



Reassessment of a fossil specimen of *Rhinella marina* (Linnaeus, 1758) (Anura: Bufonidae), from Early Pleistocene of Bolivia

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Bufonidae is a cosmopolite and speciose clade that is currently hypothesized to have originated in Gondwana around 78–99 Ma (Pramuk *et al.* 2008). The systematics of the family was assessed using morphological and molecular data, alone or in a total evidence analysis (Pramuk 2006; Pramuk *et al.* 2008; Bocxlaer *et al.* 2010; Pyron & Wiens 2011). Due to taxonomic changes, most of the South American species of *Bufo* Garsault were relocated to the genus *Rhinella* Fitzinger, currently the second most speciose genus with 92 scientifically named and valid species (Frost 2020). The species in the genus are arranged in six taxonomic groups (*crucifer*, *granulosa*, *margaritifera*, *marina*, *spinulosa*, *veraguensis* species groups [Frost 2020]); the *Rhinella marina* group is characterized by specimens with well-ossified and exostosed skull, ornamented with deep striations, pits, and rugosities (Maciel *et al.* 2010). The *Rhinella marina* group includes eleven living species, distributed in two main clades named after their geographical distribution: the north-central clade and south-central clade. The south-central clade bears the species: *R. achavali* (Maneyro, Arrieta & de Sá), *R. arenarum* (Hensel), *R. icterica* (Spix), and *R. rubescens* (Lutz). The north-central clade bears the species: *R. cerradensis* Maciel, Brandão, Campos & Sebben, *R. horribilis* (Wiegmann), *R. jimi* (Stevaux), *R. marina* (Linnaeus), *R. poeppigii* (Tschudi), *R. schneideri* (Werner), and *R. veredas* (Brandão, Maciel & Sebben).

The fossil record of the South American *Rhinella marina* group is composed of around ten specimens, ranging from the Miocene to the Pleistocene (Estes & Wassersug 1963) and recovered in Colombia, Bolivia, Peru, Brazil, and Argentina. There are fossil representatives of four extant species: *Rhinella arenarum* (Báez & Nicoli 2004; Pérez-Ben *et al.* 2014, 2019b; Cruz *et al.* 2018), *Rhinella jimi* (Araújo-Júnior & Moura 2014; Araújo-Júnior *et al.* 2016), *Rhinella marina* (as *Bufo marinus*; Estes & Wassersug [1963]; Vergnaud-Grazzini [1968]), and *Rhinella schneideri* (Tomassini *et al.* 2013; Araújo-Júnior & Moura 2014; as *Bufo paracnemis* in Vergnaud-Grazzini [1968], as *Rhinella* cf. *R. pisanoi* in Tomassini & Montalvo [2013]). And an exclusively fossil species, *Rhinella loba* Pérez-Ben *et al.*, 2019a.

Vallinoto *et al.* (2010) proposed that the *R. marina* is a group of cryptic species, embracing three lineages, one occurring in the Amazonian Basin (former *R. marina marina*), one in Peru (former *R. marina marina*), and one in Central America/Ecuador (former *R. marina horribilis*). The Central America/Ecuador clade is proposed to have diverged from the clade including the other two lineages (Amazonian Basin and Peru lineages) in the Middle Miocene (Vallinoto *et al.* 2010), and was resurrected in species level as *Rhinella horribilis* by Acevedo *et al.* (2016), based on molecular and morphological data. The two fossil specimens assigned to *R. marina*, i.e. *R. marina* UC 41159 (Estes & Wassersug 1963) from the Miocene of Colombia and *Rhinella marina* (Vergnaud-Grazzini 1968) from the Early Pleistocene of Bolivia were proposed to be closely related to the lineage occurring in the Peru/Amazon Basin (i.e. subspecies *marina*). The authors also mention morphological differences between specimens under the *R. marina* complex (i.e. *R. m. horribilis* and *R. m. marina*).

A collection number was never attributed to the fossil specimen of *Rhinella marina* described by Vergnaud-Grazzini (1968), neither was it referred to be deposited in a collection. For decades, it was considered lost, but when trying to attend a request on another fossil, the curator of the Muséum National d'Histoire Naturelle—Paris, France, found Vergnaud-Grazzini's fossils stored in the mammal collection of the museum. Vergnaud-Grazzini (1968) also states that there were twenty fragmentary fossil remains that were probably from the same individual. Part of the material originally described

are: presacral vertebra I, sacral vertebra, right scapula, and left ilium. All the fossil material was recovered, except for the left ilium, which remains lost.

Considering the brief description of the specimen in Vergnaud-Grazzini (1968), the recent updates of the taxonomy of the genus, and the opportunity the rediscovery brought, we reassess the fossil, herein referred to as MNHN *Rhinella* sp. from Bolivia (Fig. 1), presenting a detailed description of the skull. We also compare the skull of the fossil to those of other species in the *R. marina* group, infer the phylogenetic positioning of MNHN *Rhinella* sp. Bolivia based on the phylogeny of Maciel *et al* (2010) and Pramuk (2006) and comment the status of fossil specimens of genus *Rhinella* from South America.

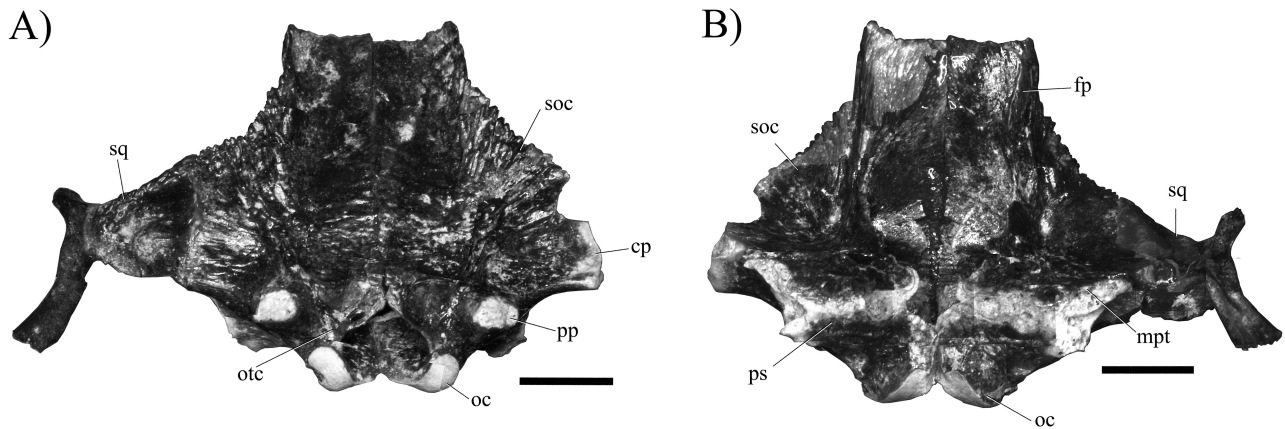


FIGURE 1. Skull of the fossil specimen of MNHN *Rhinella* sp. Bolivia from Tarija, Bolivia. A) dorsal view; B) ventral view. Abbreviations: cp, crista parotica, fp, frontoparietal, mpt, medial ramus of the pterygoid, oc, occipital condyle, otc, otoccipital, pp, prootic process, ps, parasphenoid, soc, supraorbital crest, sq, squamosal. Scale bars equal 10mm.

We compared the skull morphology of the fossil MNHN *Rhinella* sp. Bolivia to μ CT-scanned specimens and museum specimens of living and fossil species of genus *Rhinella* (Appendix I). The specimen MNHN *Rhinella* sp. Bolivia was coded in the data matrix of Pramuk (2006), with the complementation of the OTUs (operational taxonomic units) of Maciel *et al.* (2010) (who coded the same characters of Pramuk [2006] for species of the *R. marina* group). The fusion of these two data matrices allows inferring the phylogenetic allocation of the fossil MNHN *Rhinella* sp. Bolivia as a member of the *R. marina* group and its relationships within the group. We provide the combined data matrix in the Supplementary Data 1. We ran a phylogenetic analysis on TNT v 1.1 (Goloboff *et al.* 2008) using the morphological character subset of Pramuk (2006), submitting 5,000 replications to the tree bisection—reconnection branch swapping method (TBR), retaining 10 trees per replication. Intraspecific polymorphic characters were coded as missing values. The morphometric measurements were made using ImageJ Fiji (Schindelin *et al.* 2012).

The general morphology and morphological measurements of the fossil specimen MNHN *Rhinella* sp. Bolivia are consistent with those of individuals of *R. marina* species group. Our analysis generated 204 trees retained with the best score of 533 steps (CI = 0.226; RI = 0.668). The topology of the majority-rule consensus with 50 percent-cut recovers MNHN *Rhinella* sp. Bolivia as member of the *R. marina* species group (Fig. 2). The single unequivocal synapomorphy proposed for the *R. marina* group is the articulation between the medial ramus of the pterygoid and parasphenoid alae formed by a jagged or “scalloped” suture (Pramuk 2006). Unfortunately, the character is not visible in the fossil, as the parasphenoid and pterygoid are synostotically sutured, preventing the recognition of the particular morphology of the medial ramus of the pterygoid. However, MNHN *Rhinella* sp. Bolivia has a set of diagnostic characters that only occur in species of the *R. marina* group: presence of a ‘broad’ skull, supraorbital crests well-defined, heavy cranial ornamentation, anterior margins of frontoparietals nearly transverse, and otic rami of squamosals expanded and overlying the crista parotica broadly, contacting the frontoparietals medially (Pramuk 2006).

The relationship of MNHN *Rhinella* sp. Bolivia within the *R. marina* group is not resolved; from the 83 characters, it was possible to code only 13, due to the fragmentary condition of the fossil (Figs. 1, 2).

Systematic paleontology

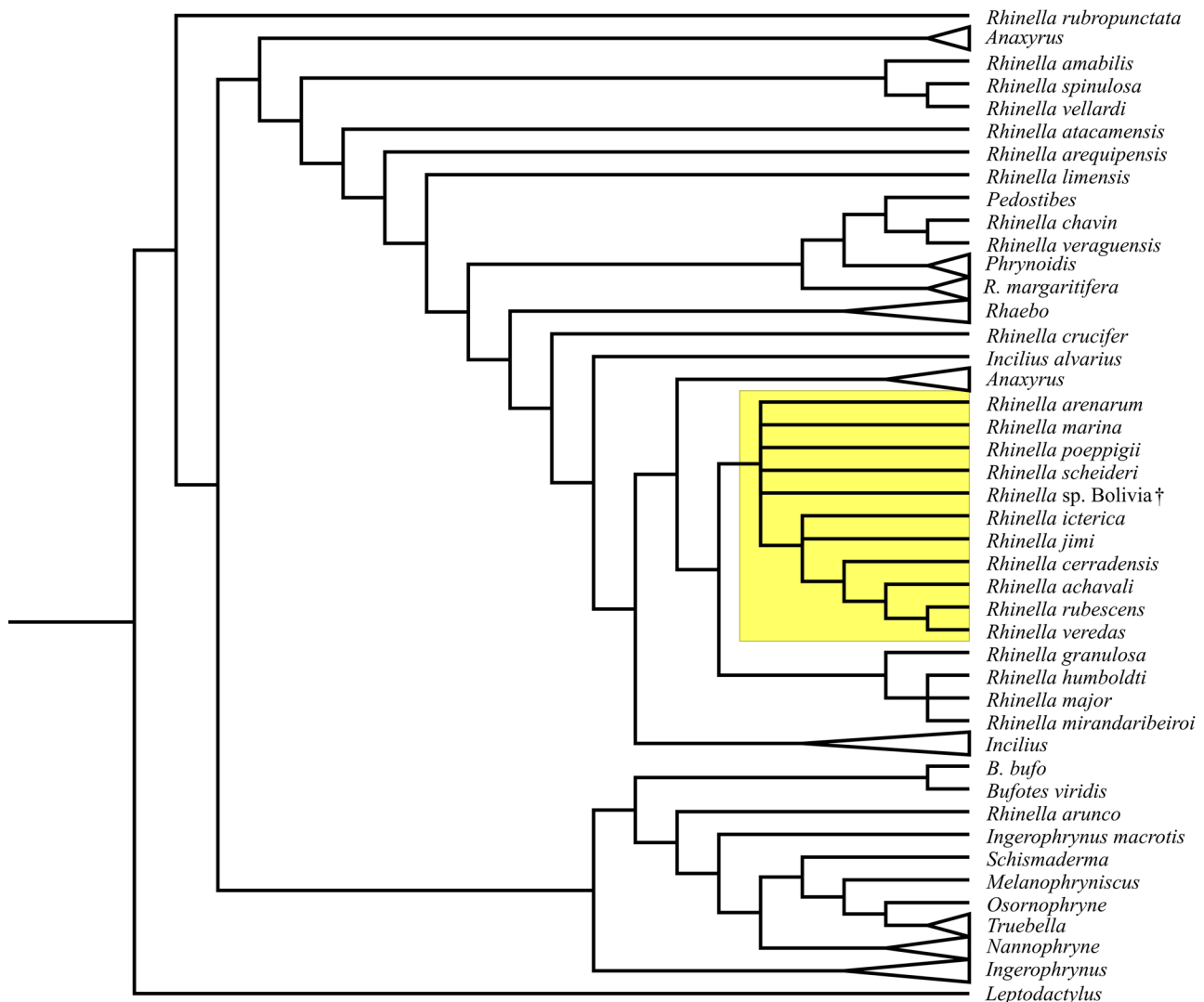


FIGURE 2. Majority-rule consensus of 204 most-parsimonious trees. Yellow box indicates the *Rhinella marina* species group. Cross-mark indicates the fossil specimen MNHN *Rhinella* sp. from Early Pleistocene of Tarija, Bolivia.

Anura Duméril
Neobatrachia Reig
Bufonidae Gray
***Rhinella* Fitzinger**

Type Species. *Oxyrhynchus proboscideus* (Spix) (currently *Rhinella proboscidea* [Frost *et al.* 2006]). Extant taxon, Neotropical distribution.

***Rhinella* sp. indet. (*Rhinella marina* species group) (Fig. 1)**

Referred Specimen Unnumbered Specimen from the Muséum National d’Histoire Naturelle (MNHN), skull partially preserved. The preserved bones are the frontoparietals, squamosals (left otic ramus, left zygomatic and left ventral rami), parasphenoid (only the alae), the pterygoid (only the medial ramus sutured with the parasphenoid alae), and otoccipital.

Locality and Age Department of Tarija, Bolivia, Early Pleistocene of Tarija Formation (Marshall & Sempere 1991).

Distribution Bolivia.

Description. The fossil specimen MNHN *Rhinella* sp. from Tarija, Bolivia is an adult based on the degree of mineralization of otoccipital and the degree of ornamentation in the frontoparietal, squamosal, and otoccipital. MNHN *Rhinella*

sp. Bolivia is composed of a skull partially preserved, with frontoparietal and otoccipital well preserved and the squamosal, parasphenoid and pterygoid as fragmentary elements. The frontoparietals, otoccipital, and left squamosal are well preserved, enabling an inference of the skull maximum width. We measured the left side of the skull (19.8mm) from the lateral-most portion of squamosal to the suture of frontoparietals (at the level of crista parotica) and mirrored it to infer the approximate maximum-width of the skull (39.6mm).

The frontoparietals, otoccipital, and left squamosal display exostosis on its dorsal surface. At the level of the crista parotica, the rugosities pattern is transversely oriented. The supraorbital crest of the frontoparietals and the posterior region of the skull (above the crista parotica) are heavily ornamented with striations, pits, and rugosities, and other portions of the skull (i.e. anterior and medial portion of frontoparietals) are mainly smooth (Fig. 1A). The frontoparietals are medially in contact; this suture was glued together incorrectly, producing an artifact: in ventral view, it is clear that the occipital condyles are extremely juxtaposed, instead of separated by the corpus of the parasphenoid. The suture between each frontoparietal and the corresponding nasal is not preserved, but the morphology of the anterior edge of frontoparietal is transverse. The postorbital portion (9.5mm; at the level of crista parotica) of the frontoparietal is wider than the interorbital portion (4.3mm; located anterior to the supraorbital crest). On the posterior portion of frontoparietal, the occipital artery canal is open, and the processus posterior of the frontoparietal does not overlap the otoccipital, forming a poorly-developed post-temporal fenestra (Fig. 1A). The frontoparietal maximum-length is 16.5mm.

The prootics and exoccipitals are completely fused, forming well-ossified otoccipitals. The left squamosal was glued together with the otoccipital and frontoparietal. However, the posterior-most portion of the otic ramus of the squamosal is not perfectly preserved and lacks the portion that sutures with the otoccipital. The result is that the two bones were not glued correctly, producing an artifact. The otic ramus is enlarged, and in contact with the posterolateral margin of the frontoparietal, forming a continuous temporal arcade. The maximum length of the otic ramus is 6.6mm. The zygomatic ramus of the squamosal is free from the ventral ramus of the maxilla and is chisel-shaped. In posterior view, the ventral ramus of the squamosal is ventrolaterally angled. In ventral view, the parasphenoid is a fragmentary element, with only the alae partially preserved (i.e. the parasphenoid corpus and the distal portions of the alae were lost). The alae of the parasphenoid are anterolaterally directed (Fig. 1B). At the point of contact of the frontoparietal and the posteromedial edge of the prootic there is the posterior prootic process, that is rounded and moderately prominent, and was damaged by abrasion. The occipital condyles are closely juxtaposed, but this condition could be due to an artifact. The pterygoids articulated with the skull bones are preserved in the fossil only as the medial ramus of the pterygoid, which is fused to each ala of the parasphenoid and extends medially along approximately half the length of the parasphenoid ala.

We noted the degree of ornamentation may vary intraspecifically in *R. poeppigii* and *R. marina*, as well as Pérez-Ben *et al.* (2014) also pointed to *R. arenarum*. In *R. poeppigii*, the frontoparietals are almost smooth dorsally, except for the supraorbital crest, presenting a striated pattern of ornamentation; the striated pattern can be marked (usnm: 346830) or inconspicuous (usnm: 346829). In *R. marina*, the dorsal surface of frontoparietal can be well-ornamented with pits, striations, and rugosities (YPM HERA 009560, 009561) ornamented with pits and striations (omnh 46722) or smooth with striations (uf:herp 172560). Estes & Wassersug (1963) suggests the species *R. horribilis* (former *R. m. horribilis*) presents “thin, fine, almost pitted sculpture, and relatively low cranial crests”. Acevedo *et al.* (2016), when revalidating *R. horribilis* to species status, focused on cranial morphometric differences between *R. marina* and *R. horribilis* and did not evaluate the diagnostic characters proposed by Estes & Wassersug (1963).

MNHN *Rhinella* sp. Bolivia differs from *R. poeppigii* in the ornamentation of the dermal skull bones, by presenting pits, striations, and rugosities on the frontoparietal, squamosal, and otoccipital. In *R. poeppigii*, the ornamentation is weak and striated. MNHN *Rhinella* sp. Bolivia differs from *R. arenarum* and *R. ictérica* by the presence of a wide concavity in the posterior portion of the otic ramus of the squamosal, bordered by the postorbital crest. The presence of this concavity is shared by *R. poeppigii*, *R. schneideri*, and *R. marina*, and this concavity is an attachment point for nuchal muscles (Estes & Wassersug 1963). MNHN *Rhinella* sp. Bolivia additionally differs from *R. schneideri* by presenting a slightly inclined supraorbital crest, while in *R. schneideri* this crest is the most inclined of all species in the *R. marina* group. MNHN *Rhinella* sp. Bolivia differs from *R. cerradensis* by the presence of a wide concavity in the posterior portion of the otic ramus of the squamosal; this concavity is narrow in *R. cerradensis*. We did not have access to specimens of *R. jimi* and *R. veredas*, but data in the literature (i.e. Maciel *et al.* 2010) allow to infer that MNHN *Rhinella* sp. Bolivia differs from *R. jimi* and *R. veredas* in presenting a developed supraorbital flange on frontoparietal, while this flange is inconspicuous in *R. jimi* and *R. veredas*.

MNHN *Rhinella* sp. Bolivia differs from *Rhinella loba* MMP 1003, from the Pliocene of Argentina, in morphometrical differences of frontoparietal (length = 4.4 mm and width = 16.1 mm, in MNHN *Rhinella* sp. Bolivia; length = 10.1

mm and width = 18.6 mm in *Rhinella loba* MMP 1003 [Pérez-Ben *et al.* [2019a]] and on the degree of skull ornamentation. The skull of *Rhinella* sp. Bolivia is ornamented with pits, striations, and rugosities, while in *R. loba* it is heavily ornamented with tuberculated pattern.

The fossil specimen MNHN *Rhinella* sp. Bolivia resembles the fossil specimen *R. marina* UC 41159 (from the Miocene of Colombia) and extant *R. marina* in the presence of the transverse ornamentation-pattern on the skull roof at the region of the crista parotica. The specimen MNHN *Rhinella* sp. Bolivia differs from the extant species of our comparative scope (except for *R. marina* YPM HERA 009560, 009561) in presenting an open occipital artery-canal and differs from all species of *R. marina* group in our scope (except for *R. marina* UC 41159 and *R. marina* YPM HERA 009560, 009561) in presenting an otoccipital not covered by the processus posterior of the frontoparietal. Consequently, the post-temporal fenestra is inconspicuous.

Even with the inclusion of this fossil in a phylogenetic analysis, it is difficult to infer the relationship between the fossil and the other species in the *R. marina* group and to propose if this specimen is a fossil representative of a living species or a new fossil species. At the taxonomic level, it is also difficult to understand the osteological differences between species of the *Rhinella marina* species group and trace the intraspecific variation present in some nominal species, especially within the *R. marina* lineages (Vallinoto *et al.* 2010). We propose the fossil specimen MNHN *Rhinella* sp. Bolivia is closely related to the species of the *R. marina* group, but its species-level identification is not possible.

The fossil record of Bufonidae in South America consists mostly of fragmentary, isolated bones, preventing proper identification to the species level (Pérez-Ben *et al.* 2014). In contrast to the fragmentary nature of these fossils, many specimens were assigned to species without adequate justification (i.e. Tomassini & Montalvo 2013; Tomassini *et al.* 2013; Araújo-Júnior & Moura 2014; Cruz *et al.* 2017). Many specimens presented in the aforementioned works are post-cranial remains that do not bear apomorphies or diagnostic characters for species or genus assignment. We propose the fossil specimens associated to the family should be thoroughly reviewed.

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APPENDIX I. Specimens examined. Institutional Abbreviations—ASUnB Antonio Sebben collection in Universidade de Brasilia; IRSNB Institut Royal des Sciences Naturelles del Belgique, Brussels, Belgium; KUVV Museum of Natural History, University of Kansas, USA; MMP Museo Municipal de Ciencias Naturales Lorenzo Scaglia, Mar del Plata, Argentina; omnh Oklahoma Museum of Natural History, Norman, Oklahoma, USA; PVL, Paleontolog a de Vertebrados, Instituto Miguel Lillo, Tucum n, Argentina; uf:herp University of Florida, Florida Museum of Natural History, Gainesville, Florida, USA; UC University of California, Oakland, California, USA; usnm National Museum of Natural History, Washington, DC, USA; YPM HERA Yale Peabody Museum of Natural History, New Haven, Connecticut, USA.

Fossil specimens:

Rhinella sp. PVL 6450; *Rhinella arenarum* MMP 5119, PVL 2197; *Rhinella loba* MMP 1003; *Bufo marinus* (= *Rhinella marina*) UC 41159; *Bufo spinulosus* (= *Rhinella spinulosa*) KUVV 49566.

Specimens examined through μ CT-scanned images:

Atelopus oxyrhynchus uf:herp:93190; *Melanophryniscus stelzneri* uf:herp:63183; *Rhaebo blombergi* uf:herp:104602; *Rhinella arenarum* usnm 70620, 70622; *Rhinella beebei* IRSNB:17147; *Rhinella castaneotica* usnm 518807, 518809; *Rhinella crucifer* usnm 70613, 70614; *Rhinella dapsilis* usnm 196951, 201814; *Rhinella granulosa* usnm 566017, 566018; *Rhinella icterica* usnm 100954, 100957; *Rhinella marina* omnh 46722, uf:herp 172560; *Rhinella poeppigii* usnm 346829, 346830; *Rhinella schneideri* usnm 281765.

Cleaned and stained:

Rhinella cerradensis ASUnB 2553, 2554; *Rhinella marina* YPM HERA 009560, 009561, 009726, 009754, 009760, 010391.

SUPPLEMENTARY DATA 1

Data matrix

All species coded are obtained in Pramuk (2006), with the exception of the OUT obtained from Maciel *et al.* (2010), that are marked with number-sign (#).

Anaxyrus americanus

020001010200002000100100001001021201200000001002100210110001001210021110120000?0000

Anaxyrus boreas

0100110002000010000021000000010211010000000100210021011100100101000020012000010000

Anaxyrus cognatus

01000101120000200000010000101102120120000000100210021011010100021102011022100010000

Anaxyrus debilis

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Anaxyrus exsul

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Anaxyrus fowleri

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Anaxyrus punctatus

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Anaxyrus quercicus

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Anaxyrus terrestris

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Anaxyrus woodhousii

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B. bufo

00000100031000100000010001101102000100100010000110021121000100100000000012?00000000

Bufotes viridis

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Duttaphrynus melanostictus

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Incilius alvarius

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Incilius coccifer

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Incilius coniferus

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Incilius luetkenii

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Incilius valliceps

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Ingerophrynus macrotis

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Leptodactylus

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Melanophryniscus

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Nannophryne cophotis
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Nannophryne corynetes
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Nannophryne variegatus
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Osornophryne
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Pedostibes
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Phrynooidis juxtasper
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R. margaritifera 1
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R. margaritifera 2
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Rhaebo blombergi
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Rhaebo caeruleostictus
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Rhaebo guttatus
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Rhaebo haematiticus
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 #*Rhinella achavali*
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Rhinella amabilis
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Rhinella arenarum
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Rhinella arequipensis
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Rhinella arunco
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Rhinella atacamensis
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 #*Rhinella cerradensis*
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Rhinella chavin
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Rhinella crucifer
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Rhinella granulosa
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Rhinella humboldti
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 #*Rhinella icterica*
 02000101210200200020010000110212100110000010000001001021110100111111110022000010100
 #*Rhinella jimi*
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