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CARLOS HENRIQUE LUZ NUNES DE ALMEIDA

PHYLOGENETIC RECONSTRUCTION BASED ON INTERNAL AND EXTERNAL MORPHOLOGY OF GENUS *Thoropa* COPE, 1865 (ANURA, CYCLORAMPHIDAE)

RECONSTRUÇÃO FILOGENÉTICA COM BASE EM MORFOLOGIA INTERNA E EXTERNA DO GÊNERO Thoropa *COPE, 1865 (ANURA, CYCLORAMPHIDAE)*

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INSTITUTO DE BIOLOGIA

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Dissertation presented to the Biology Institute of Universidade Estadual de Campinas as part of the requirements for obtaining the Master title in Animal Biology in Animal Biodiversity Area

Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título Mestre em Biologia Animal, na área de Biodiversidade Animal

ORIENTADOR: PROF. DR. LUÍS FELIPE DE TOLEDO RAMOS PEREIRA
CO-ORIENTADOR: PROF. DR. RENATO NEVES FEIO

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELO ALUNO CARLOS HENRIQUE LUZ NUNES DE ALMEIDA, E ORIENTADA PELO PROF. DR. LUÍS FELIPE DE TOLEDO RAMOS PEREIRA.

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RESUMO

Os anfíbios da família Cycloramphidae são endêmicos da porção oriental do Brasil e alguns estão ameaçados ou em perigo de extinção. Apesar de ser uma família com poucos grupos taxonômicos, apenas três gêneros (*Cycloramphus*, *Thoropa* e *Zachaenus*), até o momento não há uma proposta filogenética para o gênero *Thoropa* que abranja todas as espécies. Então, propomos a reconstrução filogenética de *Thoropa* usando como grupo esterno, uma espécie do gênero *Hylodes* (Hylodidae), duas do gênero *Cycloramphus* e uma espécie do gênero *Zachaenus*. Definimos 86 caracteres baseados em osteologia, ecologia e morfologia externa de adultos e larvas. Neste contexto realizamos a descrição da forma larval de *Cycloramphus rhyakonastes*, a qual foi utilizada como grupo esterno. A filogenia proposta é baseada em critérios de máxima parcimônia. Como duas das árvores resultantes indicam parafilia, propomos aqui um novo gênero para as espécies de menor porte, separando-as das espécies maiores.

Palavras chave: Árvore filogenética, canto de anúncio, ecologia, máxima parcimônia, morfologia, novo gênero.

ABSTRACT

The phylogenetic resolution of Neotropical amphibians increased considerably in recent decades, providing great understanding of the relationships of higher taxa, such as the relationships between families and genera. After such improvement, amphibian systemticians are now focusing their efforts on lower taxa, such as the relationships within congeneric species. Under such context we did a phylogenetic reconstruction of the genus *Thoropa*, for which no comprehensive phylogeny is available. We examined all species of the genus including two species with extinct populations and possible new species. We adopted the criteria of maximum parsimony to analyze 86 characters based on osteology, external morphology, behavior, bioacoustics, and tadpole external morphology of at least one specimen per species. As the resulting topology of *Thoropa* tree indicates a possible case of paraphyly, we propose a new genus, assuring the monophyly of *Thoropa* in all possible scenarios.

Keywords: Advertisement call, ecology, phylogenetic tree, maximum parsimony, morphology, new genus.

SUMÁRIO

Este trabalho é dedicado à minha família, aos meus ex-professores e aos amigos que sempre me incentivaram e me apoiaram em minha busca pelo conhecimento.

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INTRODUÇÃO GERAL

Atualmente a família Cycloramphidae possui 36 espécies distribuídas em três gêneros, *Cycloramphus* Tschudi 1838 com 28 espécies, *Thoropa* Cope, 1865 com seis espécies e *Zachaenus* Cope 1866 com duas espécies (Caramaschi & Sazima 1984; Frost 2014; Cocroft & Heyer 1988; Verdade 2005; Feio & Caramaschi 2006; Lingnau *et al.* 2008). A maioria destes anfíbios são endêmicos da Mata Atlântica, ocorrendo do estado da Bahia ao estado do Rio Grande do Sul (Haddad & Sazima 1989; Siqueira *et al.* 2006; Lingnau *et al*. 2008).

As espécies da família Cycloramphidae diferem em seus modos reprodutivos. Há espécies que depositam seus ovos no solo, como *Zachaenus* e parte das espécies de *Cycloramphus*, onde os embriões se desenvolvem e as larvas são endotróficas. Outras espécies depositam ovos na margem ao longo de riachos, como *Thoropa* e parte das espécies de *Cycloramphus*, e suas larvas ficam aderidas às rochas em áreas borrifadas em riachos. Estas larvas são exotróficas (Verdade 2005; Heyer & Crombie 1979, Verdade & Rodrigues 2003; Giaretta & Cardoso 1995).

Para o gênero *Thoropa* são conhecidas as sete espécies a saber: *T. miliaris* (Spix 1824), *T. petropolitana (Wandolleck 1907)*, *T. taophora* (Miranda-Ribeiro 1923), *T. lutzi* Cochran 1938, *T. megatympanum* Caramaschi & Sazima 1984, *T. saxatilis* Cocroft & Heyer 1988 (Caramaschi & Sazima 1984; Frost 2014; Cocroft & Heyer 1988; Feio & Caramaschi 2006) e da Juréia uma espécie sendo descrita que tem afinidade com *T. taophora*. O tamanho médio dos indivíduos adultos varia de 28 a 102 mm e ocorrem do nível do mar até cerca de 1.500 m de altitude (Cocroft & Heyer 1988; Feio *et al.* 2006).

Uma das espécies, *T. petropolitana*, está aparentemente extinta na sua localidade tipo (Petrópolis, Rio de Janeiro) onde foram abundantes e fácil de encontrar até 1979

(Heyer 1988). Além desta, a população de *T. lutzi* da Serra dos Órgãos (localidade tipo) está aparentemente extinta, não sendo encontrado há vários anos (Izechsohn & Carvalho-e-Silva, 2000). De acordo com a lista de espécies ameaçadas atual (DOU 2014) esta espécie está classificada como dados insuficientes (DD). Todavia, no estado do Espírito Santo é possível encontrar uma população associada a esta espécie, a qual está em perigo de extinção (EN), segundo a lista vermelha do estado, dadas as consequências de atividades antrópicas. Portanto, além de *Thoropa* ser um gênero com poucas espécies, com modos reprodutivos especializados, endêmica da porção oriental do país, duas das sete conhecidas contam com populações desaparecidas.

Isso por si só torna o grupo apropriado para investigações aprofundadas, em especial sobre sistemática, a qual ainda não é totalmente conhecida. Assim, decidimos no presente estudo realizar uma proposta filogenética para o grupo que abranja todas as espécies, baseando-se em dados osteológicos, ecológicos, e de morfologia externa de adultos e da forma larval. Neste contexto, aproveitamos também para descrever uma das larvas especializadas da família Cycloramphidae (Anexo III), que será utilizada como grupo externo na reconstrução filogenética de *Thoropa*.

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ARTIGO

PHYLOGENETIC RECONSTRUCTION BASED ON INTERNAL AND EXTERNAL MORPHOLOGY OF GENUS *Thoropa* COPE, 1865 (ANURA, CYCLORAMPHIDAE)

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Abstract

The phylogenetic resolution of Neotropical amphibians increased considerably in recent decades, providing great understanding of the relationships of higher taxa, such as the relationships between families and genera. After such improvement, amphibian systemticians are now focusing their efforts on lower taxa, such as the relationships within congeneric species. Under such context we did a phylogenetic reconstruction of the genus *Thoropa*, for which no comprehensive phylogeny is available. We examined all species of the genus including two species with extinct populations and possible new species. We adopted the criteria of maximum parsimony to analyze 86 characters based on osteology, external morphology, behavior, bioacoustics, and tadpole external morphology of at least one specimen per species. As the resulting topology of *Thoropa* tree indicates a possible case of paraphyly, we propose a new genus, assuring the monophyly of *Thoropa* in all possible scenarios.

Key-words: Advertisement call, ecology, phylogenetic tree, maximum parsimony, morphology, new genus

Introduction

A phylogenetic tree is made based on the comparison of characters such as morphology (Delfino & Smith 2012), acoustic parameters (Goicoechea *et al.* 2010), DNA sequences (Pyron & Wiens 2011), or a combination of characters from multiple sources, for example a combination of molecular, ecological and morphological data, which is termed trees of total evidence, based on integrative taxonomy (Padial *et al*. 2010). The generation of phylogenetic trees can be based on phenetic analysis, where a distance matrix of the selected characters is made by grouping those with less difference and represented in a phenogram; or in a cladistic analysis, in which the trees are generated by the methods of maximum likelihood, bayesian inference and maximum parsimony (Amorim 2002; Buso 2005). The maximum likelihood method is based generally on molecular data in reconstructing the phylogeny by maximizing the likelihood of the observed data (Santos *et al.* 2009). Usually the bayesian inference is adopted for complex models (Nylander *et al.* 2004). The maximum parsimony method is based on the concept that the best hypothesis to explain a process is simpler. Thus, we adopt as more parsimonious that tree with the smallest number of evolutionary changes (Pinteiro *et al.* 2005; Vallinoto *et al.* 2009).

In the last years, one of the most widely used phylogenetic methods for amphibians has been the maximum parsimony, as observed in studies by Frost *et al.* (2006) and Faivovich *et al.* (2010). Chaparro *et al.* (2007) used the maximum likelihood method and the method of maximum parsimony, and one of the most recent work, Faivovich *et al.* (2012) applied the maximum parsimony and the bayesian method. Pyron & Wiens (2011) used the maximum likelihood method. Independently of the different methods that are applied in current systematics, the amphibian taxonomy is reaching a greater stability than in the previous decades.

Although a plethora of methods is available, the maximum parsimony is the most suitable for studies based on morphological datasets. For example, osteological characters have been used as a baseline for the phylogenetic proposals of several anuran genera, such as *Telmatobius* Wiegmann 1834 (Barrionuevo 2013), *Stumpffia* Boettger 1881 (Klages *et al.* 2013), and *Pseudopaludicola* Miranda-Ribeiro 1926 (Cardozo & Suárez 2012). An advantage of using morphological with respect to molecular data is the possibility of evaluating a larger amount of taxa, since morphology is less expensive to access than molecular data and allows the inclusion of preserved specimens and also extinct species (Wiens 2000; Giribet 2015). Molecular studies could collaborate with the understanding of the evolution of the genus. For example, Fitzpatrick *et al.* (2009) published the genetic relationships of three close-related species: *T. miliaris*, *T. taophora* and *Thoropa* sp. (aff. *taophora*). However, so far we are not able to target the endangered or already extinct species with molecular analyses, as we lack DNA samples from those lineages.

The genus *Thoropa* currently allocated in Cycloramphidae family contains six species, *T. miliaris* (Spix 1824), *T. petropolitana* (Wandolleck 1907), *T. taophora* (Miranda-Ribeiro 1923), *T. lutzi* Cochran 1938, *T. megatympanum* Caramaschi & Sazima 1984 and *T. saxatilis* Cocroft & Heyer 1988 (Caramaschi & Sazima 1984; Frost 2014; Cocroft & Heyer 1988; Feio, Napoli & Caramaschi 2006). The genus, endemic to the eastern portion of the Brazil, Cerrado and Atlantic Forest, from Bahia to Rio Grande do Sul (Siqueira *et al.* 2006; Haddad *et al.* 2013; Frost 2014).

The coloration of the species was selected to resemble rocks and the surrounding background where they live (Izecksohn & Carvalho-e-Silva 2001). These amphibians are small to medium sized (28-102 mm) and occur in altitudinal gradient, from sea level to 1500 m of elevation (Cocroft & Heyer 1988; Feio, Napoli & Caramaschi 2006; Frost

2014). At least *Thoropa taophora* is polygamous, with some recent observations showing two females sharing breeding sites (Muralidhar *et al.* 2013). They can stand salty water on rocks shore where they prey marine invertebrates (Sazima 1971; Abe & Bicudo 1991). As an adaptation to marine shore environment, these animals have higher osmotic concentration of plasma, musculature and urine (Abe & Bicudo 1991). *Thoropa* lay eggs on rocks with a thin layer of water. Tadpoles are semi-terrestrial (Giaretta & Facure 2004) and cannibalistic (Muralidhar *et al.* 2013). These tadpoles have a depressed shape, long tail and bulging eyes. The movement is achieved using the oral apparatus and the ventral disk and the tail is long and vigorous (Bokermann 1965). Therefore, the genus is unique in several aspects from the ecological point of view.

The population of *Thoropa lutzi* is categorized as data deficient (DD). In the state of Espírito Santo, this species is endangered (EN). The type population of *T. petropolitana* was apparently extinct (EN). *Thoropa saxatilis*, from the south Brazil, is vulnerable (VU). The other three known species not are under extinction risk. Therefore, the genus is also unique in the sense that half of its known diversity is endangered.

To sum up, the ecology of this genus includes some exclusive reproductive and physiological specializations, it is an irreplaceable group with few species, of which some are extinct or endangered. Therefore, it is an evolutionary lineage that urges further investigation. In particular the systematic relationships of the congeneric species are so far not understood. Thus, we propose reconstructing its phylogeny based on post-metamorphic osteology and external morphology, tadpole external morphology, and some behavioral traits.

The aim of this study is the phylogeny of *Thoropa* based on morphological and ecological data comprising all species and testing the monophyly of the genus.

Methods

We examined animals deposited in scientific collections, including adult males of the genus *Thoropa*, *Cycloramphus*, *Zachaenus* and *Hylodes*, totaling 11 lineages. We analyzed at least one specimen (microtomography or color-stained animals) of each species for osteology and at least three (Table S1). Analyses were performed by direct comparison of species. The distribution of *Thoropa* genus is restrict to the Atlantic Forest and Cerrado, eastern portion of Brazil (Fig. 1). When the distribution of the species is wide, we used samples of individuals from different localities, except for *Cycloramphus eleutherodactylus* that may consist a species complex (Verdade 2005).

We examined specimens from the Brazilian museums: Museu de Zoologia “Prof. Adão José Cardoso”, Unicamp, Campinas, São Paulo (ZUEC), Célio F. B. Haddad Amphibian Collection, Departamento de Zoologia, Unesp, Rio Claro, São Paulo (CFBH); Museu Nacional, Rio de Janeiro (MNRJ); Museu de Zoologia João Moojen de Oliveira and Universidade Federal de Viçosa, Minas Gerais (MZUFV). Advertisement calls of adtult males were obtained from the following sound archives: Fonoteca Neotropical Jacques Vielliard, Unicamp, Campinas, São Paulo (FNJV).

Anatomical characters based on the osteology and external morphology were used for the construction of the phylogeny, following standards nomenclature proposed by Cei (1980), Heyer *et al.* (1990), and Lynch & Duellman (1997). As external groups (outgroups) we used *Hylodes phyllodes* Heyer & Cocroft 1986 (Hylodidae), *Cycloramphus rhyakonastes* Heyer 1983, *Cycloramphus eleutherodactylus* (Miranda-Ribeiro 1920) and *Zachaenus parvulus* (Girard 1853) (Cycloramphidae). The analyzed species are presented in Table S1. The characters and states were defined as shown in Table S2.

Osteology. Most of the species were color-stained (see detailed methods in appendix I) in to access the skeleton. However, for endangered species (*Thoropa lutzi* and *T. petropolitana*) we did microtomographies, avoiding destruction of rare collection materials (Fig. S1) (Table S3).

Osteological measurements (Fig. 2 A, B and C) followed the terminology of Duellman & Trueb (1994) and were used together in the form of the following proportions: SKL/MIL, SKW/MIL, SKW/SKL, LV2/MIL, LSV/MIL, LPA/SKL, WFP/SKL, WFF/SKL, LSP/MIL and PBL/SKL. Abbreviations for all measurements are presented in Tables S3 and S4. Furthermore, we characterized the skull shape, shape of the anterior apical process of the frontoparietal, nasal shape, lateral process of the nasal, and terminal phalanx shape.

The morphometric measurements of bones in color-stained specimens were made with a digital caliper with 0.05 mm precision and a Zeiss stereomicroscope Stemi DV4. For morphometric measurements of osteology in 3D microtomography images we used the Skyscan-1173 Bruker® microtomograph and MicroDicom Viewer software.

External morphology. Measurements of external morphology (Fig. 2 D and E) were used in the form of the following proportions: HW/SVL, HL/SVL, HL/HW, THL/SVL, TBL/SVL, THL/TBL, FL/SVL, ED/SVL, END/SVL, TD/SVL, TD/HW, HnL/SVL, ED/HW and END/HW, following the terminology of Cei (1980) (Tables S4 and S5). As additional characters we also made use of the callus in front of the tympanum, tympanum visibility, evidence of supra-tympanic membrane, skin rugosity on dorsal view, skin rugosity on lateral view, disposition of nuptial spines on thumb, iris reticulation, fingers length formula, toes length formula, toes fimbriae, toes membrane, vocal sac, and tympanum diameter relative to the eye diameter. Measurements were made with a digital caliper with 0.05 mm precision and a Zeiss stereomicroscope Stemi DV4.

For each dimensional and proportional character of the osteology and external morphology we used the mean among all individuals of each species, both for the ingroup and outgroup. These values were used as a reference for the definition of the character states.

Behavior. Ecological characters were also used, such as: reproductive mode (*sensu* Haddad & Prado, 2005), and advertisement call parameters (dominant frequency and number of notes per call).

Advertisement calls. We analyzed the calls using the software Raven Pro 64 1.5 with default configurations of window and overlap depending of the quality of record and based on the terminology of Toledo *et al.* (2014) and Peloso *et al.* (2014). We described the calls of five species in detail, with two populations of *T. lutzi*. We considered five bioacoustical characters: peak of dominant frequency, minimum and maximum frequency (extracted from the spectrogram), call duration (measured in the oscillogram) and number of notes per call. We did not include characters based on pulses (pulses/note and pulse duration), because it is subjected to larger amount of error. We also did not include intervals between calls and notes, because these variables are subjected to climate differences and social context.

Tadpole external morphology. We analyzed the following characters: body shape in dorsal view, body shape in lateral view, lower jaw sheath, oral disc, eye position, nostrils position, labial tooth raw formula (LTRF), spiracle position, abdominal disc, abdomen flap, pattern of color on dorsal tail region, size of the dorsal tail fin, size of the ventral fin tail and size of the vent tube (see appendix III). The position of spiracle, vent tube, labial tooth row formula (LTRF) and the measurements were taken on the preserved tadpoles following the terminology of McDiarmid & Altig (1999). Additionally, we included the belly flap measurement following Heyer *et al*. (1990). Except for *Cycloramphus eleutherodactylus*, *Zachaenus parvulus* and *Thoropa* sp. (aff. *taophora*), which was not analyzed due to lack material in collections.

Phylogenetics. Phylogenetic relationships among the species of the genus *Thoropa* were assessed by cladistic using the principle of maximum parsimony. We did not assign weight to the characters. In cases where a character was not known or was not applied, the state was coded with a question mark "?" or hyphen "-". The characters were coded in a matrix, using the Mesquite 2.75 software (Maddison & Maddison 2011).

We analyzed the matrix (Tables S2, S6) using TNT 1.1 software (Goloboff *et al.* 2008) set to 10,000 trees, in memory, rooting the external group, and using the "New Technology Search" with pre-existing configuration with addition of the parameters Ratchet and Drift looking for the minimum length of three times and after collapsing the trees, in the construction of trees the data were rearranged several times. Then, we used the data from this analysis retained in the memory in a new search with Traditional Search has been made for the replacement algorithm using TBR (tree bisection reconnection) and collapsing the trees, rearranged the data again, thereby generating the most parsimonious trees. Resampling analysis was done using the methods of Bootstrap and Jacknife with 10,000 replications, which generated trees with their values.

For consensus tree, the trees was temporarily collapsed when minimum branch length was = 0, using strict consensus. After that, we did the Bootstrap and Jacknife analysis. We used in TNT software the Bremer Supports with 100 steps for suboptimal and using the Traditional Search, stopping when maxtrees hit and collapsing trees after the search.

Results

Our matrix comprises 86 characters of which 25 are of osteology, 40 of external morphology, seven of ecology and bioacoustics, 14 of external morphology of tadpoles (Table S2).

We analyzed matrix using the "New Technology Search" resulted in 315,291 rearrangements and using the "Traditional Search" with branch swapping (TBR) resulted in 2,822 rearrangements with five equally parsimonious trees (Fig. S2).

Of the five trees, according to the known systematic, we choose one monophyletic and another paraphyletic, the most appropriate according to the known evolutionary history of the genus *Thoropa*. Comparing the Bootstrap and Jackknife values we opt for the paraphyletic tree, rather than the monophyletic one, because those values were higher in the paraphyletic tree (Fig. 3). The characters that support the differences (number of steps and rooting in nodules) between monophyletic and paraphyletic trees are: 11, 12, 13, 39, 42, 56, 57, 82 and 84 (see table S2). We built a consensus tree using the same data for five most parsimonious trees, using five steps to suboptimal, resulting in 80 trees. Based on the analysis of Bootstrap and Jackknife for consensus tree we have the confirmation of frequencies (Fig. 4). The Bremer Supports in 2,384 trees show how longer should be the branches before node collapses (Fig. 5).

According to our analysis the most parsimonious evolutionary history occurred in two different lineages or clades. The dichotomy shown is supported in part by Bremer Support on branch with value four, but the values of Bremer Support is not shown for small species of *Thoropa*, represented by a polytomy.

The advertisement calls of *Thoropa* species are different, especially when comparing the clades of the smaller to the larger species. Calls of *T. lutzi* and *T.*

petropolitana are simple, with of only one note, whereas the calls of the other species are composed: *T. megatympanum* has three notes, *T. taophora* has four notes, and *T. miliaris* three to five notes (Fig. 6).

The advertisement call of *Thoropa lutzi* from municipality of Cataguases, Minas Gerais state is single (with one note) with a mean of 1,246 pulses, and short (the mean duration is 434 ms). The dominant frequency is 2.07 kHz, the mean minimum frequency is 1.58 kHz and the mean maximum frequency is 6.94 kHz. The interval between pulses and pulse duration is about 1 ms (Fig. 6A, Table 1). One male was recorded (FNJV 31426); air temperature 20ºC. Another individual from Morro do Sumaré, municipality of Rio de Janeiro (type locality), state of Rio de Janeiro was recorded (FNJV 31426). Its advertisement call is single and has a mean duration of 191.8 ± 5.8 ms (range: 182 – 197), mean interval between calls has a mean duration of 26 seconds; notes had a mean of 371.6 ± 25.17 (range: 345 – 411) pulses per note. Mean peak of dominant frequency is 1.78 kHz, mean minimum frequency is 0.24 ± 0.08 kHz (range: 0.15 – 0.37) and the mean maximum frequency is 13.11 ± 0.65 kHz (range: 12.21 – 11.38) (Fig. 6B, Table 1).

The advertisement call of *Thoropa petropolitana* has a mean duration of 39.5 ± 5.06 ms (range: 36 – 47), mean interval between calls of about 22 seconds, and each call consists of a single note with a mean of 12 ± 1.09 (range: 11 – 14) pulses per note. The mean peak of dominant frequency is 0.75 kHz, the mean minimum frequency is 0.11 ± 0.43 kHz (range: 0.08 – 0.16) and the mean maximum frequency is 15.46 ± 0.96 kHz (range: 13.98 – 16.50) (Fig. 6C, Table 1). One male was recorded in the municipality of Petrópolis, Rio de Janeiro state (FNJV 31759).

The advertisement call of *Thoropa megatympanum* has a mean duration of 284.33 ± 7.55 ms (range: 273 – 295), mean interval between calls of about 11 seconds, and each call consists of three notes, the first note with a mean of 213 ± 9.33 (range: 199 – 227) pulses per note; the second and third notes with a mean of 158 ± 1.78 (range: 155 – 160) pulses per note. The mean peak of dominant frequency is 2.64 ± 0.96 kHz (range: 1.21 – 3.45), the mean minimum frequency is 0.26 ± 0.05 kHz (range: 0.21 – 0.34) and the mean maximum frequency is 6.76 ± 0.54 kHz (range: 6.02 – 7.58) (Fig. 6D, Table 1). One male was recorded in the municipality of Grão Mogol, Minas Gerais state; air temperature 20ºC (FNJV 31421).

The advertisement call of *Thoropa taophora* is composed of four notes with a mean of 79 ± 2 (range: 76 – 80) pulses per note. The mean duration of call in ms is 278 ± 26.22 (range: 239 – 300) and the mean interval between the calls is 9.35 ± 3.30 (range: 6.05 – 12.66) seconds. The peak of dominant frequency is 0.689 kHz, the mean minimum frequency is 0.05 ± 0.07 kHz (range: 0 – 0.15) and the mean maximum frequency is 5.91 ± 0.78 kHz (range: 5.08 – 7.07) (Fig. 6E, Table 1). One male was recorded in Boracéia, São Paulo state (Heyer *et al*. 1990).

The advertisement call of *Thoropa miliaris* is composed of three to five notes. The first note with a mean of 122 ± 38.2 (range: 92 – 165) pulses per note; the other notes with a mean of 81.67 ± 2.52 (range: 79 – 84) pulses per note. The mean duration of call in ms is 301 ± 13.74 (range: 122 – 418) and the mean interval between the calls is 10 ± 17.91 (range: 0.23 – 36.76) seconds. The mean peak of dominant frequency is 1.59 ± 0.83 kHz (range: 0.37 – 2.25), the mean minimum frequency is 0.14 ± 0.53 kHz (range: 0.09 – 0.18) and the mean maximum frequency is 19.52 ± 1.18 kHz (range: 18.51 – 21.21) (Fig. 6F, Table 1). One male was recorded from Morro do Sumaré, municipality of Rio de Janeiro, state of Rio de Janeiro (FNJV 31770).

Apomorphies. Based on our characters were found the following apomorphies:

Thoropa taophora: Proportion among eye-nostril distance and head width (END/HW) > 24.38, this character (52) belongs to external morphology and its value is less than expected, making it unique; Vent tube, character 86, belongs to tadpole external morphology, being not visible it is unique. See table 2.

Thoropa megatympanum: Proportion among tibia length and snout-vent length (TBL/SVL) > 54.33, character 43, of external morphology, the value is less than expected and is exclusive; Proportion among foot length and snout-vent length (FL/SVL) > 75.16 character 45 of external morphology, its value is less than expected and unique. See table 2.

Thoropa saxatilis: The character 22 of the osteology is exclusive, the apical anterior process of frontoparietal has a presence of dual process; Proportion among head length and head width (HL/HW) > 78.64, character (41) belongs to external morphology and its value is less than expected, making it unique; Eyes position character 77 that belongs to tadpole external morphology. Being dorsolateral this character is unique; Labial tooth raw formula (LTRF), character 79 of tadpole external morphology, unique for 1(1)/3(1). See table 2.

Thoropa petropolitana: Proportion among skull width and mandible-ischium length (SKW/MIL) > 32.42, character 12 of osteology, its value is more than expected; Proportion among eye diameter and snout-vent length (ED/SVL) > 14.11, character 46 of the external morphology, exclusive; Proportion among tympanum diameter and snout-vent length TD/SVL > 6.91, external morphology, character 48, its value is less than expected; Iris not reticulated, exclusive, character 59 of the external morphology; Jaw sheat lower part of the tadpole external morphology. The character 75 represent short and narrow with rounded tip. See table 2.

Thoropa lutzi: Skin on dorsal view, character 56 of the external morphology that presents many apparent glands, exclusive character. See table 2.

Taxonomic history of Thoropa petropolitana *and* T. lutzi. Wandolleck (1907) described a new species and named it *Hylodes petropolitanus* presenting the external morphology and part of the very well detailed osteology through colorful illustrations and photographs. Miranda-Ribeiro (1923) commented that *Hylodes petropolitanus* were actually juveniles of *Ololigon abbreviatus*. Noble (1925) suggested that *Hylodes petropolitanus* should be included in the *Borborocoetes* genus. Miranda-Ribeiro (1926) reaffirms, now using the genera *Ololygon*, that *O. petropolitanus* is a synonym of *Ololygon miliaris*. Noble (1927) observed morphological similarities between larvae and reproduction of *Hylodes petropolitanus* and *Borborocoetes miliaris* and included a synonymy *Hylodes petropolitanus* in the genus *Borborocoetes*, as *Borborocoetes petropolitanus*. Müller (1927) suggested that *Eleutherodactylus petropolitanus* was a synonym of *Hylodes petropolitanus*. Noble (1931) observed proximity between species *Borborocoetes miliarius* and *Hylodes petropolitanus*.

Cochran (1938) described *Thoropa lutzi* with a brief diagnosis. Myers (1946) questioning the identity of some individuals of *Thoropa lutzi* and placed them in the genus *Eupsophus* (as *E. lutzi*). Lutz (1954) did not accept the genus *Ololygon* for these specimens, because *Thoropa* was described earlier. Lutz (1954) referred at that time to *Thoropa miliaris* and *Thoropa petropolitana*, questioning the validity of *Thoropa lutzi*. Cochran (1955) adopted the genera *Eupsophus* Fitzinger for *Eupsophus lutzi*, *Eupsophus miliaris* and *Eupsophus petropolitanus*. A decade later, Bokermann (1966) commented on the type locality of Brazilian amphibians and referred to these species as *Thoropa*: *Thoropa lutzi*, *Thoropa miliaris* and *Thoropa petropolitana*. Lutz (1972) did a comment on *Thoropa miliaris*, *Thoropa petropolitana* and *Thoropa lutzi*, and this latest recognizes as a synonym of *Thoropa miliaris*. Lynch (1972) presented results of osteological studies, recognizing the genera *Eupsophus*, *Ischnocnema* and *Thoropa* and was convinced that the species *Thoropa lutzi*, *Thoropa miliaris* and *Thoropa petropolitana* were properly allocated (see also Table S7).

Taxonomic implications. As a consequence of the observed paraphyletism we opted for placing the smaller species (currently *T. petropolitana* and *T. lutzi*) in a new genus. This clade is the one that should be renamed as the clade with the larger species contains *T. miliaris*, the first *Thoropa* species described (Spix 1824). As we could not revalidate any of the generic names already attributed for the species of *Thoropa* (Table S7), we hereby propose a new genus. This genus is supported by the phylogeny and also by morphological, bioacoustical and geographical data (see below).

"Thoropa" (pequena), *new genus*

Type species. Thoropa petropolitana (Wandolleck, 1907).

Diagnosis. The genus *"Thoropa" (pequena)* is diagnosed by the following combination of synapomorphic characters: i) width of the frontoparietal fenestra larger than 1.19 mm; ii) the proportion of length of the vertebra II / mandible to ischium length larger than 22.34; iii) eye to nose distance > 4.18 mm; iv) tympanum diameter > 3.08 mm; v) absence of callus in front of the tympanum; vi) few or no glands in the lateral skin; vii) fingers length formula as I<II<IV<III; viii) aggregation in small groups; ix) the advertisement call is

simple, with of only one note, whereas the calls of the other species are composed; x) very evident abdominal disc in tadpoles, with folds exceeding lateral margins of the body; xi) bilobed abdominal flap in tadpoles.

Species within the genus "Thoropa" (pequena) *gen. nov.*

"Thoropa" (pequena) petropolitana (Wandolleck, 1907) *comb. nov.*

"Thoropa" (pequena) lutzi Cochran, 1938 *comb. nov.*

Comparison with the genus Thoropa. The *"Thoropa" (pequena)* genus differs from *Thoropa* genus in morphological and osteological aspects as presently listed. However, what highly notable is the size difference between the genus, species of *"Thoropa" (pequena)* are very small and quite slender does not exceed 22 mm in length, while the smallest species of the genus *Thoropa* has the maximum size about 40 mm and the largest 78 mm.

The advertisement calls of both genera are different. The advertisement call of *"Thoropa" (pequena)* consists of only one note (single) and in case of *"Thoropa" (pequena) lutzi* the call is longer than the call of *Thoropa* species, which in turn have three to five notes (composed) depending of the species. Likewise, the advertisement call of *"Thoropa" (pequena) petropolitana* that is a short single note repeated at irregular intervals ranging from 22 seconds. The number of notes in *Thoropa* species varies among species, *T. megatympanum* has three, *T. taophora* four and seven to twelve to *T. miliaris*. These calls resemble a goat voice and it is very evident that they consist of many notes and they are repeated at irregular intervals, sometimes beyond a minute (Bokermann 1965). The frequencies of call of *"Thoropa" (Pequena) lutzi* from Minas Gerais state varies from 1.58

kHz up to 6.94 kHz, while *"Thoropa" (Pequena) lutzi* from Rio de Janeiro state varies from 0.24 kHz up to 13.11 kHz, indicating the possibility of different species. The frequencies of *T. taophora* varies between 0.05 kHz up to 5.91 kHz, mean interval between calls is 9. 35 seconds and the frequencies of call of *T. miliaris* varies between 0.14 kHz up to 19.52 kHz in low pitched and the frequencies of call of *T. megatympanum* varies between 0.26 kHz up to 6.76 kHz, the first note of call is composed by a mean of 213 pulses while from the second and third notes are composed by a mean of 158 pulses. The advertisement call of *T. megatympanum* resembles the call of *T. taophora* and *T. miliaris*, but is shorter and repeated at shorter intervals of approximately 11 seconds. In *T. miliaris* the first note of call is composed by a mean of 122 pulses while from the second to last note are composed by a mean of 81.67 pulses and the notes are grouped by pairs.

The genus *Thoropa* occurs from sea level in case of *T. miliaris*, *T. taophora* and *Thoropa* sp. (aff. *taophora*), and often breeding in rock shores (Sazima 1971; Abe & Bicudo 1991). According to Feio (2002) *T. miliaris* can be found up to 1500 meters of elevation. The species of *"Thoropa" (pequena)* genus they are found above 650 meters of elevation (Santa Teresa - ES), not occurring at sea level.

With respect to larval form, the genus *"Thoropa" (pequena)* present a very evident abdominal disc, with folds that exceed the lateral margins of the body, not exceeding lateral margins in tadpoles of *Thoropa* genus.

Miniaturization. We tested the hypothesis that the small size of the two species of *"Thoropa" (pequena)* is an instance of miniaturization and was not found osteological effects of miniaturization such as: fused bones or bones lost. Then, the fact of species of

"Thoropa" (pequena) be smaller than those of *Thoropa*, based in Yeh (2002) and Trueb & Alberch (1985) about miniaturization in amphibians, this is not a case of miniaturization.

Conservation

Independently of being a distinct genus, both species of *"Thoropa" (pequena)* are threatened due to unknown factors. Although new populations of *"Thoropa" (pequena) lutzi* have been discovered in the last years, additional investigation is necessary to assure the correct specific identification, because they could also belong to different species, not described yet. Carvalho-e-Silva *et al*. (2000) referring to amphibians threatened with extinction in in the state of Rio de Janeiro, cite *Thoropa lutzi* in category "critically endangered - CR" arguing that the species has not been seen for years. Caramaschi *et al*. (2000) on amphibians threatened with extinction in Rio de Janeiro refers to the decline and disappearance of *"Thoropa" (pequena) lutzi* and include the category "presumably endangered" in Rio de Janeiro. In the state of Espírito Santo in accordance with the Espécies da Fauna Ameaçadas de Extinção no Estado do Espírito Santo (Passamani & Mendes 2007), this species is categorized as endangered (EN) with suggestions of population decline due to anthropogenic activities. In the state of Minas Gerais two new population was discovered (R. N. Feio personal communication) without data about its status yet. Then, in accordance with the Brazilian most recent redlist (DOU 2014) this species is categorized as data deficient (DD).

Since the end of the twentieth century, there is a concern about the species of the genus *"Thoropa" (pequena)*, mainly in the state of Rio de Janeiro, where they have not been found more for a long time. Heyer (1988) mentioned that *"Thoropa" (pequena) petropolitana* was common in rocky cliffs in the Serra of Orgãos, Teresópolis, Rio de Janeiro. However it have never been found again since 1979. Caramaschi *et al*. (2000) on amphibians threatened with extinction in Rio de Janeiro refers to the decline and disappearance of *"Thoropa" (pequena) petropolitana* and include the category "endangered" in Rio de Janeiro. Actually being categorized as endangered (EN) by the Brazilian redlist of threatened species (DOU 2014).

Thoropa saxatilis in the state of Rio Grande do Sul and Santa Catarina in accordance of Brazilian redlist of threatened species (DOU 2014) is categorized as vuklnerable (VU) with suggestions of population decline due to anthropogenic activities.

Finally, the population of *"Thoropa" (pequena) lutzi* in the state of Espírito Santo and two new population recently discovered at the state of Minas Gerais need further investigation, in our view, they are different species of *"Thoropa" (pequena) lutzi* and *"Thoropa" (pequena) petropolitana*. It is our understanding that *"Thoropa" (pequena) lutzi* the state of Espírito Santo is endangered due to human activities, possibly their habitat is becoming extinct, and new species of the state of Minas Gerais for now we know nothing. So more attention is needed for these species in order to prevent them from avoiding the same status as the original species in the state of Rio de Janeiro.

Concluding remarks

There are no previous phylogenetics studies of the genus *Thoropa*. The exception is a phylogeographic study with *Thoropa miliaris* and *T. taophora* based on molecular data (Fitzpatrick *et al.* 2009). *Thoropa miliaris* and *T. taophora* are also terminals of a recent amphibian phylogeny (Pyron & Wiens 2011), and *T. miliaris* is represented in the amphibian tree of life of Frost *et al.* (2006). All these previous studies showed the same tree arrangement as ours. Therefore, at least for these three *Thoropa* species and for the position of *Thoropa* in relation to *Cycloramphus* and *Hylodes* the integrative set of data we used matches previous molecular phylogenies.

We believe it will be quite interesting a Cycloramphidae family phylogeny that can, probably, better shows the position of the species within the family and also will be possible to test the monophyly of the genus *Thoropa* and *"Thoropa" (pequena)* regarding the genus *Cycloramphus*. Indeed, Verdade (2005) shows that *T. miliaris* is a sister group of *Cycloramphus* and also suggests the synonymization of *Zachaenus* with *Cycloramphus*. Hereby, our results also corroborate with such proposition, however our dataset is limited.

It is necessary more data from the external morphology, osteology and molecular to investigation of populations of *"Thoropa" (pequena) lutzi* from Minas Gerais and Rio de Janeiro states, because the analysis of the advertisement calls indicated that these populations may not be the same species.

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Tables

Table 1. Advertisement call parameters of species of the genus *Thoropa*. Values presented as mean ± standard deviation (range; sample size). When only one value is presented the sample size is equal to 1.

Parameter / Species	***Thoropa megatympanum***	***Thoropa miliaris***	***Thoropa taophora***	***Thoropa petropolitana***	***Thoropa lutzi***	***Thoropa lutzi***
Call duration (ms)	284.33 ± 7.55 (273 – 295); 3	301 ± 13.74 (122 – 418); 5	278 ± 26.22 (239 – 300); 3	39.5 ± 5.06 (36 – 47); 4	434	191.8 ± 5.80 (182 – 197); 5
Pulse duration (ms)	3; 47	1; 12	2; 28	0.3; 10	1; 10	1; 12
Peak of dominant frequency (kHz)	2.641 ± 0.96 (1.206 – 3.445); 5	1.593 ± 0.83 (0.375 – 2.250); 4	0.689; 3	0.750; 5	2.067	1.781; 5
Minimum frequency (kHz)	0.259 ± 0.05 (0.208 – 0.335); 5	0.139 ± 0.53 (0.09 – 0.186); 4	0.051 ± 0.07 (0 – 0.154); 3	0.110 ± 0.43 (0.08 – 0.16); 5	1.581	0.238 ± 0.08 (0.149 – 0.372); 5
Maximum frequency (kHz)	6.764 ± 0.54 (6.023 – 7.576); 5	19.525 ± 1.18 (18.512 – 21.209); 4	5.909 ± 0.78 (5.081 – 7.074); 3	15.462 ± 0.96 (13.985 – 16.499); 5	6.939	13.115 ± 0.65 (12.207 – 11.384); 5
Notes per call	3; 5	4.25 ± 0.96 (3 – 5); 4	5 ± 1.73 (4 – 7); 3	1	1	1
Pulses per note, except in the first note of *T. miliaris* and *T. megatympanum*	158 ± 1.78 (155 – 160); 3	81.67 ± 2.52 (79 – 84); 3	79 ± 2 (76 – 80); 4	12 ± 1.09 (11 – 14); 5	1246	371.6 ± 25.17 (345 – 411); 5
Pulses per note in the first note	213 ± 9.33 (199 – 227); 3	122 ± 38.2 (92 – 165); 3	–	–	–	–
Interval between calls (s)	10.96 ± 0.24 (10.53 – 11.42); 4	10 ± 17,91 (0.23 – 36.76); 4	9.35 ± 3.30 (6.05 – 12.66); 2	21.50 ± 4.28 (14.62 – 27.50); 4	–	26.56 ± 9.05 (14.98 – 45.99); 5
Interval between notes of the same call (ms)	0.49 ± 0.05 (0.37 – 0.59); 15	0.09 ± 0.04 (0.05 – 0.18); 8	0.75 ± 0.03 (0.68 – 0.82); 8	–	–	–
Interval between pulses of the same note (ms)	3.3 ± 0.5 (2 – 4); 14	0.1; 8	5.43 ± 0.49 (5 – 6); 7	0.1; 10	1 ± 0 (0.98 – 1.02); 10	1 ± 0 (0.98 – 1); 12
Source	Present study	Present study	Present study	Present study	Present study	Present study
Record by	Toledo, L. F.	Bokermann, W. C. A.	Heyer, W. R.	Bokermann, W. C. A.	Clodoaldo, L. A.	Bokermann, W. C. A.
Voucher Nbr	FNJV 31421	FNJV 31770	–	FNJV 31759	FNJV 31426	FNJV 31772

Table 2. List of apomorphies presented by genus *Thoropa*.

Species	Apomorphy	Morphology	Character	State	
Thoropa taophora	END/HW > 24.38	External	52	0	Not
	Vent Tube	Tadpole External	86	0	Not visible
T. megatympanum	TBL/SVL > 54.33	External	43	0	Not
	FL/SVL > 75.16	External	45	0	Not
T. saxatilis	Apical anterior process of frontoparietal	Osteo	22	1	Presence of dual process
	HL/HW > 78.64	External	41	0	Not
	Eyes Position	Tadpole External	77	1	Dorsolateral
	LTRF	Tadpole External	79	2	1(1)/3(1)
T. petropolitana	SKW/MIL > 32.42	Osteo	12	1	Yes
	ED/SVL > 14.11	External	46	0	Not
	TD/SVL > 6.91	External	48	0	Not
	Iris	External	59	0	Not reticulated
	Jaw sheat lower part	Tadpole External	75	2	Short and narrow with rounded tip
T. lutzi	Skin on dorsal view	External	56	1	Present many apparent glands

Figure legends

Figure 1. *Thoropa* species distribution in the eastern portion of Brazil, including the locations analyzed with materials (colored circles) and type localities (black symbols).

Figure 2. Color-stained *Thoropa miliaris* (MZUFV 4123) showing the characters measured in the present study. Overview of the animal in dorsal view (A), structure of the skull in ventral (B) and dorsal views (C).

Figure 3. Topologies obtained from maximum parsimony analysis represented by a monophyletic and a paraphyletic tree rooted in *Hylodes phyllodes*. The values in the branches on the left side indicate Bootstrap values and right side Jackknife values (P = 36) with 10.000 repetitions. The lengths of the branches contain no information. The illustrations do not match the natural size of the animals.

Figure 4. Strict consensus of 5 parsimonious trees (2,479 steps) from the total evidence analysis. The values in the branches on the left side indicate Bootstrap values and right side Jackknife values (P = 36) with 10.000 repetitions. The lengths of the branches contain no information. The illustrations do not match the natural size of the animals.

Figure 5. The most parsimonious tree of the cladistic analysis with 11 taxa and 86 characters. The values represent the Bremer Supports, which show how much longer should be the tree before a particular node collapses. The illustrations do not match the natural size of the animals.

Figure 6. Spectrogram (above) and waveform (below) of the advertisement calls of *T. lutzi* from the state of Minas Gerais (A) (brightness = 63; contrast = 71; FFT = 312), *T. lutzi* from the type locality in the state of Rio de Janeiro (B) (brightness = 45; contrast = 61; FFT = 600), *T. petropolitana* from state of Rio de Janeiro (C) (brightness = 43; contrast = 58; FFT = 115), *T. megatympanum* from the state of Minas Gerais (D) (brightness = 65; contrast = 74; FFT = 414), *T. taophora* from the state of São Paulo (E) (brightness = 76; contrast = 78; FFT = 270), and *T. miliaris* from the type locality in the state of Rio de Janeiro (F) (brightness = 42; contrast = 62; FFT = 161).

Figures

Figure 1

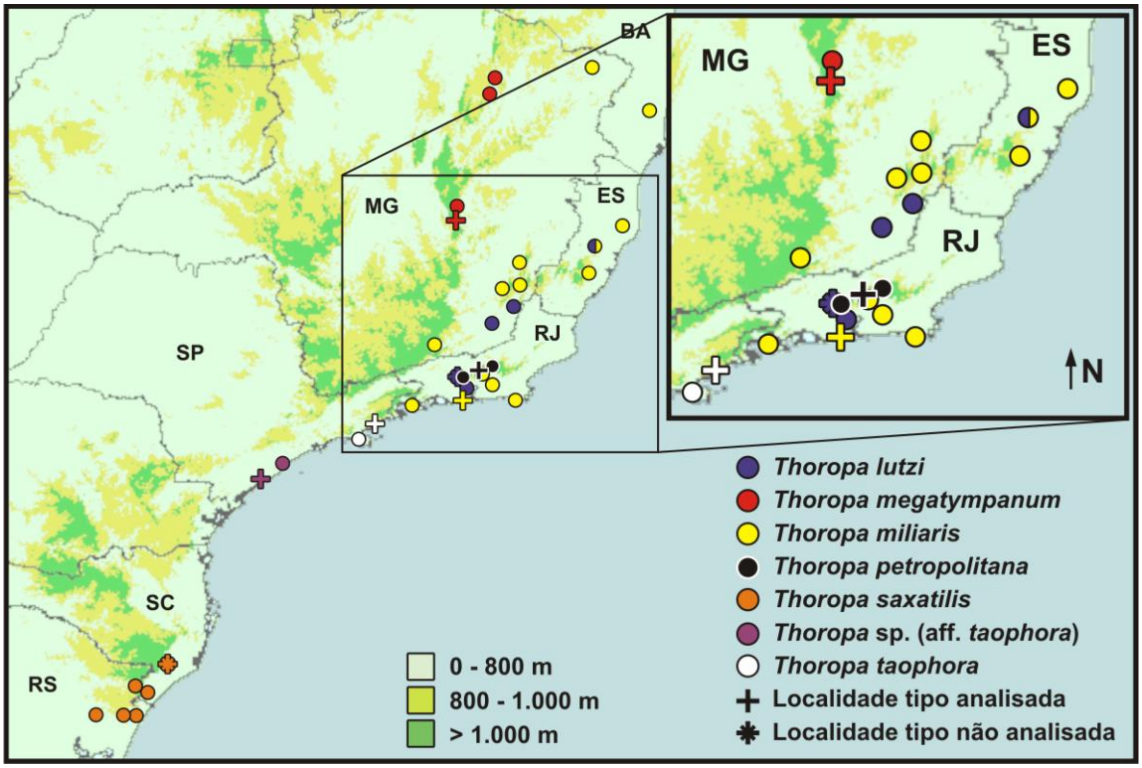


Figure 2

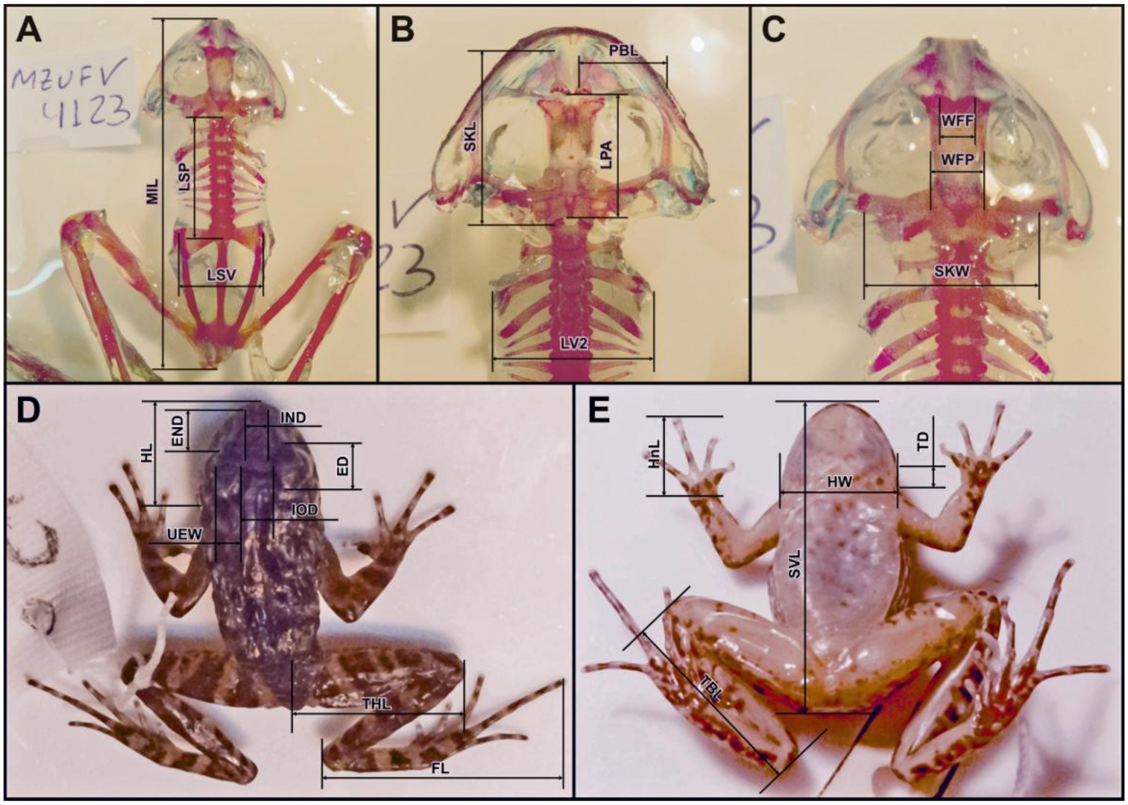
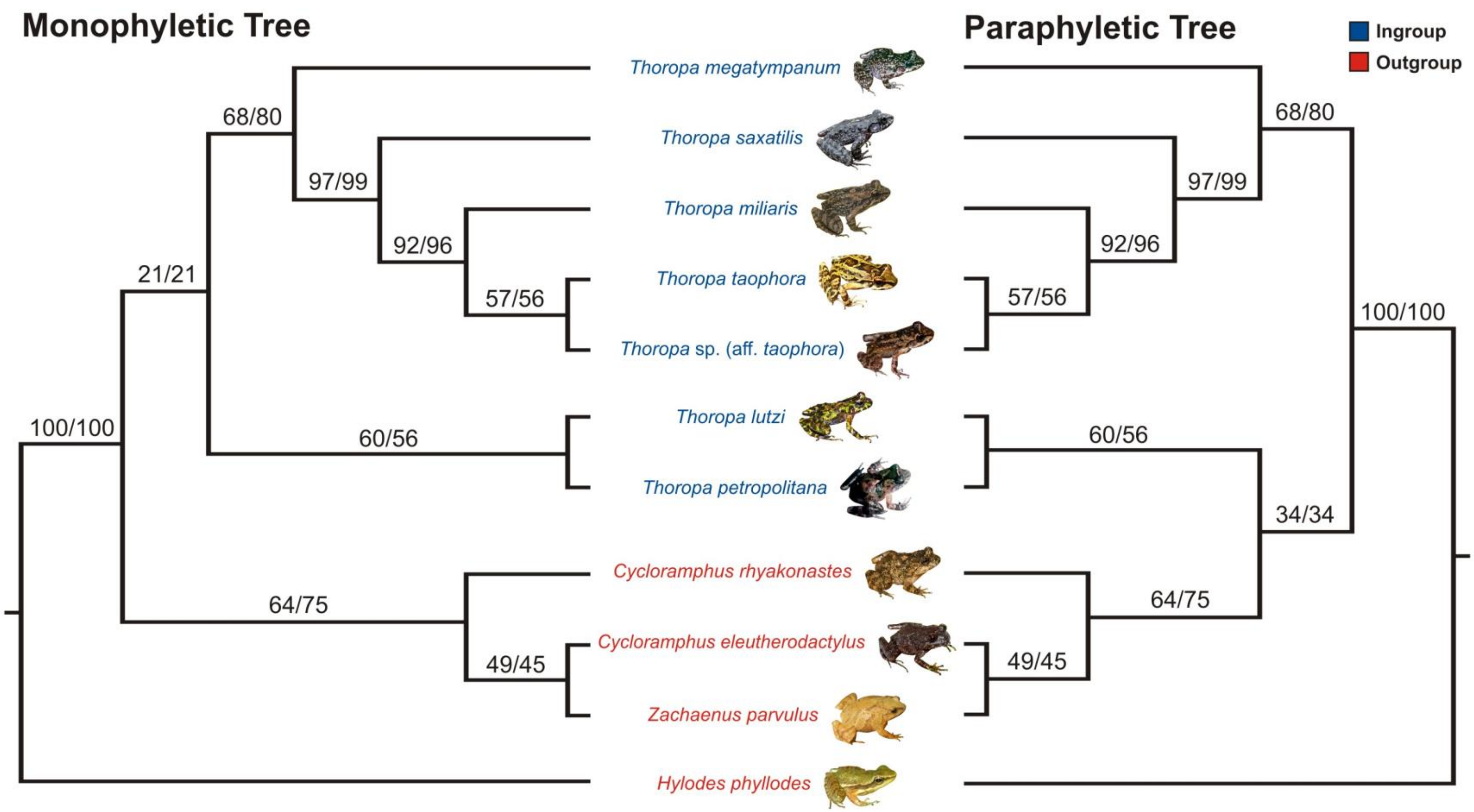
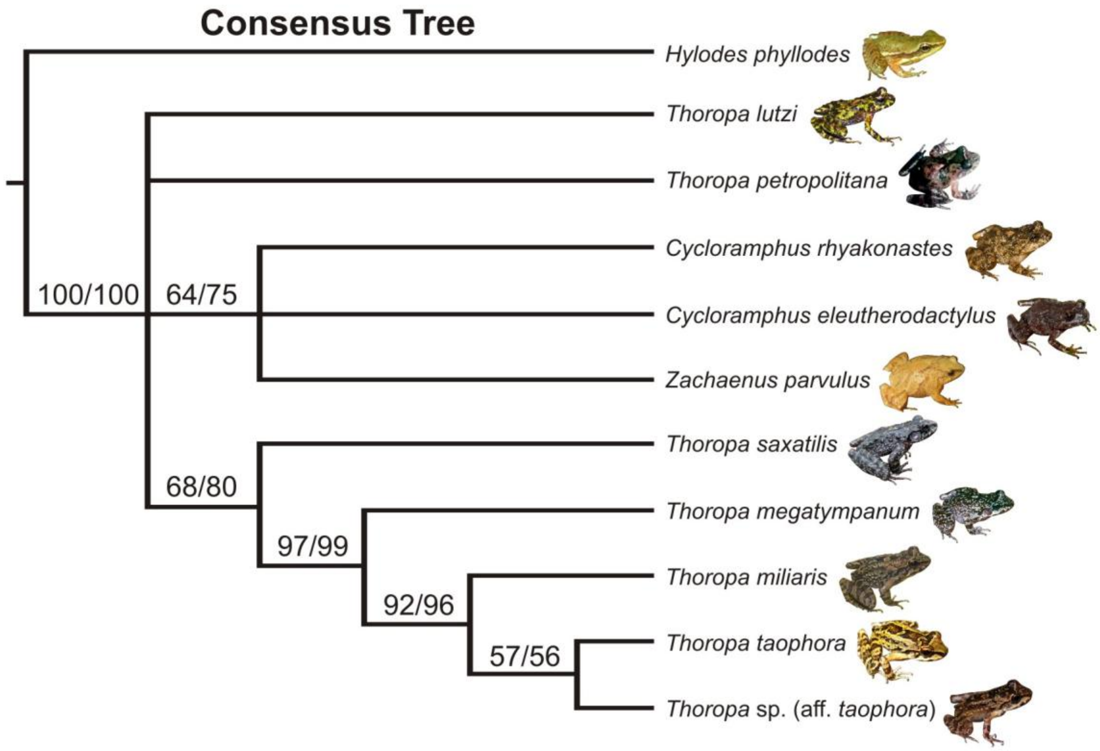

A
MZUFV
4123
MIL
LSP
LSV
B
PBL
SKL
LPA
LV2
C
WFF
WFP
SKW
D
HL
END
IND
ED
IOD
UEW
THL
FL
E
HnL
TD
HW
SVL
TBL

Figure 3



Monophyletic Tree
Paraphyletic Tree
Ingroup
Outgroup
Thoropa megatympanum
Thoropa saxatilis
Thoropa miliaris
Thoropa taophora
Thoropa sp. (aff. taophora)
Thoropa lutzi
Thoropa petropolitana
Cycloramphus rhyakonastes
Cycloramphus eleutherodactylus
Zachaenus parvulus
Hylodes phyllodes
68/80
97/99
92/96
57/56
21/21
100/100
60/56
64/75
49/45
68/80
97/99
92/96
57/56
100/100
60/56
34/34
64/75
49/45

Figure 4



Consensus Tree
Hylodes phyllodes
Thoropa lutzi
Thoropa petropolitana
Cycloramphus rhyakonastes
Cycloramphus eleutherodactylus
Zachaenus parvulus
Thoropa saxatilis
Thoropa megatympanum
Thoropa miliaris
Thoropa taophora
Thoropa sp. (aff. taophora)
100/100
64/75
68/80
97/99
92/96
57/56

Figure 5

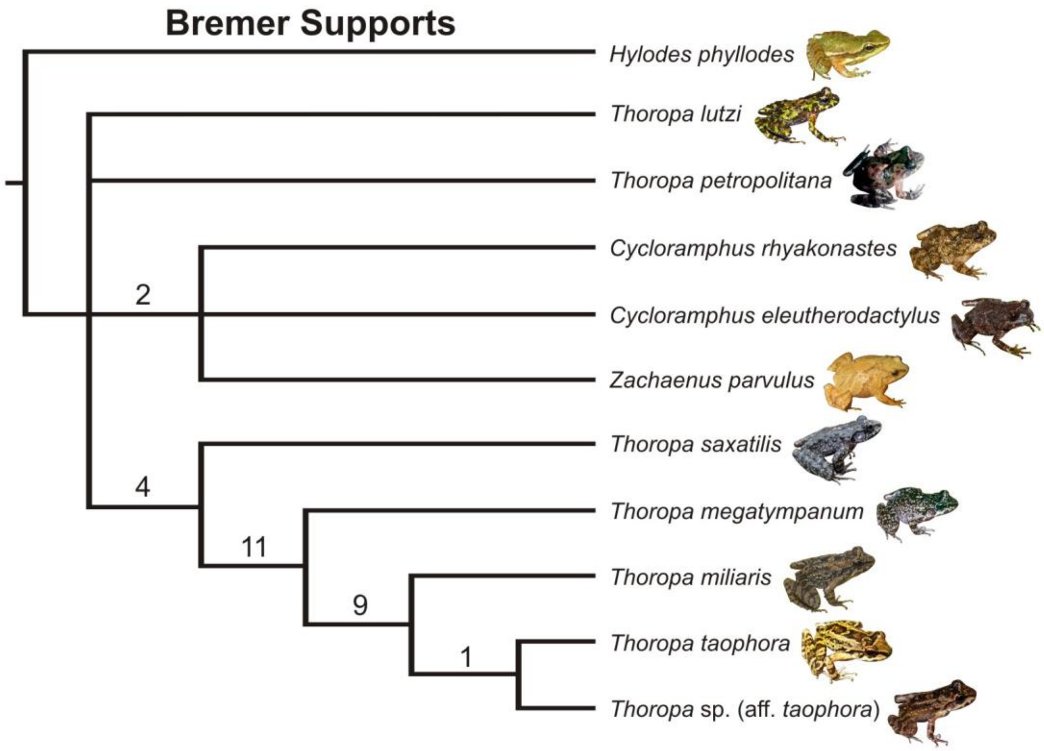
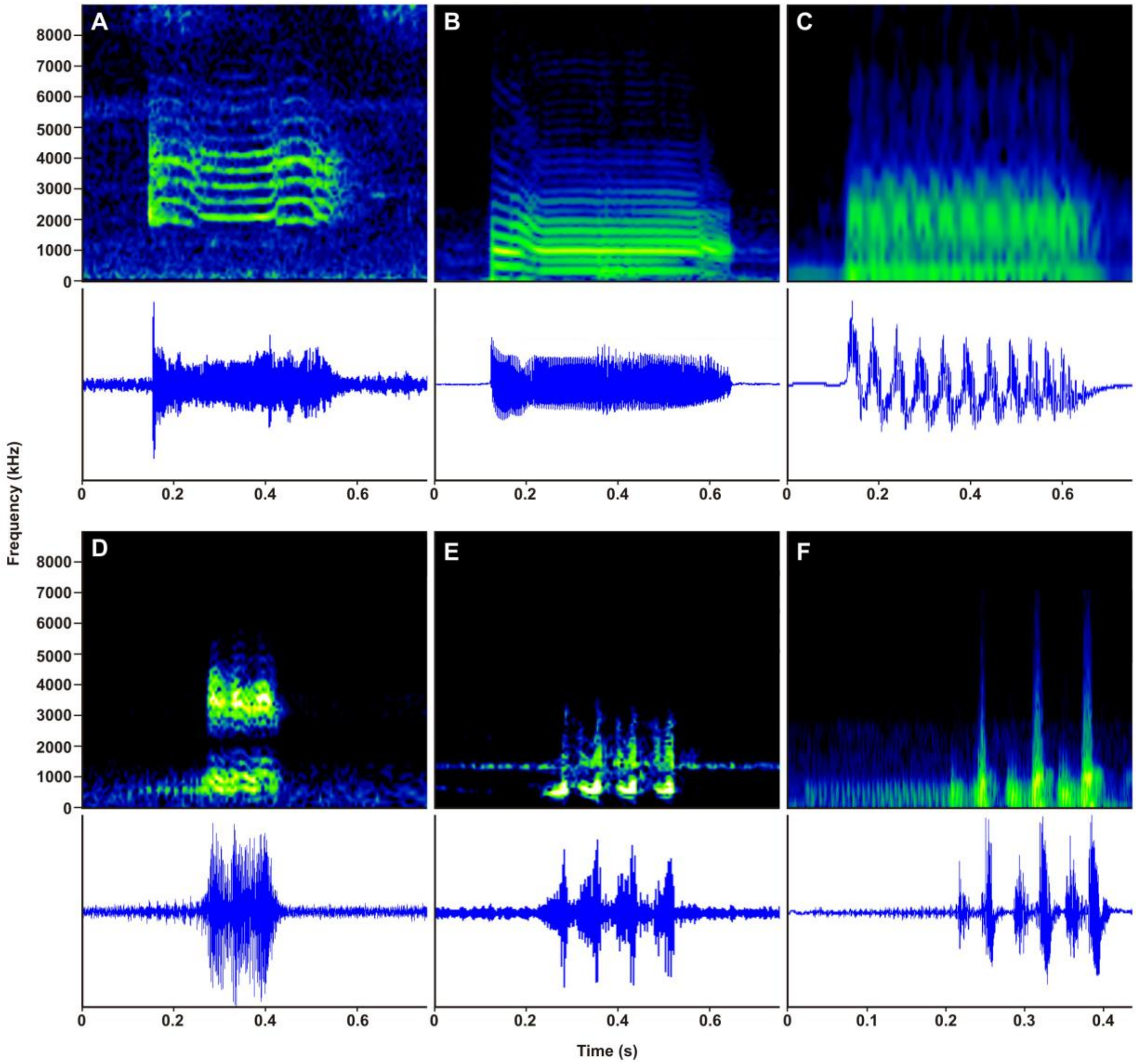

Bremer Supports
Hylodes phyllodes
Thoropa lutzi
Thoropa petropolitana
Cycloramphus rhyakonastes
Cycloramphus eleutherodactylus
Zachaenus parvulus
Thoropa saxatilis
Thoropa megatympanum
Thoropa miliaris
Thoropa taophora
Thoropa sp. (aff. taophora)
2
4
11
9
1

Figure 6


A
B
C
D
E
F
8000
7000
6000
5000
4000
3000
2000
1000
0
Frequency (kHz)
0
0.2
0.4
0.6
0.1
0.3
Time (s)

Supplementary Material

Table S1. Species currently recognized and number of individuals analyzed.

Species	Osteology	External Morphology
Hylodes phyllodes	02	20
Cycloramphus rhyakonastes	01	03
Cycloramphus eleutherodactylus	01	20
Zachaenus parvulus	01	20
Thoropa miliaris	06	20
Thoropa taophora	03	27
Thoropa sp. (aff. *taophora*)	01	06
Thoropa megatympanum	03	20
Thoropa saxatilis	02	10
Thoropa lutzi	02	14
Thoropa petropolitana	02	20

Table S2. Characters and states data used in the matrix generated by Mesquite 2.75 software.

NBR	CHARACTERS	STATE 0	STATE 1	STATE 2	STATE 3	STATE 4
OSTEOLOGY						
01	MIL > 38.05 mm	No	Yes			
02	SKL > 11.71 mm	No	Yes			
03	SKW > 12.22 mm	No	Yes			
04	LV2 > 8.40 mm	No	Yes			
05	LSV > 7.77 mm	No	Yes			
06	LPA > 8.48 mm	No	Yes			
07	WFP > 3.07 mm	No	Yes			
08	WFF > 1.19 mm	No	Yes			
09	LSP > 12.19 mm	No	Yes			
10	PBL > 4.84 mm	No	Yes			
11	SKL/MIL > 30.84	No	Yes			
12	SKW/MIL > 32.42	No	Yes			
13	SKW/SKL > 105.07	No	Yes			
14	LV2/MIL > 22.34	No	Yes			
15	LSV/MIL > 20.43	No	Yes			
16	LPA/SKL > 72.97	No	Yes			
17	WFP/SKL > 27.58	No	Yes			
18	WFF/SKL > 9.18	No	Yes			
19	LSP/MIL > 32.01	No	Yes			
20	PBL/SKL > 39.82	No	Yes			
21	Skull shape	Longer than wide	Wider than long		Width equal to the length	
22	Apical anterior process of frontoparietal	Presence of simple process (1 tip)	Presence of dual process		Multiple process	
23	Nasal	Longer than wide	Wider than long		Width equal to the length	
24	Nasal lateral process	Not present	Present		Scarcely perceptible	
25	Terminal phalanx	Normal	"T" shape			
EXTERNAL MORPHOLOGY						
26	SVL > 41 mm	No	Yes			
27	HW > 17.22 mm	No	Yes			
28	HL > 13.34 mm	No	Yes			
29	THL > 21.50 mm	No	Yes			
30	TBL > 22.46 mm	No	Yes			
31	FL > 30.85 mm	No	Yes			
32	HnL > 11.77 mm	No	Yes			

33	IND > 4.45 mm	No	Yes		
34	END > 4.18 mm	No	Yes		
35	IOD > 4.21 mm	No	Yes		
36	ED > 5.84 mm	No	Yes		
37	UEW > 3.57 mm	No	Yes		
38	TD > 3.08 mm	No	Yes		
39	HW/SVL > 41.23	No	Yes		
40	HL/SVL > 31.88	No	Yes		
41	HL/HW > 78.64	No	Yes		
42	THL/SVL > 52.01	No	Yes		
43	TBL/SVL > 54.33	No	Yes		
44	THL/TBL > 96.07	No	Yes		
45	FL/SVL > 75.16	No	Yes		
46	ED/SVL > 14.11	No	Yes		
47	END/SVL > 9.97	No	Yes		
48	TD/SVL > 6.91	No	Yes		
49	TD/HW > 17.45	No	Yes		
50	HnL/SVL > 28.68	No	Yes		
51	ED/HW > 34.93	No	Yes		
52	END/HW > 24.38	No	Yes		
53	Callus in front of the tympanum	Not present	Present		
54	Tympanum visibility	Not visible externally	Visible externally		
55	Supra-tympanic membrane	Not apparent	Evident		
56	Skin on dorsal view	Few glands or nothing	Many apparent glands		
57	Skin on lateral view	Few glands or nothing	Many apparent glands		
58	Disposition of nuptial spines on thumb	No spines	Spread, small spines	Two groups, small spines	One group, large spines
59	Iris	Not reticulated	Reticulated		
60	Fingers length formula	II<I<IV<III	I<II<IV<III	II<IV<I<III	II<I<III<IV
61	Toes length formula	I<II<V<III<IV	I<II<III<V<IV	I<II<V<III<IV	
62	Toes fimbriae	Not present	Present		
63	Toes membrane	Not present	Present		
64	Vocal sac	Simple	Bilobate		
65	Tympanum diameter relative to the eye	Small, less than 50% of the eye size	Medium, less than 75% of the eye size	Large, more than 75% of the eye size	
BEHAVIOR					
66	Reproductive mode	In the water	On the soil		

67	Aggregation mode	Solitary	Small group			
68	Song - Dominant frequency (approximately)	> 400 Hz	> 1000 Hz	> 1500 Hz	> 3000 Hz	
69	Song - Maximum frequency (approximately)	> 5000 Hz	> 6000 Hz	> 10000 Hz		
70	Song - Minimum frequency (approximately)	> 0.50 Hz	> 0.200 Hz	> 0.300 Hz		
71	Canto - Duration	> 30 ms	> 150 ms	> 200 ms	> 300 ms	
72	Song - Number of notes	One note	Three notes	Four to seven notes	More than seven notes	
TADPOLE EXTERNAL MORPHOLOGY						
73	Body shape (dorsal view)	Oval	Elliptic			
74	Body shape (lateral view)	Not depressed	Depressed	Very depressed		
75	Jaw sheat lower part	Short and wide	Long and narrow with rounded tip	Short and narrow with rounded tip	"V" shape	
76	Oral disc	Large with lips underdeveloped	Large with lips well developed			
77	Eyes position	Dorsal	Dorsolateral	Lateral		
78	Nares position	Snout eye half distance	Close to the eyes			
79	LTRF	2/3	2(1-2)/3(1)	1(1)/3(1)		
80	Spiracle position	Indistinct	Anterior median	Sinister	Posterior median	
81	Abdominal disc	No abdominal disc	Slightly evident	Very evident (folds exceed laterals)		
82	Abdominal flap	No flap	Not bilobed	Shallowly bilobed	Bilobed	
83	Padrão de cor da região dorsal da cauda	Not disruptive	Disruptive			
84	Size of the dorsal fin of the tail	1/2 of the tail	1/3 of the tail	1/4 of the tail	1/5 of the tail	Full tail
85	Size of the ventral fin of the tail	1/2 of the tail	3/4 of the tail	4/5 of the tail	Full tail	
86	Vent tube	Not visible	Short and median			

Table S3. Species examined, number of individuals (n) and average measures ± standard deviation of osteology. See table S4 for abbreviations of characters.

	T. taophora n = 3	*Thoropa* sp. (aff. *taophora*) n = 1	*T. miliaris* n = 6	*T. megatympanum* n = 3	*T. petropolitana* n = 2	*T. saxatilis* n = 2	*T. lutzi* n = 2	*Z. parvulus* n = 1	*H. phyllodes* n = 2	*C. rhyakonastes* n = 1	*C. eleutherodactylus* n = 1
MIL	62.71 ± 12.39 (53.55 – 76.81)	66.10	53.52 ± 6.82 (44.97 – 62.57)	33.54 ± 4.88 (29.99 – 39.11)	17.05 ± 1.82 (15.76 – 18.34)	38.14 ± 0.24 (37.97 – 38.31)	20.56 ± 1.24 (19.68 – 21.44)	26.86	27.59 ± 0.14 (27.49 – 27.69)	41.35	31.15
SKL	18.27 ± 4.56 (15.08 – 23.50)	19.91	17.03 ± 1.64 (14.94 – 19.52)	10.40 ± 1.33 (9.61 – 11.93)	5.23 ± 0.64 (4.78 – 5.68)	11.93 ± 0.11 (11.85 – 12.01)	5.78 ± 0.42 (5.49 – 6.08)	8.93	8.10 ± 0.12 (8.01 – 8.18)	12.67	10.55
SKW	19.58 ± 4.47 (16.17 – 24.64)	19.95	16.41 ± 1.97 (13.60 – 18.78)	10.34 ± 1.60 (9.30 – 12.19)	5.85 ± 0.75 (5.32 – 6.38)	11.92 ± 0.09 (11.86 – 11.99)	5.98 ± 0.24 (5.81 – 6.15)	10.49	7.82 ± 0.04 (7.79 – 7.84)	15.06	11.01
LV2	13.77 ± 4.20 (10.58 – 18.53)	13.68	11.09 ± 2.04 (8.57 – 13.60)	7.49 ± 1.93 (6.27 – 9.72)	4.19 ± 0.29 (3.99 – 4.40)	8.12 ± 0.20 (7.98 – 8.26)	4.86 ± 0.48 (4.52 – 5.20)	6.70	4.97 ± 0.01 (4.96 – 4.97)	10.2	7.32
LSV	12.01 ± 3.18 (9.66 – 15.63)	14.33	10.69 ± 2.00 (7.84 – 12.72)	6.21 ± 0.93 (5.64 – 7.28)	4.06 ± 0.35 (3.81 – 4.31)	7.76 ± 0.36 (7.51 – 8.02)	3.86 ± 0.11 (3.78 – 3.94)	6.05	4.69 ± 0.07 (4.64 – 4.74)	9.67	6.12
LPA	13.27 ± 2.28 (11.47 – 15.84)	14.86	11.70 ± 1.30 (9.91 – 13.16)	6.56 ± 0.73 (6.07 – 7.40)	4.02 ± 0.45 (3.71 – 4.34)	8.07 ± 0.08 (8.02 – 8.13)	4.76 ± 0.52 (4.39 – 5.13)	5.84	5.16 ± 0.01 (5.15 – 5.17)	9.14	9.93
WFP	4.16 ± 0.85 (3.58 – 5.14)	4.49	4.13 ± 0.55 (3.55 – 4.88)	2.95 ± 0.13 (2.84 – 3.10)	1.72 ± 0.19 (1.59 – 1.86)	3.57 ± 0.07 (3.52 – 3.62)	1.95 ± 0.02 (1.94 – 1.97)	2.00	2.79 ± 0.24 (2.62 – 2.96)	3.52	2.49
WFF	2.13 ± 0.77 (1.30 – 2.82)	2.19	2.53 ± 0.21 (2.23 – 2.82)	2.29 ± 0.06 (2.26 – 2.36)	0 ± 0	2.33 ± 0.13 (2.24 – 2.43)	0.65 ± 0.04 (0.62 – 0.68)	0.01	0 ± 0	0.18	0.82
LSP	21.82 ± 6.17 (17.05 – 28.79)	21.88	16.20 ± 2.34 (13.67 – 19.52)	10.54 ± 1.78 (9.43 – 12.60)	5.81 ± 0.64 (5.36 – 6.26)	12.26 ± 0.55 (11.87 – 12.65)	7.35 ± 0.81 (6.78 – 7.93)	7.14	10.14 ± 0.26 (9.95 – 10.32)	12.18	8.81
PBL	7.40 ± 0.92 (6.85 – 8.47)	9.56	7.31 ± 1.09 (5.91 – 8.87)	4.11 ± 0.69 (3.63 – 4.91)	1.61 ± 0.04 (1.58 – 1.64)	5.52 ± 0.03 (5.50 – 5.54)	2.00 ± 0.04 (1.98 – 2.03)	3.70	2.44 ± 0.17 (2.32 – 2.56)	4.89	4.67
SKL/MIL	28.96 ± 1.41 (28.13 – 30.59)	30.12	31.94 ± 1.53 (29.99 – 34.22)	31.06 ± 0.85 (30.50 – 32.04)	30.65 ± 0.45 (30.32 – 30.97)	31.28 ± 0.49 (30.93 – 31.63)	28.12 ± 0.33 (27.89 – 28.35)	33.24	29.34 ± 0.29 (29.13 – 29.54)	30.64	33.86
SKW/MIL	31.11 ± 0.94 (30.19 – 32.07)	30.18	30.69 ± 1.05 (29.07 – 32.29)	30.82 ± 0.46 (30.29 – 31.16)	34.27 ± 0.73 (33.75 – 34.78)	31.26 ± 0.44 (30.95 – 31.57)	29.10 ± 0.59 (28.68 – 29.52)	39.05	28.33 ± 0.27 (28.13 – 28.51)	36.42	35.34
SKW/SKL	107.49 ± 2.78 (104.85 – 110.40)	100.20	96.27 ± 5.55 (90.33 – 103.24)	99.27 ± 2.73 (96.77 – 102.17)	111.81 ± 0.73 (111.29 – 112.3)	99.95 ± 0.18 (99.83 – 100.08)	103.49 ± 3.31 (101.15 – 105.82)	117.46	96.55 ± 1.87 (95.23 – 97.87)	118.86	104.36
LV2/MIL	21.66 ± 2.23 (19.75 – 24.12)	20.69	20.60 ± 1.31 (19.05 – 22.42)	22.13 ± 2.51 (19.89 – 24.85)	24.65 ± 0.94 (25.31 – 23.99)	21.29 ± 0.65 (20.83 – 21.75)	23.61 ± 0.91 (24.25 – 22.96)	24.94	18.00 ± 0.12 (17.91 – 18.07)	24.67	23.49
LSV/MIL	18.99 ± 1.20 (18.03 – 20.34)	21.67	19.93 ± 2.48 (17.43 – 24.25)	18.51 ± 0.58 (17.89 – 19.03)	23.83 ± 0.48 (24.17 – 23.50)	20.36 ± 1.07 (19.60 – 21.12)	18.79 ± 0.59 (18.37 – 19.20)	22.52	17.00 ± 0.34 (16.75 – 17.24)	23.39	19.64

LPA/SKL	73.67 ± 8.25 (67.40 – 83.02)	74.63	68.91 ± 7.04 (64.88 – 83.18)	63.15 ± 1.13 (62.02 – 64.28)	77.01 ± 0.85 (77.61 – 76.40)	67.68 ± 0.01 (67.67 – 67.69)	82.16 ± 3.12 (79.96 – 84.37)	65.39	63.75 ± 0.77 (63.20 – 64.29)	72.14	94.12
WFP/SKL	22.93 ± 0.96 (21.87 – 23.74)	22.55	24.28 ± 1.94 (21.88 – 26.70)	28.62 ± 2.35 (25.98 – 30.48)	33.00 ± 0.37 (33.26 – 32.74)	29.92 ± 0.31 (29.70 – 30.14)	33.86 ± 2.08 (32.40 – 35.33)	22.39	34.45 ± 2.46 (32.70 – 36.18)	27.78	23.6
WFF/SKL	12.73 ± 6.67 (5.53 – 18.70)	10.99	14.91 ± 1.27 (13.22 – 17.13)	22.26 ± 2.15 (19.78 – 23.62)	0 ± 0	19.56 ± 0.94 (18.90 – 20.23)	11.23 ± 0.08 (11.18 – 11.29)	0.11	0 ± 0	1.42	7.77
LSP/MIL	34.42 ± 2.85 (31.83 – 37.48)	33.10	30.23 ± 1.00 (29.53 – 32.18)	31.39 ± 1.28 (29.91 – 32.21)	34.07 ± 0.09 (34.01 – 34.13)	32.14 ± 1.65 (30.98 – 33.31)	35.71 ± 1.79 (34.45 – 36.98)	26.58	36.74 ± 1.14 (35.93 – 37.54)	29.46	28.28
PBL/SKL	41.30 ± 4.80 (36.04 – 45.42)	48.01	42.86 ± 3.88 (39.44 – 48.76)	39.45 ± 1.69 (37.77 – 41.15)	30.96 ± 2.96 (33.05 – 28.87)	46.27 ± 0.20 (46.12 – 46.41)	34.72 ± 1.89 (33.38 – 36.06)	41.43	30.13 ± 1.65 (28.96 – 31.29)	38.60	44.26

Table S4. Anatomical abbreviations adopted in the present study for external morphology and osteology.

External Morphology		**Osteology**	
SVL	Snout to vent length	MIL	Mandible to ischium length
HW	Head width	SKL	Skull length
HL	Head length	SKW	Skull width
THL	Thigh length	LV2	Length of vertebra II
TBL	Tibia length	LSV	Length of sacral vertebra
FL	Foot length	LPA	Length of parasphenoid
HnL	Hand length	WFP	Width of frontoparietal
IND	Internarial distance	WFF	Width of frontoparietal fenestra
END	Eye to nose distance	LSP	Length of spine
IOD	Interorbital distance	PBL	Palatal bone length
ED	Eye diameter		
UEW	Upper eyelid width		
TD	Tympanum diameter		

Table S5. Species, number of individuals (n) and average measures ± standard deviation of external morphology. See table S4 for abbreviations of characters.

	T. taophora **n = 27**	***Thoropa* sp. (aff. *taophora*)** **n = 6**	***T. miliaris*** **n = 6**	***T. megatympanum*** **n = 20**	***T. petropolitana*** **n = 20**	***T. saxatilis*** **n = 10**	***T. lutzi*** **n = 14**	***Z. parvulus*** **n = 20**	***H. phyllodes*** **n = 20**	***C. rhyakonastes*** **n = 3**	***C. eleutherodactylus*** **n = 20**
SVL	77.35 ± 8.73 (58.93 – 92.11)	61.13 ± 5.82 (53.20 – 68.37)	56.54 ± 6.04 (47.00 – 68.44)	39.74 ± 6.62 (28.73 – 51.28)	18.88 ± 1.98 (15.15 – 21.92)	46.55 ± 5.92 (38.32 – 53.80)	21.83 ± 0.78 (20.83 – 23.40)	26.02 ± 3.06 (20.80 – 32.08)	26.85 ± 1.40 (24.40 – 29.77)	40.35 ± 2.51 (37.93 – 42.94)	35.75 ± 5.48 (28.84 – 47.30)
HW	32.67 ± 3.73 (25.05 – 39.16)	25.88 ± 2.96 (21.70 – 29.67)	24.51 ± 3.24 (20.26 – 31.31)	16.83 ± 3.23 (11.02 – 21.46)	6.81 ± 0.91 (5.35 – 8.97)	19.76 ± 2.33 (16.73 – 23.52)	7.57 ± 0.30 (7.17 – 8.17)	12.71 ± 1.50 (10.21 – 16.01)	8.34 ± 0.40 (7.80 – 9.09)	17.70 ± 0.16 (17.54 – 17.85)	16.63 ± 2.93 (13.56 – 22.74)
HL	25.84 ± 3.33 (18.73 – 32.42)	21.65 ± 3.48 (16.99 – 25.71)	19.38 ± 1.81 (15.35 – 21.88)	14.09 ± 2.58 (9.23 – 18.96)	5.79 ± 0.66 (4.79 – 7.18)	14.19 ± 2.27 (10.34 – 17.00)	6.21 ± 0.44 (5.59 – 7.10)	6.86 ± 0.68 (5.57 – 8.17)	8.66 ± 0.69 (7.19 – 10.35)	11.84 ± 0.58 (11.44 – 12.51)	12.20 ± 1.54 (9.45 – 14.60)
THL	40.32 ± 4.84 (31.50 – 48.06)	34.02 ± 3.66 (28.55 – 38.12)	30.82 ± 3.34 (25.63 – 37.85)	20.44 ± 3.56 (15.35 – 26.87)	9.99 ± 1.14 (7.43 – 12.88)	25.48 ± 3.11 (21.52 – 30.28)	11.38 ± 0.47 (10.57 – 12.14)	12.42 ± 1.81 (10.32 – 16.53)	13.42 ± 0.66 (11.94 – 14.54)	20.74 ± 0.27 (20.57 – 21.05)	17.45 ± 1.91 (14.88 – 22.12)
TBL	43.65 ± 5.01 (31.02 – 51.59)	35.26 ± 3.50 (30.57 – 39.67)	32.34 ± 3.53 (26.88 – 38.06)	20.88 ± 3.29 (15.07 – 26.46)	10.82 ± 1.11 (8.27 – 13.41)	27.01 ± 2.80 (22.52 – 30.76)	12.44 ± 0.56 (11.13 – 13.37)	11.77 ± 1.28 (9.76 – 14.42)	14.37 ± 0.89 (12.07 – 16.22)	20.04 ± 0.49 (19.57 – 20.55)	18.49 ± 2.16 (15.03 – 22.70)
FL	58.81 ± 6.71 (40.66 – 70.22)	46.69 ± 4.25 (41.04 – 51.55)	43.03 ± 4.48 (34.02 – 49.98)	27.86 ± 4.73 (18.83 – 36.62)	14.54 ± 2.02 (10.4 – 18.1)	37.03 ± 4.21 (29.97 – 43.31)	17.27 ± 0.94 (15.96 – 18.77)	17.35 ± 2.37 (12.45 – 21.92)	20.27 ± 1.03 (18.10 – 21.95)	29.72 ± 0.33 (29.35 – 29.96)	26.78 ± 3.20 (22.04 – 32.40)
HnL	22.48 ± 2.81 (16.46 – 26.78)	17.91 ± 1.92 (15.96 – 20.36)	15.70 ± 1.68 (13.06 – 18.00)	10.95 ± 2.22 (7.22 – 14.82)	5.15 ± 0.68 (3.98 – 6.69)	14.44 ± 1.73 (11.35 – 17.11)	6.63 ± 0.60 (5.54 – 7.56)	6.47 ± 1.14 (5.00 – 8.60)	8.85 ± 0.63 (7.93 – 10.14)	10.71 ± 0.23 (10.57 – 10.98)	10.13 ± 1.37 (8.03 – 12.03)
IND	8.01 ± 1.23 (5.50 – 10.08)	7.02 ± 0.74 (6.08 – 7.90)	6.26 ± 0.66 (5.28 – 7.61)	4.47 ± 0.87 (3.18 – 5.62)	2.07 ± 0.20 (1.78 – 2.67)	4.45 ± 0.87 (3.22 – 6.20)	2.16 ± 0.18 (1.79 – 2.38)	2.95 ± 0.58 (2.27 – 4.55)	3.85 ± 0.23 (3.39 – 4.26)	4.30 ± 0.26 (4.01 – 4.50)	3.37 ± 0.63 (2.50 – 4.66)
END	7.39 ± 1.03 (5.46 – 9.32)	6.70 ± 0.46 (6.00 – 7.17)	6.27 ± 1.06 (3.33 – 8.80)	4.42 ± 0.84 (3.17 – 5.92)	1.78 ± 0.17 (1.50 – 2.08)	5.36 ± 0.70 (4.32 – 6.43)	1.94 ± 0.12 (1.75 – 2.13)	2.26 ± 0.32 (1.76 – 3.00)	2.01 ± 0.15 (1.71 – 2.31)	4.27 ± 0.23 (4.14 – 4.54)	3.53 ± 0.73 (2.37 – 4.66)
IOD	7.55 ± 1.00 (5.83 – 9.06)	5.46 ± 0.88 (4.17 – 6.69)	5.98 ± 0.67 (4.65 – 7.35)	4.32 ± 0.79 (3.13 – 5.70)	1.72 ± 0.26 (1.25 – 2.27)	4.86 ± 0.67 (3.80 – 6.00)	1.82 ± 0.12 (1.60 – 2.00)	3.43 ± 0.63 (2.44 – 5.22)	2.70 ± 0.46 (1.96 – 4.20)	4.51 ± 0.01 (4.50 – 4.52)	3.97 ± 0.63 (2.88 – 5.30)
ED	10.78 ± 1.30 (8.83 – 14.01)	8.45 ± 0.88 (6.82 – 9.23)	7.96 ± 0.79 (6.66 – 9.57)	5.66 ± 1.03 (3.88 – 7.80)	2.50 ± 0.27 (2.19 – 2.94)	7.17 ± 0.79 (6.30 – 8.34)	3.11 ± 0.27 (2.55 – 3.50)	2.75 ± 0.54 (2.00 – 3.76)	3.89 ± 0.27 (3.46 – 4.46)	6.91 ± 0.06 (6.84 – 6.95)	5.04 ± 0.94 (4.03 – 6.99)
UEW	6.44 ± 0.80 (5.24 – 8.08)	5.20 ± 0.49 (4.57 – 5.93)	5.06 ± 0.60 (4.06 – 6.70)	3.62 ± 0.75 (2.43 – 4.60)	1.55 ± 0.23 (1.16 – 1.98)	4.36 ± 0.53 (3.66 – 5.11)	1.93 ± 0.24 (1.46 – 2.27)	1.70 ± 0.35 (1.09 – 2.31)	2.48 ± 0.21 (1.99 – 2.78)	3.73 ± 0.35 (3.42 – 4.11)	3.17 ± 0.51 (2.47 – 4.23)
TD	6.69 ± 0.77 (4.64 – 8.09)	4.84 ± 0.38 (4.17 – 5.10)	5.30 ± 0.69 (4.06 – 6.23)	4.38 ± 1.06 (2.53 – 6.14)	1.27 ± 0.18 (1.02 – 1.76)	6.10 ± 0.89 (4.56 – 7.55)	1.59 ± 0.13 (1.40 – 1.75)	0 ± 0	2.16 ± 0.15 (1.82 – 2.43)	0 ± 0	1.57 ± 0.43 (0.84 – 2.36)
HW/SVL	42.25 ± 1.32 (40.20 – 44.38)	42.26 ± 1.07 (40.78 – 43.39)	43.04 ± 2.62 (38.56 – 47.90)	42.21 ± 2.24 (37.99 – 45.02)	36.04 ± 2.58 (32.77 – 41.97)	42.52 ± 1.63 (39.02 – 44.91)	34.72 ± 1.33 (32.18 – 36.86)	48.95 ± 2.88 (43.69 – 56.34)	31.12 ± 1.23 (29.31 – 34.62)	43.99 ± 2.92 (41.24 – 47.06)	46.46 ± 2.91 (41.01 – 52.26)

HL/SVL	33.40 ± 1.95 (30.76 – 36.90)	35.27 ± 3.06 (31.93 – 40.02)	34.03 ± 2.69 (28.03 – 39.86)	35.74 ± 2.57 (31.63 – 40.36)	30.79 ± 3.40 (27.11 – 42.09)	30.38 ± 1.53 (26.98 – 32.43)	28.47 ± 2.07 (24.53 – 32.10)	26.48 ± 2.09 (23.48 – 31.58)	32.29 ± 2.42 (27.90 – 39.48)	29.37 ± 0.71 (28.80 – 30.16)	34.40 ± 3.38 (28.11 – 40.57)
HL/HW	79.12 ± 4.94 (71.19 – 90.70)	83.36 ± 5.43 (77.46 – 92.41)	79.27 ± 8.21 (61.40 – 91.57)	85.28 ± 5.49 (73.50 – 93.12)	85.43 ± 6.67 (71.57 – 100.29)	71.61 ± 5.41 (61.80 – 81.61)	82.15 ± 7.04 (69.03 – 95.81)	54.20 ± 4.39 (46.50 – 63.89)	103.42 ± 6.54 (89.09 – 120.48)	66.90 ± 3.37 (64.08 – 70.63)	74.32 ± 8.71 (58.48 – 89.27)
THL/SVL	52.11 ± 1.86 (49.03 – 56.28)	55.62 ± 2.04 (53.66 – 59.34)	54.22 ± 2.77 (49.71 – 59.54)	51.55 ± 2.11 (47.92 – 54.61)	52.95 ± 3.35 (46.81 – 58.75)	54.83 ± 2.34 (49.02 – 57.59)	52.15 ± 1.56 (49.34 – 54.01)	47.78 ± 4.13 (36.87 – 52.87)	50.19 ± 2.87 (44.10 – 54.72)	51.50 ± 2.64 (49.02 – 54.28)	49.24 ± 3.99 (42.10 – 56.01)
TBL/SVL	56.43 ± 1.98 (52.64 – 61.41)	57.66 ± 0.64 (56.92 – 58.60)	57.02 ± 3.22 (49.95 – 61.92)	52.70 ± 1.92 (50.04 – 57.06)	57.39 ± 3.14 (51.92 – 62.66)	58.20 ± 2.31 (54.15 – 61.10)	57.01 ± 2.13 (52.73 – 59.76)	45.49 ± 3.98 (36.29 – 53.36)	53.90 ± 3.16 (43.93 – 58.13)	49.78 ± 2.78 (46.57 – 51.59)	52.06 ± 3.57 (42.59 – 57.76)
THL/TBL	92.38 ± 2.81 (87.80 – 101.55)	96.47 ± 3.12 (93.39 – 101.27)	95.22 ± 4.07 (85.86 – 101.80)	97.87 ± 3.51 (92.13 – 105.45)	92.31 ± 4.32 (79.18 – 99.28)	94.26 ± 3.62 (87.13 – 98.73)	91.56 ± 3.51 (85.37 – 96.67)	105.38 ± 8.61 (85.28 – 116.95)	93.13 ± 5.22 (84.32 – 107.70)	103.52 ± 2.96 (100.09 – 105.25)	94.61 ± 4.91 (86.15 – 106.33)
FL/SVL	76.07 ± 3.27 (69.00 – 83.59)	76.41 ± 1.38 (74.67 – 78.60)	75.64 ± 4.93 (66.70 – 84.49)	70.65 ± 3.63 (64.39 – 76.74)	77.13 ± 8.16 (54.27 – 87.44)	79.69 ± 2.62 (76.01 – 84.03)	79.13 ± 3.35 (74.57 – 85.74)	66.91 ± 7.12 (46.54 – 79.13)	75.83 ± 3.43 (69.39 – 82.18)	73.83 ± 3.99 (69.51 – 77.37)	75.44 ± 6.01 (64.33 – 87.24)
ED/SVL	13.98 ± 1.10 (11.46 – 16.39)	13.83 ± 0.71 (12.81 – 14.52)	14.08 ± 0.98 (12.65 – 16.21)	14.05 ± 1.22 (11.32 – 16.46)	13.30 ± 1.17 (11.01 – 15.41)	15.49 ± 1.30 (12.02 – 16.78)	14.24 ± 1.11 (12.10 – 15.98)	10.53 ± 1.30 (8.83 – 12.93)	14.50 ± 0.89 (13.61 – 16.49)	17.17 ± 1.06 (16.18 – 18.29)	14.09 ± 1.03 (12.79 – 16.19)
END/SVL	9.56 ± 0.86 (7.94 – 12.08)	10.99 ± 0.40 (10.48 – 11.54)	11.40 ± 1.54 (5.40 – 13.09)	11.09 ± 0.65 (9.85 – 12.32)	9.50 ± 0.84 (8.10 – 11.18)	11.51 ± 0.57 (10.58 – 12.59)	8.89 ± 0.53 (8.16 – 9.74)	8.72 ± 1.01 (5.63 – 10.73)	7.54 ± 0.55 (6.68 – 8.97)	10.64 ± 1.20 (9.64 – 11.96)	9.84 ± 1.01 (7.75 – 11.98)
TD/SVL	8.70 ± 1.00 (6.33 – 11.18)	7.93 ± 0.42 (7.37 – 8.62)	9.20 ± 1.11 (7.97 – 11.86)	10.69 ± 1.46 (8.80 – 13.84)	6.72 ± 0.50 (6.06 – 8.11)	13.11 ± 1.11 (11.89 – 15.51)	7.29 ± 0.56 (6.53 – 8.20)	0 ± 0	8.04 ± 0.44 (7.45 – 9.04)	0 ± 0	4.38 ± 0.92 (2.55 – 6.06)
TD/HW	20.60 ± 2.38 (14.89 – 26.41)	18.77 ± 1.09 (16.98 – 20.15)	21.33 ± 2.18 (18.39 – 26.64)	25.47 ± 2.87 (20.28 – 32.55)	18.69 ± 1.51 (14.82 – 20.85)	30.84 ± 2.59 (27.25 – 36.37)	21.03 ± 1.62 (18.34 – 23.38)	0 ± 0	25.76 ± 1.41 (23.30 – 28.69)	0 ± 0	9.40 ± 1.67 (5.84 – 12.14)
HnD/SVL	29.05 ± 1.33 (26.72 – 31.33)	29.31 ± 1.60 (27.31 – 31.69)	27.48 ± 1.75 (23.88 – 31.75)	27.72 ± 1.82 (25.13 – 31.47)	27.35 ± 3.20 (22.99 – 36.35)	31.06 ± 1.41 (29.22 – 34.10)	30.38 ± 2.33 (26.41 – 33.55)	24.85 ± 2.95 (20.65 – 31.14)	33.14 ± 1.80 (29.42 – 35.89)	26.64 ± 2.16 (24.66 – 28.94)	28.51 ± 2.46 (22.52 – 32.79)
ED/HW	33.09 ± 2.50 (27.77 – 38.09)	32.71 ± 1.56 (30.53 – 34.73)	32.74 ± 2.61 (28.40 – 37.97)	33.59 ± 3.12 (26.09 – 38.71)	37.03 ± 3.66 (27.06 – 41.86)	36.40 ± 2.32 (30.82 – 39.35)	41.09 ± 3.89 (33.42 – 47.69)	21.58 ± 3.02 (17.46 – 29.49)	46.53 ± 3.30 (40.00 – 53.60)	39.04 ± 0.19 (38.87 – 39.24)	30.38 ± 2.14 (25.36 – 33.91)
END/HW	22.63 ± 2.01 (18.77 – 28.23)	26.02 ± 1.39 (24.16 – 27.64)	26.48 ± 3.72 (11.49 – 29.12)	26.51 ± 1.84 (22.20 – 29.31)	26.45 ± 2.85 (21.29 – 32.40)	27.10 ± 1.46 (25.60 – 30.17)	25.64 ± 1.68 (23.08 – 27.77)	17.83 ± 1.78 (11.87 – 20.22)	24.16 ± 1.48 (21.67 – 28.20)	24.14 ± 1.13 (23.37 – 25.43)	21.25 ± 2.39 (17.24 – 28.68)

Table S6. Matrix made using the Mesquite 2.75 software composed by 86 characters and 11 terminal taxa.

H. phyllodes	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 1 2 1 0 0 0 0 0 0 0 0 0
	0 0 0 0 0 1 1 0 0 0 1 1 0 1 1 1 1 0 0 1 0 0 1 0 0 1 2 1 0 1 1 0 0 ?
	? ? ? 3 1 0 3 0 0 0 0 2 0 0 0 4 3 0
C. eleutherodactylus	0 0 0 0 0 1 0 0 0 0 1 1 0 1 0 1 0 0 0 1 1 0 1 1 0 0 0 0 0 0 0 0 0 0
	0 0 0 0 1 1 0 0 0 0 1 1 0 0 0 0 0 0 1 1 1 0 0 0 1 1 0 0 0 ? 0 1 0 ?
	? ? ? 0 ? ? ? ? ? ? ? ? ? ? ? ? ? ?
C. rhyakonastes	1 1 1 1 1 1 1 0 0 1 0 1 1 1 1 0 1 0 0 0 2 2 1 1 0 0 0 0 0 0 0 0 0 0
	1 1 1 - 1 0 0 0 0 1 0 1 1 - - 0 1 0 - 0 0 1 1 0 1 3 1 0 1 ? 0 0 0 ?
	? ? ? 0 1 1 1 1 1 0 0 2 1 1 ? 0 0 ?
Z. parvulus	0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 0 0 0 0 1 1 0 1 1 0 0 0 0 0 0 0 0 0 0
	0 0 0 - 1 0 0 0 0 1 0 0 0 - - 0 0 0 - 0 0 0 0 0 1 2 0 0 0 ? 0 1 0 ?
	? ? ? 2 ? ? ? ? ? ? ? ? ? ? ? ? ? ?
T. taophora	1 1 1 1 1 1 1 1 1 1 0 0 1 0 0 1 0 1 1 1 1 0 2 2 1 1 1 1 1 1 1 1 1 1
	1 1 1 1 0 1 1 0 1 0 1 1 0 1 1 1 1 0 1 1 1 0 1 1 1 0 0 0 0 0 1 0 0 0
	0 0 2 2 0 2 0 1 0 0 0 0 1 2 1 2 3 0
T. sp. (aff. *Taophora*)	1 1 1 1 1 1 1 1 1 1 0 0 0 0 1 1 0 0 1 1 1 0 2 2 1 1 1 1 1 1 1 1 1 1
	1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 0 1 1 1 0 0 0 0 0 1 0 0 0
	? ? ? 2 0 ? ? ? ? ? ? ? ? ? ? ? ? ?
T. miliaris	1 1 1 1 1 1 1 1 1 1 1 0 0 0 1 0 0 1 0 1 1 0 2 2 1 1 1 1 1 1 1 1 1 1
	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 0 0 1 1 1 1 0 1 1 1 0 0 0 0 0 1 0 0 2
	2 0 3 2 0 2 0 1 0 0 1 2 1 2 1 3 1 1
T. megatympanum	0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 1 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 1
	0 0 0 1 0 1 1 0 0 1 0 1 1 1 1 0 1 1 1 1 1 0 1 1 1 0 0 0 0 0 2 0 ? 1
	1 0 2 1 1 2 0 1 0 1 1 2 1 ? 0 0 3 1
T. petropolitana	0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 0 1 0 1 0 0 1 1 0 0 0 0 0 0 0 0 0
	0 0 0 0 0 0 1 1 1 0 1 0 0 0 1 0 1 1 0 1 1 0 0 3 0 1 1 0 0 0 1 0 1 3
	2 0 0 0 1 2 2 1 0 0 1 0 2 3 1 2 0 1
T. lutzi	0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 1 0 1 0 1 0 0 1 1 0 0 0 0 0 0 0 0 0
	0 0 0 0 0 0 1 0 1 0 1 1 0 1 1 1 1 1 0 1 1 1 0 2 1 1 1 0 0 0 1 0 1 0
	1 0 3 0 1 2 0 1 0 0 1 2 2 3 1 1 2 1
T. saxatilis	0 0 0 0 1 0 1 1 0 1 1 0 0 0 1 0 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1
	1 1 1 1 1 0 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 0 1 0 0 0 2 0 ? ?
	? ? ? ? 0 2 0 1 1 1 2 0 1 ? 0 1 0 1

Table S7. Names originally used and valid combinations for the species involved with the genus *Thoropa* Cope, 1965 (Feio 2002).

Utilização original	**Combinação atual**
Rana miliaris Spix 1824	*Thoropa miliaris* (Spix, 1824)
Cystignathus missiessi Eydoux & Souleyet, 1842	*Thoropa miliaris* (Spix, 1824)
Eupsophus fuliginosus Fitzinger, 1860	*Thoropa miliaris* (Spix, 1824)
Ololygon abbreviatus Fitzinger, 1861	*Thoropa miliaris* (Spix, 1824)
Cystignathus discolor Reinhardt & Lütken, 1861	*Thoropa miliaris* (Spix, 1824)
Thoropa missiessi Cope, 1865	*Thoropa miliaris* (Spix, 1824)
Hylodes abbreviatus Hensel, 1867	*Thoropa miliaris* (Spix, 1824)
Cystignathus (Eupsophus) fuliginosus Steindachner, 1869	*Thoropa miliaris* (Spix, 1824)
Ololygon abbreviatus Steindachner, 1869	*Thoropa miliaris* (Spix, 1824)
Ololygon miliaris Peters, 1872	*Thoropa miliaris* (Spix, 1824)
Thoropa miliaris Boulenger, 1882	*Thoropa miliaris* (Spix, 1824)
Borborocoetes miliaris Boulenger, 1891	*Thoropa miliaris* (Spix, 1824)
Hylodes miliaris Wandolleck, 1907	*Thoropa miliaris* (Spix, 1824)
Hylodes petropolitanus Wandolleck, 1907	*Thoropa petropolitana* (Wandolleck, 1907)
Eleutherodactylus miliaris Müller, 1927	*Thoropa miliaris* (Spix, 1824)
Eleutherodactylus petropolitanus Müller, 1927	*Thoropa petropolitana* (Wandolleck, 1907)
Ololigon abbreviatus Miranda-Ribeiro, 1923	*Thoropa miliaris* (Spix, 1824)
Ololigon abbreviatus petropolitana Miranda-Ribeiro, 1923	*Thoropa petropolitana* (Wandolleck, 1907)
Ololigon abbreviatus taophora Miranda-Ribeiro, 1923	*Thoropa miliaris* (Spix, 1824)
Ololigon abbreviatus abbreviata Miranda-Ribeiro, 1923	*Thoropa miliaris* (Spix, 1824)
Ololigon miliaris Miranda-Ribeiro, 1926	*Thoropa miliaris* (Spix, 1824)
Ololigon miliaris petropolitana Miranda-Ribeiro, 1926	*Thoropa petropolitana* (Wandolleck, 1907)
Ololigon miliaris taophora Miranda-Ribeiro, 1926	*Thoropa miliaris* (Spix, 1824)
Ololigon miliaris abbreviata Miranda-Ribeiro, 1926	*Thoropa miliaris* (Spix, 1824)
Borborocoetes petropolitanus Noble, 1927	*Thoropa petropolitana* (Wandolleck, 1907)
Platymantes abbreviatus Luederwaldt, 1929	*Thoropa miliaris* (Spix, 1824)
Hylodes abbreviatus tanophora Luederwaldt, 1929	*Thoropa miliaris* (Spix, 1824)
Hylodes brieni White, 1930	*Thoropa miliaris* (Spix, 1824)
Eupsophus miliaris Parker, 1932	*Thoropa miliaris* (Spix, 1824)
Eupsophus miliaris Miranda-Ribeiro, 1937	*Thoropa miliaris* (Spix, 1824)
Thoropa lutzi Cochran, 1938	*Thoropa lutzi* Cochran, 1938
Eupsophus miliaris Cochran, 1955	*Thoropa miliaris* (Spix, 1824)
Eupsophus lutzi Cochran, 1955	*Thoropa lutzi* Cochran, 1938
Eupsophus petropolitanus Cochran, 1955	*Thoropa petropolitana* (Wandolleck, 1907)
Thoropa megatympanum Caramaschi & Sazima, 1984	*Thoropa megatympanum* Caramaschi & Sazima, 1984
Thoropa saxatilis Cocroft & Heyer, 1988	*Thoropa saxatilis* Cocroft & Heyer, 1988

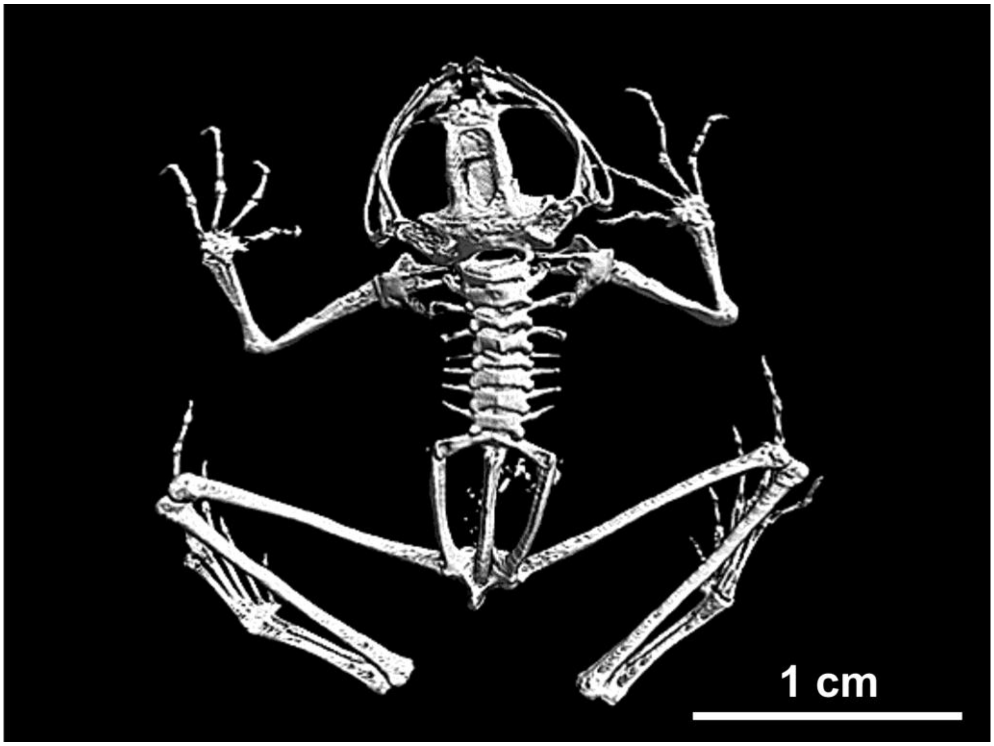


Figure S1. Skeleton of *Thoropa lutzi* (ZUEC 10687) from Magé, state of Rio de Janeiro, generated by microtomography.

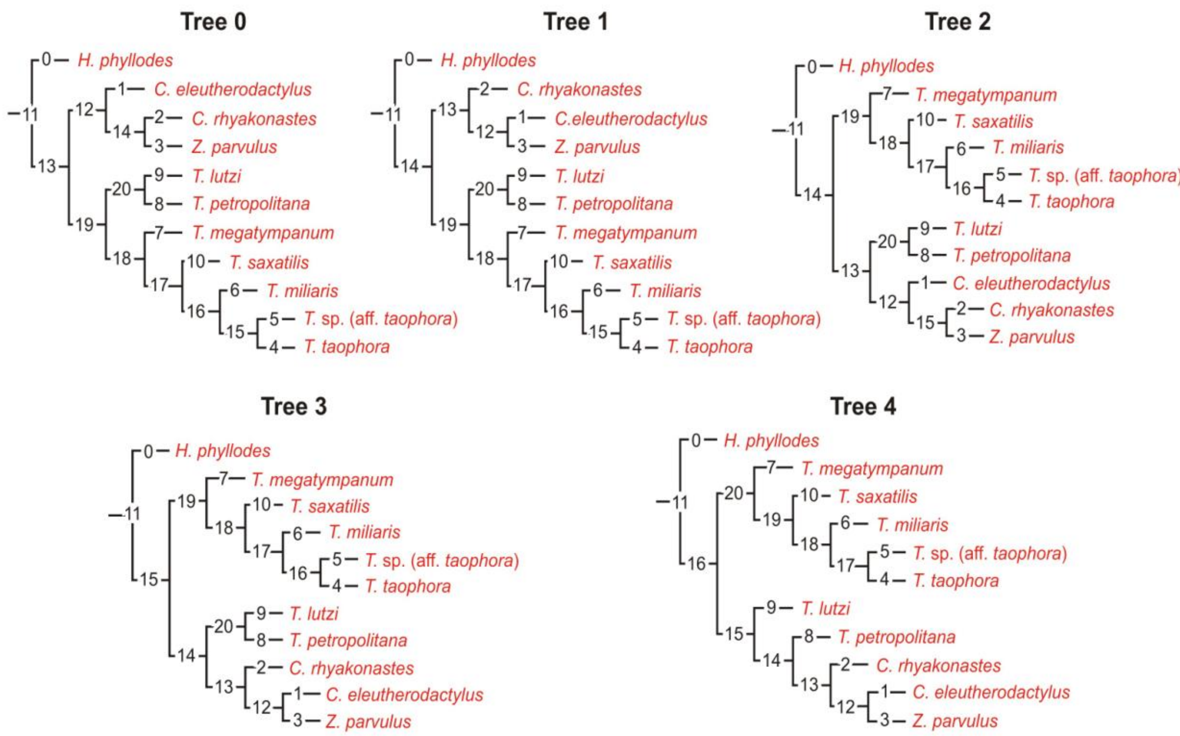


Figure S2. The five most parsimonious trees generated by TNT 1.1 software using the matrix with 11 taxa and 86 characters generated by Mesquite 2.75 software. Values are number of branches.

APPENDICES

Appendix I. Color-staining method for preparing osteological material.

Photo archive. Photographs were taken to file the characteristics of external morphology and patterns of coloration, enabling the possibility in future studies to confirm the identification of the same.

Removing of the skin. The skin and the viscera of the specimens were removed with the help of scalpels and dissection instruments and stored with their respective voucher number, or need for future studies to confirm the identification.

Washing. The specimen fixed and without skin was submitted to a bath in distilled water for 24 hours to remove excess fixative substances (formaldehyde and ethanol).

Transparency of the musculature. The specimen was immersed in a solution of 20% KOH until the muscle was transparent.

Coloration of bone elements. The specimen was immersed in a solution of ethanol with Alizarin PA (Alizarin Red S) for 24 hours or until the bones are red or reddish-purple. The solution was prepared with alizarin adding gradually the ethanol until it presented a deep purple color. Then, when the process of rehydration of the specimen at intervals of three hours' soaking in solutions of 95% ethanol, 70%, 50% and 30%, respectively. After this procedure, the specimen was immersed in three baths of distilled water with change intervals of three hours each.

Removing the muscle tissue. This process, when necessary, aimed to visualize the skeletal elements that were covered or wrapped by non-transparent muscles. Therefore, it was removed manually with the aid of tweezers and scissors (surgical).

Final bleaching and storage. Colored bones, the specimen was washed in ethanol PA for 5 to 10 minutes to remove the excess alizarin red. Then, was transferred to a vessel containing the KOH solution used previously and glycerin to 25% every 24 hours was replaced with solutions of 50% glycerin and 75%, respectively. After the last solution, the sample was placed on 100% glycerin with a small amount of thymol and stored. Glycerin also assists in clarifying some tissues and thymol prevents contamination and spread of fungi.

Appendix II. List of the adult individuals analyzed for osteology and external morphology.

a) Osteology by means of color-staining, microtomography and radiology:

Thoropa taophora (3 indivíduos): ZUEC 172, 1730, 1738 Ubatuba, São Paulo; *Thoropa* sp. (aff. *taophora*) (1 indivíduo): ZUEC 17335 Iguape - SP; *Thoropa miliaris* (6 indivíduos): MZUFV 3661, 4123, 4129 Araponga - MG; 4095 Lima Duarte, Minas Gerais; 3922 Alto Caparaó, Minas Gerais; 4148 Almenara, Minas Gerais; *Thoropa megatympanum* (3 indivíduos): MNRJ 22910 Botumirim, Minas Gerais; ZUEC 2103, 2319 Jaboticatubas, Minas Gerais; *Thoropa petropolitana* (2 indivíduos): MNRJ 23344, 23357 Teresópolis, Rio de Janeiro; *Thoropa saxatilis* (2 indivíduos): CFBH 30384 Maquiné, Rio Grande do Sul; 30397 Riozinho, Rio Grande do Sul; *Thoropa lutzi* (2 indivíduos): ZUEC 10687, 10688 Magé, Rio de Janeiro; *Zachaenus parvulus* (1 indivíduo): ZUEC 936 Rio de Janeiro, Rio de Janeiro; *Cycloramphus rhyakonastes* (Grupo *C. fuliginosus*) (1 indivíduo): ZUEC 17673 São João da Graciosa, Paraná; *Cycloramphus eleutherodactylus* (Grupo *C. eleutherodactylus*) (1 indivíduo): ZUEC 2724 Santo André, São Paulo; *Hylodes phyllodes* (2 indivíduos): ZUEC 17259, 17276 Biritiba-Mirim, São Paulo.

b) External morphology:

Thoropa taophora (25 indivíduos): CFBH 10417 Ilha Bela - São Paulo; ZUEC 14, 629, 899, 900, 1105, 1111, 1274, 1336, 1340, 1484, 1512, 1519, 1528–29, 1532, 1537, 1738, 1745, 1858, 2020, 2141, 2625, 2627, 9007 Ubatuba - São Paulo; *Thoropa* sp. (aff. *taophora*) (6 indivíduos): CFBH 29707–08, 29710–11 Peruíbe - São Paulo; ZUEC 17326, 17335 Iguape - São Paulo; *Thoropa miliaris* (20 indivíduos): CFBH 25110–11, 25117 Domingos Martins - Espírito Santo; 26379 Linhares - Espírito Santo; MNRJ 5831

Teresópolis - Rio de Janeiro; 9347, 9350 Ilha Mangaratiba - Rio de Janeiro; 23530, 23587 Sinonésia - Minas Gerais; 22866, 26499–500 Rio de Janeiro - Rio de Janeiro; 27500–01 Braga - Minas Gerais; 46861 Itamaraju - Bahia; 53500 Cachoeira de Macacu - Rio de Janeiro; 76758, 76760, 84656 Saquarema - Rio de Janeiro; 84073 Santa Tereza - Espírito Santo; *Thoropa megatympanum* (20 indivíduos): CFBH 789, 791 Santana do Riacho - Minas Gerais; 10194, 10196 Grão Mogol - Minas Gerais; MNRJ 26160, 26162–63 Jaboticatubas - Minas Gerais; 22906–09, 22913 Botumirim - Minas Gerais; MZUFV 4115 Jaboticatubas - Minas Gerais; ZUEC 2102, 2202, 2319 Santana do Riacho - Minas Gerais; 2841–42, 3957, 15938 Jaboticatubas - Minas Gerias; *Thoropa petropolitana* (20 indivíduos): MNRJ 23060, 25950–51, 25954–56 Teresópolis - Rio de Janeiro; 23344, 23351, 23355–360 Petrópolis - Rio de Janeiro; 24148–49, 24151–53, 24158 Tinguá - Rio de Janeiro; *Thoropa saxatilis* (10 indivíduos): CFBH 3198 Cambará do Sul - Rio Grande do Sul; 30311–13 Sapiranga - Rio Grande do Sul, 30379 Maquiné - Rio Grande do Sul; 30397, 30403, 30406–07 Riozinho - Rio Grande do Sul; MNRJ 25000 Praia Grande - Santa Catarina; *Thoropa lutzi* (14 indivíduos): MNRJ 1373 Santa Tereza - Espírito Santo; MZUFV 13820–29, 13837–39 Cataguases e Prado de Minas - Minas Gerias; *Zachaenus parvulus* (20 indivíduos): CFBH 251 Rio de Janeiro - Rio de Janeiro; 10120 Petrópolis - Rio de Janeiro; MNRJ 273 Santa Tereza - Espírito Santo; 43394 Cachoeira de Macacu - Rio de Janeiro; 49637, 50226 Ilha Grande - Rio de Janeiro; 64671–72 Nova Iguaçu - Rio de Janeiro; 78051 Rio Claro - Rio de Janeiro; 73792, 84200 Petrópolis - Rio de Janeiro; 86312–13 Teresópolis - Rio de Janeiro; ZUEC 2891 Nova Iguaçu - Rio de Janeiro; 936, 3712–13, 3813–14 Rio de Janeiro - Rio de Janeiro; 9548 Ubatuba - São Paulo; *Cycloramphus rhyakonastes* (3 indivíduos): ZUEC 17672, 20139, 20152 São João da Graciosa - Paraná; *Cycloramphus eleutherodactylus* (20 indivíduos): MNRJ 60512–13, 60517, 60532–38 Teresópolis - Rio de Janeiro; ZUEC 2033 Rio de Janeiro - Rio de Janeiro; 6367, 2120, 2723–24, 3533, 6468–69 Santo André - São Paulo; 10072 Rio Verde - São Paulo; 12087 Ribeirão Grande - São Paulo; *Hylodes phyllodes* (20 indivíduos): ZUEC 1839 Ubatuba - São Paulo; 6411 Salesópolis - São Paulo; 6989 Arapeí - São Paulo; 7170 Parati - Rio de Janeiro; 6797, 8420 Santo André - São Paulo; 10091 Mogi das Cruzes - São Paulo; 17200 Cubatão - São Paulo; 17313 Iguape - São Paulo; 17258, 20023, 20048, 20050–51, 20058, 20079 Bertioga - São Paulo; 20032–33, 20035, 20080 Biritiba-Mirim - São Paulo.

Appendix III.

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The Semiterrestrial Tadpole of *Cycloramphus rhyakonastes* Heyer, 1983 (Anura, Cycloramphidae)

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The tadpole of *Cycloramphus rhyakonastes*

ABSTRACT.–Frogs in the genus *Cycloramphus* are endemic to the Brazilian Atlantic Forest Domain and many are threatened or endangered. *Cycloramphus* species lay eggs either on moist rocks or in moist soil cavities on the ground. The tadpoles of only eight of the 28 recognized *Cycloramphus* species are known. Herein we describe the tadpole of *C. rhyakonastes* based on specimens collected at the type locality in southern Brazil. *Cycloramphus rhyakonastes* is an aquatic breeder with semiterrestrial tadpoles that live on moist rocks within the splash zone of high gradient streams. We compare the *C. rhyakonastes* tadpole with all congeneric tadpoles described to date and discuss adaptations in this specialized tadpole and those of other species that adhere to rocky substrates.

Key words: Amphibia; Aquatic breeding guild; Morphology; Endangered species; Stream-dwelling.

The family Cycloramphidae includes 36 species in three genera (Caramaschi and Sazima, 1984; Frost, 1985; Cocroft and Heyer, 1988; Verdade, 2005; Feio and Caramaschi, 2006; Lingnau et al., 2008), of which the most speciose is the genus *Cycloramphus* Tschudi, 1838. The currently recognized 28 species of *Cycloramphus* are endemic to the Brazilian Atlantic Forest Domain, ranging from the state of Rio Grande do Sul to the state of Bahia (Heyer, 1983a; Haddad and Sazima, 1989; Verdade, 2005; Lingnau et al., 2008). *Cycloramphus* species differ in their reproductive modes, either 1) laying eggs on moist rocks, with tadpoles adhering to rocks in splash zones along the stream, or 2) depositing eggs in moist soil, where embryos develop terrestrially (Heyer and Crombie, 1979; Giaretta and Cardoso 1995; Verdade and Rodrigues, 2003; Verdade, 2005). Based on these characteristics, *Cycloramphus* species are categorized into aquatic and terrestrial breeding guilds (Verdade, 2005).

Thus far, larvae are described for only eight of the 28 species: *C. boraceiensis* (Heyer, 1983a), *C. brasiliensis* (Steindachner, 1864), *C. fuliginosus* (Tschudi, 1838), *C. izecksohni* (Heyer, 1983b), *C. lithomimeticus* (Silva and Overnay, 2012), *C. lutzorum* (Heyer, 1983a), *C. stejnegeri* (Noble, 1924), and *C. valae* (Heyer, 1983a). Here, we describe the tadpole of *Cycloramphus rhyakonastes* (Heyer, 1983a), an aquatic breeding species known from the localities of Banhado, Morretes, and Marumbi in the state of Paraná, and Rio Vermelho in the state of Santa Catarina (Heyer, 1983a), southeastern Brazil, and compare it to all congeneric tadpoles described to date. We discuss adaptations of this and other specialized tadpoles that adhere to rocky substrates.

METHODS

We collected two tadpoles, two metamorphs, and six adults of *C. rhyakonastes* in the night of 09 Jan 2013, at the type locality, a stream (25º21'38.18"S, 48º52'43.31"W, 470 m a.s.l.) (Fig. 1) that crosses the Estrada da Graciosa, in the district of São João da Graciosa, municipality of Morretes, State of Paraná, southern Brazil. Specimens are accessioned in the Museu de Zoologia "Prof. Adão José Cardoso" (ZUEC) at Universidade Estadual de Campinas (Unicamp), Campinas, Brazil (ZUEC 20107; 20139-42; 20151-2).

The tadpole description is based on two specimens (ZUEC 20107) at Stage 25 (Gosner 1960). Labial tooth row formula (LTRF) and 15 measurements were taken on the preserved tadpoles following the terminology of McDiarmid and Altig (1999): body length; body height; body width; tail length; maximum tail height; tail muscle height; tail muscle width; total length; oral disc width; internarial distance; interorbital distance; eye diameter; nostril diameter; eye–nostril distance; and nostril–snout distance. The abdominal flap and spiracle were stained with water-diluted crystal violet for visualization; spiracle terminology follows McDiarmid and Altig (1999) and abdominal flap and dorsal outline of snout terminology follow Heyer et al. (1990). Measurements were taken with a digital caliper (0.1 mm precision) using a stereomicroscope. Larval measurements are given in Table 1.

RESULTS

External morphology.–The larvae are depressed dorsoventrally and elongated, with body longer than wide. The lateral line is absent. The body is depressed (body height/body width = 0.61 – 0.62), elliptical in dorsal and ventral views (Fig. 2A, B). The body length is 24 % of total length (Table 1). The belly is flattened with a long, shallowly bilobed, and truncate posterior flap, which hides the vent tube. This flap extends from the back of the mouth to the beginning of the tail musculature (Fig. 2B) and is free on its posterior portion (Fig. 2E). The snout is oval in dorsal view and sloped in lateral view. The nostrils are small, rounded, dorsally located, nearer to the eyes than to the tip of the snout without ornamentation on margins of nares. The eyes are small (eye diameter/body width = 0.29) and dorsolaterally oriented. The spiracle is internal, sinistral at midbody, translucid, and with a round opening (Fig. 2A, B). The tail musculature is evident. The tail fins are low, translucent, with some light brown spots, and restricted to the distal half of the tail length (Fig. 3A, B, C). The oral disc is ventral and almost as broad as the head anteriorly. The papillae occur in a single row laterally and posteriorly. The dental formula (LTRF) is 2/3 (Fig. 2D). The first and second anterior tooth rows A1 and A2 are complete; A2 is slightly longer than A1; the three posterior tooth rows P3 are shorter than P1 and P2 that are approximately of equal length. The upper jaw sheath is well defined with lateral processes that are wider than the lateral processes of the lower jaw sheath. The lower jaw sheath is wedged and narrower than the upper jaw sheath. The lower jaw also presents a ventral extension (Fig. 2D). Larval measurements are presented in Table 1.

Comparison with other species of Cycloramphus *larvae*.–The tadpole of *Cycloramphus rhyakonastes* differs from other described congeners in having a long and posteriorly-free truncated abdominal flap (Table 2). Only *C. boraceiensis* and *C. brasiliensis* have the same LTRF as *C. rhyakonastes*. The ventral tail fin, present on the distal half of the tail, distinguishes the tadpole of *C. rhyakonastes* from *C. boraceiensis, C. izecksohni, C. stejnegeri*, and *C. valae* (Table 2).

Coloration in preservative.–In dorsal view the body is dark brown and the tail is banded brown and beige. Ventrally, the body and tail are light cream in color, with no markings. The viscera are visible and intestines are sinistral (Fig. 2B, 3B).

Natural History.–Tadpoles are semiterrestrial and were found adhered to moist rocks at the stream margins, right above the water line. Tadpoles are highly eucryptic (*sensu* Toledo and Haddad, 2009) and resemble the rocks to which they adhere. Two recently metamorphosed froglets collected at the same site measured 6.17 and 7.57 mm in SVL (ZUEC 20107).

DISCUSSION

All collected *Cycloramphus rhyakonastes* specimens (tadpoles, metamorphs, and adults) were located on the light colored portions of wet rocks in the splash zone of the stream (Fig. 1). In these microhabitats their coloration matched the background very closely. We did not test for microhabitat selection, but this behavior has been observed in other anurans (Wente and Phillips, 2005) and presumably improves crypsis (e.g., Toledo and Haddad, 2009; Thibaudeau and Altig, 2012).

Among anurans, tadpoles that adhere to rocks in streams can be classified into three ecomorphological groups: 1) suctorial, as in tadpoles of *Ascaphus truei* (Ascaphidae) that have a ventrally flattened body with a large suctorial oral disc, two biserial upper rows of teeth adapted to grip on rocks, and complete marginal papillae (Gaige, 1920; Stephenson, 1951; Hawkins et al., 1988; McDiarmid and Altig, 1999). 2) gastromyzophorous, those that use a modified belly as a sucker to maintain their position in fast-flowing water, as in tadpoles of the bufonid *Atelopus* (McDiarmid and Altig, 1999) and the ranid *Amolops* (McDiarmid and Altig, 1999), species of *Atelopus* and *Amolops* use muscles and tendons to raise the body and create suction with the belly (McDiarmid and Altig, 1999). 3) semiterrestrial, such as tadpoles of the dicroglossids *Nannophrys*, petropedetids *Petropedetes*, and cycloramphids *Thoropa*, and *Cycloramphus* spp. that have the oral disc distended, protruding and forming a sucker, with marginal papillae with an anterior gap (McDiarmid and Altig, 1999). In *Thoropa miliaris* the edges of the oral disc have many small papillae that provide adherence and rows of small keratinous hooks that hold the tadpole on the rock surface (Barth, 1956); these adaptations are not observed in *Cycloramphus*. The elongated body flap in semiterrestrial tadpoles of *Cycloramphus* does not appear to have a suctorial function, but the larger surface area provided by the flap may increase tadpole adherence to wet surfaces. These semiterrestrial ecomorphological adaptations are observed both in *Thoropa* and *Cycloramphus* (both Cycloramphidae).

Some larvae of the genus *Cycloramphus* are very hard to find. We only collected two individual tadpoles of *C. rhyakonastes* in a total of 8 field trips (over the last 5 years) to this stream. This may explain why only a third of the *Cycloramphus* larvae have been described.

Additional tadpole sampling effort should take place at type-localities. Tadpoles of *Cycloramphus* are variable, and clearly show species-specific characters. Thus, more larval descriptions have the potential to improve our knowledge about relationships within this group (Verdade, 2005).

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Captions for figures

FIG.1.–Splash zone at the type locality of *Cycloramphus rhyakonastes* showing coloration of the rocks and microhabitat where tadpoles were collected. The arrow indicates the brighter parts of the rocks where we found most of the specimens.

FIG. 2.– Dorsal (A), ventral (B), and lateral (C) views. Close up of oral apparatus (D) of tadpole of *Cycloramphus rhyakonastes.*

FIG. 3.–Tadpole of *Cycloramphus rhyakonastes*, stage 25, (ZUEC 20107) in dorsal (A), ventral (B), and lateral (C) views. Sinistral intestines and sinistral spiracle (D) and abdominal flap (E).

Table 1.–Measurements of two *Cycloramphus rhyakonastes* tadpoles at Gosner stage 25. Values presented in millimeters as mean ± standard deviations (range).

Characteristic	**Measurement**
Body length (BL)	5.46 ± 0.24 (5.18 – 5.74)
Body height (BH)	1.77 ± 0.03 (1.73 – 1.81)
Body width (BW)	2.88 ± 0.09 (2.77 – 2.99)
Tail length (TAL)	17.03 ± 0.65 (16.27 – 17.79)
Maximum tail height (MTH)	1.46 ± 0.09 (1.37 – 1.56)
Tail muscle width (TMW)	1.38 ± 0.03 (1.34 – 1.41)
Total length (TL)	22.49 ± 0.89 (21.45 – 23.53)
Oral disc width (ODW)	1.47 ± 0.1 (1.35–1.59)
Internarial distance (IND)	0.77 ± 0.04 (0.72 – 0.81)
Interorbital distance (IOD)	0.97 ± 0.00 (0.97 – 0.97)
Eye diameter (ED)	0.84 ± 0.03 (0.8 – 0.88)
Nostril diameter (ND)	0.07 ± 0.01 (0.06 – 0.08)
Eye-nostril distance (END)	0.59 ± 0.02 (0.56 – 0.61)
Nostril-snout distance (NSD)	0.82 ± 0.13 (0.67 – 0.97)

Table 2.–Comparative morphology of tadpoles of species *Cycloramphus*. BS = body shape; ESD = eye to snout distance; LTRF = labial tooth row formula. Dashes (–) indicate that information was not available. Modified from Lima et al., 2010.

Species	**Stage**	**TL (mm)**	**BL (% of TL)**	**Dorsal BS**	**Lateral BS**	**ED (% of BL)**	**Position of nares**	**Abdominal flap**	**Spiracle**	**Vent Tube**	**Ventral tail fin**	**LTRF**	**Source**
C. rhyakonastes	25	22.4	24	elliptical	depressed	15	half ESD	shallowly bilobed or not	single sinistral	not visible	distal half	2/3	Present study
C. boraceiensis	42	27.2	23	–	–	18	half ESD	bilobed	not visible	not visible	–	2(2)/3, 2/3(1)	Heyer, 1983a
C. boraceiensis	37	30.7	23	elliptical	depressed	22	half ESD	bilobed	–	not visible	distal third	2/3, 2(2)/3(1)	Lima et al., 2010
C. brasiliensis	41	37.5	25	–	depressed	17	–	–	single sinistral	not visible	distal half	2/3(1)	Heyer, 1983a
C. fuliginosus	41	43.5	19	–	depressed	12	–	shallowly bilobed or not	single sinistral	median	distal half	2/3	Heyer, 1983a
C. izecksohni (described as *C. duseni)*	41	32.0	23–28	–	depressed	13–18	–	shallowly bilobed or not	single sinistral	median	distal half	2/3	Heyer, 1983a
C. izecksohni	34–42	22.0–28.9	26–29	elliptical	very depressed	12–16	closer to eyes	shallowly bilobed or not	–	–	distal fourth	2(2)/3(1)	Lima et al., 2010
C. lithomimeticus	30	21.4	18	–	–	17	–	shallowly bilobed	not visible	–	distal half	2(2)/3(1)	Da Silva & Ouvernay, 2012
C. lutzorum	36-43	26.2-26.9	23-31	elliptical	depressed	15	half ESD	shallowly bilobed or not	dual lateral	median	distal half	2(2)/3	Lima et al., 2010
C. stejnegeri	31	25.2	–	–	not depressed	–	–	–	not visible	–	distal third	–	Heyer, 1983a
C. valae	36	29.3	24–29	–	–	16–19	half ESD	shallowly bilobed	not visible	–	distal half	2(2)/3(1)	Heyer, 1983b
C. valae	35	26.4	25	oval	depressed	16	half ESD	shallowly bilobed	–	median	distal third	2(2)/3(1)	Lima et al., 2010

Figure 1

Figure 2

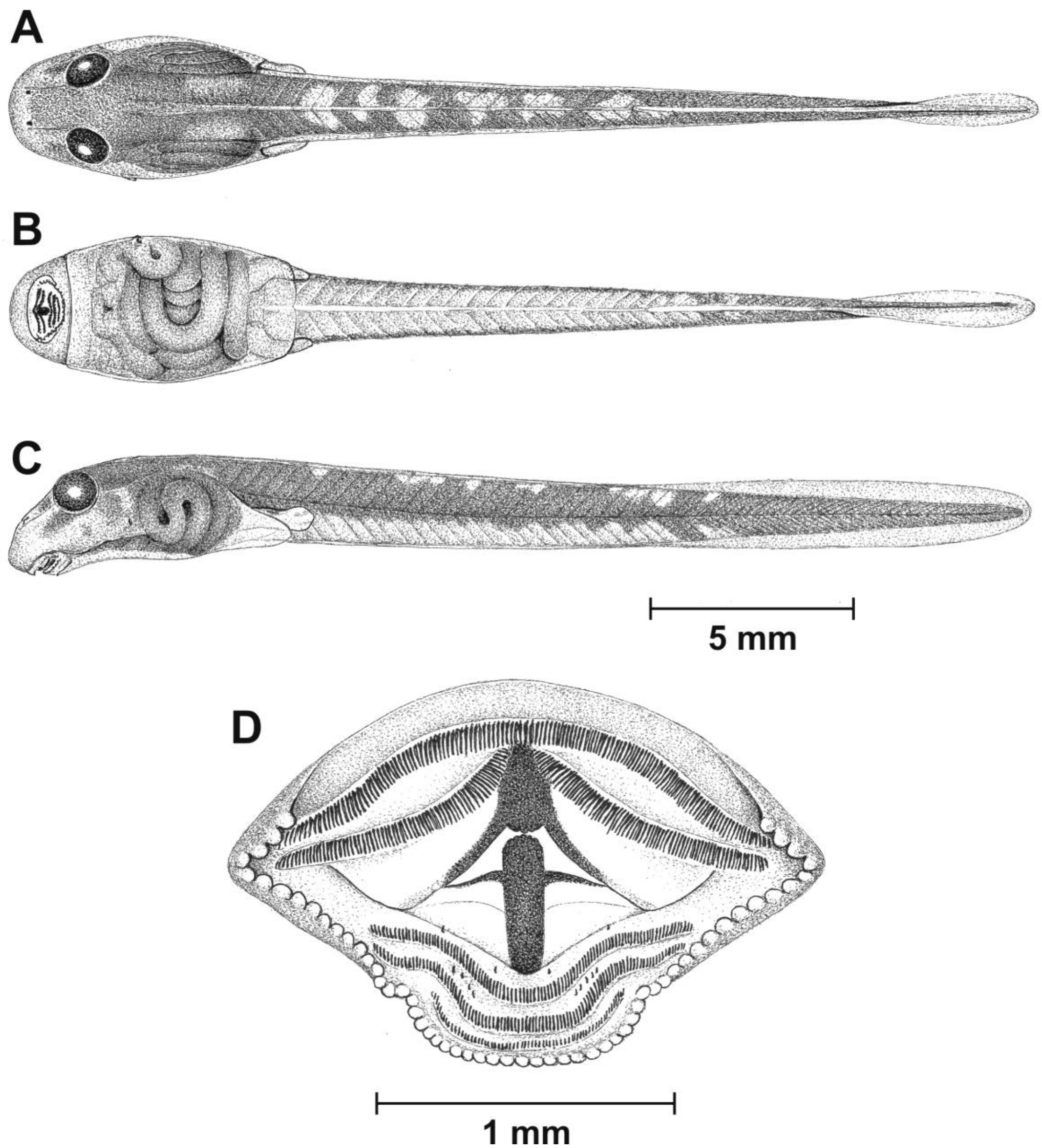
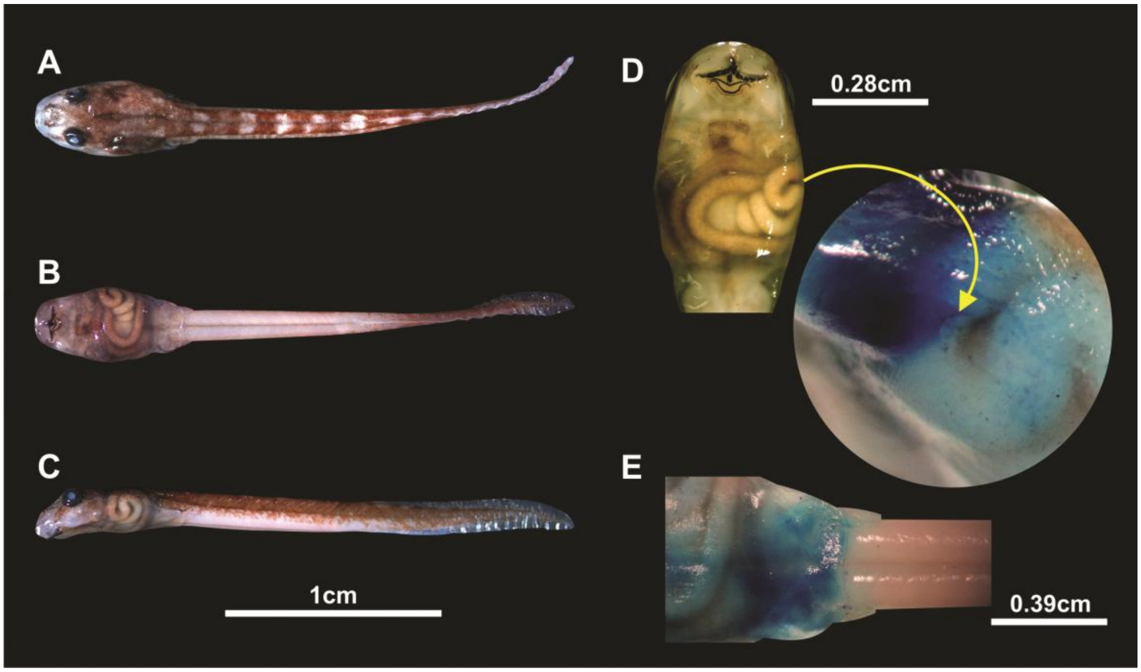


Figure 3



Conclusões Gerais

Este estudo forneceu importantes informações sobre a família Cycloramphidae e proporcionou o entendimento das relações filogenéticas entre as espécies dos gêneros *Thoropa* e *"Thoropa" (pequena)*. A filogenia e a análise dos cantos das espécies permitiu que cheguemos às seguintes conclusões:

• Caracteres da ecologia, osteologia e morfologia externa de adultos e da forma larval são fontes importantes de informação filogenética, principalmente porque com esses dados foi possível a reconstrução filogenética do gênero *Thoropa* e a separação do gênero *"Thoropa" (pequena)*;

• A árvore mais parcimoniosa encontrada e que representa a filogenia deste trabalho sugere o parafiletismo indicando que o grupo *"Thoropa" (pequena)* é grupo irmão de *Thoropa*;

• No caso da primeira linhagem, o gênero *Thoropa*, a espécie mais basal é *T. megatympanum* indicando que a fragmentação da Mata Atlântica pode ter contribuído para a especiação deste gênero. Assim, *T. megatympanum* é mais basal que *T. saxatilis*, que é mais basal do que *T. miliaris*, e que é mais basal do que *T. taophora* e *Thoropa* sp. (aff. *taophora*).

Considerações Finais

A principal razão para continuar a trabalhar com dados morfológicos é resolver relações filogenéticas de táxons fósseis ou animais extintos conservados em formalina, a filogenia molecular ainda é limitada neste aspecto. E foi o caso deste trabalho, no qual temos uma espécie aparentemente extinta, *"Thoropa" (pequena) petropolitana*, e outra, *"Thoropa" (pequena) lutzi*, categorizada como DD (Dados Deficientes), com poucos exemplares conservados em museus. Desta última, a população original está aparentemente extinta, mas são conhecidas três novas populações duas no estado de Minas Gerais e outra no estado do Espírito Santo.

Este estudo filogenético com o uso da morfologia elucidou algumas questões importantes sobre a história evolutiva do gênero *Thoropa*, e proporcionou a necessidade de adotar e propor o gênero *"Thoropa" (pequena)* para as espécies menores. Sendo assim, este estudo deixa algumas questões em aberto, como:

As populações de *"Thoropa" (pequena) lutzi* nos estados de Minas Gerais e Espírito Santo são uma nova espécie?

Os resultados obtidos em uma análise filogenética molecular seriam os mesmos?