of eyes (AMD), tympanum diameter (TD), hand length (HAL), forearm plus hand arm length (Arm), forearm length (FAL), upper arm length (Uarm), foot length (FL), tarsus plus the foot, leg length (Leg), tarsus length (TAL), thigh length (TBL) and shank length (ShL). A principal component analysis (PCA) was used in order to reduce the number of independent morphometric variables and to evaluate the distribution of specimens along morphometric space represented by principal components. This procedure was applied in order to verify the existence of clusters of specimens corresponding to the groups assigned according to taxon or locality. As differences in body size can heavily influence variation in external morphometric characters, we repeated the PCA analysis considering the residuals of a regression of 18 morphometric variables against SVL. PCA analyses were done in Past 2.16 (Hammer 2001).

We studied the morphology of tadpoles following Altig & McDiarmid (1999), with the additions of Gonçalves (2014). We measured 21 morphometric variables in preserved tadpoles using a digital caliper or a stereo microscope Zeis SV11 with graduated lenses (precision 0.1 mm). Variables were: total length (TL), internarinal distance (IND), interorbital distance (IOD), snout length (SnL), eye diameter (ED), eye-naris distance (EN), head width (HW), body width (BW), body height (BH), body length (BL), tail length (TAL), maximum tail height (MTH), tail muscle height (TMH), tail muscle width (TMW), height of upper tail fin (Utail), height of lower tail fin (Ltail), snout-spiracle distance (SSD), spiracle length (SpL), height of spiracle (HS), spiracle width (SW), maximum width of nostril (MWN) and mouth width (MW).

Morphometric characters were also used in a PCA in order to evaluate the presence, in morphometric space, of clusters corresponding to assigned groups. We repeated the PCA analysis considering the residuals of a regression of 20 morphometric variables against TL.

Because we used data of *Hypsiboas caipora* from Antunes *et al.* (2008) and they included fewer morphometric variables than us for adults and tadpoles, we performed the same analyses explained above, but only considering the subset of variables included in their study.

Analysis of acoustic data

Recordings were analyzed in Raven 1.4. (Hawthorne 2011). Calls were selected by visual inspection of the recordings sonograms and variables were measured independently for each specimen when recordings included more than one calling male. Advertisement calls of the species analyzed follow the pattern described by Garcia *et al.* (2003) for *Hypsiboas joaquini* and are formed by a single multi-pulsed note, emitted sporadically at irregular time intervals. From each call, we measured number of pulses (NP), call duration (CD), time from the start of a call to the pulse with highest energy (RTB), duration of the pulse with highest energy (DPH), lower frequency of the pulse with highest energy (LFP), upper frequency of the pulse with the highest energy (UFP), dominant frequency of the pulse with highest energy (DF), frequency bandwidth of the pulse with the highest energy (FBW), time between the start of the pulse with highest energy to the peak of the following pulse (AP), time from the pulse peak of the pulse previous to the pulse with highest energy to the pulse peak of the pulse following that with highest energy (BAP). Lower and upper frequencies of the pulse with highest energy were measured 20 dB below the dominant frequency in order to avoid overlap with background noise. Temporal variables were measured from oscillograms and spectral variables were measured from power spectrum graphs (Fig. 2). Spectral analysis applied Blackman window type, size

10 ms window size and 80% overlap. We conducted a PCA on the acoustic variables in order to reduce the number of independent variables and to evaluate the existence of clusters of individuals corresponding to taxa or localities in acoustic space. Because we used data of *Hypsiboas caipora* from Antunes *et al.* (2008) and they included fewer acoustic variables than us, we performed the same analyses explained above but with the subset of variables included in Antunes *et al.* (2008), in this new PCA.

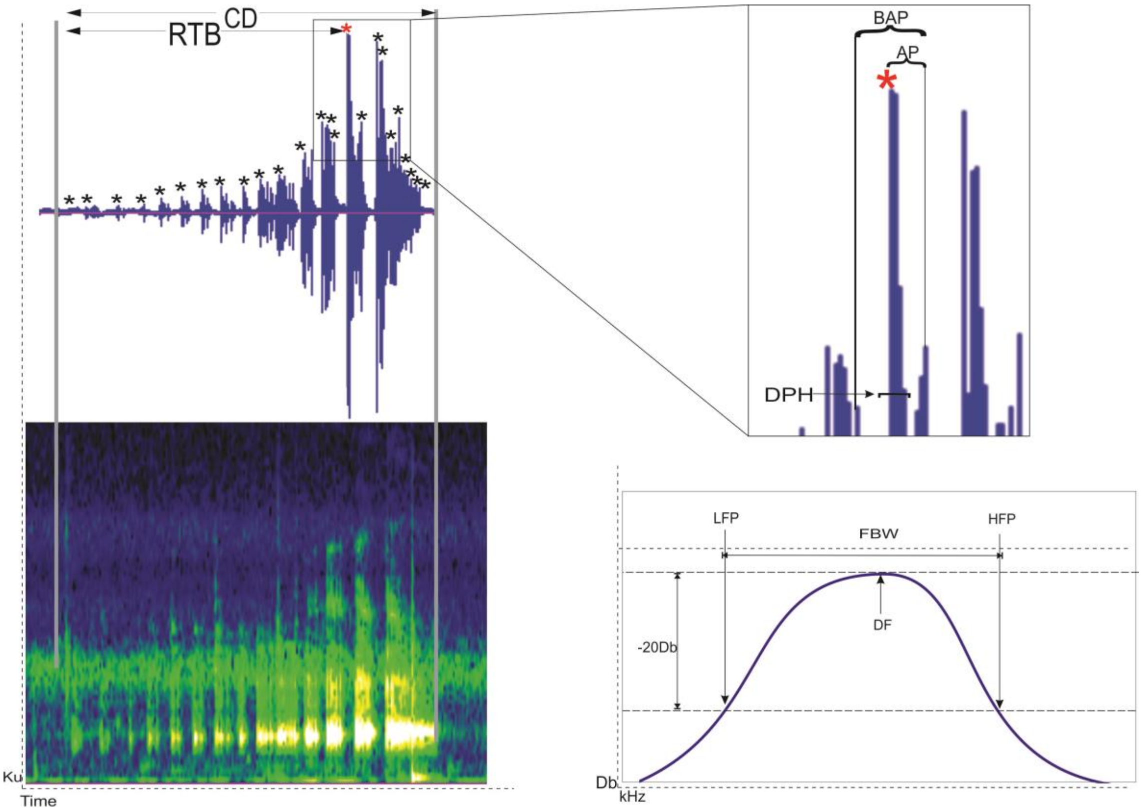

Figure 5. Oscilogram (top left and right), spectogram (bottom left), and power spectrum (bottom right) of the advertisement call of *Hypsiboas joaquini* illustrating the different acoustic variables used in this study. Asterisks indicate pulses and red asterisk marks the pulse with the highest intensity. Variables are defined in Material and methods.

Molecular data acquisition

Genomic DNA was extracted from tissue samples with DNeasy DNA extraction kits (Qiagen Inc., Germany) following the manufacturer’s recommendations. We amplified fragments of the mitochondrial 16S rDNA and 12S rDNA regions using the primers 16S AR and 16S BR (~598 pb, Palumbi *et al*. 1991) and 12S t-Phe-frog and 12S-frogR (~560 pb, Wiens *et al*. 2005) via polymerase chain reaction (PCR). PCR reactions used 8 µL ddH$_2$0, 1.25 µL of a 2µM solution of each primer, 12.5 µL Hot Star Taq Master Mix Kit (Qiagen Inc., Germany) and 2 µL of genomic DNA (about 30 ng/µL). Reactions were set with an initial denaturing step of 30 s at 94°C, followed by 35 or 45 cycles of amplification (94°C for 30 seconds, 50–55°C for 60 s, 72°C for 60 s), with a final extension step at 72ºC for 6 minutes. Amplification products were checked on 1% agarose gel. PCR products were purified and sequenced by Macrogen Inc., Seoul, South Korea. Sequences were obtained in both directions with the same primers used for PCR amplification and subjected to BLAST searches (Altschul *et al.* 1997) in GenBank to verify that the desired sequences had been amplified. Resulting sequences were checked in Sequencher 4.5 (Gene Codes, Ann Arbor, MI, USA) and all mutations or inconsistencies were verified against the original chromatograms. Homologous sequences belonging to samples of taxa included in the *Hypsiboas pulchellus* group were obtained from Genbank. To root all trees, we selected *H. faber*, a species of the sister clade of *H. pulchellus* group according to Faivovich *et al*. (2005).

Genetic distances and phylogenetic analyses

Sequences of each mitochondrial region were aligned separately using Muscle (Edgar 2004) as implemented in Mega 6.06 (Tamura *et al.* 2013) and applying default parameters. We calculate uncorrected and Kimura-2-parameters pairwise genetic distances (Kimura 1980; Nei & Kumar 2000) among the comparative units described above in Mega 6.06 (Tamura *et al.* 2013). Distance calculations were based exclusively on the 16S rDNA fragment, which is commonly used in amphibian species identification via DNA barcodes (Malkmus & Kosuch 2000; Ziegler 2002; Ziegler & Vences 2002; Steinke *et al.* 2005).

We used the concatenated sequence dataset (16S rDNA + 12S rDNA, 1105 bp) in order to estimate a maximum parsimony phylogenetic tree. Phylogenetic analyses were performed in TNT (Goloboff *et al.* 2008) using New Tecnology Searches at maximum level, finding the shortest tree a minimum of 10 times. Clade support was estimated by jackknife resampling with 1000 replicates, 50 Random Addition Sequences (RAS), and 50 Tree Bissection Reconnection (TBR).

Results

Morphological analyses

Table 1 provides a summary of the discrete external morphological characters observed, where 12 characters are identical among all samples. Observed morphological variation is composed of two structural and ten pigmentation characters.

Snout shape in lateral view is overlapping among taxonomic units, inasmuch as it is polymorphic in specimens of *Hypsiboas curupi* and *H. stellae*. Although topotypes of *H. stellae* and *H. curupi* have hypertrophied forearms, specimens of both species, but from other localities, do not. A metallic coloration of dorsal surfaces is only present in *H.* aff.2 *joaquini* and in *H.* cf. *joaquini*, however, this character in *H.* aff.2 *joaquini* is only present in juveniles. The three irregular longitudinal bars on dorsum are absent in *H.* aff. *curupi* and *H. stellae* but the character is polymorphic in *H. caipora*, *H. curupi*, and *H.* cf. *joaquini*. Flanck background coloration is also polymorphic within some comparative units, but it is dark in *H.* aff. *curupi*, *H.* aff. joaquini, and withish in *H. joaquini*, and *H. stellae*. The size of lateral spots only allows differentiation between *H. caipora* and *H.* aff. *curupi* (small) with *H. semiguttatus* (large). Spots on the hidden surface of thighs are variable within some comparative units but in *H. curupi* and *H. stellae* are present, and in *H. caipora*, *H.* aff. *curupi,* and *H. semiguttatus* are absent. The presence of spots on the cloacal region is unique of *H.* aff. *joaquini* and *H. joaquini*. In *H. curupi*, *H.* aff.2 *joaquini*, and *H.* cf. *joaquini* there is a white band on the upper side of the cloacal region, which is absent in the other taxonomic units. The presence of a white band on thighs is polymorphic in *H.* aff. *joaquini*, *H.* aff.2 *joaquini*, and *H.* cf. *joaquini*. However, its presence is constant in *H. curupi* and *H. semiguttatus*, which allows differentiation from *H.* aff. *curupi*, *H. joaquini*, and *H. stellae*. The absence of a white band on tibia of *H.* aff. *curupi*, *H. joaquini*, and *H. stellae* allows differentiation from the other taxonomic units. Also, the presence of a tarsal white band on *H.* aff. *joaquini*, *H.* aff.2 *joaquini*, and *H.* cf. *joaquini* differentiates these taxonomic units from the others.

Table 1. Variation among morphologic characters of adult males according to the comparative units defined in this study. Polimorphic characters within a comparative unit are separated by a slash (/) and variable characters are in bold face. Data from *Hypsiboas caipora* taken from Antunes et al. (2008).

	_H._ aff. _curupi_	**_H._ aff. _joaquini_**	**_H._ aff.2 _joaquini_**	**_H. caipora_**	**_H. curupi_**	**_H._ cf. _joaquini_**	**_H. joaquini_**	**_H. semiguttatus_**	**_H. stellae_**
Body shape	Robust	Robust	Robust	Robust	Robust	Robust	Robust	Robust	Robust
Snout in dorsal view	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded
Snout in lateral view	**Rounded**	**Rounded**	**Rounded**	**Rounded**	**Rounded/ Truncated**	**Rounded**	**Rounded**	**Rounded**	**Rounded/Truncated**
Pupil orientation	Horizontal	Horizontal	Horizontal	Horzintal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal
Translucid eyelid	Present	Present	Present	Present	Present	Present	Present	Present	Present
Extension of labial band	To the arm	To the arm	To the arm	To the arm	To the arm	To the arm	To the arm	To the arm	To the arm
Tympanic annulus	Present	Present	Present	Present	Present	Present	Present	Present	Present
Tympanic fold	Evident	Evident	Evident	-	Evident	Evident	Evident	Evident	Evident
Lips color	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish
Forearm shape	**Robust**	**Robust**	**Robust**	**-**	**Hypertrophied/Robust**	**Robust**	**Robust**	**Robust**	**Hypertrophied/Robust**
Prepollex	Present	Present	Present	-	Present	Present	Present	Present	Present
Belly color	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream
Metalic coloration on dorsum	**Absent**	**Absent**	**Present/Absent**	**-**	**Absent**	**Present**	**Absent**	**Absent**	**Absent**
Three irregular longitudinals bars on dorsum	**Absent**	**Present**	**Present**	**Present/ absent**	**Present/absent**	**Present/absent**	**Present**	**Present**	**Absent**
Flanks background color	**Dark**	**Dark**	**Whitish/dark**	**-**	**Whitish/dark**	**Whitish/dark**	**Whitish**	**Whitish/dark**	**Whitish**
Lateral stripe	Present	Present	Present	Present	Present	Present	Present	Present	Present
Size of lateral spots	**Small**	**Large/small**	**Large/medium/ small**	**Small**	**Medium/small**	**Medium/small**	**Medium/small**	**Large**	**Medium/small**
Spots on hidden surface of thighs	**Absent**	**Present/Absent**	**Present/Absent**	**Absent**	**Present**	**Present/Absent**	**Present/Absent**	**Absent**	**Present**
Spots on front surface of thighs	Absent	Absent	Absent	-	Absent	Absent	Absent	Absent	Absent
Spots on cloacal region	**Absent**	**Present**	**Absent**	**-**	**Absent**	**Absent**	**Present**	**Absent**	**Absent**
White stripe on cloacal region	**Present**	**Absent**	**Present**	**-**	**Present**	**Present**	**Absent**	**Absent**	**Absent**
White band on thighs	**Absent**	**Present/ Absent**	**Present/ Absent**	**-**	**Present**	**Present/ Absent**	**Absent**	**Present**	**Absent**
White band on tibia	**Absent**	**Present**	**Present**	**-**	**Present**	**Present**	**Absent**	**Present**	**Absent**
White band on tarso	**Absent**	**Present**	**Present**	**-**	**Absent**	**Present**	**Absent**	**Absent**	**Absent**

Table 2 summarizes the morphometric variation of adult males of the compared taxonomic units (data on female morphometrics is included in Appendix 5). A PCA on 19 external morphometric measurements of adult males recovered a first principal component (PC-1) that accounted for 71.8% of the total morphometric variation among samples. All variables have positive loadings on PC-1, suggesting that it is possibly related to size variation among specimens (Appendix 6). The groups compared herein largely overlap in this size-related axis (Fig. 3). A PCA on the residuals of regressions between 18 morphometric variables and SVL generated a PC-1 and PC-2 (Fig. 4) that explained 27.2% and 14.9% of the total morphometric variation among samples respectively (Appendix 7). Residual variation in HW and ShL have high loadings on PC-1, whereas PC-2 accounted for residual variation in FAL.

When we included *Hypsiboas caipora,* a PCA on 15 external morphometric measurements of adult males recovered a first principal component (PC-1) that accounted for 70.6% of the total morphometric variation among samples. All variables have positive loadings on PC-1, suggesting that it is possibly related to size variation among specimens (Appendix 8). *Hypsiboas caipora* does not overlap with *H. curupi* and *H.* aff.2 *joaquini* in in this size-related axis of PC-1 (Fig. 5). A PCA on the residuals of regressions between 14 morphometric variables and SVL generated a PC-1 and PC-2 that explained 23.7% and 14.8% of the total morphometric variation among samples respectively (Appendix 9). Residual variation in tranversal head measurements (HW, IOD) and TBL have high loadings on PC-1, whereas PC-2 accounted for residual variation in NS. *Hypsiboas caipora* is the only species that does not overlap in morphometric space (Fig. 6).

Of the 25 discrete morphological characters (Table 3) studied in tadpoles only two are variable among taxonomic units. Tadpoles of *Hypsiboas* aff.2 *joaquini* and *H. curupi* are unique by lacking a medial gap on the papillae on anterior labium (Fig. 7). Also, the tooth row formula of *H.* aff.2 *joaquini* is unique by having larger number of tooth rows, four anterior and six posterior, both with a medial gap (P1). When compared to the other taxonomic units, tadpoles of *H. curupi* and *H. stellae,* which share the same tooth formula, have a intermediate number of tooth rows. The remaining taxonomic units have two anterior tooth rows and three to four posterior rows.

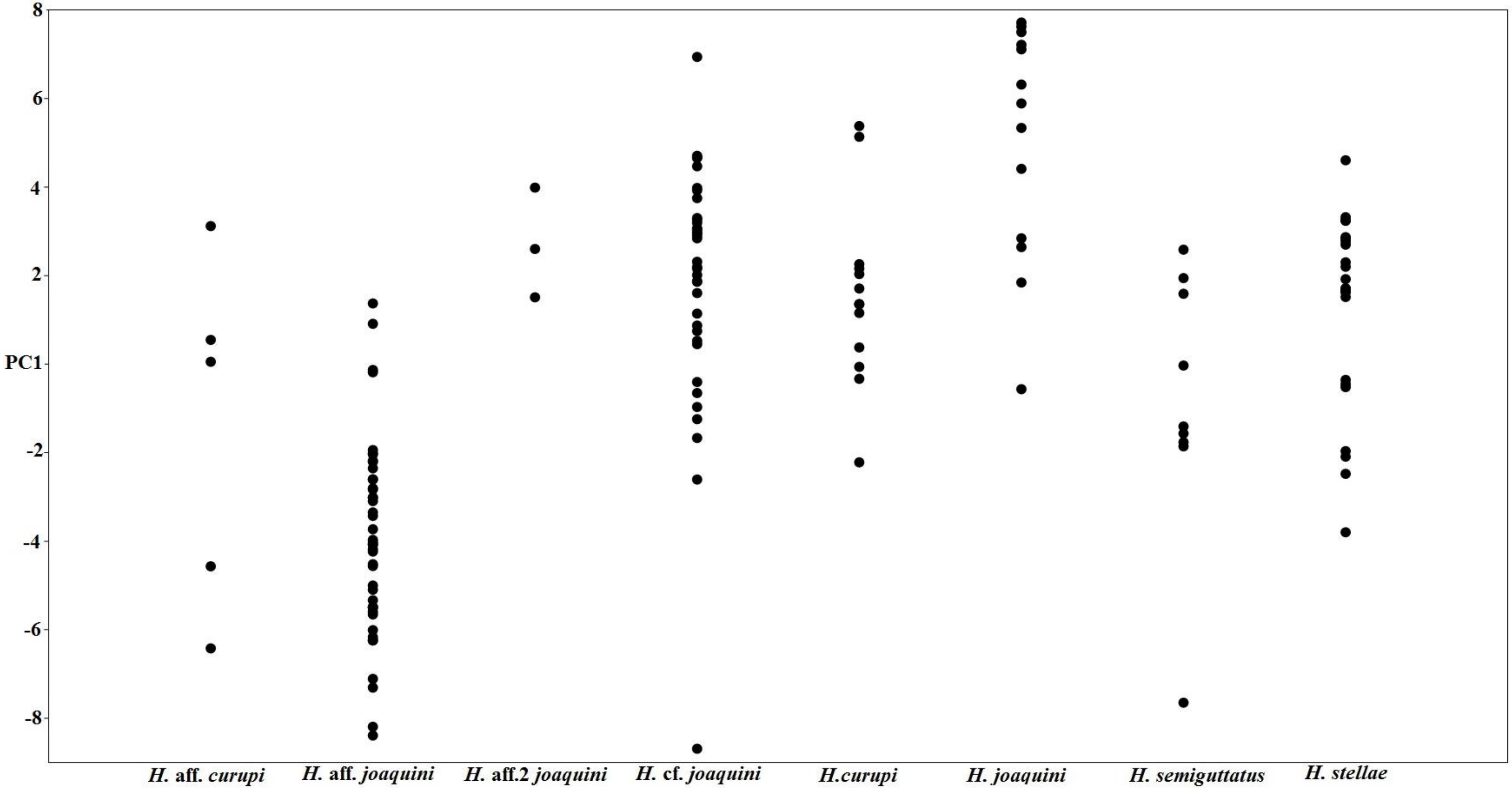

Figure 6. Variation of the first principal component (PC1) of 19 morphometric variables among adult males of the comparative units defined in this study. Each dot represent an adult male specimen within each of the comparative units. The first principal component explains 74.9% of the total variance.

Table 2. Variation of morphometric variables, minimum–maximum (mean± standard deviation), of adult males according to the comparative units defined in this study. Data from *Hypsiboas caipora* taken from Antunes *et al*. (2008).

Taxa	***H. caipora***	**_H._ aff. _curupi_**	**_H._ aff. _joaquini_**	**_H._ aff. _2 joaquini_**	***H. curupi***	**_H._ cf. _joaquini_**	***H. joaquini***	***H. semiguttatus***	***H. stellae***
N	12	5	45	3	20	40	14	9	12
AMD	3.7–4.4 (4±0.19)	3.7–4.5 (4.2±0.3)	2.9–5.1 (3.8±0.5)	3.9–5.2	3.6–5.1 (4.5±0.4)	3–5.1 (4.4±0.4)	4.4–6.2 (5.1±0.6)	3.2–5.1 (4.3±0.6)	3.6–4.6 (4.1±0.4)
Arm	–	15.8–22.7 (19.6±3.2)	8.4–22.7 (16.5±2.5)	22.8–23.9	19.9–25.1 (22.1±1.6)	12.4–26 (21.7±2.4)	20–29.8 (25±2.5)	14.4–23.3 (19.7±2.7)	18.3–24.4 (22±1.8)
ED	–	4.2–6.5 (5.1±0.9)	3.3–5.4 (4.2±0.6)	4.3–5.6	4.2–6.2 (5.1±0.6)	3.7–5.8 (5±0.5)	4.1–6.9 (5.5±0.7)	3.6–5.2 (4.3±0.6)	4.3–6 (5.1±0.6)
EN	2.2–2.7 (2.45±0.15)	2.5–3.9 (3.3±0.6)	2–4.8 (3.1±0.5)	3.6–3.8	2.9–4.4 (3.4±0.4)	2.5–6.1 (3.7±0.7)	2.8–4.7 (3.8±0.5)	2.7–3.9 (3.4±0.4)	2.9–5.8 (3.7±0.8)
FAL	5.7–6.8 (6.24±0.31)	6.5–10.5 (9.2±1.7)	6.1–10.6 (8.2±0.9)	10.4–12.1	8.9–11.7 (10.2±0.8)	6.2–13 (10.4±1.1)	9.1–13.8– (11.7±1.3)	7.1–11.5 (9.4±1.3)	8.5–11.6 (10.4±0.8)
FL	13.1–16.6 (14.36±1.04)	13.7–20.3 (16.5±2.7)	10–19.2 (15±2)	18.4–19.6	16.2- 21.2 (18.6±1.2)	10.9–23.8 (19.8±2.3)	17.3–27.8 (22.5±2.8)	12.7–21.2 (17.9±2.5)	15–21.5 (18.3±1.9)
HAL	10.3–12 (11±0.4)	9.5–14.9 (12.3±2.3)	8.5–13.6 (10.6±1.2)	13.7–14.3	12–15.2 (13.7±0.8)	7.8–16.5 (13.6±1.7)	11.9–18 (15.9±1.6)	8.6–14.1 (11.9±1.7)	10.7–15.1 (13.5±1.3)
HL	10.5–12.3 (11.4±0.5)	9.9–14.8 (12.4±2)	8.8–13.6 (11.4±1.1)	13.6–14.4	12.7–16.5 (14.4±0.9)	6.7–16.5 (13.5±1.5)	12.8–17.1 (15.3±1.2)	8.1–14.5 (12.3±2)	11–14.8 (13.6±1.2)
HW	10.9–12.7 (11.85±0.53)	9–13.8 (11.6±2)	7.8–13.3 (10.1±1.1)	13.1–13.1	11–15.3 (13.7±1.1)	8.2–15.3 (13.1±1.3)	12.4–18.2 (14.8±1.5)	8.4–14.3 (12.2±1.9)	10.2–15.7 (13.1±1.8)
ID	2.4–2.9 (2.67±0.18)	2.4–3.5 (3±0.5)	2.4–3.7 (3±0.3)	3.3–3.9	2.8–4 (3.4±0.3)	2.8–4.3 (3.6±0.3)	2.9–4.6 (3.8±0.4)	2.6–3.6 (3.2±0.3)	2.7–3.9 (3.3±0.4)
IOD	6.2–6.8 (6.5±0.23)	3.4–4.6 (4±0.5)	2.4–4.8 (3.7±0.5)	5–5.9	3.2–5.3 (4.5±0.5)	3.3–5.7 (4.3±0.6)	3.3–6.6 (4.9±0.8)	3.4–4.2 (3.9±0.3)	3.7–4.8 (4.2±0.4)
Leg	–	23–33.9 (27.8±4.5)	19–32.5 (24.9±2.9)	30.2–32.7	27.8–35.7 (31.5±1.9)	19.5–38.9 (32.4±4.2)	28.9–44.6 (36.6±4)	20.6–34.6 (28.5±4.8)	25.5 –35.2 (30.8±2.8)
NS	1.3–1.6 (1.5±0.09)	4.3–5.9 (5.2±0.6)	3.5–5.6 (4.4±0.5)	5.5–5.9	3.8–6.6 (5.4±0.6)	3.2–6.28 (5.3±0.7)	5.4–6.7 (6.1±0.4)	3.9–5.7 (5±0.6)	4.2–6.1 (5.4±0.7)
ShL	15.5–18.4 17.2±0.84)	16.2–22.9 (19.2±2.9)	13–21.2 (17.2±1.8)	21.9–23.6	19.4–25 (21.9±1.5)	13.8–26.5 (22.5±2.2)	19.2–29 (24.7±2.4)	15.1–25.1 (20.7±2.9)	17.5–24.2 (21.4±2)
SVL	29.7–35.1 (32.36±1.73)	31.4–44.5 (38±5.8)	28.4–42.5 (34.8±3.5)	42.4–43.6	35.8–46.8 (41.7±2.6)	26.7–50.6 (43.6±4.3)	40.2–54.3 (47.6±4.6)	30.7–45.1 (39.4±4.4)	34.2–45.9 (41.4±4.1)
TAL	10.2–11.9 (10.98±0.53)	10.2–14.4 (12.2±1.8)	8.5–14.6 (11.3±1.3)	13.9–15.3	11.5–15.2 (13.8±0.9)	8.7–19.7 (14.5±1.7)	13.2–17.8 (15.5±1.3)	9.7–16 (13.5±1.8)	10.8–15 (13.3±1.4)
TBL	16–18.8 17.62±0.83)	14.9–21.7 (18.2±3.1)	13.3–22.7 (17.5±1.9)	21.3–22.7	19.7–25 (22.4±1.4)	14.3–27.7 (22.6±2.5)	19.3–29.6 (25.5±2.9)	13.3–24.1 (20.1±3.3)	17.9–33.7 (22.7±4.0)
TD	1.3–1.8 (1.45±0.1)	1.5–2.2 (1.9±0.3)	1.4–2.8 (2±0.3)	2.0–2.6	1.6–2.8 (2.3±0.3)	1.5–2.7 (2.2±0.3)	2.2–3.1 (2.6±0.2)	1.3–2.2 (1.8±0.3)	1.4–2.8 (2.3±0.4)
Uarm	–	7.6–11.6 (9.8±1.8)	3.3–10.5 (8.4±1.3)	12.1–14.1	10–13 (11.7±0.9)	7.3–13.6 (11.2±1.3)	9.8–15.2 (13.1±1.6)	6.6–12.2 (10.5±1.8)	9.1–13.3 (11.2±1.0)

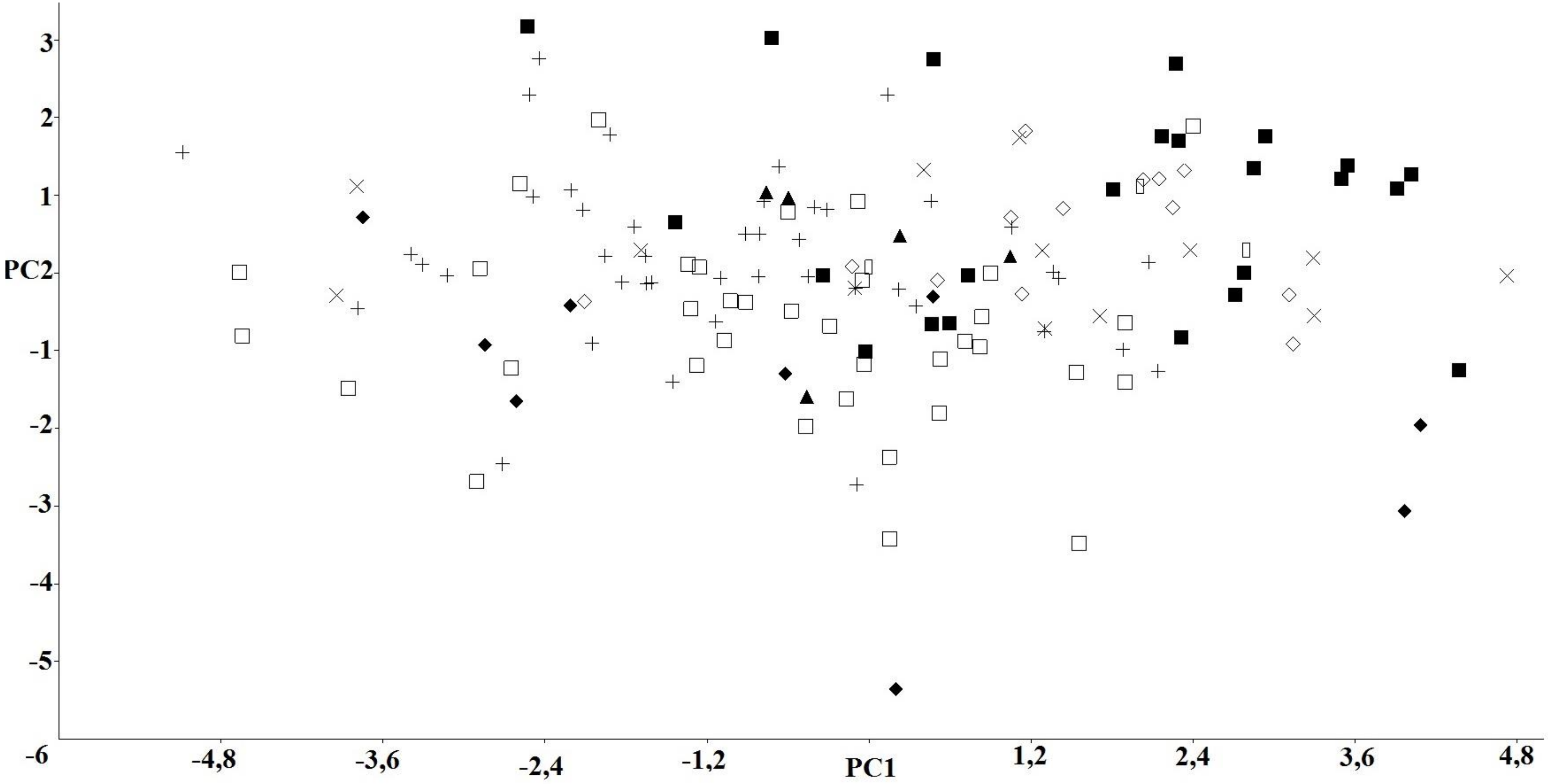

Figure 7. First two principal components (PC) of the residuals of a correlation with SVL of adult male body measurements of comparative units defined in this study. PC 1 and 2 represent 24.8% and 9.8% of total variance, respectively. Filled triangle *Hypsiboas* aff. *curupi*, cross *H.* aff. *joaquini*, rectangle *H.* aff. 2 *joaquini*, square *H.* cf. *joaquini*, lozenge *H. curupi*, x *H. joaquini*, filled lozenge *H. semiguttatus*, and filled square *H. stellae.*

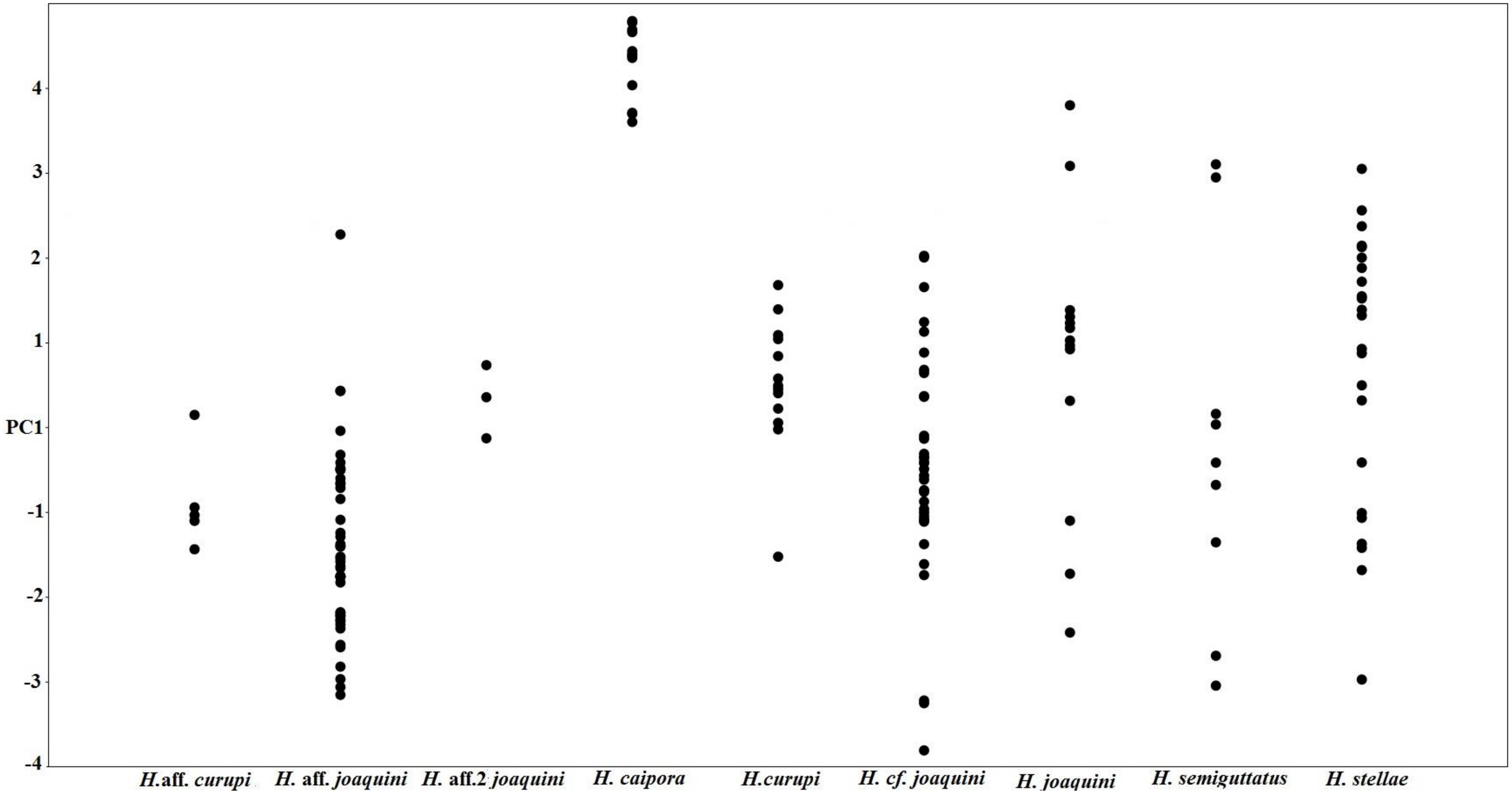

Figure 8. Variation of the first principal component (PC1) of 15 morphometric variables among adult males of the comparative units defined in this study. Each dot represent an adult male specimen within each of the comparative units. The first principal component explains 70.6% of the total variance. Data from *Hypsiboas caipora* taken from Antunes *et al*. (2008).

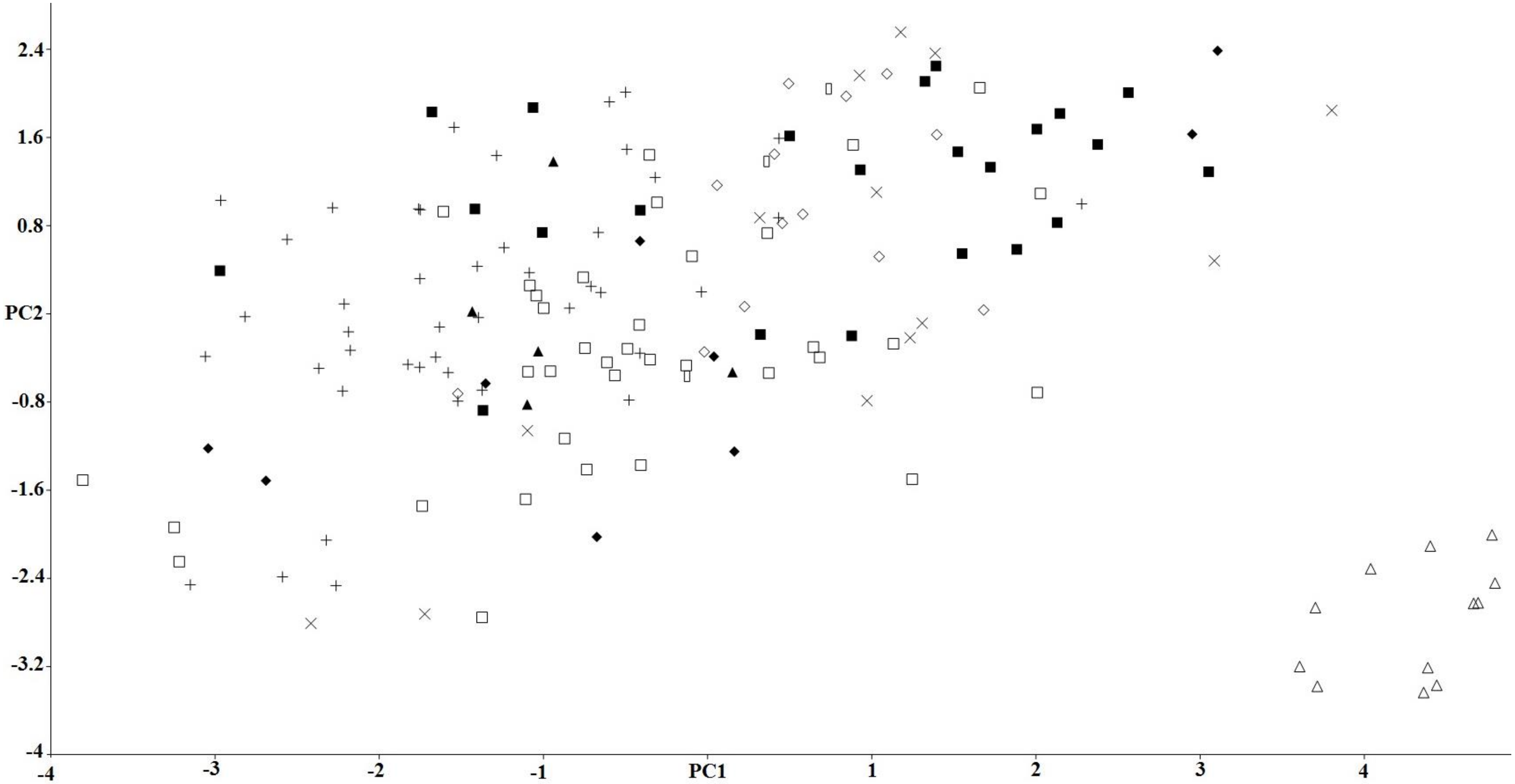

Figure 9. First two principal components (PC) of the residuals of a correlation with SVL of adult male body measurements of comparative units defined in this study. PC 1 and 2 represent 27.2% and 14.9% of total variance, respectively. Triangle *Hypsiboas caipora*, filled triangle *H.* aff. *curupi*, cross *H.* aff. *joaquini*, rectangle *H.* aff. 2 *joaquini*, square *H.* cf. *joaquini*, lozenge *H. curupi*, x *H. joaquini*, filled lozenge *H. semiguttatus*, and filled square *H. stellae.* Data from *Hypsiboas caipora* taken from Antunes *et al*. (2008).

Figure 10. Diversity of tadpole morphology among studied comparative units showing oral disc (left figures) and pairs of live and preserved specimens (top and bottom respectively, of right figures). (A) *Hypsiboas* aff.2 *joaquini*; (B) *Hypsiboas* aff. *joaquini*; (C) *Hypsiboas stellae.*

Table 4 summarizes morphometric variation among tadpoles of the studied comparative units. A PCA on the 22 external morphometric measurements of tadpoles recovered a PC-1 that accounted for 62.9% of the total morphometric variation among samples.

All variables have positive loadings on PC-1, suggesting that it possibly relates to size variation among tadpoles (Appendix 10). Tadpoles of *Hypsiboas curupi* did not overlap in morphometric space with those of *H. joaquini* and *H. semiguttatus* (Fig. 8). Also, *H. semiguttatus* just overlap along PC-1 with *H.* aff. *joaquini* and *H.* aff.2 *joaquini*. When we included *H. caipora* (12 morphometric variables available), PC-1 accounted for 66.9% of total morphometric information. All loadings had positives values

(Appendix 11). The single tadpole of *H. caipora* does not overlap with *H.* aff. *joaquini*, *H. joaquini*, and *H. semiguttatus* (Fig. 9).

Table 3. Variation among morphological characters of tadpoles according to the comparative units defined in this study. Variable characters are in bold face. Data from *Hypsiboas caipora* taken from Antunes et al. (2008).

	H.* aff. *joaquini	***H.* aff.2 *joaquini***	***H. caipora***	***H.* cf. *joaquini***	***H. curupi***	***H. joaquini***	***H. semiguttatus***	***H. stellae***
Anal tube and tail welded	Present	Present	Present	Present	Present	Present	Present	Present
Anteroventral oral disc position	Present	Present	Present	Present	Present	Present	Present	Present
Apofise in the nostril	Present	Present	Present	Present	Present	Present	Present	Present
Body ovoid on upper view	Present	Present	Present	Present	Present	Present	Present	Present
Dextrally orientated anal tube	Present	Present	Present	Present	Present	Present	Present	Present
Dorsally depressed globular body	Present	Present	Present	Present	Present	Present	Present	Present
Dorsolateral eye	Present	Present	Present	Present	Present	Present	Present	Present
Dorsolateral position of nostril	Present	Present	Present	Present	Present	Present	Present	Present
Dorsolateral spiracle	Present	Present	Present	Present	Present	Present	Present	Present
Fin form a Wide arc	Present	Present	Present	Present	Present	Present	Present	Present
Free spiracle condition	Present	Present	Present	Present	Present	Present	Present	Present
Gap on marginal papillae in the tip of mouth	**Present**	**Absent**	**Present**	**Present**	**Absent**	**Present**	**Present**	**Present**
Lateral fold of the oral disc	Present	Present	Present	Present	Present	Present	Present	Present
Lateral line	Present	Present	Present	Present	Present	Present	Present	Present
Muscle until the end of tail	Present	Present	Present	Present	Present	Present	Present	Present
Muscle with a gradual thinning	Present	Present	Present	Present	Present	Present	Present	Present
Nostril borders thin and complete	Present	Present	Present	Present	Present	Present	Present	Present
One row of marginal papillae	Present	Present	Present	Present	Present	Present	Present	Present
Reniform nostrils	Present	Present	Present	Present	Present	Present	Present	Present
Snout rounded on dorsal view	Present	Present	Present	Present	Present	Present	Present	Present
Snout rounded on lateral view	Present	Present	Present	Present	Present	Present	Present	Present
Starting of fin on body	Present	Present	Present	Present	Present	Present	Present	Present
Submarginal papillae	Present	Present	Present	Present	Present	Present	Present	Present
Tail ending on tip	Present	Present	Present	Present	Present	Present	Present	Present
Tooth row formula	**2(2)/3(1)**	**4(4)/6(1)**	**2(2)/4(1)**	**2(2)/3(1)**	**3(1,3)/5(1)**	**2(2)/3-4(1)**	**2(2)/4(1)**	**3(1,3)/5(1)**

Table 4. Variation of morphometric variables, minimum–maximum (mean± standard deviation), of tadpoles according to the comparative units defined in this study. Data from *Hypsiboas caipora* taken from Antunes *et al*. (2008).

Taxa	*Hypsiboas* aff. *joaquini* (N=15)	*Hypsiboas* aff2. *Joaquini* (N=15)	*H. caipora* (N=1)	*Hypsiboas curupi* (N=3)	*Hypsiboas* cf. *joaquini* (N=15)	*Hypsiboas joaquini* (N=20)	*Hypsiboas semiguttatus* (N=4)	*Hypsiboas stellae* (N=18)
BH	5–9.1 (7.3±1.2)	6.1–10 (7.7±1.3)	8.7	5.3–9.1	5.2–9.3 (7.4±1)	5.9–7.5 (6.7±0.4)	9.1–11.6 (10.7±1.1)	2.9–9 (5.2±2)
BL	11.7–17.5 (14.7±1.5)	7–20 (14.9±3.5)	14.2	14.5–16.3	9.1–17.2 (13.7±2.1)	10.8–39 (15.5±7.8)	18.1–19.7 (18.6±0.7)	6.2–16.3 (10.3±3.2)
BW	5.9–12.2 (8.6±1.4)	6.4–12 (8.4±1.6)	9.1	7.3–10.5	4.7–9.2 (7.7±1.1)	7.1–8.5 (7.8±0.3)	10.5–12.8 (11.6±0.9)	2.9–8.3 (5.4±1.7)
ED	1.5–2.4 (1.9±0.3)	1.3–3.7 (2.1±0.6)	1.8	2–2.5	1.3–2.3 (1.8±0.3)	1.1–2.2 (1.5±0.3)	1.9–2.5 (2.1±0.3)	0.9–3.1 (1.3±5.6)
EN	1.3–2.6 (1.7±0.4)	1.2–2.8 (1.9 -0.5)	2.6	1.5–1.9	1–1.9 (1.4±0.2)	1.3–2.1 (1.6±0.2)	2.2–4.1 (2.8±0.9)	0.5–2.1 (1±0.5)
HW	6.1–9 (7.1±0.8)	6.3–10.9 (8.4±1.4)	-	6.9–8.4	4.5–7.7 (6.3±0.8)	5.2–7.1 (6.1±0.4)	8–10 (9.1±0.8)	2.8–8.4 (5.1±1.7)
IND	1.8–3.8 (2.3±0.5)	1.8–4.1 (3±0.6)	2.5	2.4–2.8	1.3–2.4 (1.8±0.3)	1.3–2.5 (1.9±0.3)	2.7–3.9 (3.2±0.5)	1.4–2.9 (2±0.5)
IOD	2.6–4.4 (3.3±0.5)	2.8–5.1 (4±0.7)	4.2	3.1–3.6	2.3–3.8 (3.1±0.4)	2.5–3.4 (2.9±0.2)	3.6–4.9 (4.3±0.6)	1.1–4.5 (2.3±1)
Ltail	1–2.9 (2 0.5)	1.5–3 (2.1±0.4)	-	1.5–2.7	1.1–2.5 (2±0.4)	1.2–2.6 (2±0.4)	3.5–4 (3.7±0.2)	1–2.9 (1.7±0.7)
MTH	6.7–10.5 (8.4±1)	3.1 – 6.4 (5.2±0.9)	9.5	6.5–10.5	4.7–9.5 (7.7±1.5)	3.3–7.8 (6.5±1.2)	10.3–14.3 (12±1.7)	3.3–5.3 (2.8±1.2)
MW	3.1–4.1 (3.5±0.3)	4.6–8.9 (6.6±1)	4.5	5.9–6.2	2.8–4.4 (3.7±0.5)	2.8–4.3 (3.6±0.4)	4.5–5.9 (5.1±0.6)	2–5.9 (3.3±1.4)
MWN	0.5–2.8 (1.2±0.7)	0.6–1 (1±0.2)	0.5	0.4–0.9	0.5–1 (0.8±0.1)	0.5–2.2 (1±0.3)	0.6–1.2 (1±0.3)	0.1–1.2 (0.4±0.3)
SnL	1.5–4.7 (2.4±0.8)	0.3–5.1 (2.9±1.4)	-	2.5–2.8	1.3–2.3 (1.8±0.3)	1.3–2.1 (1.7±0.2)	2.2–3.3 (2.8±0.5)	0.7–2.8 (1.3±0.5)
HS	0.8–2.2 (1.6±0.5)	1.3 –3.9 (2.5±0.7)	-	1.8–2.4	1–2.3 (1.5±0.4)	1.4–2.8 (1.9±0.3)	2.4–2.9 (2.6±0.2)	0.8–2.4 (1.3±0.6)
SpL	0.9–4.4 (2.3±1.1)	1–3.5 (2.9±0.8)	-	1.8–3.5	1.4–3.4 (2.5±0.6)	1.4–3.4 (2.5±0.5)	2–4.2 (3.1±1.1)	0.5–2.2 (1.4±0.5)
SSD	8.3–12.6 (10±1.1)	7.8–14.8 (10.6±1.8)	-	10.1–10.6	6.5–12 (9.6±1.6)	7.3–9.8 (8.8±0.6)	11.8–14.6 (13.1±1.2)	3.9–11.7 (6.8±2.4)
SW	0.6–1.6 (1.1±0.3)	0.7–1.8 (1.2 -0.2)	-	0.7–1	0.8–1.4 (1.1±0.2)	0.8–1.5 (1.1±0.1)	1–1.4 (1.2±0.1)	0.3–2.7 (0.7±0.3)
TAL	23–40.1 (28.6±4.2)	18.9–38.7 (27.2±5.4)	20.8	24–31.1	17.8–27.9 (24.3±3.4)	18.8–27.4 (25±2)	34.2–38.8 (37.2±2)	9.9–36.2 (19±7.9)
TL	35.5–56.4 (42.9±5)	29.96–52.1 (41.5±7)	34.1	38.4–45.8	26.4–44.8 (38.1±5.4)	29.9–40 (37.4±2.2)	53.4–57 (55.1±1.8)	16.3–53 (28.8±11)
TMH	3.2–7.1 (4.5±1.2)	6.8–10 (8.3±0.9)	-	5.4–5.7	2.5–4.8 (3.9±0.8)	2.4–4.9 (3.3±0.6)	5.6–7.2 (6.1±0.7)	1.3–10.5 (6±2.3)
TMW	3.2–10.1 (4.4±2)	2 – 7.1 (3.8±1.3)	-	2–4.6	2.2–4.6 (3.3±0.7)	2–4.2 (2.6±0.5)	4.3–5.4 (4.6±0.5)	0.9–4.3 (1.9±1)
Utail	1.4–4.6 (2.7±0.7)	1.8–3.3 (2.4±0.5)	-	1.2–3.8	1.1–3.7 (2.9±0.7)	1.6–2.9 (2.3±0.3)	4–4.4 (4.3±0.2)	1–3.8 (2.2±0.8)

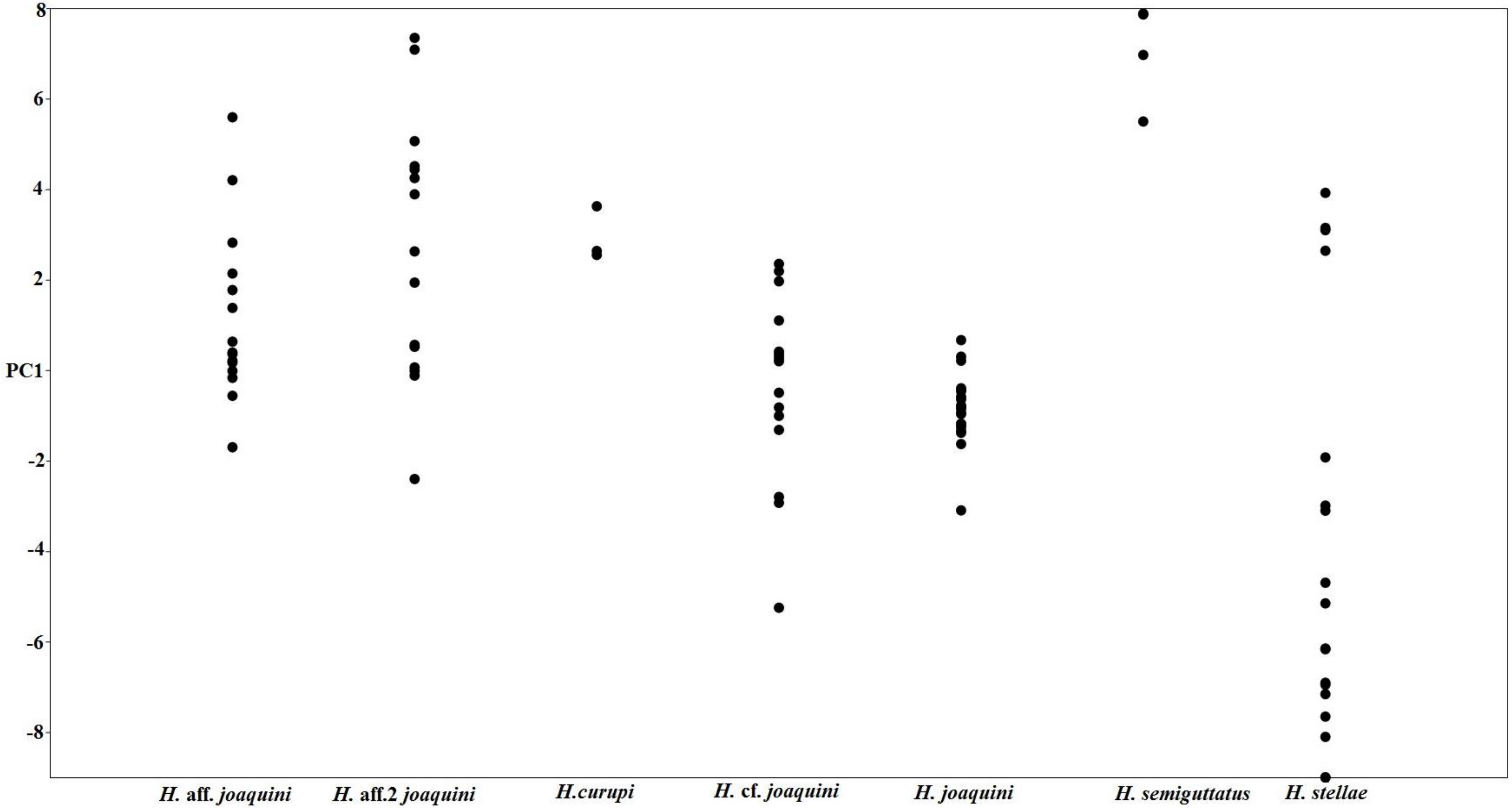

Figure 11. Variation of the first principal component (PC1) of 22 morphometric variables among tadpoles comparative units defined in this study. Each dot represent a tadpole specimen within each of the comparative units. The first principal component explains 62.9% of the total variance.

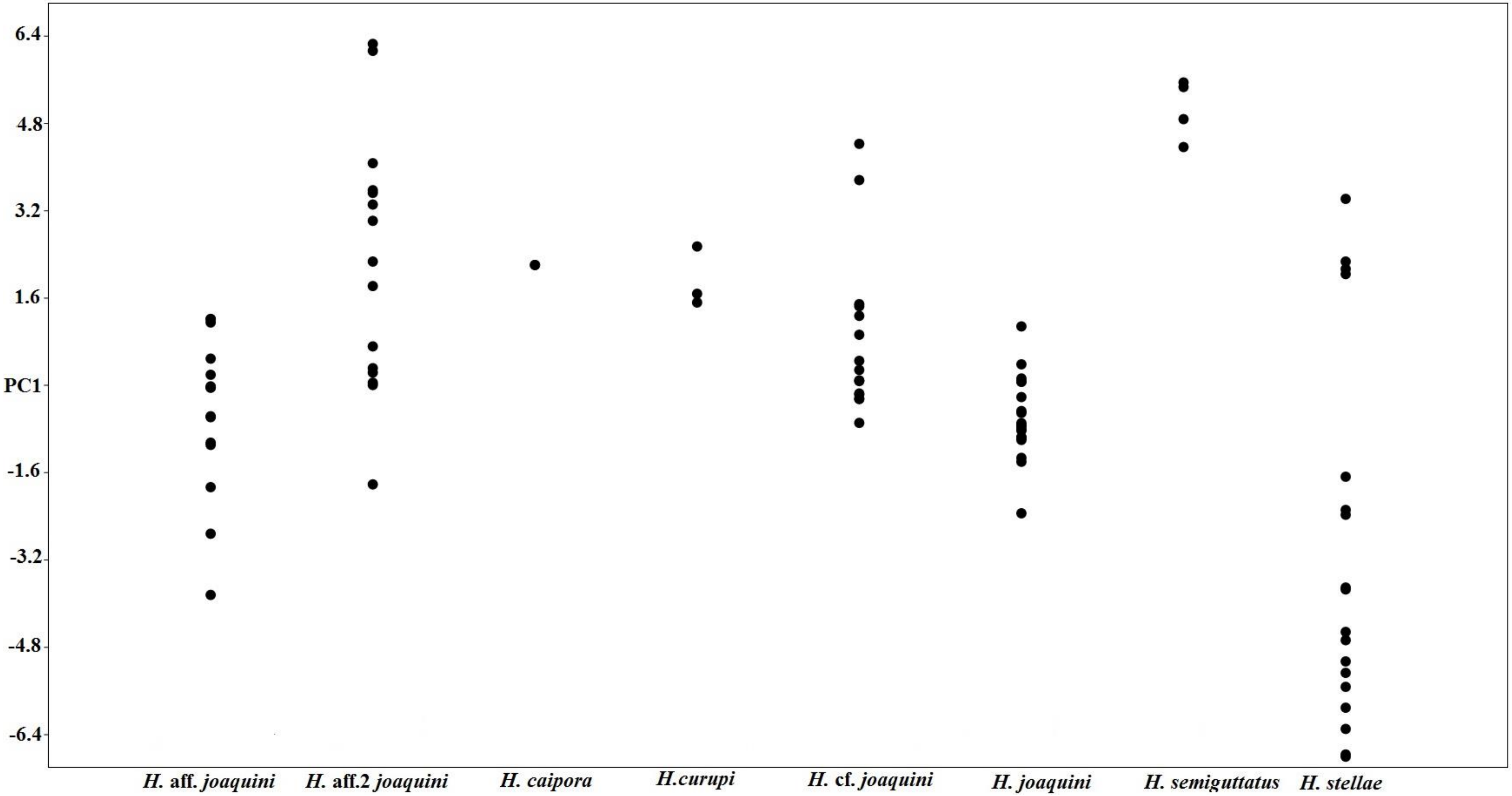

Figure 12. Variation of the first principal component (PC1) of 12 morphometric variables among tadpoles of the comparative units defined in this study. Each dot represent an adult male specimen within each of the comparative units. The first principal component explains 66.9% of the total variance. Data from *Hypsiboas caipora* taken from Antunes *et al*. (2008).

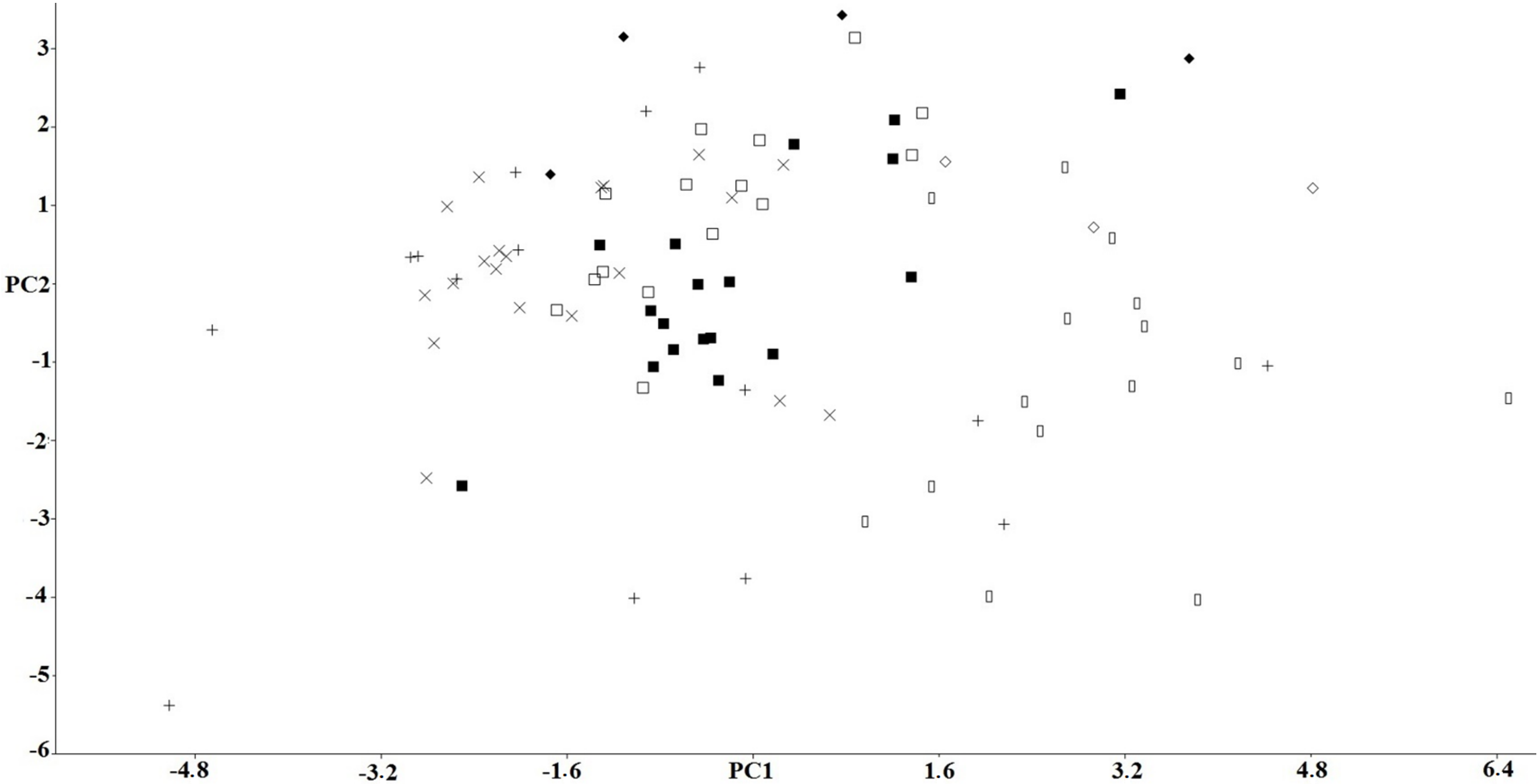

Figure 13. First two principal components (PC) of the residuals of a correlation with TL of tadpole body measurements of comparative units defined in this study. PC 1 and 2 represent 23.0% and 14.7% of total variance, respectively. Filled triangle *Hypsiboas* aff. *curupi*, cross *H*. aff. *joaquini*, rectangle *H.* aff. 2 *joaquini*, square *H*. cf. *joaquini*, lozenge *H. curupi*, x *H. joaquini*, filled lozenge *H. semiguttatus,* and filled square *H. stellae.*

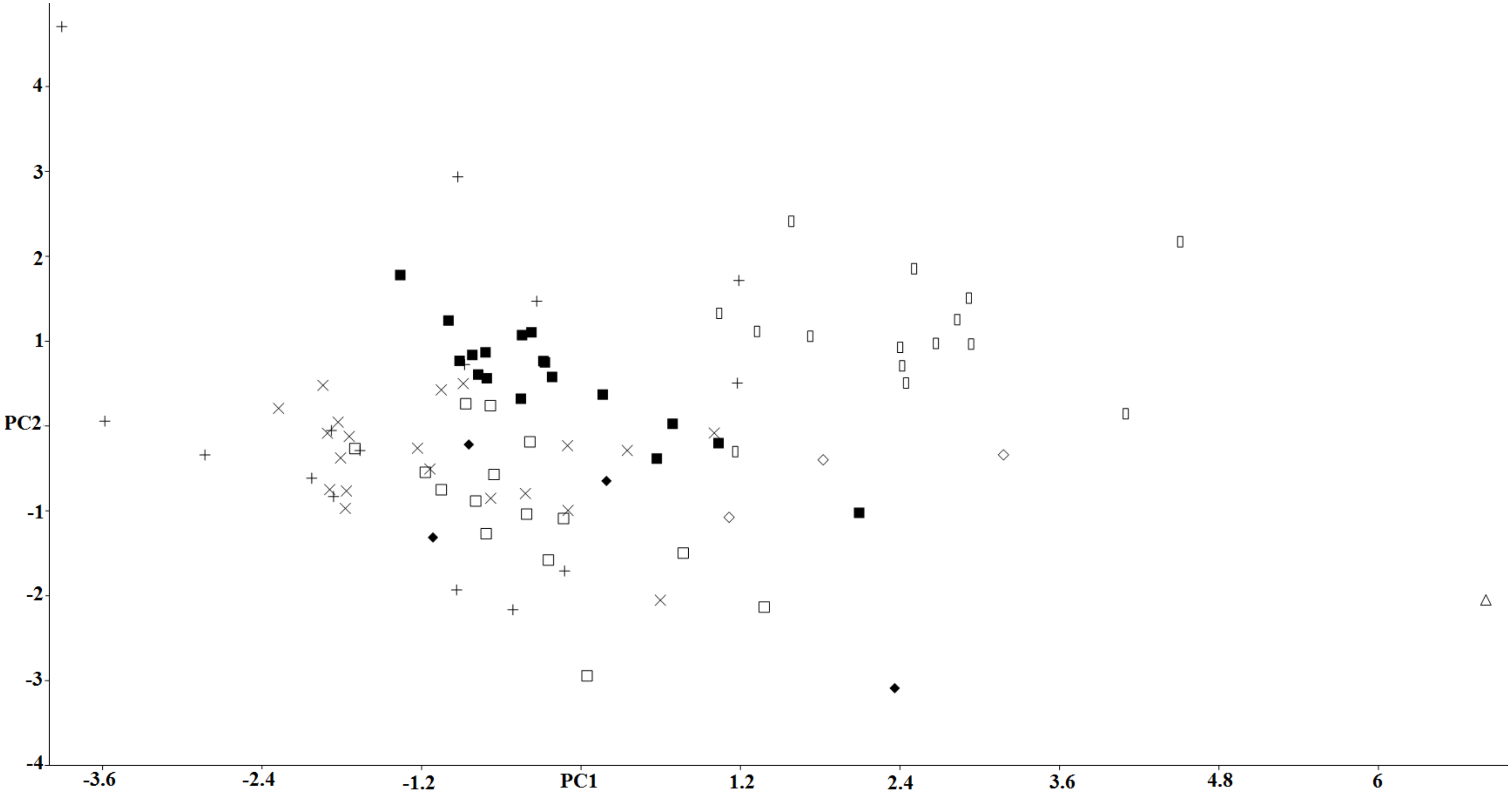

Figure 14. First two principal components (PC) of the residuals of a correlation with TL of tadpole body measurements of comparative units defined in this study. PC 1 and 2 represent 29.3% and 14.0% of total variance, respectively. Triangle *Hypsiboas caipora*, filled triangle *H.* aff. *curupi*, cross *H*. aff. *joaquini*, rectangle *H.* aff. 2 *joaquini*, square *H*. cf. *joaquini*, lozenge *H. curupi*, x *H. joaquini*, filled lozenge *H. semiguttatus*, and filled square *H. stellae.* Data from *Hypsiboas caipora* taken from Antunes *et al*. (2008).

A PCA on the residuals of regressions between the 21 morphometric variables of tadpoles and their TL recovered a PC-1 and PC-2 that explained 23.0% and 14.7% of the total morphometric variation among tadpoles respectively (Appendix 12). Residual variation in width measurements (HW, IOB and MW) had high loadings on PC-1, whereas PC-2 accounted for residual variation in measurements of TL. However, all tadpoles of all groups largely overlapped in size-free morphometric space (Fig. 10). When we included *H. caipora* (11 morphometric variables), PC-1 and PC-2 explained 29.3% and 14% of the total information respectively (Appendix 13). Residuals variation of IOD, MW, and MTH have higth values in PC-1 and IND in PC-2. *Hypsiboas caipora* is the only isolated taxa in morphometric space (Fig. 11).

Acoustic analysis

Because the sources of advertisement call recordings were heterogeneous (see Material and Methods), the number of recorded males per taxonomic unit and the number of calls analyzed for each male varied (2–34 males per comparative unit and 1–23 calls per recorded male). The diversity of recording sources also implied that many call samples lack information on SVL of the vocalizing male or air temperature at the time of recording, so we were not able to consider effects of these covariates in the results described below. Sample sizes, average values, and variation of acoustic parameters are presented in Table 5.

A PCA on the ten acoustic variables measured from advertisement calls recovered a PC-1 and PC-2 that accounted, respectively, for 56.0% and 17.2% of the total acoustic variation. Except for LFP, DF, and NP all the remaning the acoustics variables had high loadings on PC-1. The variables on PC-2 (Appendix 14) UFP, FBW, and DF. Calls of all taxa overlapped in acoustic space defined by PC-1 and PC-2, except calls of *Hypsiboas stellae*, which formed a well-defined cluster along PC-1 (Fig. 13). Calls of *H. stellae* are characterized by longer duration, with longer pulses more scattered along the call. They are also emitted at a lower dominant frequency than other taxa. Calls emitted by males of the remaining comparative units were spectrographically very similar and overlapped in relation to all temporal and spectral parameters estimated from calls.

Table 5. Variation, followed by mean±standard deviation, of studied acoustic variables. Sample size (N) refers to number of specimens and number of total calls analyzed. Data from *Hypsiboas caipora* taken from Antunes *et al*. (2008).

Taxa	***H.* aff. *joaquini*** (N=33/132)	***H.* aff.2 *joaquini*** (N=12/89)	***H. caipora*** (N=1/17)	***H. curupi*** (N=2/21)	***H.* cf. *joaquini*** (N=25/37)	***H. joaquini*** (N=12/53)	***H. semiguttatus*** (N= 3/7)	***H. stella*** (N=12/14)
AP (s)	0.002–0.05 (0.01±0.009)	0.007 –0.028 (0.013±0.005)	–	0.006	0.003–0.03 (0.009±0.005)	0.008–0.06 (0.025±0.021)	0.009–0.01 (0.013±0.02)	0.01–0.17 (0.22±0.09)
BAP (s)	0.006–0.2 (0.03±0.03)	0.021 –0.068 (0.044±0.012)	–	0.012–0.015	0.012–0.068 (0.03±0.02)	0.023–0.16 (0.056±0.04)	0.07–0.37 (0.105±0.01)	0.005–0.03 (0.026±0.1)
CD (s)	0.2–0.8 (0.4±0.1)	0.2–0.7 (0.5±0.1)	0.1–0.41 (0.24±0.08)	0.25–0.26	0.2–0.6 (0.4±0.1)	0.4–0.9 (0.5±0.1)	0.2–0.4 (0.3 ± 0.1)	0.9–1.8 (1.3 ± 0.3)
DF (Hz)	2170–2670.1 (2389.5±122.7)	1609.4 –2153.3 (1910±151.6)	2500–2930 (2679±108.5)	2222–2264.3	1173.7–2343.8 (2128.5±228.2)	1656.3–2584 (2110.4±268.2)	1500–2124.6 (1746 ± 268.6)	1205.9–1808.8 (1521.7 ± 177.4)
DPH (s)	0.005–0.020 (0.009±0.003)	0.006–0.021 (0.013±0.005)	– –	0.007 –0.008	0.004–0.037 (0.012±0.008)	0.003–0.014 (0.009±0.003)	0.004–0.005 (0.004 ± 0)	0.017–0.080 (0.035 ± 0.017)
FBW (Hz)	428.6–1684.7 (866.1±325.2)	517.3–1439 (1080.9±252.5)	– –	244.4 –375	605.9–1688.5 (1215.6±290.8)	638.2–1420.8 (1014±255.9)	973–1274.1 (1072.6 ± 138.5)	1046–1901 (1370 ± 266.2)
LFP (Hz)	1389.2–2262.9 (1875±254.7)	880.5–1625.6 (1327±230.6)	1330–1790 (1600±127.9)	2092.7–2101.4	823–1980.3 (1462.4±283.6)	803.1–2002.7 (1508±347.7)	717.7– 1556.9 (1167.8 ± 346.9)	760 – 1031 (915 ± 87.4)
NP (Unid.)	12–50 (38.6±8.1)	25–45 (35±5.9)	14–48 (31.8±9.6)	35–48	9–47 (21.7±10.8)	11–57 (37.8±14.1)	36–47 (41.5 ± 4.7)	7–14 (11.3 ± 2.9)
RTB (s)	0.1–0.5 (0.3±0.1)	0.1–0.6 (0.4±0.1)	–	0.2	0.2–0.5 (0.3±0.1)	0.3–0.6 (0.4±0.1)	0.1–0.3 (0.2 ± 0.1)	0.5–1.2 (0.9 ± 0.3)
UFP (Hz)	2408.9–3177.3 (2741.1±164.5)	2096.5–2926.1 (2407.9±235.5)	3170–3900 (3553±191.1)	2337.1 –2476.4	2379.3–3166 (2678±166.4)	2193.1–3095.7 (2522±259.7)	1991.9–2529.9 (2240.4 ± 221.6)	1996 – 2715 (2286 ± 236)

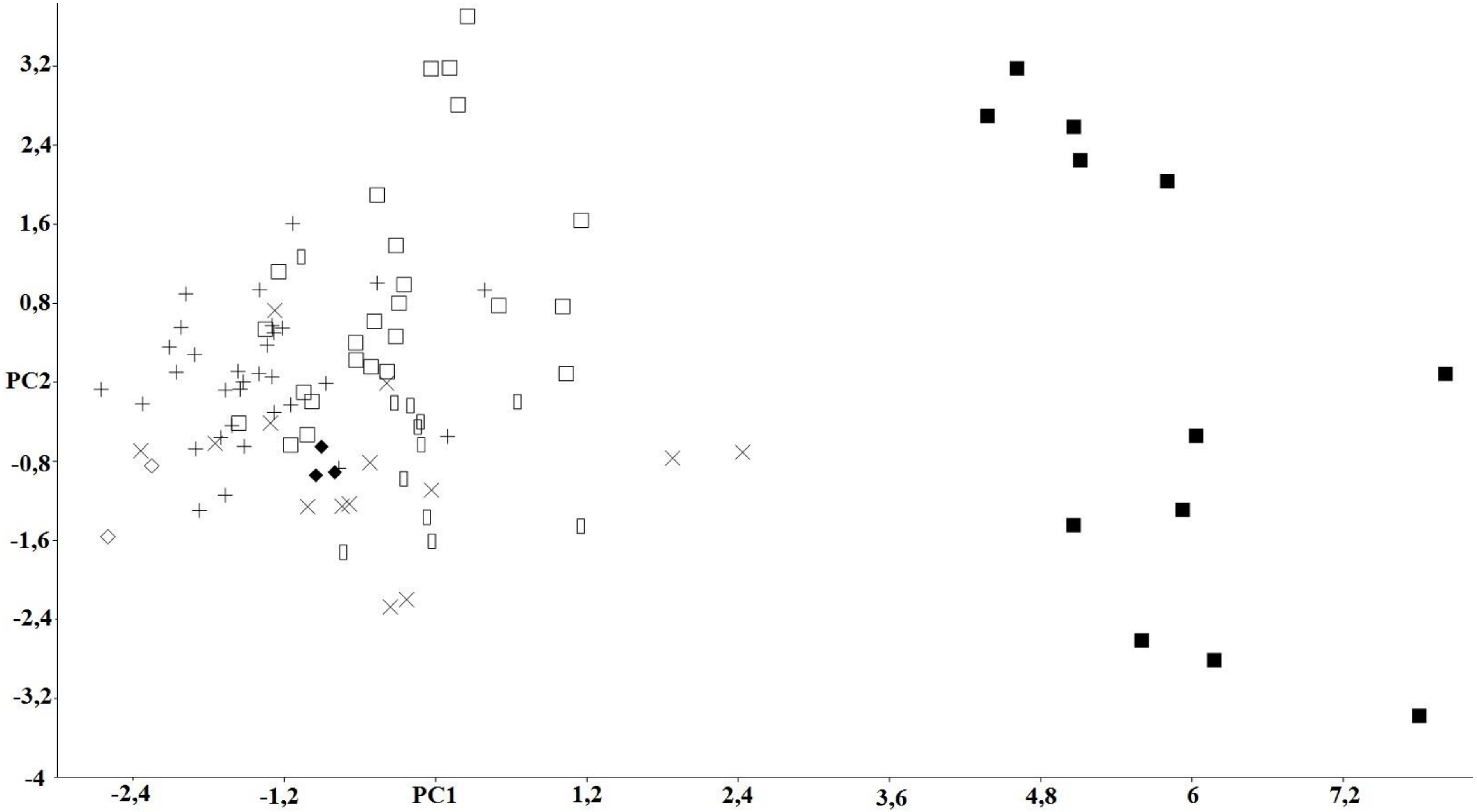

Figure 15. First two principal components (PC) of 10 bioacoustic variables of the advertisement call of comparative units defined in this study. PC 1 and 2 represent 56.0% and 17.2% of total variance, respectively. Filled triangle *Hypsiboas* aff. *curupi*, cross *H.* aff. *joaquini*, rectangle *H.* aff. 2 *joaquini*, square *H.* cf. *joaquini,* lozenge *H. curupi,* x *H. joaquini*, filled lozenge *H. semiguttatus,* and filled square *H. stellae.*

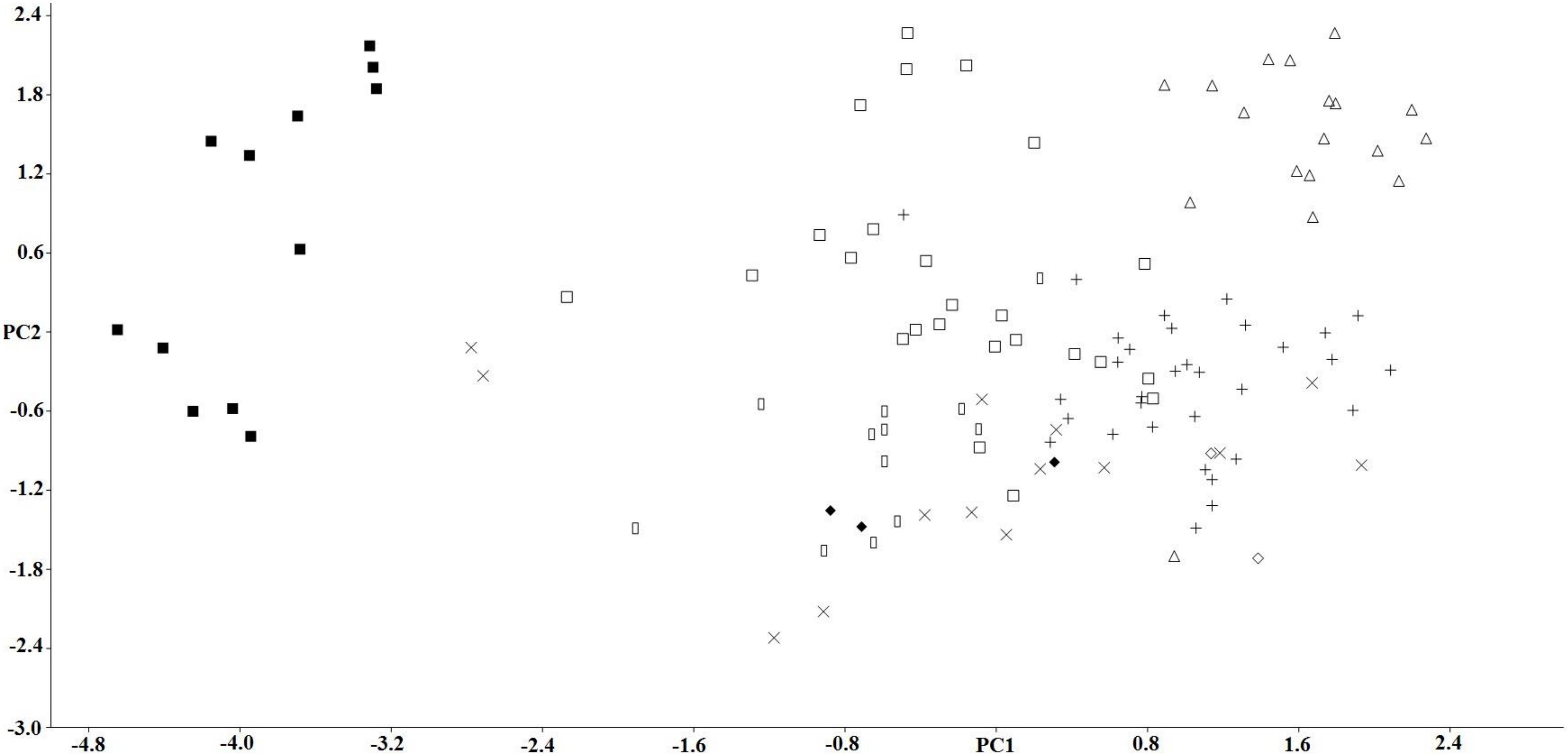

Figure 16. First two principal components (PC) of 10 bioacoustic variables of the advertisement call of comparative units defined in this study. PC 1 and 2 represent 56.0% and 17.2% of total variance, respectively. Triangle *Hypsiboas caipora*, cross *H.* aff. *joaquini*, rectangle *H.* aff. 2 *joaquini*, square *H.* cf. *joaquini*, lozenge *H. curupi*, x *H. joaquini*, filled lozenge *H. semiguttatus*, and filled square *H. stellae*. Data from *Hypsiboas caipora* taken from Antunes *et al*. (2008).

When we included *Hypsiboas caipora* (five acoustic variables available) we recovered a PC-1 and PC-2 that accounted, respectively, for 62.8% and 34.1% of the total acoustic variation (Appendix 15). Excepted for CD, all the others acoustics variables have high loadings on PC-1. Again, the only non-overlapping call was that of *H. stellae* (Fig. 14).

Phylogenetic analyses and genetic distances

The aligned and concatenated dataset had 1106 characters. Tree searches recovered 1853 most parsimonious trees of 604 lenght. The strict consensus tree recovered a clade that only includes all the comparative units studied [Jacknife (JK) = 87]. This monophyletic group, which we call the *Hypsiboas semiguttatus* clade, is sister of the remaining sampled species of the *H. pulchellus* group (JK = 30). Within the *H. semiguttatus* clade, *H. stellae* is recovered as the sister taxon (JK = 95) of a clade that includes the other comparative units (JK=44). This latter clade includes a basal politomy formed by *H. caipora* (represented by a single sample from GenBank), a monophyletic *H. semiguttatus* (JK = 93), a monophyletic *H. curupi* (JK = 96), and a poorly resolved clade (JK = 94) that includes *H.* aff. *curupi*, *H. joaquini*, *H.* aff. *joaquini*, *H.* aff. 2 *joaquini*, and *H.* cf. *joaquini*. These last five comparative units are rampantly non-monophyletic, the few resolved clades show no geographic structure, and examination of branch lengths revealed little or no differentiation among sequences of terminals.

Genetic distances among the compared taxonomic units are summarized in Table 6. As expected from the phylogenetic analysis, the taxonomic units *Hypsiboas* aff. *curupi*, *H. joaquini*, *H.* aff. *joaquini*, *H.* aff.2 *joaquini*, and *H.* cf. *joaquini* exhibit very low genetic distances (0.0–1.5%). The genetic distances between members of the afore mentioned species and the sister taxa *H. curupi* is more marked (2.1–3.0%).

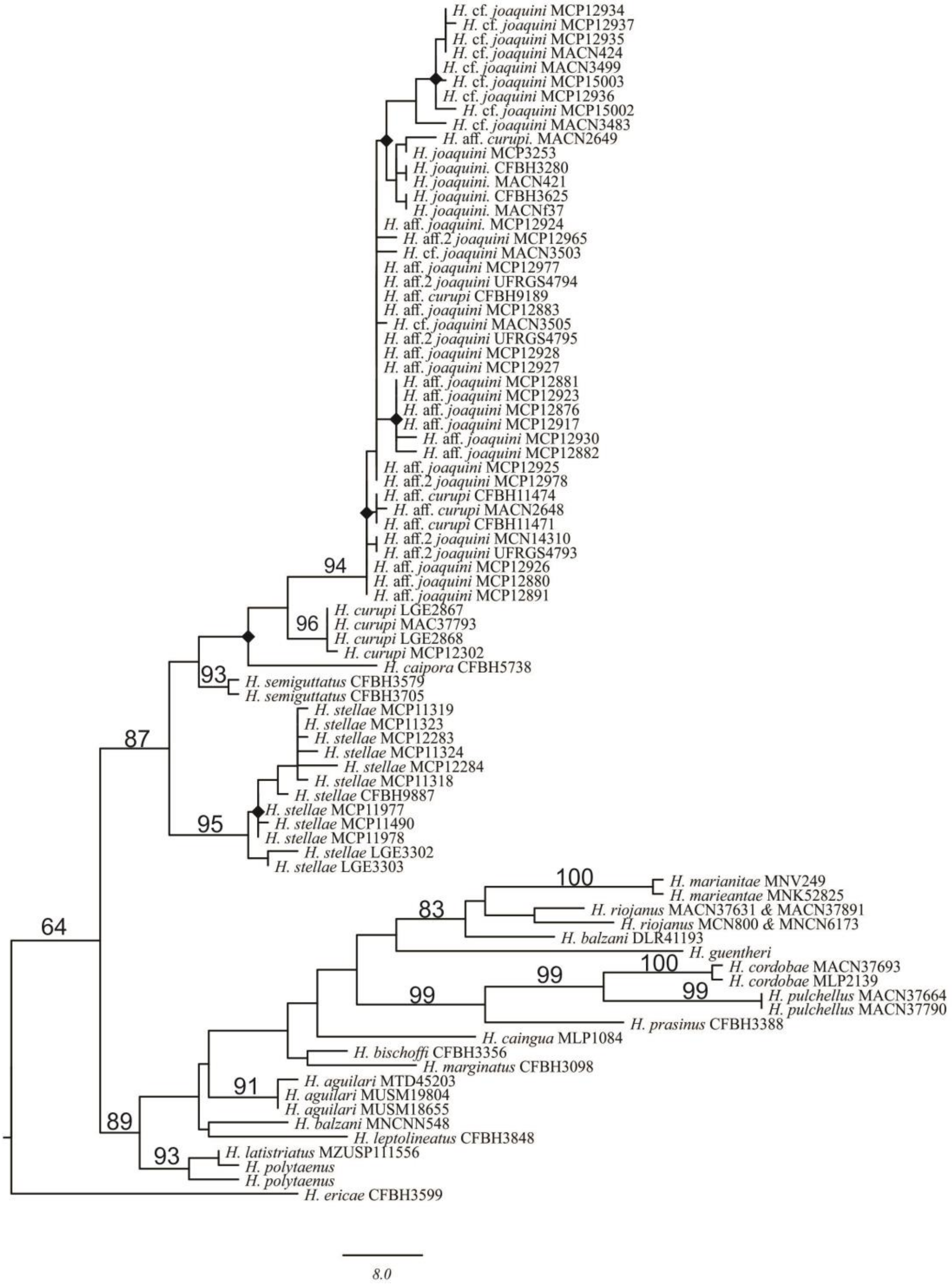

Figure 17. Phylogenetic relationships of *Hypsiboas pulchellus* species group inferred from parsimony analysis of a similarity alignment of DNA sequences of mitochondrial genes 12S and 16S. This topology reflects one of the 1853 most parsimonious trees (tree costs = 604 steps). Numbers on branches represent jackknife proportions and black dots on nodes indicate collapsed clades in the strict consensus. The root (*Hypsiboas faber*) was cropped for ease of representation.

Table 6. Percentage of genetic distances of comparative units analyzed in this study and calculated based on a similarity alignment of DNA sequences of the gene 16S. Diagonal intra-unit distances, while inter-unit K2P and uncorrected pairwise genetic distances are above and below the diagonal respectively. Sample size for each comparative unit is in parentheses.

	H. aff. *joaquini* (n=16)	*H.* aff.2 *joaquini* (n=6)	*H.* cf. *joaquini* (n=11)	*H.* aff. *curupi* (n=5)	*H. caipora* (n=1)	*H. curupi* (n=4)	*H. joaquini* (n=5)	*H. semiguttatus* (n=2)	*H. stellae* (n=12)
H. aff. *joaquini*	0.0–06	0.0–0.5	0.1–1.5	0.2–0.6	3.9–4.9	2.3–2.7	0.2–0.6	4.0	3.9–5.1
H. aff.2 *joaquini*	0.0–0.2	0.0–0.4	0.0–1	0.0–0.4	3.9–4.1	2.3–2.7	0.2–0.4	4.3	3.9–4.7
H. cf. *joaquini*	0.0–0.2	0.0–0.01	0.0–1.5	0.0–0.9	3.5–4.1	2.1–3.1	0.1–1.1	3.7–4.7	3.3–5.1
H. aff. *curupi*	0.0–0.6	0.0–0.4	0.0–1	0.0–0.2	4.0	2.3–2.5	0.2–0.4	4.0	3.9–4.9
H. caipora	3.7–4.1	3.7–3.9	3.5–4.3	3.7	–	3.7–3.9	4–4	2.8	3.8–4.5
H. curupi	2.3–2.8	2.3–2.6	2.1–3.0	2.3–2.6	3.5–3.7	0.0–0.2	2.1–2.3	3.9–4.1	3.9–4.9
H. joaquini	0.2–0.6	0.1–0.4	0.1–1	0.1–0.4	4.0	2.1–2.3	0.0	4.3	3.9–4.7
H. semiguttatus	3.9–4.1	3.9–4.1	3.0–4.0	4.0	2.8	4.0	4.0	–	2.9–4.2
H. stellae	3.7–4.3	3.7–4.7	3.2–4.7	3.7–4.7	3.5–4.3	3.5–4.7	3.7–4.5	2.8–4.1	0.0–1.7

Discussion

Intraspecific phenotypic polymorphisism or genetically cryptic species

Our phylogenetic analyses of DNA sequences revealed a surprising pattern. All individuals assigned to populations representing potentially new species (*i.e.*, *H.* aff. *curupi*, *H.* aff. *joaquini*, *H.* aff. 2 *joaquini*, *H.* cf. *joaquini*) cluster together with *H. joaquini* sensu stricto (s. str.). Furthermore, this clade show very short branches, zero in some cases causing polytomies, and no coherent geographic structure of haplotypes. On the other hand, all currently recognized species of the *H. semiguttatus* clade are monophyletic with jackknife values > 90 (note that we only included one specimen of *H. caipora*). The topology and branch lengths observed among *H. joaquini* s. str. and associated samples indicate that they form a single species, with a wider distribution than previously thought. However, one should be cautious because speciation can occur in the face of gene flow, given sufficient strong selection against hybrids (Nosil 2008), and incomplete lineage sorting can blur the historical relationships among species, inasmuch as the history of genes may not reflect the history of species (Page & Charleston 1998). In these situations, detecting species limits using DNA sequences can prove difficult, one could say that they are “genetically cryptic” species. For example, Jungfer *et al*. (2013) discovered that *Osteocephalus taurinus* is parapyletic with respect to *O. oophagus*, although they show marked phenotypic differences (paired lateral sacs and pond breeding and single vocal sac and breeding in bromeliads, respectively). When we compared the phenotypic data of adult males, tadpoles, and advertisement calls of *Hypsiboas joaquini* s. str. with those of *H*. aff. *curupi*, *H*. aff. *joaquini*, *H*. aff. 2 *joaquini*, *H*. cf. *joaquini* and interesting pattern emerges.

Regarding the morphology of adults, only some coloration characteristics seem to be variable among populations but fixed within units of comparison. For example, the presence of spots on the cloacal region diagnoses *Hypsiboas joaquini* s. str. and *H*. aff. *joaquini* from *H*. aff. *curupi*, *H*. aff. *joaquini*, *H*. aff. 2 *joaquini*, *H*. cf. *joaquini*. However, coloration is polymorphic among these frogs and diagnoses would depend on the combination of multiple color related characters.

Our morphometric analyses of adult males indicate that all units of comparison overlap on morphometric space except for specimens of *H. caipora*. We found similar results in our morphometric analyses of tadpoles, no differentiation across morphospace despite small sample size of some taxonomic units (*i.e*., *H. caipora*, *H. curupi*, and *H. semiguttatus*). The results of our analyses of bioacoustic data also reveal a general pattern of overlap, the only exception is the advertisement call of *H. stellae*, which is well differentiated in the acoustic space. On the other hand, the external morphological characteristics of the oral disc of tadpoles is intriguing. The only tadpoles that lack a gap on marginal papillae in the tip of mouth are those of *H. curupi* and *H*. aff. 2 *joaquini*. Furthermore, the tooth row formula of *H. caipora, H. joaquini* s. str. *H*. aff. *joaquini*, *H*. cf. *joaquini* is 2(2)/3–4(1), of *H. curupi* and *H. stellae* is 3(1,3)/5(1), and of *H*. aff. 2 *joaquini* is 4(4)/6(1). Thus, the tadpole of *H*. aff. 2 *joaquini* is unique among representatives of the *H. semiguttatus* clade. The obvious difference in the morphology of the oral disc of *H*. aff. 2 *joaquini* deserves explanation. Below we discuss three possible scenarios.

A cursory comparison of the habitat characteristics in which the two types of tadpoles are found reveals informative differences. Specimens of *Hypsiboas* aff. 2

joaquini inhabit the headwaters of Rio dos Sinos, RS, Brazil. It is fast flowing river, with a substrate of large rocks, and inmerse in dense primary Atlantic forest. Tadpoles were found in pools at the bottom of high cascades (> 10 m). This envioroment contrasts with our other collecting sites, which are slow flowing rivulets of the table mountains of southern Brazil, the surrounding vegetation is composed of grasslands and bushes, and there are no waterfalls. One can think that this marked differences in habitat might imply different biotic and abiotic selective preasures leading to the observed divergence. Local adaption to the unique conditions of the headwaters of Rio dos Sinos can therefore explain the acute differences. Local adaption can be seen as an initial stage of speciation, inasmuch as it requires a degree of evolutionary indepence either by reduced geneflow due to isolation or by strong selective pressures reducing the fitness and frequency of hybrids. However, phenotypic divergence does not necessarely imply independent evolutionary lineages. Phenotypic plasticity is a well-known phenomenom affecting the development of tadpoles (Relyea & Werner 2000). Several examples exist on the literature providing empirical evidence of plasticity of tadpoles when developing under different environmental conditions such as presence/absence of predators, seasonality, gradient distribution, the architecture of aquatic environment, light-induced and changes in physical and chemical environmental conditions (e.g., Thurnheer & Reyer 2001; Bridge 2002; Eterovick *et al*. 2010; Calich *et al*. 2012; Beaty *et al*. 2015). Although it would involve a radical change in oral morphology, we cannot discard that the observed differences in tadpoles of *H*. aff. 2 *joaquini* are due to phenotypic plasticity. The possible effect of phenotypic plasticity can be empirically tested. Egg clutches from both types of environments can be raised inside cages allowing water and nutrient flow in the different habitats, with appropriate controls, and tadpoles can be examined for differences. Nonetheless, with the data at hand, we are inclined to favor a scenario of local adaption. Despite our efforts, our tadpole sample size is moderate (N = 15–20) for *Hypsiboas joaquini*, *H*. aff. *joaquini*, *H*. aff. 2 *joaquini*, *H*. cf. *joaquini* (we did not examined tadpoles of *H*. aff. *curupi*). We do not think that problems with sample size suffies to explain the breadth of the observed differences inasmuch as among the four comparative units, the only variation (besides that of *H*. aff. 2 *joaquini*) among qualitative characters is found within *H. joaquini* s. str., with tadpoles having three or four posterior tooth rows. Furthermore, we sampled tadpoles of *H*. aff. 2 *joaquini* in two different occasions (2015 and 2016) so we can most likely discard that we have studied specimens from a single clutch.

Taxonomic implications

Our interpretation of the variation observed in DNA sequences, adult and tadpole morphology, and advertisement calls of *Hypsiboas* aff. *curupi*, *H*. aff. *joaquini*, *H*. aff. 2 *joaquini*, and *H*. cf. *joaquini* is that all these units of comparison belong to the same species, *H. joaquini*. This has important taxonomic implications. *Hypsiboas joaquini* was redescribed and characterized so it did only reflect variation at the type locality (Garcia *et al*. 2003). This was a necessary step at the time, but that required further research because several populations do not match the diagnosis of topotypic *H. joaquini* (see Introduction). According to our study, *H. joaquini* sensu this work has a large geographical distribution (Fig. 1) reaching the western portion of SC and the southern corner of the Serra do Mar (the most austral portion of the Atlantic forest). The phenotypic variation of *H. joaquini* is also much larger than initially thought and its diagnosis needs to be updated. Table 7, which summarizes the most relevant diagnostic characters among species of the *H. semiguttatus* clade, indicate that *H. joaquini* sensu this work cannot be diagnosed solely on the basis of the studied phenotypic characters from the other species of the clade.

Table 7. Summary of diagnostic characters among species of the *Hypsiboas semiguttatus* clade according to the taxonomy recommended in this study.

	H. caipora	***H. curupi***	***H. joaquini***	***H. semiguttatus***	***H. stellae***
Tadpoles, tooth row formula	2(2)/4(1)	3(1,3)/5(1)	2(2)/3-4(1); 4(4)/6(1)	2(2)/4(1)	3(1,3)/5(1)
Tadpoles, Gap on marginal papillae in the tip of mouth	Present	Absent	Absent/present	Present	Present
Spots on hidden surface of thighs	Absent	Present	Absent/Present	Absent	Present
Three irregular longitudinal bars on dorsal	Absent/present	Absent/present	Absent/present	Present	Absent
Size of lateral spots	Small	Medium/small	Large/medium/small	Large	Small
White band on thighs	-	Present	Abset/present	Present	Absent

White band tibia	-	Present	Abset/present	Present	Absent

Morphology of the oral disc allows differentiation from *H. curupi* and *H. stellae* but our knowledge on the variation of this structure seems to be too precarious. Advertisement calls only seems diagnostic for *H. stellae*. As things stand, people interested in identifying specimens (either live or preserved) of these species will need to resort to DNA sequences, inasmuch as variation within *H. joaquini* seems to encompass that exhibited by the other species.

Phylogenetic relationships

Faivovich *et al*. (2004, 2005) found that *Hypsiboas curupi* (as *H*. sp. 1 and *H*. sp. 7, respectively), *H. joaquini*, and *H. semiguttatus* belong to the *H. pulchellus* species group and are more closely related among each other than with any other species of *Hypsiboas*. Antunes *et al*. (2008) added *H. caipora* to this clade of southeastern species. Wiens *et al*. (2010) reached the same results regarding the relationships of these species applying a maximum likelihood analysis of a similarity alignment of an augmented dataset of Faivocich *et al*. (2005) and Wiens *et al*. (2005). However, *H. caipora* was not included in Wiens *et al*. (2010) analysis and the relationship of the clade containing *H. curupi*, *H. joaquini*, and *H. semiguttatus* within the *H. pulchellus* species group was different from those reported by Faivovich *et al*. (2004, 2005) and Antunes *et al*. (2008). Pyron & Wiens (2011) provided additional evidence of the monophyly of this group, although they did not include *H. caipora* and *H. curupi*. Duellman *et al*. (2016), in their study of legacy DNA sequences from GenBank, reported an unespected result. Although they included data of *H. caipora*, *H. curupi*, *H. joaquini*, and *H. semiguttatus*, they found *H. curupi* more closely related to other species of the *H. pulchellus* group (bootstrap support = 83) than to *H. caipora*, *H. joaquini*, and *H. semiguttatus*. The terminal of *H. curupi* included in Duellman *et al.* (2016: Appendix 1) is only represented by the marker CXCR4 (GenBank accession code KF751483), although there are also DNA sequences available in GenBank of the genes 12S, 16S, cytochrome b, and seventh in absentia from Faivovich *et al.* (2004, 2005). Thus, we suspect that the surprising phylogenetic position of *H. curupi* in Duellman *et al*. (2016) is the result of just sampling a single protein coding nuclear gene CXCR4, with poor phylogenetic information (see Padial *et al*. 2014 for an empirical example of a well-resolved and supported relationship of a terminal despite absence of unambiguous synapomorphies).

Our study is the first to include DNA sequences of *Hypsiboas stellae* and of several problematic populations of *H. joaquini*. We recovered *H. stellae* as the sister taxon of a clade including *H. caipora*, *H. curupi*, *H. joaquini*, and *H. semiguttatus*. Our phylogenetic result is compatible with Faivovich *et al*. (2004, 2005) and Antunes *et al*. (2008), the main difference is a polytomy in the strict consensus involving *H. caipora* and *H. semiguttatus*. This difference is probably due to our smaller character sampling. We use the name *H. semiguttatus* clade to refer to this natural group of species from southeast South America (northeastern Argentina, southeastern Brazil, and eastern Paraguay).

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Appendixes

Appendix **1.** Currently recognized species of the *Hypsiboas pulchellus* group.

Original name	**Taxonomic authority**	**Type locality**	**Current name**
Hypsiboas aguilari	Lehr, Faivovich, & Jungfer, 2010	San Alberto, Peru	*Hypsiboas aguilari*
Hyla albonigra	Nieden, 1923	Buenos Ayres, Argentina	*Hypsiboas alboniger*
Hyla balzani	Boulenger, 1898	Prov. Yungas, Bolivia	*Hypsiboas balzani*
Hypsiboas bandeirantes	Caramaschi & Cruz, 2013	Itapecerica da Serra, São Paulo	*Hypsiboas bandeirantes*
Hyla beckeri	Caramaschi & Cruz, 2004	Poços de Caldas, Minas Gerais, Brazil.	*Hypsiboas beckeri*
Hyla bischoffi	Boulenger, 1887	Taquara, Brazil.	*Hypsiboas bischoffi*
Hypsiboas botumirim	Caramaschi, Cruz & Nascimento, 2009	Botumirim, Minas Gerais, Brazil	*Hypsiboas botumirim*
Hyla buriti	Caramaschi & Cruz, 1999	Buritis, Minas Gerais, Brazil	*Hypsiboas buriti*
Hyla caingua	Carrizo, 1991	Iguazú, Misiones, Argentina	*Hypsiboas caingua*
Hypsiboas caipora	Antunes, Faivovich & Haddad, 2008	Pilar do Sul, São Paulo, Brazil	*Hypsiboas caipora*
Hyla callipleura	Boulenger, 1902	San Ernesto, Bolivia	*Hypsiboas callipleura*
Hyla polytaenia cipoensis	Lutz, 1968	Jaboticatubas, Minas Gerais, Brazil	*Hypsiboas cipoensis*
Hyla pulchella cordobae	Barrio, 1965	Santa Rosa de Calamuchita, Córdoba, Argentina	*Hypsiboas cordobae*
Hypsiboas curupi	Garcia Faivovich & Haddad, 2007	Departamento Guarany, Misiones , Argentina	*Hypsiboas curupi*
Hyla cymbalum	Bokermann, 1963	Campo Grande, São Paulo, Brasil	*Hypsiboas cymbalum*
Hyla ericae	Caramaschi & Cruz, 2000	Alto Paraíso de Goiás, Goiás, Brazil	*Hypsiboas ericae*
Hyla freicanecae	Carnaval & Peixoto, 2004	Jaqueira, Pernambuco, Brazil	*Hypsiboas freicanecae*
Hyla polytaenia goiana	Lutz, 1968	Jatobasinho, Goiás	*Hypsiboas goianus*

Original name	**Taxonomic authority**	**Type locality**	**Current name**
Hyla guentheri	Boulenger, 1886	Terra de Areia, Rio Grande do Sul, Brazil	*Hypsiboas guentheri*
Hypsiboas gladiator	Köhler, Koscinski, Padial, Chaparro, Handford, Lougheed & De la Riva, 2010	San Miguel, Departamento Cusco, Peru	*Hypsiboas gladiator*
Hypsiboas jaguariaivensis	Caramaschi, Cruz & Segalla, 2010	Jaguariaíva, Paraná, Brazil	*Hypsiboas jaguariaivensis*
Hyla raddiana joaquini	Lutz, 1968	São Joaquim, Santa Catarina, Brazil	*Hypsiboas joaquini*
Hyla latistriata	Caramaschi & Cruz 2004	Itamonte, Minas Gerais, Brazil	*Hypsiboas latistriatus*
Hyla leptolineata	Braun & Braun, 1977	Cambará do Sul, Rio Grande do Sul, Brasil	*Hypsiboas leptolineatus*
Hyla marginata	Boulenger, 1887	Taquara, Rio Grande do Sul, Brasil	*Hypsiboas marginatus*
Hyla marianitae	Carrizo, 1992	Salta, Argentina	*Hypsiboas marianitae*
Hyla melanopleura	Boulenger, 1912	Huancabamba, Peru	*Hypsiboas melanopleura*
Hyla palaestes	Duellman, De la Riva & Wild, 1997	La Mar, Peru	*Hypsiboas palaestes*
Hyla phaeopleura	Caramaschi & Cruz, 2000	Alto Paraíso, Goiás	*Hypsiboas phaeopleura*
Hypsiboas poaju	Garcia, Peixoto & Haddad, 2008	Rancho Queimado, Santa Catarina, Brazil	*Hypsiboas poaju*
Hyla polytaenia	Cope, 1870	Brazil.	*Hypsiboas polytaenius*
Hyla prasinus	Burmeister, 1856	Rio de Janeiro Brazil.	*Hypsiboas prasinus*
Hyla pulchella pulchella	Duméril & Bibron, 1841	Montévidéo, Uruguay	*Hypsiboas pulchellus*
Hyla riojana	Koslowsky, 1895	Rioja, Argentina	*Hypsiboas riojanus*
Hyla secedens	Lutz, 1963	Barro Branco, Rio de Janeiro, Brazil	*Hypsiboas secedens*
Hyla semiguttata	Lutz, 1925	São Bento do Sul, Santa Catarina, Brazil	*Hypsiboas semiguttatus*
Hypsiboas stellae	Kwet, 2008	Sinimbú, Rio Grande do Sul, Brazil	*Hypsiboas stellae*
Hyla stenocephala	Caramaschi & Cruz, 1999	Poços de Caldas, MG, Brazil	*Hypsiboas stenocephalus*

Appendix 2. Sources of advertisement calls analyzed in this study. Recordings obtained from our fieldwork are in bold. Data from *Hypsiboas caipora* were taken from Antunes *et al*. (2008).

Taxon	**Locality**	**Voucher**	**Recording Code/Publication**	**Sample size**	**Hour**	**Temperature**
Hypsiboas aff. *joaquini*	São Francisco De Paula/RS	No	7 a 13	5/43	No	No
		No	8b02	5/34	04:30	10.5
		No	9b14	3/15	23:15	21
		MCP 12891	**12891_12917**	1/1	22:00	10
		No	**1130**	1/1	23:00	20
		No	**1128**	2/4	23:00	20
		No	**1126**	3/4	23:00	20
		No	**1122**	2/2	23:00	20
		No	**1123**	2/2	23:00	20
		No	**1129**	1/1	23:00	20
		No	**1124**	3/16	23:00	20
		No	**1125**	3/17	23:00	20
Hypsiboas cf. *joaquini*	São José dos Ausentes/RS	No	**SJA aff1**	1/1	21:00	12
		MCP 12937	**12937**	1/1	21:00	12
		MCP 12934	**12934**	1/1	21:00	12
		No	**SJA aff3**	1/1	21:00	12
		No	**119**	1/1	22:00	12
		No	**116**	2/2	22:00	12
	Cambará do Sul/RS	No	Voucher	3/4	No	No
		UFMG 10008	100008	2/12	21:00	15
		No	**1134**	1/1	22:00	13
		No	**1135**	1/1	22:00	13
		No	**1146**	1/1	22:00	13
		No	**1147**	1/2	22:00	13

Taxon	**Locality**	**Voucher**	**Recording Code/Publication**	**Sample size**	**Hour**	**Temperature**
		No	**1148**	4/8	22:00	13
		No	**1149**	1/1	22:00	13
		No	**1150**	3/3	22:00	13
		No	**1157**	1/1	09:00	22
Hypsiboas joaquni	Urubici/SC	No	21b1 Axel	1/1	23:00	18.5
		No	21 a 16	1/13	No	No
		No	FNRJ	2/4	20:45	14.5
		No	5b36	4/22	No	No
		No	9B07	1/3	23:40	19.7
		No	9b08	3/10	No	No
Hypsiboas caipora	Pilar do Sul/SP	No	Ago03 (nota1)	No	No	No
		No	Ago03(nota2)	No	No	No
		No	1adez03	No	No	No
		No	1bdez03	No	No	No
		No	1cdez03	No	No	No
		No	2bdez03	No	No	No
		No	2cdez03	No	No	No
		No	out04	No	No	No
		No	16out04	No	No	No
		No	10/nov/04	No	No	No
		No	11nov2004canto2	No	No	No
		No	12nov04canto1	No	No	No
		No	12/nov/04	No	No	No
		No	1/11/2004canto1	No	No	No
		No	1nov04canto2	No	No	No
		No	1nov04canto3	No	No	No
		No	2nov04canto1	No	No	No
		No	2nov04canto2	No	No	No
Hypsiboas semiutattus	Ponta Grossa/PR	No	Semigutattus Diego	2/4	No	No
	Serra do Mar/PR	No	Semiguttatus Axel	1/3	23:00	18
Hypsiboas curupi	San Pedro/Misiones/ Argentina	No	Curupi Diego	2/21	22:30	21
Hypsiboas stellae	Sinimbú/RS	No	pard2	3/4	No	No
		No	pard17 a 12	2/2	No	No
		No	12 a 13	2/3	No	No
		No	16b2	2/5	No	No
Hypsiboas aff2. joaquini	Caraá/RS	No	Nascente	1/1	16:00	No

Taxon	Locality	Voucher	Recording Code/Publication	Sample size	Hour	Temperature
		No	**1186**	1/1	15:00	28
		No	**1191**	1/1	15:00	28
		No	**1192**	1/1	15:00	28
		No	**1193**	1/1	15:00	28
		No	**1194**	1/1	15:00	28
		No	**1195**	6/81	15:00	28

Appendix 3. Specimens studied in this work. Specimens collected during our field expeditions are in bold face.

Taxon	Locality	Voucher	Sex/Stage
Hypsiboas curupi	Cuartel Rio Victoria-Guarany/Misiones/Argentina	MNRJ 59355**	Adult/male
Hypsiboas stellae	25 de Mayo/ Misiones/Arg	LGE 3303	Adult/male
Hypsiboas stellae	25 de Mayo/ Misiones/Arg	LGE 3305	Female
Hypsiboas stellae	25 de Mayo/ Misiones/Arg	LGE 3305	Female
Hypsiboas cf. stellae	Agudo/RS	ZUFSM 2373	Adult/male
Hypsiboas bischoffi	Anita Garibaldi/SC	MCP 8992	Adult/male
Hypsiboas bischoffi	Barracão/RS	MCP 714	Adult/male
Hypsiboas bischoffi	Barracão/RS	MCP 715	Adult/male
Hypsiboas curupi	Bom Progresso/RS	MCP 12299	Adult/male
Hypsiboas curupi	Bom Progresso/RS	MCP 12300	Adult/male
Hypsiboas curupi	Bom Progresso/RS	MCP 12301	Adult/male
Hypsiboas curupi	Bom Progresso/RS	MCP 12302	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP 2454	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP 481	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP 2457	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB 2927	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB 2928	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB 2931	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB 2932	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB 2937	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	CFBH 3052	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	CFBH 3082	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	CFBH 3135	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	CFBH 3138	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP 3227	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP 3292	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP 3295	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP 3296	Adult/male

Taxon	Locality	Voucher		Sex/Stage
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	3431	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB	9688	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB	9690	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB	9693	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB	9745	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB	9746	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB	11304	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	12336	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	12338	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	12339	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	12340	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	13337	Adult/male
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	UFMG-Amp	1251b	Tadpoles
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	UFMG-Amp	923b	Tadpoles
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	481	Female
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	3294	Female
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCN/FZB	9692	Female
Hypsiboas cf. *joaquini*	Cambará do Sul/RS	MCP	9723	Female
Hypsiboas bischoffi	Campo Belo do Sul/SC	MCP	10465	Adult/male
Hypsiboas bischoffi	Campo Belo do Sul/SC	MCP	10471	Adult/male
Hypsiboas aff. *curupi*	Campos Novos/SC	CFBH	23856	Adult/male
Hypsiboas aff. *curupi*	Campos Novos/SC	CFBH	23857	Adult/male
Hypsiboas aff. *curupi*	Campos Novos/SC	CFBH	23855	Female
Hypsiboas pulchellus	Candiota/RS	MCP	3951	Adult/male
Hypsiboas pulchellus	Candiota/RS	MCP	5420	Adult/male
Hypsiboas bischoffi	Canoinha/SC	MCP	2104	Female
Hypsiboas aff.2 *joaquini*	Caraá/RS	UFRGS	7184	Adult/male
Hypsiboas aff.2 *joaquini*	Caraá/RS	UFRGS	7185	Adult/male
Hypsiboas aff.2 *joaquini*	Caraá/RS	UFRGS	7186	Adult/male
Hypsiboas aff.2 *joaquini*	Caraá/RS	MCN/FZB	14310	Female
Hypsiboas aff2. *joaquini*	Caraá/RS	MCP	12965	Tadpoles
Hypsiboas aff2. *joaquini*	Caraá/RS	MCP	12978	Tadpoles
Hypsiboas* aff2. *joaquini	**Caraá/RS**	**MCP**	**13172**	**Adult/male**
Hypsiboas* aff2. *joaquini	**Caraá/RS**	**MCP**	**13210**	**Tadpoles**
Hypsiboas* aff2. *joaquini	**Caraá/RS**	**MCP**	**13211**	**Tadpoles**
Hypsiboas* aff2. *joaquini	**Caraá/RS**	**MCP**	**13202**	**Tadpoles**
Hypsiboas* aff2. *joaquini	**Caraá/RS**	**MCP**	**13212**	**Tadpoles**
Hypsiboas cf. *joaquini*	Caracol/RS	MNRJ	22561	Adult/male
Hypsiboas stellae	Caxias do Sul/RS	MCP	11490	Adult/male
Hypsiboas curupi	Derrubadas/RS	ZUFSM	2230	Adult/male
Hypsiboas curupi	Derrubadas/RS	ZUFSM	2231	Adult/male
Hypsiboas curupi	Derrubadas/RS	ZUFSM	2233	Adult/male
Hypsiboas curupi	Derrubadas/RS	ZUFSM	2235	Adult/male
Hypsiboas curupi	Derrubadas/RS	ZUFSM	2236	Adult/male

Taxon	**Locality**	**Voucher**		**Sex/Stage**
Hypsiboas curupi	Derrubadas/RS	ZUFSM	8141	Tadpoles
Hypsiboas curupi	Derrubadas/RS	ZUFSM	8150	Tadpoles
Hypsiboas curupi	Derrubadas/RS	MCP	2232	Female
Hypsiboas curupi	Derrubadas/RS	MCP	2234	Female
Hypsiboas curupi	Derrubadas/RS	MCP	2237	Female
Hypsiboas stellae	Dois Lajeados/RS	MCP	9887	Adult/male
Hypsiboas pulchellus	Dom Feliciano/RS	MCP	4033	Adult/male
Hypsiboas guentheri	Dom Pedro de Alcântara/RS	MCP	11152	Adult/male
Hypsiboas guentheri	Dom Pedro de Alcântara/RS	MCP	11222	Adult/male
Hypsiboas curupi	General Manuel Bergano, Misiones, Arg	LGE	2869	Female
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	2867	Adult/male
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	2868	Adult/male
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	2871	Adult/male
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	2872	Adult/male
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	2894	Adult/male
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	3372	Adult/male
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	3924	Adult/male
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	3925	Adult/male
Hypsiboas curupi	General Manuel Bergano/Misiones/Arg	LGE	3934	Adult/male
Hypsiboas stellae	Guaporé/RS	UFRGS	5330	Adult/male
Hypsiboas stellae	Guaporé/RS	UFRGS	5331	Adult/male
Hypsiboas stellae	Guaporé/RS	UFRGS	5332	Adult/male
Hypsiboas cf. *stellae*	Ibarama/RS	ZUFSM	7189	Tadpoles
Hypsiboas stellae	Ibarama/RS	ZUFSM	3781	Adult/male
Hypsiboas curupi	Ita/SC	MCP	2379	Female
Hypsiboas curupi	Ita/SC	MCP	8671	Adult/male
Hypsiboas curupi	Ita/SC	MCP	8672	Adult/male
Hypsiboas stellae	Lagoa Vermelha/RS	MCP	11977	Adult/male
Hypsiboas stellae	Lagoa Vermelha/RS	MCP	11978	Adult/male
Hypsiboas marginatus	Lauro Muller/SC	MCP	12345	Adult/male
Hypsiboas bischoffi	Lebon Regis/SC	MCP	8931	Adult/male
Hypsiboas leptolineata	Lebon Regis/SC	MCP	8889	Female
Hypsiboas guentheri	Lomba Grande/RS	MCP	9992	Adult/male
Hypsiboas guentheri	Lomba Grande/RS	MCP	9993	Adult/male
Hypsiboas marginatus	Maquiné/RS	MCP	5662	Adult/male
Hypsiboas leptolineata	Novo Hamburgo/RS	MCP	9139	Adult/male
Hypsiboas semiguttatus	Palmeira/PR	CFBH	3372	Adult/male
Hypsiboas semiguttatus	Palmeira/PR	CFBH	3373	Adult/male
Hypsiboas semiguttatus	Piraquara/PR	CFBH	3364	Adult/male

Taxon	**Locality**	**Voucher**		**Sex/Stage**
Hypsiboas semiguttatus	Piraquara/PR	CFBH	3580	Adult/male
Hypsiboas semiguttatus	Piraquara/PR	CFBH	5407	Tadpoles
Hypsiboas pulchellus	Santana do Livramento/RS	MCP	12424	Adult/male
Hypsiboas marginatus	Santo Amaro da Imperatriz/SC	MCP	8116	Adult/male
Hypsiboas marginatus	Santo Amaro da Imperatriz/SC	MCP	8117	Adult/male
Hypsiboas marginatus	Santo Amaro da Imperatriz/SC	MCP	8123	Adult/male
Hypsiboas semiguttatus	São Bento Do Sul/SC	MNRJ	22562	Adult/male
Hypsiboas semiguttatus	São Bento Do Sul/SC	MNRJ	22564	Adult/male
Hypsiboas semiguttatus	São Bento Do Sul/SC	MNRJ	68*	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	UFMG-Amp	905	Tadpoles
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	UFMG-Amp	911	Tadpoles
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	2455	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	2456	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	CFBH	3062	Female
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	CFBH	3063	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	CFBH	3076	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3210	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3211	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3212	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3263	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3266	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3270	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3297	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3303	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3419	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3439	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	3639	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4499	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4500	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4502	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4503	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4590	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4591	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4592	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4974	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4975	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4976	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4977	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	ZUFSM	6046	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	ZUFSM	6075	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	ZUFSM	6075	Tadpoles
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	ZUFSM	6081	Adult/male

Taxon	**Locality**	**Voucher**		**Sex/Stage**
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	ZUFSM	6081	Tadpoles
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	ZUEC	11250	Adult/male
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	ZUEC	11251	Adult/male
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12876**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12880**	**Adult /male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12881**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12882**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12883**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12891**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12917**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12923**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12924**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12925**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12926**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12927**	**Adult/male**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12928**	**Tadpoles**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12930**	**Tadpoles**
Hypsiboas aff. _joaquini_	**São Francisco de Paula/RS**	**MCP**	**12977**	**Tadpoles**
Hypsiboas aff. *joaquini*	São Francisco de Paula/RS	MCP	4501	Female
Hypsiboas bischoffi	São Francisco de Paula/RS	MCP	1776	Adult/male
Hypsiboas bischoffi	São Francisco de Paula/RS	MCP	10678	Adult/male
Hypsiboas bischoffi	São Francisco de Paula/RS	MCP	12051	Female
Hypsiboas leptolineata	São Francisco de Paula/RS	MCP	11471	Adult/male
Hypsiboas leptolineata	São Francisco de Paula/RS	MCP	12806	Adult/male
Hypsiboas leptolineata	São Francisco de Paula/RS	MCP	12812	Adult/male
Hypsiboas leptolineata	São Francisco de Paula/RS	MCP	11472	Female
Hypsiboas marginatus	São Francisco de Paula/RS	MCP	2415	Adult/male
Hypsiboas marginatus	São Francisco de Paula/RS	MCP	12830	Adult/male
Hypsiboas joaquini	São Joaquim/SC	MCP	8217	Adult/male
Hypsiboas joaquini	São Joaquim/SC	MNRJ	4034**	Adult/male
Hypsiboas cf. *joaquini*	São José dos Ausentes/RS	MCP	3293	Adult/male
Hypsiboas cf. *joaquini*	São José dos Ausentes/RS	ZUFSM	6062	Adult/male
Hypsiboas cf. *joaquini*	São José dos Ausentes/RS	ZUFSM	6073	Adult/male
Hypsiboas cf. _joaquini_	**São José dos Ausentes/RS**	**MCP**	**12934**	**Adult/male**
Hypsiboas cf. _joaquini_	**São José dos Ausentes/RS**	**MCP**	**12935**	**Adult/male**
Hypsiboas cf. _joaquini_	**São José dos Ausentes/RS**	**MCP**	**12936**	**Adult/male**
Hypsiboas cf. _joaquini_	**São José dos Ausentes/RS**	**MCP**	**12937**	**Adult/male**
Hypsiboas cf. *joaquini*	São José dos Ausentes/RS	MCP	1238	Female
Hypsiboas pulchellus	São Lourenço Do Sul/RS	MCP	11279	Female
Hypsiboas semiguttatus	Saraiva/SC	MNRJ	22563	Adult/male
Hypsiboas cf. *stellae*	Sinimbú/RS	MCP	4946	Tadpoles
Hypsiboas cf. *stellae*	Sinimbú/RS	MCP	4957	Tadpoles
Hypsiboas cf. *stellae*	Sinimbú/RS	MCP	7601	Tadpoles
Hypsiboas cf. *stellae*	Sinimbú/RS	MCP	13201	Tadpoles

Taxon	**Locality**	**Voucher**		**Sex/Stage**
Hypsiboas cf. *stellae*	Sinimbú/RS	MCP	13202	Tadpoles
Hypsiboas stellae	Sinimbú/RS	MCP	11317	Adult/male
Hypsiboas stellae	Sinimbú/RS	MCP	11319	Adult/male
Hypsiboas stellae	Sinimbú/RS	MCP	11322	Adult/male
Hypsiboas stellae	Sinimbú/RS	MCP	11325	Adult/male
Hypsiboas stellae	Sinimbú/RS	CFBH	25715	Adult/male
Hypsiboas stellae	Sinimbú/RS	MCP	11324	Female
Hypsiboas guentheri	Terra de Areia/RS	MCP	3299	Adult/male
Hypsiboas guentheri	Terra de Areia/RS	MCP	5196	Adult/male
Hypsiboas marginatus	Terra de Areia/RS	MCP	1635	Adult/male
Hypsiboas marginatus	Timbé do Sul/SC	MCP	12344	Adult/male
Hypsiboas guentheri	Torres/RS	MCP	10687	Adult/male
Hypsiboas guentheri	Torres/RS	MCP	10897	Adult/male
Hypsiboas joaquini	Urubicí/SC	UFMG-Amp	926	Tadpoles
Hypsiboas joaquini	Urubicí/SC	UFMG-Amp	3319	Tadpoles
Hypsiboas joaquini	Urubicí/SC	CFBH	3629	Adult/male
Hypsiboas joaquini	Urubicí/SC	CFBH	3630	Adult/male
Hypsiboas joaquini	Urubicí/SC	MCP	8245	Adult/male
Hypsiboas joaquini	Urubicí/SC	MCP	8246	Adult/male
Hypsiboas joaquini	Urubicí/SC	MCP	8247	Adult/male
Hypsiboas joaquini	Urubicí/SC	ZUEC	10515	Adult/male
Hypsiboas joaquini	Urubicí/SC	ZUEC	10516	Adult/male
Hypsiboas joaquini	Urubicí/SC	ZUEC	10517	Adult/male
Hypsiboas joaquini	Urubicí/SC	ZUEC	10518	Adult/male
Hypsiboas joaquini	Urubicí/SC	ZUEC	10519	Adult/male
Hypsiboas joaquini	Urubicí/SC	MNRJ	4033*	Adult/male
Hypsiboas joaquini	Urubicí/SC	UFMG-Amp	928a	Tadpoles
Hypsiboas joaquini	Urubicí/SC	UFMG-Amp	928b	Tadpoles
Hypsiboas joaquini	Urubicí/SC	CFBH	3624	Female
Hypsiboas aff. *curupi*	Xanxerê/SC	CFBH	21128	Adult/male
Hypsiboas aff. *curupi*	Xanxerê/SC	CFBH	21129	Adult/male
Hypsiboas aff. *curupi*	Xanxerê/SC	CFBH	21140	Adult/male

* Holotype **Paratype

Appendix 4. **Samples used for molecular analyses.**

Name	12S	16S	Voucher	Locality	Source
Hypsiboas aff. *curupi*	**-**	**X**	CFBH11474	Campos Novos/SC	This study
Hypsiboas aff. *curupi*	**-**	**X**	CFBH11471	Campos Novos/SC	This study
Hypsiboas aff. *curupi*	**X**	**X**	MACN2648	Campos Novos/SC	This study
Hypsiboas aff. *curupi*	**X**	**X**	MACN2649	Campos Novos/SC	This study
Hypsiboas aff. *curupi*	**-**	**X**	CFBH9189	Xanxere/SC	This study
Hypsiboas aff. *joaquini*	**X**	**X**	MCP12930	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**X**	**X**	MCP12881	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**X**	**X**	MCP12917	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12977-1	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12977-2	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12928	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12927	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12926	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12925	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12880	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12876	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12923	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12924	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12891	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12883	São Francisco de Paula/RS	This study
Hypsiboas aff. *joaquini*	**-**	**X**	MCP12882	São Francisco de Paula/RS	This study
Hypsiboas aff.2 *joaquini*	**X**	**X**	MCP12978	Caraá/RS	This study
Hypsiboas aff.2 *joaquini*	**X**	**X**	MCP12965	Caraá/RS	This study
Hypsiboas aff.2 *joaquini*	**-**	**X**	FZB 14310	Caraá/RS	This study
Hypsiboas aff.2 *joaquini*	**-**	**X**	UFRGS4793	Caraá/RS	This study
Hypsiboas aff.2 *joaquini*	**-**	**X**	UFRGS4795	Caraá/RS	This study

Name	**12S**	**16S**	**Voucher**	**Locality**	**Source**
Hypsiboas aff.2 *joaquini*	**-**	**X**	UFRGS4794	Caraá/RS	This study
Hypsiboas aguilari	HM444783	HM444783	MUSM19804	San Alberto, Pasco, Peru	Lehr *et al.* (2010)
Hypsiboas aguilari	KF794115	-	MTD45203	San Alberto, Pasco, Peru	Faivovich et *al.*(2013)
Hypsiboas aguilari	HM444786	-	MUSM18655	San Alberto, Pasco, Peru	Lehr *et al.* (2010)
Hypsiboas balzani	AY549323	AY549323	DLR 41193	Prov. Noryungas, La Paz, Bolivia	Faivovich *et al.* (2004)
Hypsiboas balzani	HM480433	-	MNCN548	Prov. Noryungas, La Paz, Bolivia	Kohler *et al.* (2010)
Hypsiboas bischoffi	AY549324	AY549324	CFBH 3356	Rancho Queimado/SC	Faivovich *et al.* (2004)
Hypsiboas caingua	AY549326	AY549326	MLP 1084	Posadas, Misiones, Argentina	Faivovich *et al.* (2004)
Hypsiboas caipora	EU077268	EU077268	CFBH5738	Pilar do Sul/SP	Antunes *et al.* (2008)
Hypsiboas cf. *joaquini*	**X**	**X**	MACN3483	Cambará do Sul/RS	This study
Hypsiboas cf. *joaquini*	**X**	**X**	MACN3499	Cambará do Sul/RS	This study
Hypsiboas cf. *joaquini*	**X**	**X**	MACN424	Cambará do Sul/RS	This study
Hypsiboas cf. *joaquini*	**X**	**X**	MACN3505	São Francisco de Paula/RS	This study
Hypsiboas cf. *joaquini*	**X**	**X**	MACN3503	São Francisco de Paula/RS	This study
Hypsiboas cf. *joaquini*	**X**	**X**	MCP15002	Cambará do Sul/RS	This study
Hypsiboas cf. *joaquini*	**X**	**X**	MCP15003	Cambará do Sul/RS	This study
Hypsiboas cf. *joaquini*	**X**	**X**	MCP12937	São José dos Ausentes/RS	This study
Hypsiboas cf. *joaquini*	**X**	**X**	MCP12934	São José dos Ausentes/RS	This study
Hypsiboas cf. *joaquini*	**-**	**X**	MCP12936	São José dos Ausentes/RS	This study
Hypsiboas cf. *joaquini*	**-**	**X**	MCP12935	São José dos Ausentes/RS	This study
Hypsiboas cordobae	AY549331	AY549331	MACN 37693	Villa Elena, San Luis, Argentina	Faivovich *et al.* (2004)
Hypsiboas cordobae	AY549329	AY549329	MLP2139	Tanti, Cordoba, Argentina	Faivovich *et al.* (2004)
Hypsiboas curupi	**-**	**X**	LGE2868	General Manuel Belgrano, Misiones, Argentina	This study
Hypsiboas curupi	**-**	**X**	LGE2867	General Manuel Belgrano, Misiones, Argentina	This study
Hypsiboas curupi	AY549359	AY549359	MAC37793	San Vicente, Misiones, Argentina	Faivovich *et al.* (2004)
Hypsiboas curupi	**-**	**X**	MCP12302	Bom Progresso/RS	This study
Hypsiboas ericae	AY549332	AY549332	CFBH 3599	Alto Paraiso de Goias/GO	Faivovich *et al.* (2004)
Hypsiboas faber	AY54933.1	AY54933	MACN36999	San Vicente, Misiones, Argentina	Faivovich *et al.* (2004)

Name	**12S**	**16S**	**Voucher**	**Locality**	**Source**
Hypsiboas guentheri	AY843631	AY843631	CFBH 3386	Terra de Areia/RS	Faivovich *et al.* (2005)
Hypsiboas joaquini	**X**	**X**	MACNf37	Ubirici/SC	This study
Hypsiboas joaquini	**X**	**X**	MACN421	Ubirici/SC	This study
Hypsiboas joaquini	**X**	**X**	MCP3253	Urubici/SC	This study
Hypsiboas joaquini	AY549340	AY549340	CFBH 3280	Urubici/SC	Faivovich *et al.* (2004)
Hypsiboas joaquini	AY549339	AY549339	CFBH 3625	Urubici/SC	Faivovich *et al.* (2004)
Hypsiboas latistriatus	AY549360	AY549360	MZUSP111556	Itamontes/MG	Faivovich *et al.* (2004)
Hypsiboas leptolineatus	AY549341	AY549341	CFBH 3848	São Domingos/SC	Faivovich *et al.* (2004)
Hypsiboas marginatus	-	AY549342	CFBH 3098	Sao Franciso de Paula/RS	Faivovich *et al.* (2004)
Hypsiboas marianitae	AY549344	AY549344	MNV 249	Baritu, Salta, Argentina	Faivovich *et al.* (2004)
Hypsiboas marianitae	AY549343	AY549343	MNK 52825	Caballero, Santa Cruz, Bolivia	Faivovich *et al.* (2004)
Hypsiboas polytaenius	AY843655	AY843655	CFBH 5752	Itiatia/RJ	Faivovich *et al.* (2005)
Hypsiboas polytaenus	-	AY819374	No voucher	Boraceia/SP	Wiens *et al.* (2005)
Hypsiboas prasinus	AY549347	AY549347	CFBH 3388	Rio Vermelho/SC	Faivovich *et al.* (2004)
Hypsiboas pulchellus	AY549350	AY549350	MACN37790	El Cazador, Buenos Aires, Argentina	Faivovich *et al.* (2004)
Hypsiboas pulchellus	AY549351	AY549351	MACN37664	Basavilbaso, Entre Rios, Argentina	Faivovich *et al.* (2004)
Hypsiboas riojanus	AY563626	-	MACN37891	Caspinchango, Catamarca, Argentina	Faivovich *et al.* (2004)
Hypsiboas riojanus	HM480455	-	MNCN6173	Empalme, Santa Cruz, Bolivia	Kohler *et al.* (2010)
Hypsiboas riojanus	-	AY819420	MCN 800	Vaqueros, Salta, Argentina	Faivovich *et al.* (2004)
Hypsiboas riojanus	-	AY549354	MACN 37631	Catamarca, Argentina	Faivovich *et al.* (2004)
Hypsiboas semiguttatus	-	AY549358	CFB 3705	Piraquara/PA	Faivovich *et al.* (2004)
Hypsiboas semiguttatus	AY549357	AY549357	CFB 3579	Piraquara/PA	Faivovich *et al.* (2004)
Hypsiboas stellae	**-**	**X**	LGE3302	Vila Bonita, Misiones, Argentina	This study
Hypsiboas stellae	**-**	**X**	LGE3303	Vila Bonita, Misionoes, Argentina	This study
Hypsiboas stellae	**X**	**X**	MCP11318	Sinimbú/RS	This study
Hypsiboas stellae	**X**	**X**	MCP11978	Lagoa Vermelha/RS	This study
Hypsiboas stellae	**-**	**X**	MCP12284	Sinimbú/RS	This study
Hypsiboas stellae	**-**	**X**	MCP11324	Sinimbú/RS	This study

Name	12S	16S	Voucher	Locality	Source
Hypsiboas stellae	-	X	MCP11319	Sinimbú/RS	This study
Hypsiboas stellae	-	X	MCP12283	Sinimbú/RS	This study
Hypsiboas stellae	-	X	MCP11323	Sinimbú/RS	This study
Hypsiboas stellae	-	X	MCP13201	Sinimbú/RS	This study
Hypsiboas stellae	-	X	CFBH9887	Dois Lageados/RS	This study
Hypsiboas stellae	-	X	MCP11490	Caxias do Sul/RS	This study
Hypsiboas stellae	-	X	MCP11977	Lagoa Vermelha/RS	This study

Appendix 5. Variation of morphometric variables, minimum–maximum (mean± standard deviation), of adult females according to the comparative units defined in this study. Data from *Hypsiboas caipora* taken from Antunes *et al.* (2008).

Taxa	*H. caipora*	*H.* aff. *joaquini*	*H.* aff.2 *joaquini*	*H. curupi*	*H. cf. joaquini*	*H. joaquini*	*H.stellae*	*H.* aff. *curupi*
(n)	1	2	1	4	9	1	3	1
AMD	4	3.6 – 3.8	5.5	3.8 – 5.1 (4.4±0.5)	2.9 – 5 (4.26±0.6)	5.2	4	3.8
Arm	–	15 – 18	26.4	20.8–25.6 (22.2±1.7)	12.4 – 23.9 (21.1±3.7)	29.8	20.8 – 24.3 (22.5±1.7)	18.6
ED	–	4.2 – 4.5	5.3	4.4–5.9 (5.3±0.5)	3.7 – 5.7 (4.8±0.5)	6.9	5 – 5.2 (5.1±0.1)	4.3
EN	3	2.5 – 4.5	4.1	2.9–4.4 (3.4±0.6)	2.5 – 5.5 (3.8±0.8)	4.22	3 – 4.5 (3.6±0.8)	3.2
FAL	7.7	7.7 – 8.4	13.1	9.4–11.9 (10.5±1)	6.2 – 11.2 (10.1±1.5)	13.2	9.8 – 10.6 (10.2±0.4)	8.4
FL	–	13.7 – 15.7	22.9	17.3–22.1 (19±2.1)	10.9 – 21.8 (19.2±3.2)	27.8	17,3 – 21 (19.1±1.7)	27.9
HAL	12.5	9.8 – 11.4	12	12.4–6.5 (14.5±1.8)	7.8 – 14.8 (13.3±2.8)	18	12.4 – 15.6 (14±1.6)	19
HL	13.2	11.3 – 12	15.7	12.2–16.4 (14±1.5)	6.7 – 14.7 (13.1±2.5)	16.8	12.8 – 14.9 (14.1±1.2)	10.8
HW	13.8	8.8 – 10.2	15.3	10.8–15.4 (13.1±1.6)	8.2 – 13.7 (12.2±1.6)	18.2	12.8 – 14.9 (14±1.1)	10.8
ID	3	2.5 – 3.2	4.2	3.2–3.7 (3.4±0.2)	2.9 – 4.3 (3.5±0.4)	4.6	3.2 – 3.5 (3.4±0.16)	3
IOD	7	2.6 – 4.7	5.3	3.7–5.5 (4.3±0.6)	3.3 – 4.8 (4.2±0.5)	4.5	3.7 – 4.33 (4.1±0.3)	3.6
Leg	–	22.5 – 25.8	37.4	30–37.3 (32±3.3)	19.5 – 36.3 (31 ±5.9)	44.6	29.15 – 33.95 (31.6±2.4)	27.7
NS	1.8	3.6 – 4.2	6.6	4.5–6.5 (5.4±0.6)	4.1 – 6.3 (5.6±0.6)	6.7	4.53 – 5.71 (5.25±0.63)	4.5
ShL	20.1	15.7 – 17.8	25.8	19.7–26.3 (22.1±2.3)	13.8 – 24.8 (22±3.2)	29	20.4 – 24 (22.2±1.8)	19.2
SVL	37.6	31.6 – 37.5	50.8	37–48 (40.5±3.9)	26.7 – 49 (43.5±6.5)	54.3	36.65 – 43.03 (40.81±3.6)	37.7
TAL	12.9	9.5 – 12.5	16.15	11.3–22.1 (15.1±3.5)	8.7 – 19.2 (14.3±3)	17.8	11.34 – 33.3 (19.9±12)	11.6
TBL	20.3	15.8 – 18	26.6	20.7–25.6 (22.7±1.9)	14.3 – 24.2 (22.2±3)	29.6	21.1 – 24.5 (23±1.8)	19
TD	1.7	1.9 – 2.5	2.1	2.2 – 2.7 (2.4±0.2)	1.5 – 4.8 (2.5±0.9)	3.1	2 – 2.4 (2.3±0.2)	3.2
Uarm	–	8.6 – 8.8	13	11.3 – 14.5	7.3 – 13.3	15.1	10.7 – 12	9.4

(12.5±1.3) (10.6±2) (11.2±0,7)

Appendix 6. Loading values of the five first principal components from a PCA analysis of 19 morphometric variables of adult males.

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
AMD	0.7496	-0.1026	0.1970	0.3060	0.2130
Arm	0.9548	-0.0629	-0.0302	-0.0097	-0.0093
ED	0.7268	0.0414	0.1496	0.4572	0.0636
EN	0.5906	0.6113	0.4549	-0.0763	-0.1956
FAL	0.9181	-0.0469	-0.0261	-0.0667	-0.0042
FL	0.9489	-0.0926	0.0698	-0.0214	-0.0466
HAL	0.9556	-0.0052	0.0276	0.0172	0.0083
HL	0.9343	0.0381	-0.0531	0.0707	0.0314
HW	0.9461	-0.0414	-0.0740	0.0276	-0.0007
ID	0.8237	0.1120	-0.0424	0.0929	-0.1680
IOD	0.7513	0.1127	-0.2942	-0.1989	-0.3519
Leg	0.9579	-0.0957	0.0185	-0.0115	-0.0440
NS	0.6947	0.0013	0.2259	-0.4806	0.4069
ShL	0.9743	-0.1130	0.0200	-0.0230	-0.0445
SVL	0.9728	-0.0127	0.0192	-0.0482	-0.0214
TAL	0.8969	-0.1889	0.0017	0.0146	-0.0813
TBL	0.9456	-0.0768	-0.0627	-0.0283	-0.0140
TD	0.6229	0.5037	-0.4650	0.0886	0.3382
Uarm	0.9074	-0.1655	-0.0602	-0.0978	0.0353
"Eigenvalues"	16.2723	0.4164	0.0762	0.0122	0.1151
% of total variance explained	74.8600	4.0700	3.4100	3.2600	2.8100

Appendix 7. Loading values of the five first principal components of a PCA analysis of the residuals of linear regressions between 18 morphometric variables and SVL.

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
AMD	0.3483	0.0186	0.3951	0.2673	-0.489
Arm	0.7044	0.152	-0.177	0.2209	0.1089
ED	0.3614	0.2946	0.3259	0.1043	-0.2417
EN	-0.0886	0.4103	0.2753	0.2574	0.4204
FAL	0.4498	0.0868	-0.4108	0.1832	0.1287
FL	0.4716	-0.2836	0.3656	0.2319	0.2202
HAL	0.6487	0.2422	0.227	0.2781	0.2056
HL	0.5565	0.4735	0.0538	-0.049	-0.2424
HW	0.6561	0.2339	-0.1143	-0.1281	-0.2392
ID	0.1517	0.3361	0.3634	-0.5015	0.1944
IOD	0.274	0.2647	-0.3253	-0.3233	0.4317
Leg	0.6529	-0.315	0.258	-0.0547	0.2386
NS	0.065	0.0076	-0.2727	0.5487	0.1502
ShL	0.8008	-0.3531	0.0068	-0.0701	0.0511
TAL	0.4433	-0.4412	0.126	-0.3816	-0.0394
TBL	0.6715	-0.0791	-0.1223	-0.2109	0.0338
TD	0.0968	0.6417	-0.1735	-0.1675	-0.1371
Uarm	0.5466	-0.2048	-0.5036	0.0428	-0.2404
"Eigenvalues"	7.8107	1.4852	0.2974	0.248	0.5544
% of total variance explained	24.81	9.81	7.96	7.03	6.08

Appendix 8. Loading values of the five first principal components from a PCA analysis of 15 morphometric variables of adult males including *Hypsiboas caipora*.

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
AMD	0,7412	0,1931	-0,1405	-0,3854	0,4837
EM	0,6599	-0,2709	0,6065	-0,3272	-0,09361
FAL	0,9093	-0,2291	-0,08285	0,04833	-0,09999
FL	0,9524	0,05065	-0,07584	-0,07475	-0,07684
HAL	0,948	0,1274	-0,003458	-0,01011	-0,01597
HL	0,9361	0,1007	0,0174	0,05537	0,06968
HW	0,9035	0,3044	-0,01409	0,06366	0,02248
ID	0,8578	-0,08872	0,0487	0,07801	-0,08763
IOD	0,2638	0,8846	0,27	0,1488	-0,03737
NS	0,7542	-0,5499	-0,0891	-0,01054	-0,05055
ShL	0,9716	0,08354	-0,09119	-0,02893	-0,083
SVL	0,9767	-0,02314	-0,02292	0,001034	-0,06841
TAL	0,9059	0,05427	-0,184	-0,0174	-0,1313
TBL	0,9436	0,1096	-0,07346	0,07315	-0,07139
TD	0,6912	-0,3589	0,2205	0,4407	0,3673
"Eigenvalues"	12,4152	0,3876	0,385692	0,054724	0,1271
% of total variance explained	71.81	10.19	3.87	3.33	2.96

Appendix 9. Loading values of the five first principal components of a PCA analysis of the residuals of linear regressions between 14 morphometric variables and SVL, including *Hypsiboas caipora.*

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
AMD	0,4407	0,07443	0,1208	0,4402	0,1594
EM	-0,2426	0,2348	0,4045	0,4046	0,2545
FAL	-0,2042	0,7244	-0,1902	-0,02013	-0,2518
FL	0,4819	0,1502	-0,2185	0,5691	0,08381
HAL	0,6618	0,2528	0,1996	0,2181	-0,08058
HL	0,5467	0,3271	0,4786	-0,08623	-0,06525
HW	0,8058	-0,006225	0,231	-0,1225	-0,1441
ID	0,01635	0,3565	0,2666	-0,2953	0,7483
IOD	0,6732	-0,4611	0,2473	-0,1475	-0,01827
NS	-0,5102	0,687	-0,1006	0,1323	-0,06399
ShL	0,7254	0,3363	-0,3648	0,0082	-0,02988
TAL	0,3941	0,2328	-0,5387	-0,3098	0,3519
TBL	0,6553	0,3134	-0,1289	-0,2418	-0,1624
TD	-0,2634	0,4837	0,4911	-0,2787	-0,2339
"Eigenvalues"	4,18085	3,706105	0,8978	0,27054	0,54774
% of total variance explained	27.19	14.93	10.06	7.93	6.87

Appendix 10. Loading values of the five first principal components from a PCA analysis of 22 morphometric variables of tadpoles.

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
BH	0.9217	0.2507	-0.0604	-0.0188	0.0141
BL	0.5778	-0.0072	0.2503	0.2701	0.5381
BW	0.9300	0.0970	-0.0898	0.1093	0.1280
ED	0.8053	-0.3535	0.0439	-0.1073	0.2157
EN	0.8250	0.0109	-0.0072	0.0663	-0.0459
HS	0.5570	0.5379	0.3342	-0.1174	0.1099
HW	0.9525	-0.1514	0.0230	-0.1081	-0.0491
InD	0.7300	-0.3790	-0.0201	-0.2971	-0.1359
IOD	0.9187	-0.0433	-0.0022	-0.0693	-0.1094
Ltail	0.7408	0.3808	-0.3889	-0.1094	-0.1374
MTH	0.8344	-0.2966	0.0696	0.0027	-0.1289
MW	0.7548	-0.2610	0.2427	-0.3997	-0.2194
MWN	0.4826	-0.1480	0.1568	0.6664	-0.4182
SnL	0.7456	-0.4192	0.1023	-0.1269	0.1936
SpL	0.6162	0.3036	0.2911	-0.2845	0.0365
SSD	0.9351	0.0686	-0.0091	0.1165	-0.0410
SW	0.6113	0.3584	0.5034	0.0909	-0.1297
TAL	0.9229	0.0784	0.0071	0.1657	-0.0015
TL	0.9565	0.1034	0.0250	0.1143	0.0085
TMH	0.8850	0.0665	-0.3243	-0.0325	-0.1082
TMW	0.7503	-0.3330	-0.2104	0.2727	0.2017
Utail	0.7197	0.2992	-0.5496	-0.0464	0.0996
"Eigenvalues"	17.1732	0.1632	0.3873	0.1575	0.0211
% of total variance explained	62.8800	7.2700	5.5800	4.8700	3.5300

Appendix 11. Loading values of the five first principal components from a PCA analysis of 22 morphometric variables of tadpoles including *Hypsiboas caipora*.

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
BH	0,8922	0,08612	-0,1431	-0,2866	-0,09837
BL	0,6032	0,2942	-0,4731	0,5533	0,03506
BW	0,9107	0,1674	-0,1417	-0,189	-0,01259
ED	0,826	-0,2328	-0,107	0,1253	-0,3577
EN	0,837	0,06759	-0,03796	-0,08286	0,4451
InD	0,7542	-0,4148	0,159	0,221	0,198
IOD	0,9164	-0,1024	0,03772	-0,1271	0,0482
MTH	0,8188	-0,2001	0,1507	0,1123	0,01932
MW	0,7727	-0,474	0,1488	0,06989	-0,08647
MWN	0,5087	0,5353	0,624	0,2237	-0,07545
TAL	0,9146	0,2366	-0,0181	-0,1358	-0,04021
TL	0,9417	0,1881	-0,03356	-0,1336	-0,07042
"Eigenvalues"	9,6962	0,15121	0,1657	0,35053	0,00447
% of total variance explained	66,92	8,38	6,16	5,13	3,32

Appendix 12. Loading values of the five first principal components of a PCA analysis of the residuals of linear regressions between 21 morphometric variables and TL.

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
BH	0.4308	0.6886	0.1738	0.1723	-0.1412
BL	0.0680	-0.1568	0.1138	0.3880	-0.1099
BW	0.5011	0.3140	-0.1850	0.4006	-0.1497
ED	0.5897	-0.4094	0.0165	-0.0953	-0.4092
EN	0.2889	-0.0411	-0.0783	0.0728	0.6238
HS	-0.1735	0.3953	0.5960	0.0988	-0.2628
HW	0.8840	-0.1177	0.1514	-0.0325	0.0781
InD	0.6346	-0.2923	0.0486	-0.4029	0.2257
IOD	0.7288	0.0992	0.1430	0.0950	0.2313
Ltail	0.2090	0.7395	-0.2230	-0.2286	0.3321
MTH	0.6093	-0.3642	0.0590	-0.0164	0.0083
MW	0.6975	-0.2086	0.4610	-0.2241	0.2091
MWN	-0.1001	-0.3344	-0.1690	0.4020	0.2965
SnL	0.5517	-0.4691	0.0358	-0.0532	-0.2693
SpL	-0.0577	0.1631	0.5571	-0.3869	0.0511
SSD	0.5640	0.2475	0.0241	0.5567	0.1421
SW	-0.0416	0.1698	0.6758	0.4705	0.1502
TAL	-0.2489	-0.2598	-0.3977	0.1128	0.4600
TMH	0.5828	0.4701	-0.3408	-0.1780	-0.0410
TMW	0.4464	-0.2882	-0.4956	0.2598	-0.3410
Utail	0.2806	0.7164	-0.4364	-0.1823	-0.1585
"Eigenvalues"	7.4454	1.0619	0.7301	1.2291	0.9257
% of total variance explained	23.0300	14.7300	10.7500	7.7600	7.1600

Appendix 13. Loading values of the five first principal components of a PCA analysis of the residuals of linear regressions between 21 morphometric variables and TL, including *Hypsiboas caipora.*

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
BH	0,4844	-0,7216	-0,1203	-0,1687	0,06095
BL	0,1227	-0,0676	0,3266	0,8196	0,1626
BW	0,4665	-0,6041	0,3815	0,06056	0,02702
ED	0,5986	0,2255	-0,2152	0,4361	-0,06808
EN	0,4375	0,001532	0,6588	-0,0948	-0,2411
InD	0,6463	0,4977	-0,03827	-0,01596	-0,1264
IOD	0,7743	-0,1004	0,09666	-0,3042	0,004813
MTH	0,7009	0,1998	0,0687	0,02209	0,3063
MW	0,7517	0,348	-0,206	-0,1401	-0,1067
MWN	-0,1642	0,3369	0,4231	-0,2458	0,7332
TAL	-0,325	0,2651	0,5939	-0,08817	-0,4554
"Eigenvalues"	4,4937	0,380832	1,96949	0,28062	0,297203
% of total variance explained	29,3	14	12,15	9,86	8,73

Appendix 14. Loading values of the five first principal components from a PCA analysis of ten bioacoustics variables of advertisement calls.

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
BAP	0.7021	-0.2649	0.3548	0.0601	-0.5366
AP	0.8422	-0.2558	0.1462	-0.1059	0.0795
CD	0.8909	-0.1750	0.2477	0.1728	0.1332
DF	-0.7830	0.2314	0.5160	-0.0649	0.0708
DPH	0.7707	-0.1270	0.1326	-0.4516	0.2266
FBW	0.7032	0.6579	-0.1400	0.1916	0.0118
LPF	-0.8441	-0.1952	0.4204	-0.2132	-0.0199
NP	-0.6697	-0.4268	0.1790	0.4573	0.1716
RTB	0.8594	-0.0871	0.3293	0.2117	0.1880
UFP	-0.0022	0.9085	0.3868	0.0250	-0.0089
"Eigenvalues"	2.4695	0.2660	2.5728	0.2829	0.3161
% of total variance explained	55.9700	17.2200	9.7800	5.8900	4.3400

Appendix 15. Loading values of the five first principal components from a PCA analysis of five bioacoustics variables of advertisement calls including *Hypsiboas caipora* and *H. curupi* descriptions database.

	PC-1	**PC-2**	**PC-3**	**PC-4**	**PC-5**
CD	0.6317	-0.5817	0.4042	-0.3147	0.0106
DF	0.3688	0.8744	0.2357	-0.1488	0.1472
LPF	-0.8009	-0.0381	0.5160	0.3011	0.0090
RTB	0.8972	0.2702	0.1295	0.1994	-0.2558
UFP	0.8692	-0.2622	-0.0520	0.3636	0.2022
"Eigenvalues"	1.9660	0.2626	1.2334	0.4006	0.1133
% of total variance explained	54.7410	24.9250	10.0950	7.6700	2.5632